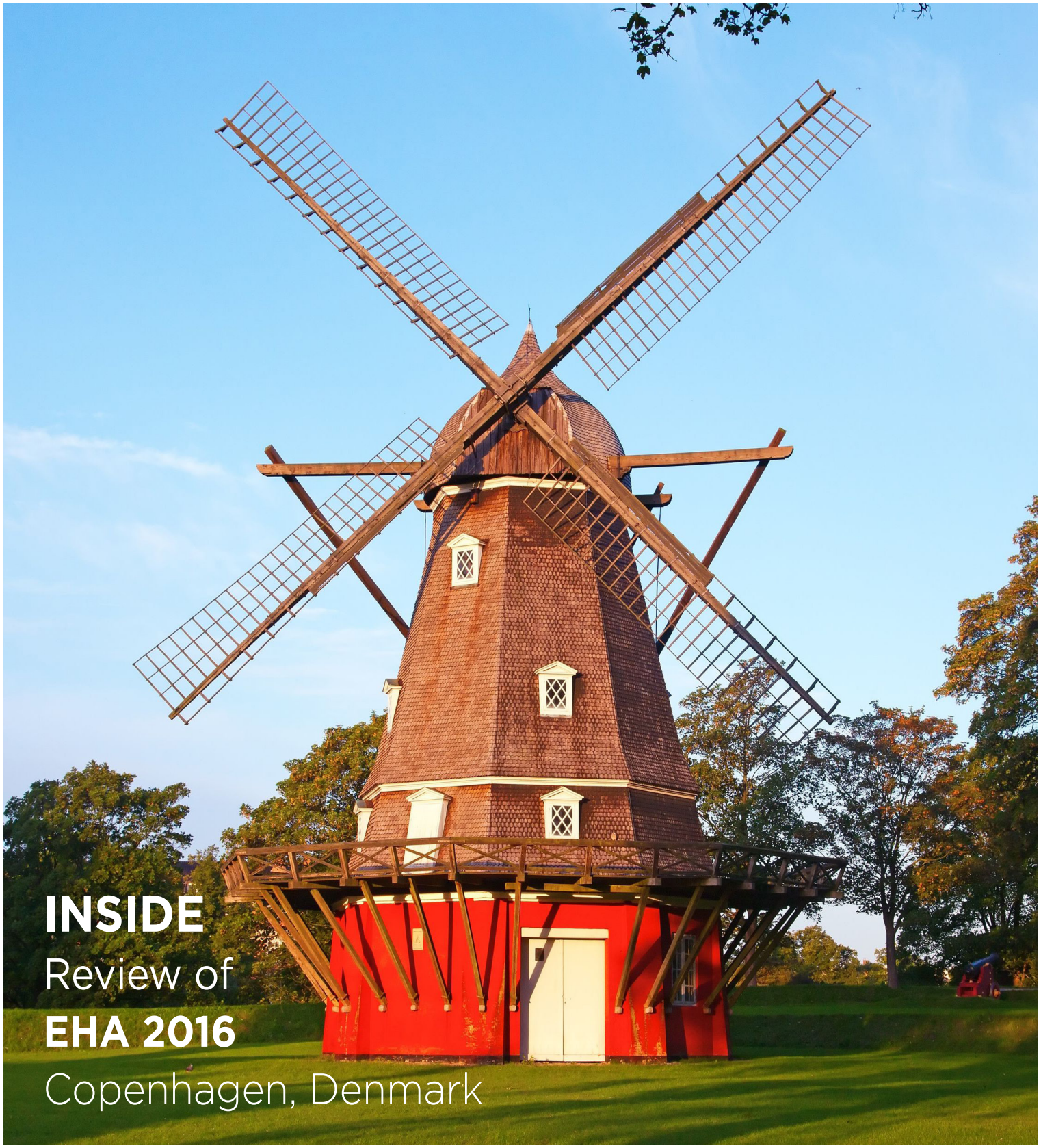
EMJ EUROPEAN MEDICAL JOURNAL

HEMATOLOGY

ISSN 2053-6631 Vol 4.1 • July 2016 • emjreviews.com



INSIDE
Review of
EHA 2016
Copenhagen, Denmark

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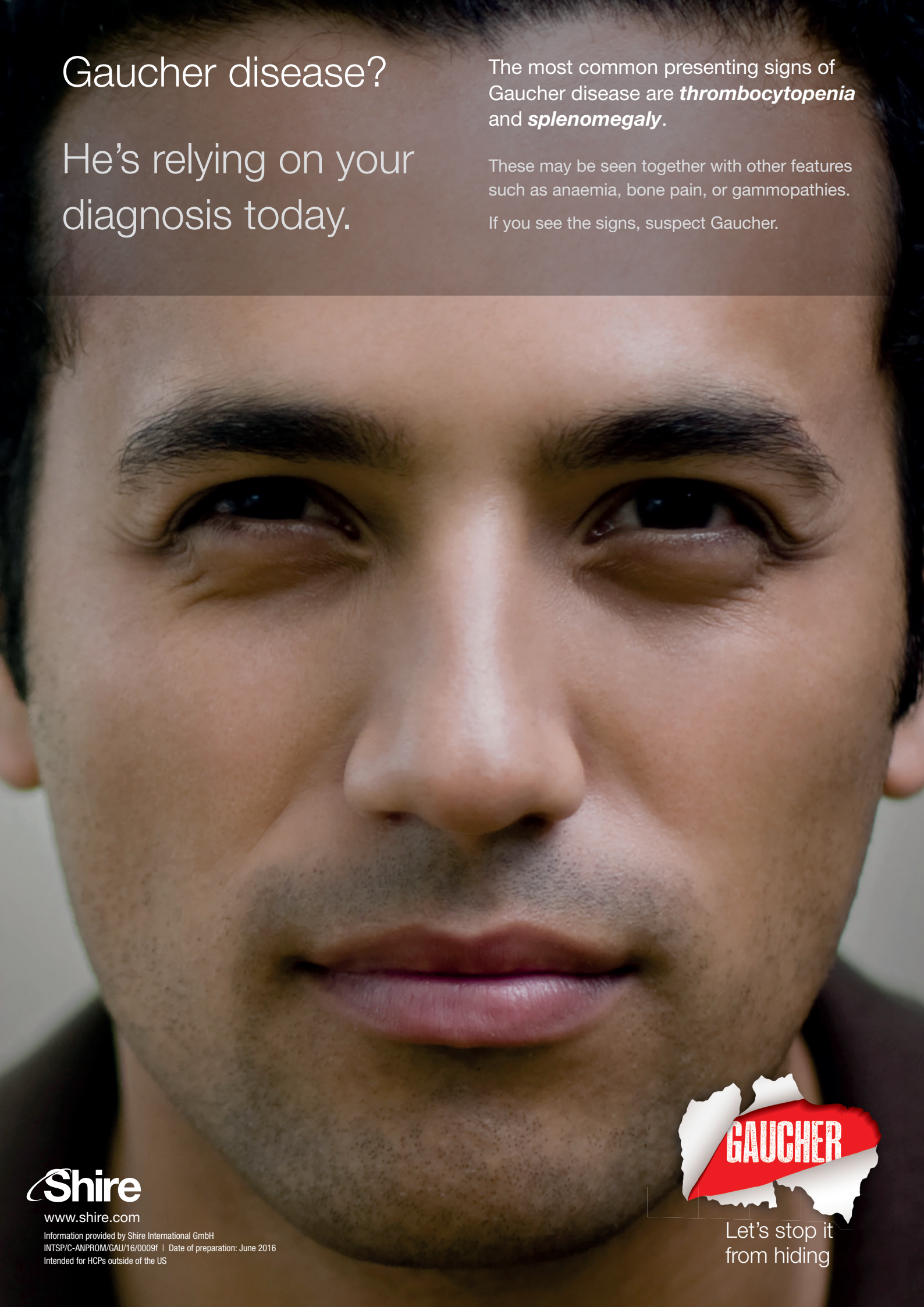
Gaucher disease?
He's relying on your diagnosis today.
The most common presenting signs of Gaucher disease are ***thrombocytopenia*** and ***splenomegaly***.
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HEMATOLOGY 4.1 | JULY 2016

Published by the European Medical Group

The MedBIC, Anglia Ruskin University, Chelmsford, CM1 1SQ

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Welcome

Welcome to this edition of our journal *European Medical Journal Hematology,* which is comprised of important haematology news, research, and breakthroughs in the form of high-quality peer-reviewed articles, insightful interviews, and a comprehensive review of one of the most important congresses of the year.

The 21st Congress of the European Hematology Association (EHA) took place in June earlier this year in the city of Copenhagen, Denmark. The event offered a substantial programme spanning many aspects of the haematology field as well as providing a welcome opportunity to interact and have discussions with experts from around the world. There was an array of research presentations, a selection of which have been summarised in our abstract reviews section.

Our coverage of the EHA 2016 congress also extends to reporting on the news of the latest research developments elsewhere in the field which were first announced at the event. The congress review section provides an informative account of these news stories. Among them are the current results from ongoing clinical trials and the use of both immunotherapy and genome sequencing to combat various blood diseases.

A collection of interviews with some of our *EMJ Hematology* board members also feature here, offering some invaluable reflections and insights from experienced and distinguished individuals working in the field.

Finally, we have a number of peer-reviewed articles which skilfully engage with and offer new insights into important areas of haematology research and practice. There is a review of the standard across Europe of service delivery to children with sickle cell disease that also highlights the lack of up-to-date and systematically collected data on the burden of this disease across the European Union. There is also a discussion of the risk of bleeding caused by direct oral anticoagulants and the approaches which can be taken by physicians to appropriately prescribe this treatment and minimise this risk. The rest of the articles included in this year's *EMJ Hematology* journal should also prompt some excellent discussion and reflection.

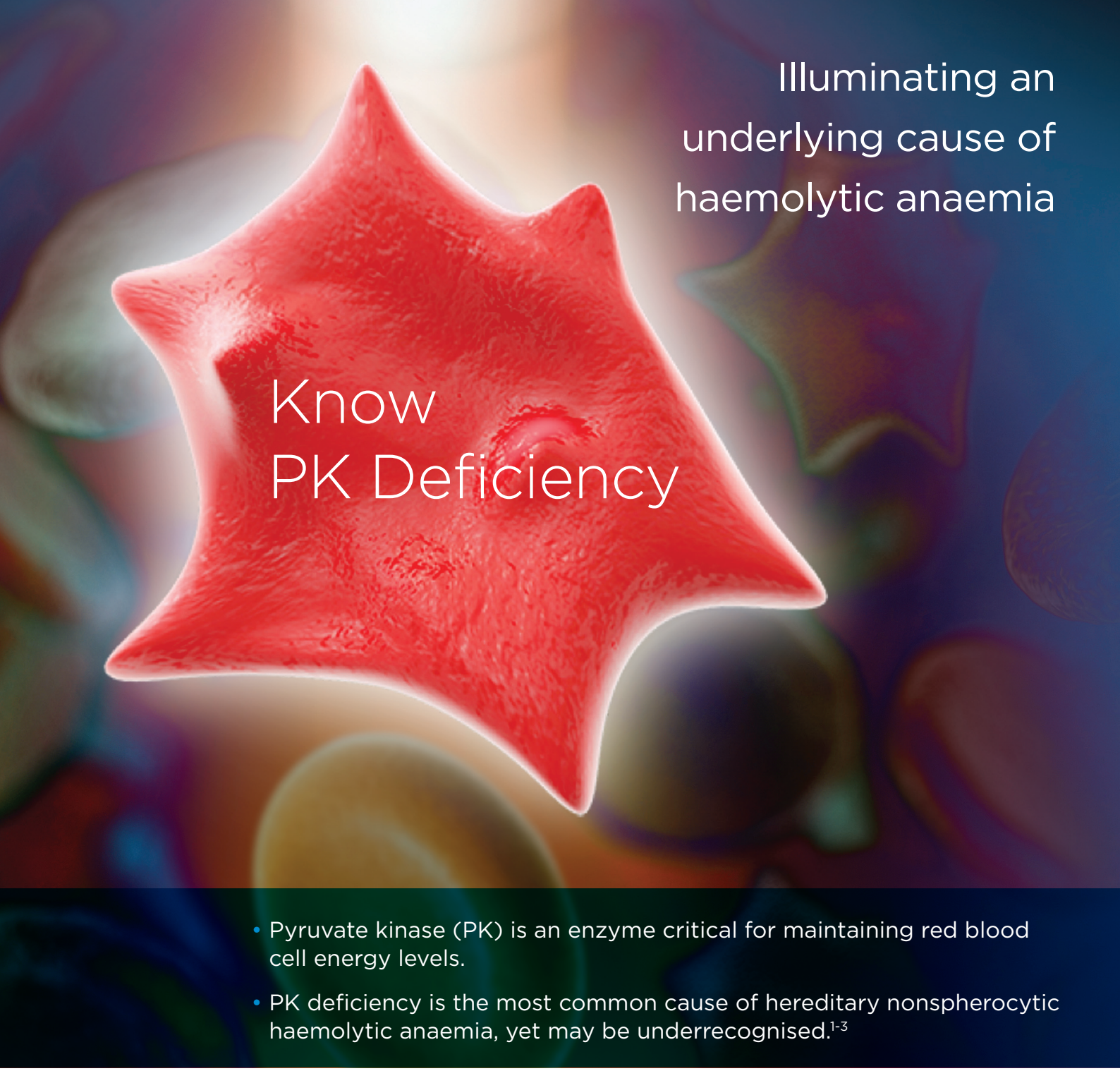
Thank you for reading *EMJ Hematology,* we hope you enjoy making your way through the journal and that you are looking forward to more discussions, development, and news from the EMJ team in next year's edition.

Spencer Gore

Director, European Medical Journal

European Medical Journal Hematology is published annually.
For subscription details please visit www.emjreviews.com

Front cover and contents photograph: Copenhagen, Denmark, home of EHA 2016.



- Pyruvate kinase (PK) is an enzyme critical for maintaining red blood cell energy levels.
- PK deficiency is the most common cause of hereditary nonspherocytic haemolytic anaemia, yet may be underrecognised.[1-3]

When to Suspect PK Deficiency

PK deficiency should be considered in patients with haemolysis and no findings suggestive of an acquired autoimmune process, red cell membrane defect, or haemoglobinopathy.[1]

Patient identification and diagnosis are taking on new importance.

Learn More at www.knowPKdeficiency.com

1. Grace RF, Zanella A, Neufeld EJ, Morton DH, Eber S, Yaish H, Glader B. Erythrocyte Pyruvate Kinase Deficiency: 2015 Status Report. Am J Hematol. 2015 Sep;90(9):825-30.

2. Zanella A, Fermo E, Bianchi P, Chiarelli LR, Valentini G. Pyruvate kinase deficiency: the genotype-phenotype association. Blood Reviews 2007: 21; 217–231.

3. Zanella A, Bianchi P. RBC pyruvate kinase deficiency: from genetics to clinical manifestations. Bailliere's Clin Haematol 2000;13:57-81.

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Foreword

Dr Emanuele Angelucci

Chairman, Hematology and Transplant Center, Ospedale Oncologico di Riferimento Regionale "Armando Businco", Cagliari, Italy.

Dear Colleagues,

Welcome to *European Medical Journal Hematology*. This issue offers a range of insightful and engaging discussions in many important areas of the haematology field. There is also a comprehensive review of the European Hematology Association (EHA) 2016 congress.

Among the articles featured in the journal, Druley explores how paediatric predisposition to leukaemia is influenced by germline genetic and epigenetic variation. This article touches on an important topic for geneticists studying paediatric leukaemia and it is valuable in highlighting new pathways for research into the disease and its treatment.

> "The journal has highlighted the important announcements and the significant research findings made at the event."

Allogeneic stem cell transplantation (alloSCT) is currently the only therapeutic approach to myelodysplastic syndrome (MDS) offering a curative option for patients. However, alloSCT is a procedure associated with a substantial risk of severe complications, presenting the persistent dilemma of gauging appropriate use. Thomas and Le Jeune discuss the latest in acute lymphoblastic leukaemia in adults. In this insightful piece they challenge a number of tricky questions in this topic.

There is also Colombatti and Sainati's timely assessment of the current management of children with sickle cell disease (SCD) across Europe. The article draws important attention to the shortcomings currently found in regards to co-ordinated action and organised data collection across varying European countries. It also offers some practical guidance to improve care in the field of paediatric SCD. This is a topic which will continue to be of fundamental importance to European haematologists in light of the increasing patient numbers caused by recent migration.

This year's EHA congress saw record-breaking success with more than 10,000 people in attendance and over 2,500 abstracts submitted. The journal has highlighted the important announcements and the significant research findings made at the event. I am confident that you will find the contents of *EMJ Hematology* to be both informative and interesting.

Emanuele Angelucci

Chairman, Hematology and Transplant Center, Ospedale Oncologico di Riferimento Regionale "Armando Businco", Cagliari, Italy.


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EHA ANNUAL CONGRESS 2016

THE BELLA CENTER,
COPENHAGEN, DENMARK
9TH-12TH JUNE 2016

Welcome to the *European Medical Journal* review of the 21st Congress of the European Hematology Association

The city of Copenhagen has been voted the go-to place for holiday makers in Denmark, and from the 9th of June and the 4 days that followed, it was without a doubt the go-to place for haematologists around the world as the 21st Congress of the European Hematology Association (EHA) 2016 took place there. The beautiful city of Copenhagen is a cultural hub with international appeal, providing the perfect environment to meet and discuss all things haematology.

The EHA congress last appeared in Copenhagen back in 2008, welcoming around 6,500 people in attendance. After its 8-year break from the city, EHA 2016 is now able to boast well over 10,000 attendees. In light of the event's popularity, the President of the EHA, Prof Tony Green, Haematology Department, University of Cambridge, Cambridge, UK, took the opportunity to express in the opening ceremony address: "We feel confident there is something for everyone." This year's congress included a record number of 2,435 submitted abstracts and 84 submitted late breaking abstracts, all highlighting the latest innovations from a vibrant community of basic, translational, and clinical researchers within the field of haematology.

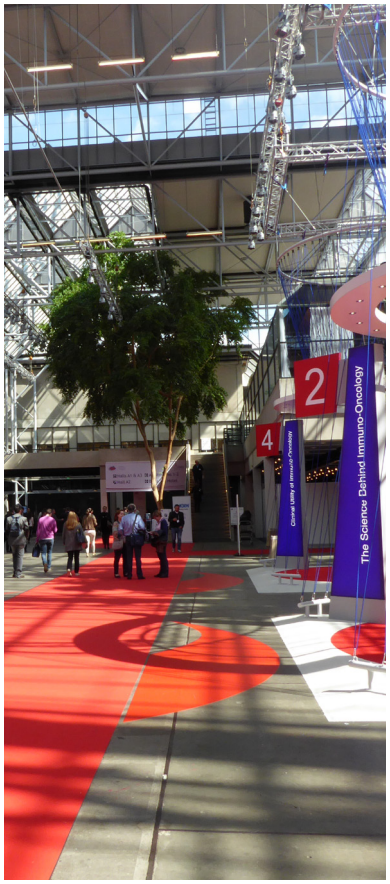
Each year, the EHA recognises important contributions made by leading haematology researchers with the José Carreras Award. This year the award was bestowed upon Prof Eva Hellström Lindberg, Sweden, in recognition of her contributions to translational and clinical research into the pathogenesis and treatment of myelodysplastic syndromes. Established in 2008, the Jean Bernard Lifetime Achievement Award honours the lifetime contributions of outstanding physicians and scientists to the advancement of haematology medicine. This year's award went to Prof Clara Camaschella, Italy, for her outstanding portfolio of work concerning the pathophysiology of inherited disorders of iron metabolism including hereditary haemochromatosis, genetic iron deficiency, and iron-loading anaemias.

Several EHA research fellowships were allocated to support researchers just beginning their careers and the winners were as follows: The John Goldman Clinical Research Fellowship: H. Poeck (Germany); the Clinical Research Fellowship:

G. Jansen (Netherlands); the Non-Clinical Junior Research Fellowship: D. Passaro (UK); the José Carreras Non-Clinical Research Fellowship: A. Nai (Italy); the Non-Clinical Advanced Research Fellowship: E. Laurenti (UK); and M. Crisan (UK). EHA President Prof Green emphasised the commitment of the organisation to the development of junior and early career researchers with the announcement of the launch of the new Clinical Research Training in Hematology programme. "This is a programme that will run throughout the coming year and is specifically targeted at young haematologists with the aim of providing them with a way of becoming specialised in the area of clinical trials," Prof Green said during the opening ceremony address.

> "This is a programme that will run throughout the coming year and is specifically targeted at young haematologists with the aim of providing them with a way of becoming specialised in the area of clinical trials."

EHA 2016 was an excellent event that brought everyone up to speed with the latest innovations and developments through its comprehensive scientific and educational programme. It brought to the forefront many of the pressing issues and promising results that have emerged from recent haematology research and practice. This includes topics such as whether leukaemia patients can ever move on from life-long tyrosine kinase inhibitor therapy, and the promising results of the high remission rates for leukaemia patients receiving the antibody drug conjugate vadastuximab talirine in a Phase I trial. These are just two amongst the many stories reported in this year's journal which reflect a brilliant year for the advancement of haematological medicine, and we are already looking forward to next year's congress, which is set to take place in the Spanish capital, Madrid.



GSGN-CD33A Antibody Drug Causing High Remissions Rates for Leukaemia Patients

SGN-CD33A (also known as vadastuximab talirine) combined with hypomethylating therapy has shown promising results for the treatment of acute myeloid leukaemia (AML) in older patients.

This is according to a EHA press release dated 11th June 2016. It reports on data from an ongoing Phase I trial evaluating the antibody drug conjugate vadastuximab talirine in concert with standard therapies (azacitidine, decitabine) in older AML patients who have declined intensive frontline therapy.

AML is an aggressive form of blood cancer which is difficult to treat in older patients because intensive chemotherapy can cause adverse effects and response rates from standard therapies are modest. SGN-CD33A is targeted to CD33, which is expressed on leukaemic blasts in nearly all AML patients, with expression remaining relatively consistent across the range of ages, risk factors, and disease characteristics. The SGN-CD33A drug conjugate is comprised of a novel antibody system stably linked to a highly potent cell-killing agent.

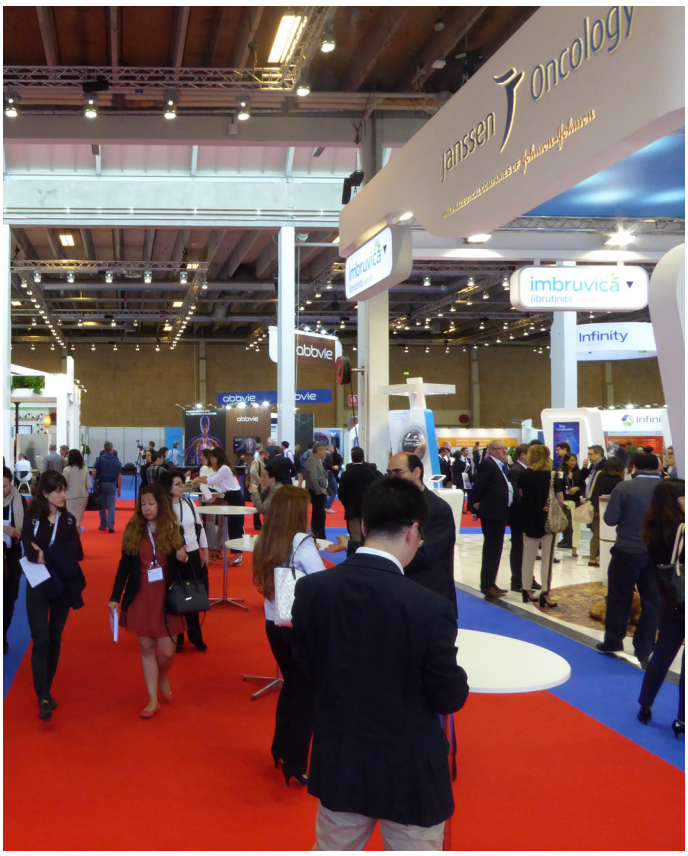
As of 11th June 2016, 53 AML patients with an average age of 75 years old have been treated with SGN-CD33A combined with either the azacitidine or the decitabine methylating agent. Efficacy evaluation was available in 49 of the patients who had received this treatment. A composite complete remission rate of 73% was measured in the group. The mortality rates were 2% at 30 days and 8% at 60 days. Grade III, or severe adverse events, occurred in at least 20% of the patients who experienced febrile neutropenia, thrombocytopenia, anaemia, and neutropenia. The combination of treatment was found to be well-tolerated by patients and yielded encouraging response rates in older AML patients, which are favourable to other hypomethylating agents in this population.

84 submitted late breaking abstracts

Results of Ongoing Gene Therapy Study for Haemophilia B Reported

RESULTS of an ongoing study have been reported evaluating the safety and tolerability of a single intravenous infusion designed to facilitate production of the Factor IX (FIX) protein, according to a EHA press release dated 11th June 2016.

Haemophilia B is a genetic bleeding disorder caused by insufficient or defective blood clotting protein FIX, which affects roughly 80,000 males worldwide. Normal clotting factor activity levels range from >40–150%. Individuals with haemophilia B have FIX activity levels of <1%. This can result in recurrent joint, muscle, and tissue bleeding, as well as life-threatening bleeding in closed spaces such as the brain.



Prophylactic FIX protein replacement has been found to be effective in patients who fully adhere to the treatment. In spite of this, the burden of once or twice weekly intravenous infusions has meant that the treatment is not yet approved for widespread use. Researchers have provided updates on an ongoing Phase I/II study which is evaluating safety and tolerability of a single intravenous infusion of the gene transfer product SPK-9001. This product has been designed to allow the body to produce its own FIX protein by transferring a functioning FIX gene into the body of the patient.

> ...patients showed FIX activity levels of 32%, 39%, 25%, and 27%, respectively. These levels are above the threshold of 12% required to reduce the risk of bleeding.

As of 11th June 2016 four participants have been followed for 7–26 weeks after a single intravenous infusion with $5x10^{11}$ vector genomes/kg of SPK-9001 without the need for immunosuppression. No serious adverse effects related to the product or procedure have been reported, including immune responses, and no patients have required steroids or other medications to suppress the immune system.

As of 22nd May 2016 patients showed FIX activity levels of 32%, 39%, 25%, and 27%, respectively. These levels are above the threshold of 12% required to reduce the risk of bleeding. The participants are no longer infusing FIX protein products and all are free from bleeding with the exception of a suspected ankle bleed in one patient 2 days after the gene transfer.

New Oral Anticoagulants Studied as Treatment for Major Bleeding

NEW oral anticoagulants (NOACs) used to treat major bleeding (MB) have been the focus of an international prospective cohort study presented at this year's EHA Congress

in Copenhagen, Denmark, according to a EHA press release dated 10th June 2016.

Death at 30 days was found to be significantly lower in patients treated with NOACs when compared with vitamin K antagonist (VKA) treatment. NOACs are presently used in clinical practice for prophylaxis of venous thromboembolism, along with prevention of stroke and systemic embolism in nonvalvular atrial fibrillation. They are associated with a favourable safety profile in Phase III randomised control trials, having lower incidences of MB compared with VKAs.

The multicentre study was performed to gauge the efficacy of NOACS against VKAs in real-life, comparing clinical presentation, management, and outcome of bleeding. The research incorporated an Italian cohort of nine hospitals and a German cohort from the Dresden NOAC registry, with a primary study outcome of in-hospital death at 30 days. Of the 874 patients hospitalised for MB, 76% of participants were treated with VKAs and 24% treated with NOACs. Incidents of MB included intracranial haemorrhage in 52% and 22% of patients on VKAs or NOACs, respectively; and gastrointestinal bleeding in 25% and 46%, respectively. Thirty-day mortality occurred in 136 patients: 17% of VKAs patients and 10% of NOAC patients.

> “The great advantage of NOACs is the reduced risk of intracranial haemorrhage, so we show that the lower mortality rate is due, not to an intrinsic capacity to reduce death between NOAC as compared with VKA patients, but to a different pattern of bleeding sites.”

Leader of the study Dr Laura Franco, Stroke Unit, Vascular and Emergency Medicine, University of Perugia, Perugia, Italy, explained that while NOAC patients have a higher rate of gastrointestinal bleeding and lower rate of intracranial haemorrhage than VKA patients, these rates differ across bleeding sites. She commented: “The great advantage of NOACs is the reduced risk of intracranial haemorrhage, so we show that the lower mortality rate is due, not to an intrinsic capacity to reduce death between NOAC as compared with VKA patients, but to a different pattern of bleeding sites.”

New Form of Invisible Inherited Thrombocytopenia Discovered

MUTATIONS in the *ETV6* gene can cause the development of a newly discovered form of inherited thrombocytopenia (IT), a EHA press release dated 10th June 2016 reported. The disorder, named ETV6-related thrombocytopenia (ETV6-RT), is characterised by a deficiency of platelets and puts patients at risk of a number of conditions.

The results of a study presented at this year’s EHA congress in Copenhagen, Denmark, which identified this particular form of IT, suggest that ETV6-RT could present in roughly 5% of patients who have been diagnosed with IT. The disorder is passed on to children if one parent possesses the faulty gene, and is particularly dangerous as its characteristics differ greatly from the recognised warning signs of IT. Firstly, it is one of a very small number of IT variants which do not cause large platelet sizes; and secondly, there is a greatly reduced occurrence of bleeding (often a mild tendency but sometimes none at all).

The risk of blood cancer is a particular concern for paediatric patients with ETV6-RT.

The risk of blood cancer is a particular concern for paediatric patients with ETV6-RT. The authors of the study noted that, together with the difficulty of diagnosis due to the aforementioned characteristics, the increased risks of developing haematological malignancies, and particularly the risk of childhood B cell acute lymphoblastic leukaemia, should not be underestimated. The authors recommend devoting more attention to detecting blood cancer than the potential bleeding complications associated with the disorder, due to the infrequency of such events, which rarely affect quality of life for patients. Their recommendation is to run genetic screening for the *ETV6* mutation in any patient who displays platelets of a normal size but who has been diagnosed with IT.

Pre-leukaemic Stem Cells may be Key to Relapse in Acute Myeloid Leukaemia

PERSISTENCE of pre-leukaemic cells after chemotherapy is associated with recurrence of acute myeloid leukaemia (AML), according to a EHA press release dated 10th June 2016. AML is an aggressive cancer; intensive chemotherapy can induce remission, however recurrence is common especially amongst older patients.

The origin of AML in some patients is a mutation in a pre-leukaemic stem cell clone, this is a stem cell with somatic mutations which can develop into cells with additional mutations or into completely normal cells. Cells with additional mutations can progress to overt AML. Although chemotherapy can induce remission from leukaemia cells, pre-leukaemic cells can remain. This research aimed to divulge whether the persistence of these pre-leukaemic stem cells after chemotherapy changes outcomes and, ultimately, whether it affects survival.

The cohort for this trial consisted of 107 patients who had entered remission during a German multicentre trial. Within these patients, 68 genes with their associated mutations were analysed at diagnosis and following treatment to see if they were cleared by chemotherapy. Remission specimens showed that, in 36% of patients, gene mutations found in leukaemia were still present, indicating the persistence of a pre-leukaemic clone. The genes found included a specific subset including: *DNMT3A*, *TET2*, *ASXL1*, and *SRSF2*. The presence of these mutations was more common in older patients.

The most important finding from this research is the fact that these persisting mutations resulted in an increased risk of disease recurrence after chemotherapy, compared with those without persisting mutations. This is a step toward understanding why so many patients relapse and how we may soon be treating them beyond the initial chemotherapy.

> “One thing that we plan to follow-up on this study, is to look at relapses in these patients and to track how often these really come from the pre-leukaemic clone.”

Dr Klaus Metzeler, University Hospital, Ludwig-Maximilians-University, München, Germany, said: “One thing that we plan to follow-up on this study, is to look at relapses in these patients and to track how often these really come from the pre-leukaemic clone. That would give us further arguments to actually treat patients who have a pre-leukaemic clone after their standard induction chemotherapy.”

Potential Alternative for Relapsed and Refractory Myeloma Patients

MYELOMA patients who have relapsed or become refractory to treatment could have access to an effective alternative following the success of the daratumumab antibody in a Phase III study.

The results of an ongoing, open-label, randomised Phase III study conducted in 18 countries and involving 569 patients have been reported in a EHA press release dated 10th June 2016. The study evaluated daratumumab, a monoclonal antibody that binds to a novel target on myeloma cells, in combination with lenalidomide and dexamethasone. This therapy was compared to a control group who received only the lenalidomide and dexamethasone. All patients involved in the study had relapsed or refractory multiple myeloma and had received at least one prior line of therapy.

Following an interim analysis, researchers found an unparalleled 63% reduction in the risk of progression or death in the group receiving daratumumab when compared to the control group. This led to an early unblinding of the study. Overall responses ($p<0.0001$) were experienced by 93% of the daratumumab group and 76% by the control group. The extent of treatment response was found to be much higher in those taking daratumumab with 76% patients experiencing a very good partial response or better ($p<0.0001$) in comparison to 44% of control patients. With regards to a complete response, more than double the number of daratumumab patients (43% versus 19%) experienced a complete response or better ($p<0.0001$).

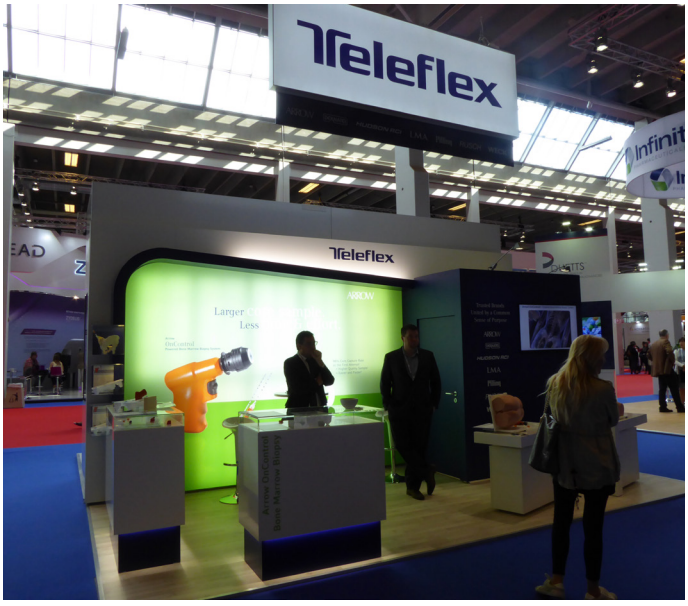
> ...the study has established a positive benefit/risk profile for daratumumab with lenalidomide and dexamethasone in multiple myeloma patients who have received at least one prior line of therapy.

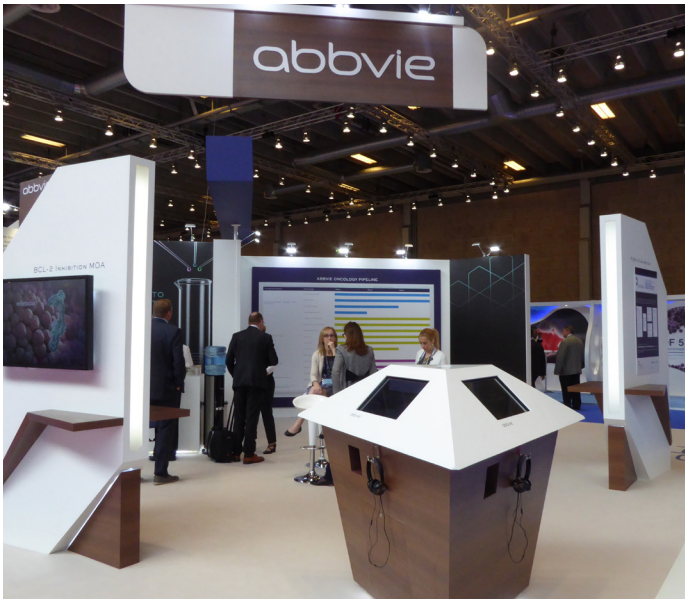
The research suggests that this combination could be an effective alternative for refractory and relapsed patients as treatment was considered to be well-tolerated by the daratumumab group with the occurrence of adverse events among patients consistent with the known profiles of these drugs. Furthermore, the study has established a positive benefit/risk profile for daratumumab with lenalidomide and dexamethasone in multiple myeloma patients who have received at least one prior line of therapy.

Immunotherapy Offers Life-Saving Approach to Leukaemia Patients

RELAPSED or refractory (r/r) patients with acute lymphoblastic leukaemia (ALL) may soon have access to a life-saving alternative treatment in the form of an immunotherapy agent. The rate of adults with ALL who achieve disease control with intense chemotherapy is as high as 90%, although only half of those responsive to the treatment will be cured. For those who are unresponsive and r/r after chemotherapy, the resulting outcome is poor, regardless of further chemotherapy or treatment such as allogenic haematopoietic stem cell transplantation. Immunotherapy,

however, offers a new treatment alternative for r/r patients by delivering antibody constructs that engage with a patient's T cells to fight ALL.





According to a EHA press release dated 10th June 2016, the results of previous Phase II trials have shown that blinatumomab, a bispecific T cell engager antibody construct, caused 43% of r/r patients with ALL to achieve disease control. It also reported on a more recent Phase III clinical trial which tested whether blinatumomab could achieve an increased survival benefit in r/r ALL patients compared to standard chemotherapy. The study involved 405 r/r ALL patients from Europe, North America, Asia, and Australia who were randomised in a 2:1 ratio to receive either blinatumomab (n=271) or a standard chemotherapy regimen (n=134). The results showed an improved overall survival of patients receiving blinatumomab.

> The results of the trial indicate that blinatumomab is the first immunotherapy agent proven to extend the life of a patient with r/r ALL when compared with chemotherapy.

The Phase II trial was stopped prematurely as the antibody treatment almost doubled the overall survival rate when compared to chemotherapy. This was witnessed in each subgroup of patients, including those who had relapsed after an allogenic haematopoietic stem cell transplantation, multiple different chemotherapy regiments, or both combined. No more severe side effects were seen after treatment with blinatumomab than chemotherapy. The results of the trial indicate that blinatumomab is the first immunotherapy agent proven to extend the life of a patient with r/r ALL when compared with chemotherapy.

Researchers Explore Effects of Leukaemia Patients Ending Life-Long Treatment

THE PROPORTION of chronic myeloid leukaemia patients maintaining a therapeutic response after stopping tyrosine kinase inhibitor (TKI) therapy has been examined in a study spanning across Europe. The study was conducted as an attempt to determine clinical and biological factors that would enable the successful ending of TKI therapy.

TKIs have significantly improved the survival rates of patients in the early chronic stages of chronic myeloid leukaemia yet this form of treatment continues for a period that spans across the lifetime of the patient. Previous research has explored the potential for patients to stop TKI therapy, revealing that 40–60% have been able to do so with a good therapy response.

According to a EHA press release dated 10th June 2016, 868 patients in 11 European countries were registered in the Phase III European Stop Tyrosine Kinase Inhibitor (EURO-SKI) study. The requirements of the study meant patients had to have at least 3 years of TKI therapy and to have had a very good response to therapy for at least 1 year prior to study entry. Patients were also required to have not failed previous TKI treatment in order to be included.

TKIs have significantly improved the survival rates of patients in the early chronic stages of chronic myeloid leukaemia yet this form of treatment continues for a period that spans across the lifetime of the patient.

The results of the study showed that 62% of the patients continued to maintain a therapeutic response 6 months after stopping the therapy. After 12 months of the therapy ending, 56% of patients continued a positive therapeutic response. The researchers also found that duration of TKI therapy as well as an encouraging treatment response prior to the end of the therapy was a predictor of whether stopping would be successful. The likelihood of a patient successfully stopping TKI therapy in relation to a patient's gender, age, or risk score was not determined by the study.

Nivolumab Benefits Patients with Relapsed Hodgkin's Lymphoma

A STUDY has been undertaken to examine the efficacy and safety of the drug nivolumab for patients with classical Hodgkin's lymphoma (cHL), according to a EHA press release dated 10th June 2016. The disease typically affects young men and women in their 30s and it is one of the most treatable types of cancer using a combination of chemotherapy and radiotherapy.

4 days

Despite this, cHL should still be a high priority, as approximately 20% of patients experience some form of relapse and will not be cured with first or second-line treatments. It hastaken over three decades for a second drug to be introduced, nivolumab, as an effective treatment for the relapse of Hodgkin's lymphoma. A few years ago, brentuximab vedotin was the first drug to be approved by the US Food and Drug Administration (FDA) for the treatment of Hodgkin's lymphoma and nivolumab is due to be the second.

> ...cHL should still be a high priority, as approximately 20% of patients experience some form of relapse and will not be cured with first or second-line treatments.

Checkmate 205, the registrational trial for the Phase II evaluation of nivolumab with cHL showed positive results. Patients with cHL, after failure of both autologous stem cell transplantation and brentuximab vedotin treatment, were treated with nivolumab. The primary endpoint of objective response rate (ORR) per an independent radiologic review committee was 66.3% and 73% by investigator response (secondary endpoint). The median response time was 2.1 months, and time of remission was 7.8 months, (95% confidence interval: 6.6 to not evaluable). At the time of analysis, 62.3% of responses were ongoing. Forty-three patients who had exhibited no response when receiving brentuximab vedotin had an impressive 72% ORR with the newer drug. The results of the study also found no significant risks of using nivolumab, as the safety profile was consistent with previously reported data of this tumour type.

Ibrutinib Performs Well in Aggressive Leukaemia Trials

POSITIVE outcomes have been observed from an integrated analysis of chronic lymphocytic leukaemia (CLL) patients treated with ibrutinib, reports a EHA press release dated 10th June 2016. CLL patients with deletion of chromosome 17p (del17p) often present with an aggressive disease and characteristically poor outcomes; survival rarely exceeds 2–3 years in patients initially treated with chemotherapy.

The European Union (EU) and USA have nuanced regulations for use of the first-in-class Bruton's tyrosine kinase inhibitor, ibrutinib, although both have approved its use for those with del17p CLL. Aiming to examine the safety and efficacy outcomes of ibrutinib in this patient population, a cross-study of three clinical trials analysed 243 individuals with del17p CLL treated with once daily ibrutinib 420 mg (n=232) or 840 mg (n=11) to the primary endpoints of progressive disease or 'unacceptable toxicity'. The median time in study was 28 months at which point overall response rate was consistent across all three trials at 84%.

With promising survival outcomes and a relatively low rate of discontinuation from adverse events the continued study of this patient group will have great significance...

Copenhagen

At a 30-month follow-up, ~55% of the participants were progression-free and 67% had survived, figures exceeding the treatment outcomes of trials investigating alternative del17p CLL therapeutic strategies. Furthermore, adverse events Grade 3 or more leading to treatment discontinuation occurred in 15% of patients (n=36). This study is ongoing, with 45% of the cohort continuing on study treatment, however it is clear from the integrated results of these clinical trials presented at EHA 2016 that ibrutinib is a potential treatment candidate for difficult-to-treat CLL populations. With promising survival outcomes and a relatively low rate of discontinuation from adverse events the continued study of this patient group will have great significance, and further clinical trials of the safety and efficacy of ibrutinib are vital.

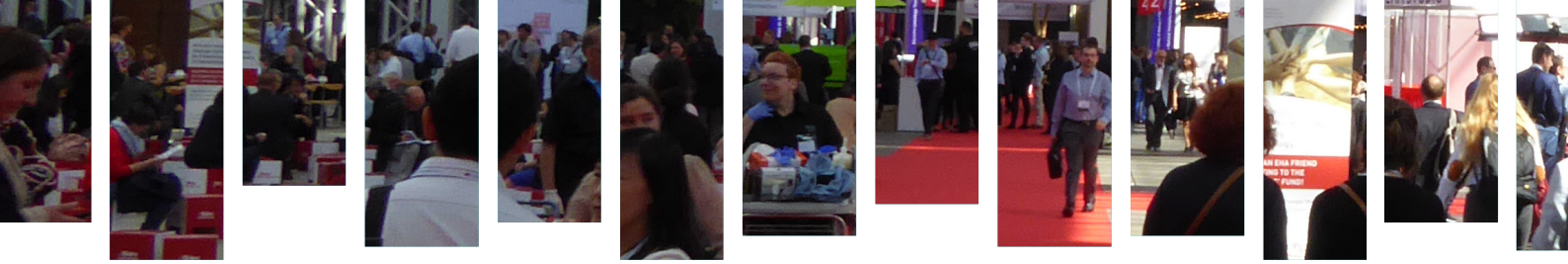
Genome Sequencing Used by Scientists to Tackle Blood Disease

RESEARCHERS around the world are currently in the process of sequencing the whole genomes of thousands of patients to identify the genetic causes of blood disorders as well as to gain insights into already known causes of some blood disorders.

Approximately three million people have a rare bleeding disorder or a disease of the platelets. Despite significant progress made in identifying the causes of many such diseases and disorders, many still remain unknown to scientists. The opportunity to generate large amounts of information to aid in future discoveries of the genetic causes of blood disorders and diseases could be a great benefit to millions of patients who experience these common life-threatening events.

According to a EHA press release dated the 11th June 2016, researchers and collaborators across the world supported by the National Institute Health Research (NIHR) BioResource for Rare Diseases in the UK are sequencing the whole genomes of thousands of patients without a genetic diagnosis. By collating the information collected from the sequencing into a research database and running computational analysis, common genetic changes can be identified, which may contribute to the disease.

Already the innovative approach has proved a success. The team have reported on the discovery of a genetic link between large platelets and deafness; a genetic change in a well-known cancer gene responsible for fragile bones; a link between scarring of bone marrow and a low platelet count; and an association between large platelets and heart rhythm problems. The team also explained that these discoveries and others have been able to benefit patients through accessible genetic testing available in the UK and elsewhere provided by thrombogenomics.org.uk.



Klaus-Michael Debatin

Chairman, Department of Paediatrics and Adolescent Medicine, University Medical Center Ulm, Ulm, Germany.

Q: You are a specialist in both haematology and paediatrics. How much does haematological practice differ between adult and paediatric populations?

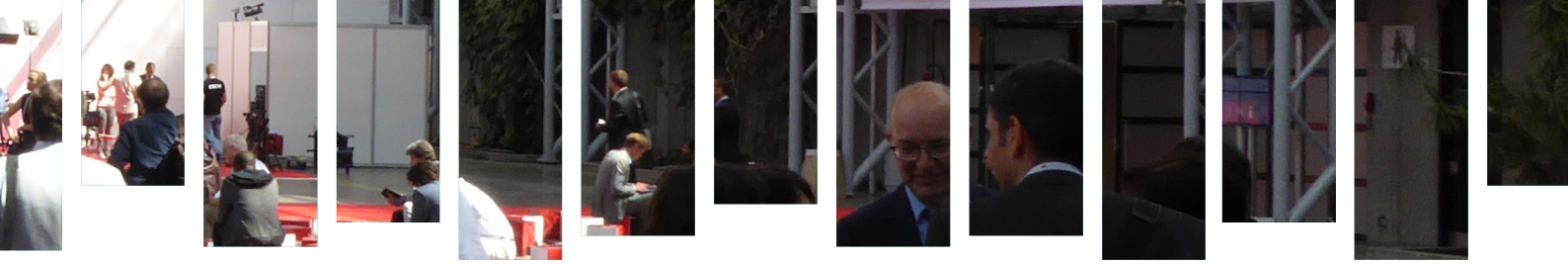
A: The spectrum of diseases is obviously different in the different age groups. Leukaemia and solid tumours have a high prevalence in the advanced age group while the frequency is low in children and adolescents, with a peak in leukaemia in early childhood. The different spectrum of disease entities is seen in leukaemia wherein childhood and adolescence acute lymphoblastic leukaemia is the most prevalent malignancy followed by Hodgkin's lymphoma and acute myeloid leukaemia. Chronic myelocytic leukaemia is rare in the young age group, while in adults, chronic myeloid leukaemia, chronic lymphatic leukaemia, and acute myeloid leukaemia are the predominant diseases in addition to a broad spectrum of different lymphomas, mostly of mature lymphoid cells. The different disease spectrum may reflect different roots of origin such as genetic susceptibility, genetic driver lesions, etc. In general, malignant disorders in childhood are highly aggressive and are unequivocally rapidly fatal in course. In addition, paediatric haematology also deals with a large variety of, in part, very rare genetic disorders with a broad spectrum of clinical phenotypes, ranging from severe combined immunodeficiency due to defects in genes of lymphoid development, to defects in immunoregulation, to bone marrow failure syndromes, and genetic causes of anaemia. The different spectrum provides an extreme challenge for individualised therapy.

> "My own area of research over the past almost 30 years has laid the basis for the development of novel therapeutic strategies..."

The enormous progress in genetic medicine provides a clear basis for understanding the diseases and developing of gene/pathway-directed therapies, both in malignant and non-malignant haematological diseases.

Q: What have been the main changes that you have witnessed since your career began in this area of medicine?

A: Over the past 30 years, the progress, especially in the treatment of childhood leukaemia has been enormous. With therapy protocols which started on the basis of trial and error, and empirical observations associated with a high burden of treatment related mortality, significant cure rates were achieved in the 1970s. Since then, treatment of childhood leukaemia and malignancies in childhood and adolescence in general has developed into one of the most significant success stories in oncology. To witness these developments directly, not only on the bedside of patients, but also on the bench of research has always been a privilege for me. The development of risk-adapted therapy protocols, where 'risk' is defined as outcome under a given therapeutic umbrella with a more and more refined risk-stratification strategy, has been the basis for this development. The identification of 'risk factors', starting from biological parameters such as age and gender, to immunophenotyping, to genotyping, to pathway-oriented translation of molecular genetic knowledge, is currently still the basis for clinical protocols that use a large extent of cytotoxic drugs that had been developed in the past without exact knowledge of their mechanism of action. Historically paediatric haematology/oncology has been concerned, in the early days, mainly with partially frustrating attempts to cure the diseases, whereas now we are more concerned



about the long-term sequelae of our patients on the background of an 80% success rate and long-term survival.

Q: What issues are currently affecting haematologists, especially those who focus on paediatric populations? What do you think could be done to address these problems?

A: In the area of malignant haematology we are particularly concerned with the long-term consequences of our successful therapies with the attempt to reduce therapy where possible and to sharpen therapeutic strategies. This includes pathway-directed approaches in areas where a cure is still not on the horizon. Genetic diseases on the other hand, to a large extent, will soon have the problem that we still cannot offer a true gene-oriented therapy, despite detailed knowledge of the genetic causes of the disease. Here, we clearly need more research on gene-correcting therapies, specifically with the knowledge that traditional approaches of gene therapy, using viral vector systems, led to a significant number of leukaemias in diseases, where haematopoietic stem cells have been gene-corrected.

Q: Your research focusses on apoptosis and tumour development. What are the latest developments in this field? Were there any particularly exciting presentations at this year's European Hematology Association (EHA) congress on current research in this area?

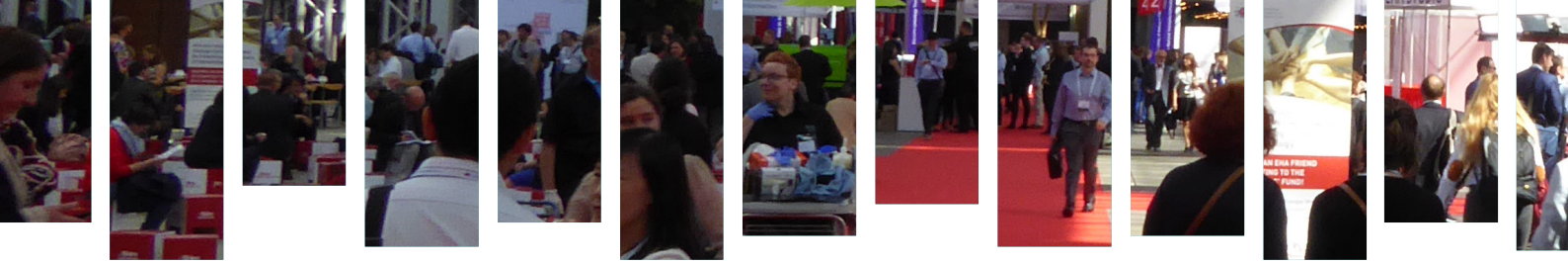
A: My own area of research over the past almost 30 years has laid the basis for the development of novel therapeutic strategies and novel compounds that specifically address cell death and survival pathways, both in tumour development as well as in therapy. These approaches include targeting anti-apoptotic *Bcl-2* family members by BH3 mimetics and the development of strategies to counteract apoptosis inhibitors. Also, *p53* is coming back into the focus as a therapeutic target by novel compounds that enable or restore deficient *p53* function. Such approaches have also been discussed in EHA meetings in the past 2 years.

Q: You have worked in Germany and the USA. How do the healthcare and research environments differ in terms of haematology between these countries?

A: I do not think that there are many differences with respect to patient treatment and outcome between Germany and the USA. As an example, Berlin-Frankfurt-Munster (BFM) protocols for treating acute lymphoblastic leukaemia developed in Germany have been, and still are, worldwide among the most successful treatment protocols and we are in good connection with our USA colleagues in balancing different treatment strategies and crossing over treatment elements that have shown superior outcome in either of these protocols. However, the difference between Germany (and Europe) and the USA is certainly in the research environment. While this is in part simply a financial issue, research budgets in the USA at comparable institutions are several fold up to 20-fold higher compared to budgets at German institutions. It is also a question of culture. Thus, the clinical researcher in the USA, although in many statements has been referred to as 'in danger' for the last few decades, is still active and many programmes at universities have a specific focus on the clinician scientist as a specific model for patient-oriented research. With the increasing knowledge in basic research related to clinical medicine, this translator position becomes even more important. Most universities in Germany have just started or built up clinical scientist programmes. In the past, like in my own career, becoming a clinical scientist has more been chance, opportunity, and individual initiative in the context of specific academic institutions or institutions funding researchers (Deutsche Forschungsgemeinschaft), than specific planning.

Q: How much progress has been made in cancer therapies in the last 10 years? What developments have had the most influence on this progress?

A: Over the past 10 years the speed of development in the -omics technologies and the discovery of genes causing specific diseases has certainly had the greatest impact on



our understanding of the nature of diseases. Incorporation of this knowledge into pathway-oriented therapies and in a deeper understanding of cell biology provide a basis for further development. However, in paediatric haematology such as in the treatment of acute lymphoblastic leukaemia, we have reached a very high level of cure rates with conventional therapy. Despite our increasing knowledge in basic research, this knowledge has not yet really translated into highly significant progress in further increasing this level. This may be partly due to the fact that identification of single-genetic lesions and single-pathway targeting by specific compounds will not solve the problem of heterogeneity of leukaemia cells and of the sheer number of deregulated pathways that occur in tumour cells. Thus, leukaemia, with rare exceptions, such as *BCR-ABL* targeting by imatinib (Glivec®) as the driver lesion, malignancies apparently need more than a driver to exhibit their malignant phenotype.

Q: How do congresses such as EHA benefit your clinical and research practice?

A: Over the years, EHA has become a central European and worldwide forum comparable (and sometimes even better) to American Society of Hematology (ASH) in discussing latest results in research and clinical practice. The quality of EHA meetings has improved greatly and the number of participants has now reached an astonishingly high level when compared to the starting years.

Q: Are there any areas in haematology which you feel are under-researched?

A: The focus on malignant haematology over the past years, has slightly put aside areas of non-malignant haematology in research and clinics, such as anaemia. However, at the moment, with a deeper understanding of the genetic causes of these diseases, the balance will swing back.

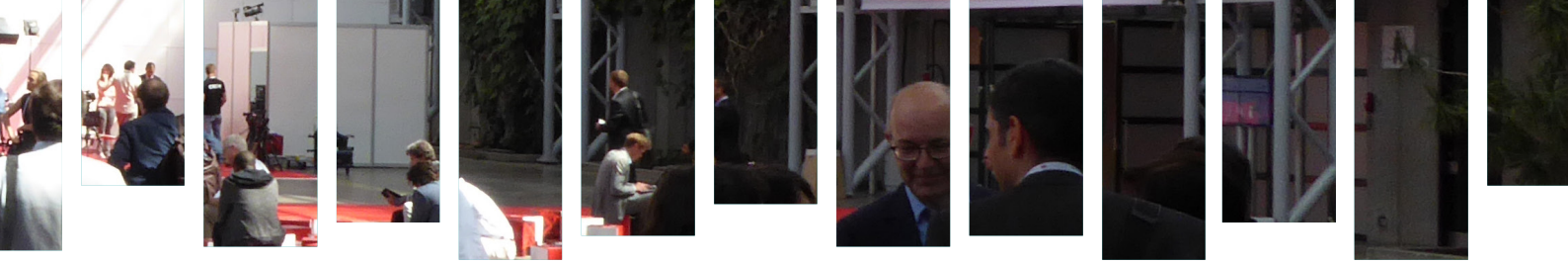
> “Anti-cancer and anti-leukaemia therapy may in fact induce cell death and this cell death is a regulated phenomenon.”

Q: What have you been most proud of in your career to date?

A: This is a really tough question. I would not say that I am proud of, but I am very glad and satisfied having had the privilege to follow a specific area of research from the very beginning over the past 30 years. When we discovered one of the key apoptosis systems, the CD95 system, we could not even spell the word ‘apoptosis’ and did not have the slightest idea that cell death is a regulated phenomenon similar to cell proliferation. At this time, in the mid-to-late 1980s, the prevailing concept in oncology was that the main characteristic of tumours is their unlimited proliferation potential. Consequently, all therapies were aimed and considered to target cell proliferation. Anti-cancer and anti-leukaemia therapy may in fact induce cell death and this cell death is a regulated phenomenon. Sensitivity or resistance of cancer and leukaemia cells may depend on their susceptibility and intactness to undergo programmed cell death or apoptosis. This was neglected because there was no concept to talk about. The focus on translation from deciphering one of the important cell death systems, to defining sensitivity and resistance of tumour cells, and more recently the development of preclinical models for leukaemia and solid tumours, is certainly what I consider my contribution to the field to be. I was always privileged to work in an area of constant development and to be part of this, both in research as well as in clinical application. This is a great satisfaction to me.

Q: Do you have advice for anyone who wishes to follow a similar career path to yours?

A: Seek and use the opportunities in research and clinical practice! Be hungry to get as much knowledge as possible on the diseases that you treat! Stay in the clinic and focus on your patients! Return to the lab and focus on your specific research and then go back to the patient! I consider this back and forth between the bench and bedside that I experienced and performed throughout my career as the most important contribution to my career path.



Ruben Mesa

Professor of Medicine, Consultant Hematologist, Chairman of the Division of Hematology and Medical Oncology, and Deputy Director, Mayo Clinic Cancer Center, Scottsdale, Arizona, USA.

Q: What attracted you to the field of haematology and oncology, and more specifically, to researching myeloproliferative neoplasms (MPNs)?

A: The tremendous unmet needs in the field of cancer is really what drew me to focus on cancer; unmet needs scientifically, in terms of understanding, in terms of new therapies, and how to best help patients. I had the opportunity to interact with patients with MPNs even in my first year of medical school at the Mayo Medical School in Rochester, Minnesota, a long-time centre of excellence for these disorders. This significant lack of understanding, unmet needs, and lack of cohesion in the MPN community attracted me greatly.

Q: How far has our understanding and treatment of MPNs improved since you first began working in this area?

A: Our understanding of MPNs has grown exponentially since I began working in this arena in 1991. Our knowledge of the molecular pathogenesis had a watershed moment with the discovery of the *JAK2 V617F* mutation in 2005. Additionally, the development of agents specifically tested for MPNs has been transformational in that in the past we would largely only be able to test agents being looked at for other disorders. Finally, we have a much greater understanding of the unmet challenges of symptom burden in these patients.

Q: What have you found to be the greatest clinical challenge facing patients with MPNs?

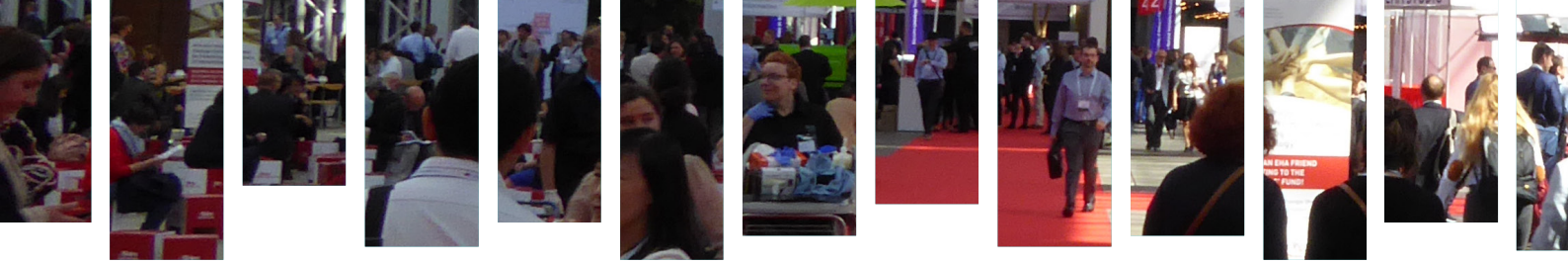
A: As we have done direct surveys of patients with MPNs, they feel that their greatest unmet need is the issue of progressive disease and the question surrounding it: how can our medical therapies in particular, which have greatly improved in their impact in preventing thrombosis, reducing splenomegaly, and improving symptoms, lead us to more definitive and longer periods of control of the disease and even disease eradication?

Q: Can you tell us more about the important role of personalised medicine in the treatment of these disorders?

A: Indeed, personalised medicine is incredibly important in heterogeneous disorders such as MPNs, which necessitate medicines to be personalised not only in terms of a very heterogeneous group of mutation profiles that may lead to alternative prognoses but also regarding how the disease affects the patient and their individual disease-related complications, symptom burden, and risk of progression.

Q: You have also highlighted the importance of allogeneic stem cell transplantation as a unique treatment for patients with a serious bone marrow disorder, myelofibrosis. As this treatment can have life-threatening side effects and is not applicable to many patients, can you speak on the future alternatives you anticipate will emerge to cure myelofibrosis?

A. I predict that the progress in myelofibrosis will be along two lines: continued improved safety of transplantation, and expansion of this potentially life-saving therapy that relies on graft versus leukaemia effect into a safer and more viable option for a greater number of patients. In parallel, I hope that further medical therapy will continue to either: 1) Control the disease indefinitely, which although not a cure would certainly be a step forward; or 2) Be able to further diminish the burden of disease for the individual so that other corresponding approaches would help eradicate the disease, whether they be based on the immune system or otherwise.



Q: What experimental treatments are you currently researching and do you expect any of these to enter clinical practice in the near future? Does it have a significant impact on the treatment of MPNs?

A: Many important therapies are in development: 1) Janus kinase inhibitors may have a more favourable impact on cytopenias, including pacritinib and momelitinib, which each have benefits in patients with thrombocytopenia and/or anaemia. I envision a situation where both of these agents would hopefully be available in the future and complement the significant benefits we have seen from ruxolitinib, in improving splenomegaly symptoms and survival; 2) PRM-151 is a particularly intriguing agent working against the fibrosing process that we hope will delay disease progression; and 3) Imetelstat is a telomerase inhibitor which has shown significant activity in early studies. Results of ongoing studies are anticipated with great interest.

Q: Have you been involved in research which has identified the impact of MPNs and sexuality and quality of life? How has this research affected patient care and do you anticipate it will affect the direction of future research in the field?

A: We currently have data on over 5,000 MPN patients from 40 countries and 15 different languages. With this cumulative information it is clear that intimacy is a major concern for patients with MPNs across the globe. Intimacy challenges include: 1) Physical limitations with intimacy, such as impotence or other issues; and 2) Intimacy challenges, which I think can be a tremendous strain for quality of life, as patients feel poorly, have pain, night sweats, and discomfort from splenomegaly. This puts a tremendous strain on intimacy as well as relationships. I am hopeful that, having identified this important need, we will be able to bring in further psychosocial support for patients with MPNs in addition to purely medical therapies, in order to better address these needs and view their care in a holistic manner.

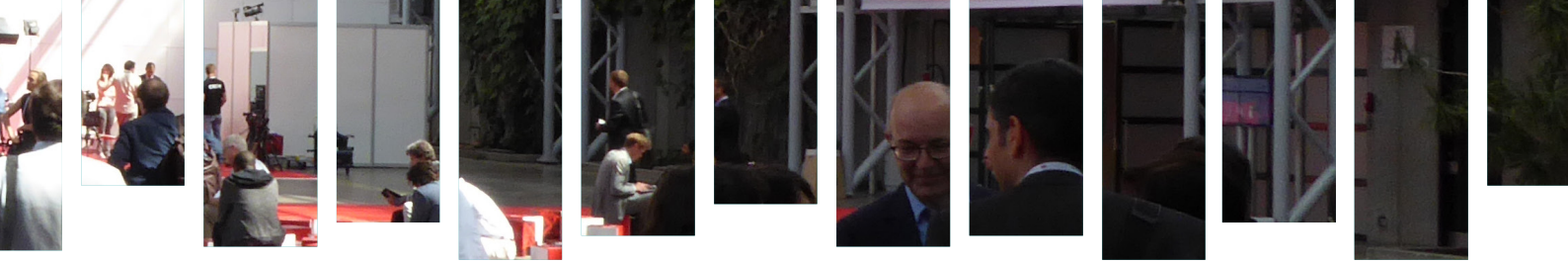
Q: What efforts are being made to raise awareness for these disorders? Could anything be improved to help patients recognise warning signs or symptoms?

A: The era of the *JAK2* mutation in particular has greatly increased the awareness about testing for MPNs amongst primary care providers, internal medicine physicians, and many others outside of the realm of haematology. I do think this has been impactful in identifying earlier cases. The disorders as a whole are uncommon enough that increasing overall awareness in the general population in regards to the signs or symptoms of MPNs would probably not be ideal, in that some of the symptoms individually can overlap with other medical disorders such as fatigue, weight loss, and night sweats. But I think it is particularly important to build awareness amongst primary care physicians and other providers who are on the frontlines of caring for patients so that when a patient has signs, symptoms, or abnormal haematologic values potentially indicative of an MPN, there is not an excess delay in their diagnosis.

Q: What advice do you have for practitioners and trainees working within the field of haematology and oncology?

A: Working in haematology and oncology is incredibly rewarding; it gives us the opportunity to learn from our patients and their loved ones as they go through an incredibly difficult journey. In parallel it is a very rewarding scientific experience in a period of tremendous advances and success. It is an emotional field for those involved and has both high points and low points that never really become easier, for example losing patients to the diseases that we have battled against together. It is important, I think, to recognise that the role of the haematologist and medical oncologist is to help patients along a difficult journey and that we hopefully leave each interaction rewarded. In some individuals we may be able to eradicate disease, in others we may not, but hopefully all patients benefit from the care that we provide.

“Working in haematology and oncology is incredibly rewarding; it gives us the opportunity to learn from our patients and their loved ones as they go through an incredibly difficult journey.”



Q: What has been the proudest achievement of your career to date? Can you tell us about your professional goals for 2016?

A: I would say the proudest achievement of my career, having been involved with many clinical trials, getting medicines registered, and caring for many patients, has been our efforts to build greater awareness regarding the burden and suffering that patients with MPNs can have as a chronic disease. Also in helping physicians around the world to be a bit more sensitive to a holistic approach and how to care for their patients. My professional goals for 2016 include trying to translate many of the efforts that we have been involved with in the clinical trial setting in ways that both become practical and helpful for individual haematology and medical oncology practices, which can be of use for the care of patients receiving standard therapies each and every day.

Emanuele Angelucci

Chairman, Hematology and Transplant Center, Ospedale Oncologico di Riferimento Regionale "Armando Businco", Cagliari, Italy.

Q: Biological therapy is proving useful in many areas of medicine; how are such therapies being implemented for patients with haematological conditions?

A: This is a difficult question. I think that biological therapy has definitely improved and treatment outcomes will further improve in many areas of medicine. In order to do this, a specific approach looking at each disease individually is necessary.

Q: In what ways has the global effort to increase the availability of treatments for haematological diseases been affected by the development of new technologies? Is the trajectory promising?

A: At the moment many of the new technologies are not widely used. However, the trajectory is promising. Special effort should be made to extend new technologies to allow patients better access. A particularly important issue is drug access in non-industrialised countries.

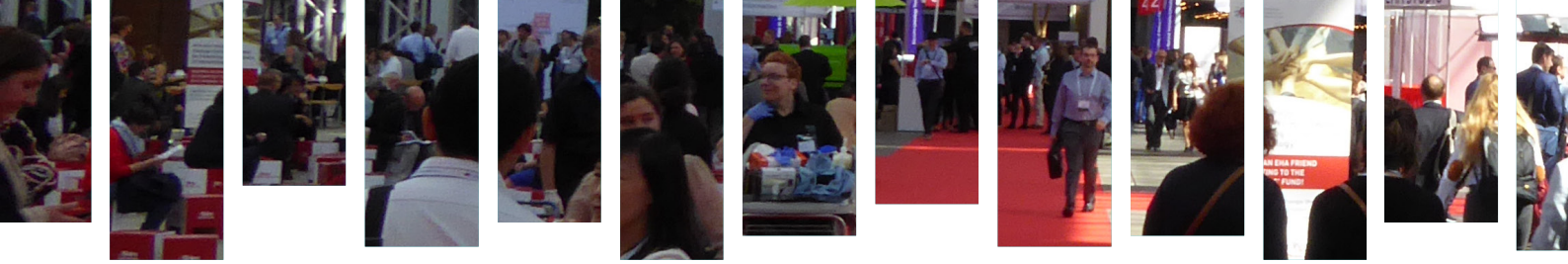
> "Genetic diseases such as thalassaemia are usually, but not always, chronic diseases which permit prolonged survival with traditional forms of treatment."

Q: Have there been any exciting developments in gene therapy in relation to your current research areas? How long before such approaches become part of daily practice?

A: Yes, indeed. Finally, after many years, gene therapy in thalassaemia seems to be close to being practical, approachable, and clinically applicable. However, two major problems still remain. Firstly, from a clinical point of view, there can be incomplete haemoglobin production after gene transfer. Secondly, there is the very high cost of the therapy itself. At the moment, the large majority of thalassaemia patients cannot approach gene therapy because of this.

Q: What are the crucial differences between genetic and acquired haematological diseases, from the point of view of the physician?

A: There are several differences that should be discussed depending on the disease. Genetic diseases such as thalassaemia are usually, but not always, chronic diseases which permit prolonged survival with traditional forms of treatment. On the other hand, acquired haematological diseases are usually, but not always, malignant diseases, mostly requiring a curative approach. Haematopoietic stem cell transplantation has changed the approach



to treating thalassaemia, introducing the idea of a cure. Gene therapy will also further change the approach.

Q: What efforts are being made to raise awareness of haematological conditions? Could anything else be done to help patients recognise warning signs or symptoms?

A: Patient associations are growing. For example, the Italian Association against Leukaemia-Lymphoma and Myeloma (AIL) is a very important association that has initiatives for raising patient awareness.

Q: In terms of the Italian healthcare system, have there been any recent developments to reduce treatment costs and improve opportunities for physicians?

A: Yes. Payment by result, cost sharing, and reimbursements have been recently introduced. The major costs in the Italian healthcare system are not the medications, as they account for no more 15% of the national healthcare system's budget. The simplification of procedures and fight against excessive bureaucracy would help reduce the cost of the healthcare system.

Q: If you could suggest one way to increase the cure rate for elderly acute leukaemia patients, what would it be?

A: At the moment I believe that the best way to treat elderly patients with acute leukaemia is to enrol patients in well designed controlled clinical trials.

Q: Are there any areas of the field of haematology that you believe are under-researched? Why is this, and how would additional research in this area benefit the field as a whole?

A: Yes there are, rare diseases and non-malignant haematology.

Q: What do you foresee as being the 'next big thing' in haematology?

A: Hopefully, to make all therapies available for any patient with a stringent clinical indication.

> "I think that biological therapy has definitely improved and treatment outcomes will further improve in many areas of medicine."

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BIOSIMILARS: SHAPING THE FUTURE IN HAEMATOLOGY

This satellite symposium took place on 10th June 2016, as a part of the 21st Congress of the European Hematology Association (EHA) 2016 in Copenhagen, Denmark

Chairperson

Robin Foà[1]

Speakers

Armando López-Guillermo,[2] Martin Schiestl,[3] Steffen Thirstrup[4]

1. Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Rome, Italy

2. Hospital Clinic, Barcelona, Spain

3. Sandoz GmbH, Kundl, Austria

4. NDA Advisory Services Ltd., London, UK

Disclosure: Prof Foà has received honoraria for advisory boards and has sat on speakers' bureaux for Roche, Genentech, Janssen Pharmaceuticals, Gilead, Sandoz Biopharmaceuticals, Pfizer, Amgen, Bristol-Myers Squibb, and Celgene. Dr López-Guillermo has received honoraria for advisory boards and has sat on speakers' bureaux for Roche, Janssen Pharmaceuticals, Gilead, Sandoz Biopharmaceuticals, Celgene, and Mundipharma. Dr Schiestl is a full-time employee of Sandoz International GmbH. Prof Thirstrup is a full-time employee of NDA Advisory Services Ltd.

Acknowledgements: Writing assistance was provided by Dr Blair Hesp CMPP of ApotheCom.

Support: The symposium was jointly sponsored by Hexal AG and Sandoz International. Authors received honoraria for preparation and delivery of their presentations. The views and opinions expressed are those of the authors and not necessarily of Hexal AG or Sandoz International GmbH.

Citation: EMJ Hematol. 2016;4[1]:30-37.

MEETING SUMMARY

Prof Robin Foà opened the symposium by highlighting how improving healthcare and an ageing population are increasing the burden on healthcare resources and creating challenges in maintaining the high level of healthcare provision that many people expect. Dr Armando López-Guillermo discussed the role of biosimilars in maintaining sustainable and affordable healthcare systems and the need to balance this against ensuring that biosimilars offer comparable efficacy and safety compared with their reference products. Dr Martin Schiestl outlined the differences in approval processes for biosimilars compared with novel biological therapies and generic versions of small-molecule drugs, and how this ensures similarity between biosimilars and their reference products. Prof Steffen Thirstrup reviewed the processes that European Union regulatory authorities undertake when deciding whether it is appropriate to extrapolate indications for biosimilars beyond a single approved indication. The meeting objectives were to discuss the role of biosimilars in meeting healthcare needs and to review what regulatory assessments biosimilars undergo prior to receiving marketing approval, and how additional extrapolated indications can be scientifically justified.

Chairperson's Introduction: How Can We Sustain Healthcare Provision?

Professor Robin Foà

Ageing societies are placing an increasing burden on healthcare resources, not only in terms of a greater number of people living longer, but declining birth rates are also resulting in populations composed of a higher proportion of older people than in the past. However, the 'biological age' of patients is also younger than it once was. For example, anyone who has lived to 70 years old can now be expected to live for another 20–25 years and these people want to maintain their quality of life as they age, which potentially means living with what may have once been a life-threatening disease that can now be managed as a chronic disease.

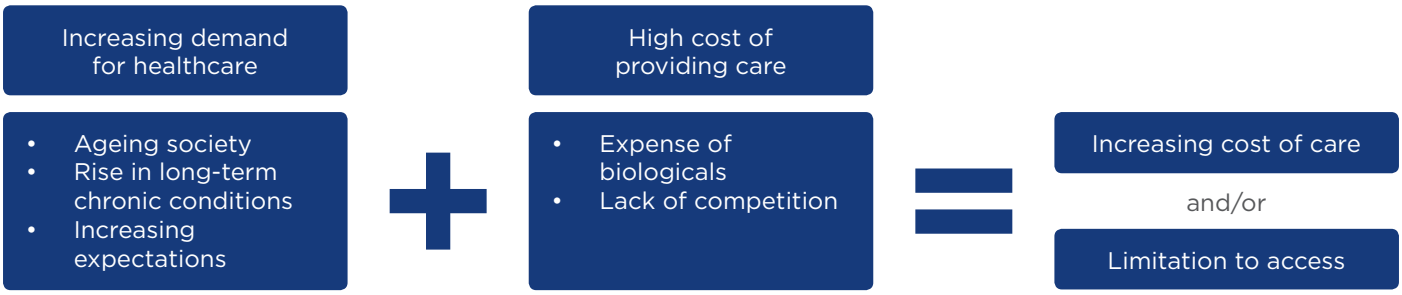

Figure 1: The challenge in sustaining healthcare provision.

Many of these diseases are managed with biological therapies that have been driven by genetic technologies that evolved from generating simple peptides in bacteria and yeasts, such as aspirin, to producing complex glycoproteins, such as monoclonal antibodies using mammalian systems. However, the costs associated with new treatments are ever-increasing and risk limiting access to treatment (Figure 1).[1] Accordingly, biosimilars, biologicals that have been shown to be highly similar to the originator or reference product in terms of the requirements for comparability, may play a role in improving access to biological treatments.

The Need for Biosimilars and the Challenge of Extrapolation: A Clinical Perspective?

Doctor Armando López-Guillermo

Biologicals have revolutionised the treatment options for many disabling and life-threatening diseases, including kidney disease, cancer, arthritis, psoriasis, and growth disorders.[2-6] The advantages of biologicals have been exemplified in haematology, where the addition of rituximab to chemotherapy in patients with B lymphoproliferative disorders has significantly improved overall survival.[7-13] Therefore, although the incidence of T cell lymphatic disorders has likely increased over the last decade, the incidence of death has decreased, which can most likely be attributed to the introduction of rituximab immunotherapy over the past few decades.[14]

However, a major limitation to the use of biologicals is their expense; biologicals account for <1% of all prescriptions, but up to 28% of prescription drug costs.[15] The rise in long-term chronic conditions treated with biologicals, alongside increasing patient expectations among ageing populations means that providing affordable and sustainable healthcare to these patients is becoming increasingly challenging.

Biosimilars may increase access to biological treatments by offering a more sustainable and affordable treatment option. Despite the affordability and comparable efficacy of biosimilars to the reference product, a primary barrier to their widespread use is clinician concerns regarding product quality, the lack of clinical and safety data, interchangeability, and extrapolated indications.[16] Although biosimilars are not exact replicas of the reference product, clinicians should understand that, before receiving marketing approval, biosimilars must undergo robust regulatory assessments and demonstrate clinical efficacy, safety, and product quality that is comparable with the original product.

Due to the complexity of monoclonal antibodies, it is not possible to generate an exact replica of a biological product.[17,18] Therefore, the goal of biosimilar development is to create a replica that is as close to the reference product as possible with no clinically meaningful differences in pharmacokinetics, pharmacodynamics, efficacy, safety, and immunogenicity.[19] To achieve this, testing of biosimilar monoclonal antibodies relies on the well-established principles of having:[19]

- An identical amino-acid sequence to the original product
- High similarity in the chemical and biophysical characteristics of the original product

Following confirmation of similarity, clinical efficacy and safety must be validated in head-to-head trials with the reference product, which are used by the European Medicines Agency (EMA) to

assess whether there are any clinically significant differences between the biosimilar and the reference product. To achieve this, validation studies must be conducted using a (preferably) double-blind, randomised, controlled trial that is adequately powered to assess non-inferiority.[20] For these studies, the most sensitive, homogenous, immunocompetent patient population should be used to detect any small product-related differences, minimise variability, and accurately assess immunogenicity.[21] Trial data for rituximab, for example, suggest that the largest effect size is seen in patients with follicular lymphoma treated with a rituximab-cyclophosphamide-vincristine-prednisone combination, making this a sensitive population for testing rituximab biosimilars.[9,22-25]

Provided a biosimilar has proven efficacious in a single, sensitive indication of the reference product, extrapolation to other approved indications of the reference product may also be justified. When considering extrapolating indications for a biosimilar, clinical experience in the indications of interest and the characteristics of the functional moieties of the biosimilar are considered against those of the reference product.[26] Additionally, the biosimilar must have the same mechanism of action for each indication as the reference product,[26] so obtaining an extrapolated indication for monoclonal antibodies with multimodal mechanisms of action can be difficult. For example, extrapolating the indication of rituximab biosimilars is challenging because different polymorphisms and mutations in B cell malignancies can influence CD20 expression, which in turn can affect affinity and response to rituximab.[27] It is also unclear whether the mechanism of action of rituximab varies depending on the lymphoma subtype or localisation. Therefore, these concerns are valid considering that there are differences in CD20 expression *in vitro*, and rituximab dose adjustment is required for different B cell malignancies, tumour burden, and phenotypes in the clinical setting.[27,28] Additionally, it is unclear whether the mechanism of action of rituximab remains the same when used in combination with chemotherapy. Therefore, in these cases additional clinical evidence may be required to support extrapolating the efficacy and safety of a rituximab biosimilar beyond its initial indication.

In conclusion, ageing populations and an increasing number of patients with chronic disease are driving a desire for increased access to biological therapies; biosimilars offer a more affordable treatment option that can increase patient access to high-quality, clinically effective therapies. Biosimilars must meet the same quality standards as novel biologicals and undergo rigorous testing before approval is granted from the EMA or US Food and Drug Administration (FDA). Although there are concerns regarding extrapolating indications beyond a single indication, each indication is systematically evaluated independently and, in the case of monoclonal antibodies with multimodal mechanisms of action, further clinical evidence is often required to justify extrapolation.

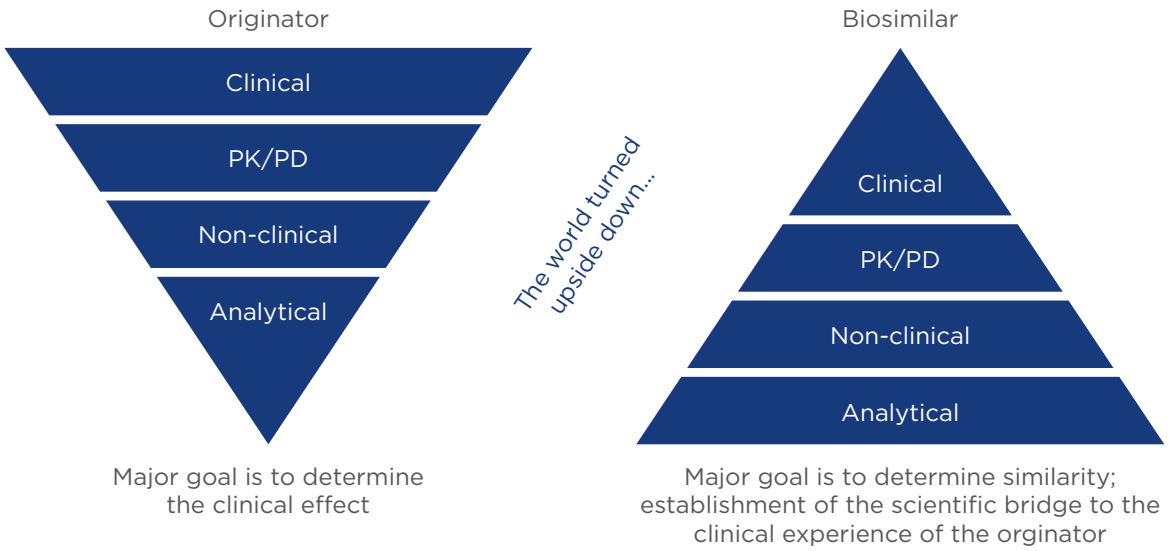

Figure 2: Comparison of the development approach for originator biologicals and biosimilars.
PD: pharmacodynamics; PK: pharmacokinetics.

The Science of Biosimilars: From Quality to Extrapolation

Doctor Martin Schiestl

Extrapolating indications for biosimilars remains controversial among clinicians. Despite regulatory bodies such as the EMA and FDA thoroughly evaluating the evidence for and against extrapolation prior to approval, some medical groups have critiqued the decisions of the EMA and expressed uncertainty about the findings, and have therefore recommended against using biosimilars in extrapolated indications.[29-31] In particular, clinician uncertainty is likely to be related to queries regarding the science behind the regulatory approval pathway for biosimilars and the way these products are developed.

Clinicians are generally familiar with the development of new products, which focusses on the clinical evidence demonstrating safety and efficacy in various indications. However, the goal in biosimilar development is to demonstrate similarity of a product containing essentially the same active ingredient as the reference product,[32] and having no clinically meaningful differences,[33] so that the clinical experience of the reference product can be applied to the biosimilar.

The most sensitive tools for demonstrating similarity are analytical methods for measuring the physicochemical and functional properties of products. Therefore, the analytical comparison sets the foundation for demonstrating biosimilarity, which is complemented by non-clinical and clinical confirmation of similarity (Figure 2).

For a biosimilar to receive marketing approval, the primary amino-acid sequence and protein folding must be identical to the reference product.[34] Other features, including post-translational modifications such as glycosylation, are required to have high similarity. However, differences are acceptable provided there is evidence to show that any differences are not clinically relevant, and it is important to note that protein glycosylation also varies from batch to batch for reference products, which is considered to be normal and is usually not problematic, provided this variability is controlled within acceptable margins.[34,35] Additionally, following approval of a reference product, it is common for the manufacturing process to be changed from time to time; for example, the cell culture media, purification methods, or manufacturing sites may be changed.[36] These changes are tightly controlled by regulatory authorities and are only approved when there is evidence that they do not result in clinically meaningful differences to the product.[37] This evidence is also based on analytical comparisons, and may be supplemented by non-clinical, or very rarely, clinical confirmation, which is then extrapolated to all indications. Therefore, the concept of extrapolation of indications for biosimilars on the basis of overall comparability is not new, given that it is already being continuously applied to reference products.

However, based on the complexity of proteins, and the many structural and functional attributes (i.e. quality attributes) that need to be compared, there is concern that minor, but clinically relevant, product differences may be overlooked. To address these concerns, each quality attribute is assessed systematically for its contribution to immunogenicity, toxicity, pharmacokinetics, and efficacy. The clinical relevance of many quality attributes of biologicals are well understood and can be assessed independently for each indication.[38-49] For example, it is well known that amino-acid sequence, protein folding, and glycosylation influence efficacy, whereas attributes such as amino-acid sequence, aggregates, host-cell proteins, and certain glycans can affect the immunogenicity of biologicals.[38-49] By understanding these features and comparing those of a biosimilar with the reference product, efficacy and immunogenicity can be controlled so that they are comparable.

Functional characterisation is also a key component in the biosimilar exercise and for elucidating structure–function relationships to increase product understanding.[50] The availability of precise bioassays allows assessment of the functional properties of biologicals and to evaluate the potential clinical impact of minor differences in certain quality attributes between the biosimilar and reference product, if they exist.[50]

As extrapolation to other indications relies on the principle of sameness between the biosimilar and the reference product, each extrapolated indication is independently evaluated on the basis of totality of available evidence and is not automatically granted for all indications of the reference product.[20,34] An extrapolated indication for a biosimilar must therefore be justified on the basis of the understanding of the mechanism of action, and the comparative analytical, non-clinical,

and clinical data between the biosimilar and the reference product.[16,26]

To conclude, for biosimilars to be approved they must undergo rigorous analytical, non-clinical, and clinical testing to establish similarity with the reference product. An extrapolated indication is systematically and independently evaluated and approved based on the totality of evidence for the biosimilar compared with the reference product.

The Evidence for Extrapolation: A Regulatory Perspective

Professor Steffen Thirstrup

As recently as 2014, both the EMA and the FDA have issued guidance on the evidence required for marketing authorisation for biosimilars,[20,34] but key regulatory requirements for assessing products, whether reference biologicals, biosimilars, or small-molecule generics, have not changed.[50] However, the type and/or amount of data required for marketing authorisation varies, depending on the type of product. The documentation required for approving a new product is considerable and extensive data documenting the product's quality (manufacturing, stability, and its controls), non-clinical safety, as well as clinical efficacy and safety must be provided for agency review. In the case of biosimilars, in addition to establishing quality, as for any other product, the manufacturer must also establish biosimilarity to the reference product in all aspects, that is, the product is as similar as current methods allow. Furthermore, clinical data must be provided in at least one indication that establishes comparable efficacy and safety with that of the reference product using sensitive endpoints. In contrast, small-molecule generics are only required to demonstrate equivalent bioavailability (bioequivalence) to claim therapeutic equivalence.[36,50]

Biosimilars are developed using a method of 'reverse engineering' as the manufacturer does not have direct access to data for the reference product at any point and, as such, a manufacturer must thoroughly analyse reference products available on the market, as well as utilise the scientific literature and general knowledge to understand how to develop cell lines and associated processes to manufacture a highly similar biological product.[50] By continually refining their manufacturing processes, biosimilar manufacturers will eventually produce a molecule that is as close to the reference product as possible.[50] Once this has been achieved, the key focus becomes characterising the biosimilar and comparing it with the reference product to provide data to support an application for marketing approval where the regulatory authorities will look to determine the overall clinical effect of the biosimilar versus the reference product.[50] The documentation supporting a request for marketing approval for a biosimilar encompasses extensive comparative detail on the physiochemical and biological properties of the biosimilar and its reference product, as well as preclinical, pharmacokinetic, and bioequivalence data. A limited amount of clinical data obtained using a sensitive endpoint in at least one indication approved for the reference product is needed to confirm no differences exist in efficacy and safety compared with the reference product.[50] Once a biosimilar has established its comparability with its reference product in at least one sensitive indication, and provided that it is scientifically justified, the biosimilar's indications can be extrapolated to all indications approved for the reference product.[50]

The regulatory concept of proving comparability is not new and is fundamental for all biologicals, as the manufacturing process for reference products can be expected to change over time as part of a normal product 'life cycle'.[36] For example, >35 changes have been made to the manufacturing process for infliximab since marketing approval was granted in 1999.[36] Even minor changes in manufacturing must undergo regulatory review, whereby the characteristics of the product pre and post-change are compared. To facilitate this, both the EMA[51] and FDA[52] have developed guidelines for demonstrating comparability following a change in manufacturing process.

These comparability guidelines are issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Committee for Medicinal Products for Human Use (CHMP) and set the data requirements for demonstrating comparability pre and post-manufacturing change for biological products, taking into account the magnitude of the change and its potential impact on the product.[51,52] This process has been labelled the 'comparability exercise', and relies on comparing physicochemical testing, biological assays, and in some cases, non-clinical and clinical data.[36]

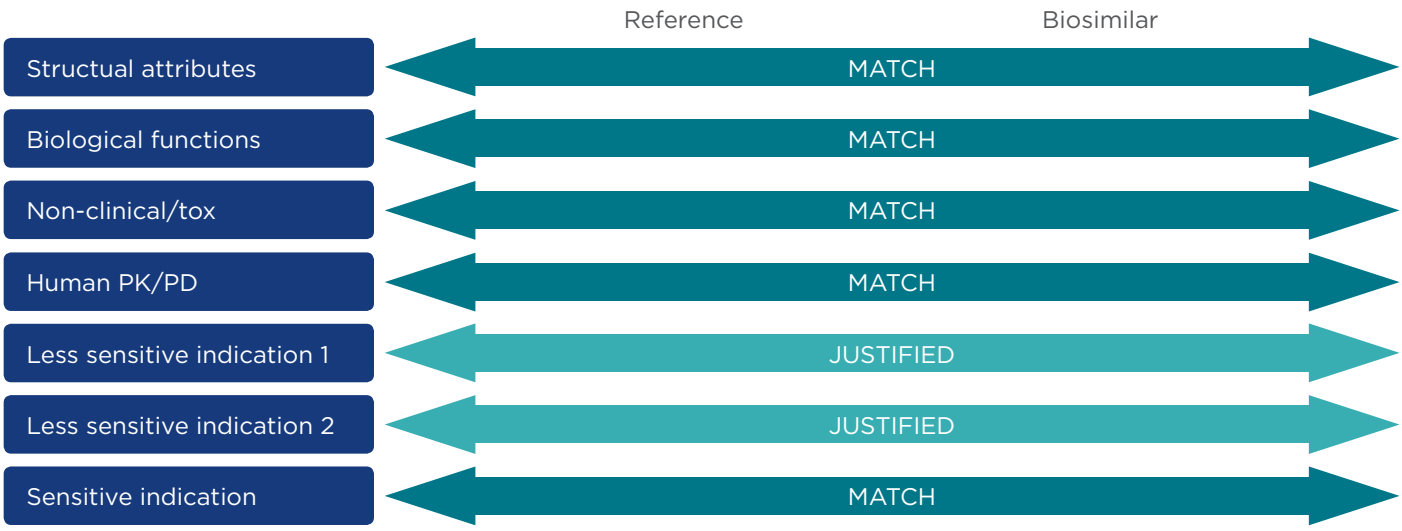

Figure 3: Key characteristics of reference products and biosimilars that are assessed during the regulatory approval process.[16,26,54]

PK: pharmacokinetics; PD: pharmacodynamics; tox: toxicity.

The ICH stipulates that comparability does not mean that these attributes are identical pre and post-change, but that they are "highly similar" and that any differences will have no impact on product efficacy or safety.[51] For example, following a manufacturing change to the rituximab reference product, which resulted in changes in glycosylation and a subsequent increase in antibody-dependent cellular toxicity potency, on assessment, regulators concluded that the benefit-to-risk profile of the product was unchanged based on the totality of evidence available through pre and post-change characterisation, and no further clinical data were required.[53]

The same scientific principles apply to the generation of a biosimilar. One of the key questions from regulators is what clinical data is needed to show comparability and provide evidence to support an extrapolated indication. A regulatory authority will review the totality of evidence gained from the comparability exercise, and assess whether safety is clearly demonstrated, an increase in immunogenicity has been excluded, and if there are any remaining uncertainties, and then identify where further data is needed.

The regulatory authorities are guided by several key principles when assessing the scientific justification for an extrapolated indication, including:

- Clinical experience with the reference product
- Established comparability
- Any differences in mechanism of action or safety (including immunogenicity) between indications
- Target receptors

An extrapolated indication is only likely to be granted for a similar or less sensitive indication or population, and this decision must be based on available clinical data (Figure 3).[26]

Therefore, extrapolating an indication is not a new process,[26] and regulatory authorities have developed rigorous and scientific guidelines for assessing the comparability of therapeutic molecules that are applicable across various scenarios from a change in manufacturing process to the generation of a biosimilar.[20,34,51,52] Extrapolation involves an extensive comparability exercise and regulatory authorities follow a key set of principles to establish stringent scientific justification before an extrapolated indication will be approved.[26]

REFERENCES

1. Baer II WH et al. Barriers to the access and use of rituximab in patients with non-Hodgkin's lymphoma and chronic lymphocytic leukemia: A physician survey. Pharmaceuticals (Basel). 2014;7(5): 530-44.

2. Cawson MR et al. Systematic review, network meta-analysis and economic evaluation of biologic therapy for management of active psoriatic arthritis. BMC Musculoskelet Disord. 2014;15:26.

3. Dutka P. Erythropoiesis-stimulating agents for the management of anemia of chronic kidney disease: past advancements and current innovations. Nephrol Nurs J. 2012;39(6):447-57.

4. Hwang IT. Efficacy and safety of growth hormone treatment for children born small for gestational age. Korean J Pediatr. 2014;57(9):379-83.

5. Nam JL et al. Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2013 update of the EULAR recommendations for the management of rheumatoid arthritis. Ann Rheum Dis. 2014;73(3):516-28.

6. Waller CF. Biosimilars and their use in hematology and oncology. Commun Oncol. 2012;9:198-205.

7. Habermann TM et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006; 24(19):3121-7.

8. Pfreundschuh M et al. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol. 2008; 9(2):105-16.

9. Coiffier B et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040-5.

10. Pfreundschuh M et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. Lancet Oncol. 2011;12(11):1013-22.

11. Marcus R et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008;26(28):4579-86.

12. Abrahamsson A et al. Real world data on primary treatment for mantle cell lymphoma: a Nordic Lymphoma Group observational study. Blood. 2014; 124(8):1288-95.

13. Hallek M et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010;376(9747): 1164-74.

14. SEER Cancer Statistics. Available at: http://seer.cancer.govt/statfacts/html/nhl.html. Last accessed: 24 June 2016.

15. Sarpatwari A et al. Progress and hurdles for follow-on biologics. N Engl J Med. 2015;372(25):2380-2.

16. Weise M et al. Biosimilars: What clinicians should know. Blood. 2012; 120(26):5111-7.

17. Revers L, Furczon E. An introduction to biologics and biosimilars. Part I: Biologics: What they are and where do they come from? Can Pharm J. 2010;143(3):184-91.

18. Reichert JM et al. European Medicines Agency workshop on biosimilar monoclonal antibodies: July 2, 2009, London, UK. MAbs. 2009;1(5):394-416.

19. Rioufol C, Sallies G. Biosimilar monoclonal antibodies in lymphoma: a critical appraisal. Expert Rev Anticancer Ther. 2015;15(5):569-78.

20. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. 2014. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/01/WC500180219.pdf. Last accessed: 24 June 2016.

21. McCamish M. EBG's perspective on draft guidance on clinical/non-clinical issues. 2016. Available at: http://www.ema.europe.eu/docs/en_GB/document_library/Presentation/2013/11/WC500154186.pdf. Last accessed: 2 June 2016.

22. Hiddemann W et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106(12):3725-32.

23. Robak T et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol. 2010;28(10):1756-65.

24. Rugo HS et al. A clinician's guide to biosimilars in oncology. Cancer Treat Rev. 2016;46:73-9.

25. Golay J et al. Lessons for the clinic from rituximab pharmacokinetics and pharmacodynamics. MAbs. 2013;5(6): 826-37.

26. Weise M et al. Biosimilars: the science of extrapolation. Blood. 2014;124(22): 3191-6.

27. Cartron G et al. Interindividual variability of response to rituximab: from biological origins to individualized therapies. Clin Cancer Res. 2011;17(1): 19-30.

28. Cartron G et al. From the bench to the bedside: ways to improve rituximab efficacy. Blood. 2004;104(9):2635-42.

29. Danese S, Gomollon F; Governing Board and Operational Board of ECCO. ECCO position statement: the use of biosimilar medicines in the treatment of inflammatory bowel disease (IBD). J Crohns Colitis. 2013;7(7):586-9.

30. Argüelles-Arias F et al. Joint position statement by "Sociedad Española de Patología Digestiva" (Spanish Society of Gastroenterology) and "Sociedad Española de Farmacología" (Spanish Society of Pharmacology) on biosimilar therapy for inflammatory bowel disease. Rev Esp Enferm Dig. 2013;105(1):37-43.

31. Shaw BE et al. Concerns about the use of biosimilar granulocyte colony-stimulating factors for the mobilization of stem cells in normal donors: position of the World Marrow Donor Association. Haematologica. 2011;96(7):942-7.

32. European Commission. What you need to know about biosimilar medicinal products. 2013. Available at: http://ec.europa.eu/DocsRoom/documents/7618/attachments/1/translations/en/renditions/pdf. Last accessed: 6 July 2016.

33. Section 7002(b)(3) of the US Affordable Care Act, adding section 351(i)(2) of the PHS Act.

34. US Food and Drug Administration. Guidance on scientific considerations in demonstrating biosimilarity to a reference product: guidance for industry (April 2015). 2015. Available at: http://www.fda.gov/downloads/DrugsGuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf. Last accessed: 24 June 2016.

35. Shiestl M et al. Acceptable changes in quality attributes of glycosylated

biopharmaceuticals. Nat Biotechnol. 2011; 29(4):310-2.

36. Schneider CK. Biosimilars in rheumatology: the wind of change. Ann Rheum Dis. 2013;72(3):315-8.

37. Windisch J. EGA's perspective on the draft quality guideline. 2013. Available at: http://www.ema.europe.eu/docs/en_GB/document_library/Presentation/2013/11/WC500154191.pdf. Last accessed: 18 March 2016.

38. Brocchini S et al. Disulfide bridge based PEGylation of proteins. Adv Drug Deliv Rev. 2008;60(1):3-12.

39. Chung CH et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med. 2008;358(11):1109-17.

40. Dashivets T et al. Multi-angle effector function analysis of human monoclonal IgG glycovariants. PLoS One. 2015;10(12): e0143520.

41. Goetze AM et al. Assessing monoclonal antibody product quality attribute criticality through clinical studies. MAbs. 2010;2(5):500-7.

42. Kennedy DM et al. Glycation of monoclonal antibodies impairs their ability to bind antigen. Clin Exp Immunol. 1994;98(2):245-51.

43. Liu H, May K. Disulfide bond structures of IgG molecules: structural variations, chemical modifications and possible impacts to stability and biological function. MAbs. 2012;4(1):17-23.

44. Markovic I. Evaluation of safety and quality impact of extractable and leachable substances in therapeutic biologic protein products: a risk-based perspective. Expert Opin Drug Saf. 2007; 6(5):487-91.

45. Rathore N, Rajan RS. Current perspectives on stability of protein drug products during formulation, fill and finish operations. Biotechnol Prog. 2008;24(3):504-14.

46. Ripple DC, Dimitrova MN. Protein particles: what we know and what we do not know. J Pharm Sci. 2012;101(10): 3568-79.

47. Wang X et al. Host cell proteins in biologics development: Identification, quantitation and risk assessment. Biotechnol Bioeng. 2009;103(3):446-58.

48. Weber CA et al. T cell epitope: friend or foe? Immunogenicity of biologics in context. Adv Drug Deliv Rev. 2009; 61(11):965-76.

49. Wright A et al. Antibody variable region glycosylation: position effects on antigen binding and carbohydrate structure. EMBO J. 1991;10(10):2717-23.

50. McCamish M, Woollett G. Worldwide experience with biosimilar development. mAbs. 2011;3(2):209-17.

51. European Medicines Agency. Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process, non-clinical and clinical issues 2007. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003935.pdf. Last accessed: 22 June 2016.

52. US Food and Drug Administration (FDA). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonised tripartite guideline: Comparability of biotechnological/biological products subject to changes in their manufacturing process Q5E 2003. Available at: http://www.fda.gov/OHRMS/DOCKETS/98fr/2004d-0118-gdl0001.pdf. Last accessed: 22 June 2016.

53. Schiestl M et al. Acceptable changes in quality attributes of glycosylated biopharmaceuticals. Nat Biotechnol. 2011; 29(4):310-2.

54. Ebbers HC. Biosimilars: in support of extrapolation of indications. J Crohns Colitis. 2014;8(5):431-5.

ADVANCES IN THE TREATMENT OF NON-HODGKIN'S LYMPHOMA: EXPLORING NEW FRONTIERS

This symposium took place on 9th June 2016, as a part of the 21st Congress of the European Hematology Association (EHA) 2016 in Copenhagen, Denmark

Chairperson

Eva Kimby[1]

Speakers

Eva Kimby,[1] Georg Lenz,[2] Catherine Thieblemont,[3] Pier Luigi Zinzani[4]

1. Hematology Center, Department of Internal Medicine, Karolinska Institute, Stockholm, Sweden

2. Universitätsklinikum Münster, Münster, Germany

3. Hôpital Saint-Louis, Paris, France

4. Institute of Haematology, "L. e. A. Seràgnoli", University of Bologna, Bologna, Italy

Disclosure: Prof Kimby is on advisory boards for Celgene, Pharmacyclics, Janssen, Baxalta, Gilead, and AbbVie and has received research support from Roche and Pfizer. Prof Lenz: Gilead (advisory board, invited speaker), Janssen (advisory board, invited speaker, grant support), Celgene (advisory board, invited speaker, grant support), Roche (advisory board, invited speaker), NanoString (advisory board). Prof Thieblemont has been an expert advisory board member for Janssen, Roche, Celgene, Gilead, Bayer. Prof Zinzani has been a speaker for Celgene, Gilead, Takeda, BMS, Janssen. Has received Honoraria from Celgene, Gilead, BMS, Jannssen, Pfizer, TG Pharmaceuticals, Roche, BMS, Bayer and has served on advisory boards for Celgene, Gilead, BMS, Janssen, Pfizer, TG Pharmaceutical, Roche, and Takeda.

Acknowledgements: Writing assistance was provided by Dr Tim Ibbotson of ApotheCom.

Support: The publication of this article was funded by Celgene. The views and opinions expressed are those of the authors and not necessarily Celgene.

Citation: EMJ Hematol. 2016; 4[1]:38-46.

MEETING SUMMARY

A recent symposium at the European Hematology Association (EHA) congress, chaired by Prof Eva Kimby, explored the changing paradigms in the treatment of non-Hodgkin's lymphoma (NHL) and the potential impact of new approaches to diagnosis and treatment. Prof Kimby opened the symposium by discussing the recent therapeutic advances in the treatment of follicular lymphoma (FL). Prof Georg Lenz then spoke about the clinical implications of diffuse large B cell lymphoma (DLBCL) diagnosis and the manner in which disease subtyping can foster effective use of targeted therapies. Prof Catherine Thieblemont presented on post-induction treatment in DLBCL, and the importance of effective treatment options to limit the number of patients who fail first-line therapy. Prof Pier Luigi Zinzani then concluded the symposium by presenting data on the new immuno-oncology treatments being evaluated in patients with relapsed or refractory NHL.

Therapeutic Advances in Follicular Lymphoma

Professor Eva Kimby

The therapeutic management of FL is complicated by the disease heterogeneity, as evidenced by the differences in histological grading, prognostic factors (such as the FL International Prognostic Index [IPI], FLIPI2, and m7-FLIPI),[1-3] and the blood and bone microenvironment, which can all vary widely between patients. Response to a specific treatment is also variable, as is the pattern of relapse. The cumulative effects of therapy such as cardiotoxicity, and the risk of transformation have to be considered when choosing therapy. IPIs are useful for predicting disease outcome at group level, but cannot identify a patient at low-risk

of disease progression, in whom the benefits of immediate induction therapy are unclear. Indeed, data from the British National Lymphoma Investigation (BNLI) indicate that a 'watchful waiting' approach in low-risk patients is associated with a median time-to-initiation of chemotherapy of 2.6 years, and that 19% of patients still do not require chemotherapy after 10 years.[4] More recent data also support the watchful waiting approach, with similar overall survival (OS) in patients receiving rituximab induction with maintenance, and those who undergo watchful waiting.[5]

The importance of rituximab maintenance in patients who show an initial response to first-line rituximab plus chemotherapy is well established.[6] Data from the PRIMA trial[7] indicated that only 18% of patients will have disease progression during an initial 2-year rituximab maintenance therapy period and that 75% of patients receiving rituximab-based maintenance therapy will remain progression-free at 3 years, compared with 58% of those undergoing observation (stratified hazard ratio: 0.55; 95% confidence interval: 0.44–0.68; p<0.0001). Although these benefits of rituximab are well established in patients with FL in the first-line setting, there remains substantial debate regarding the most effective accompanying chemotherapy regimen. A comparison of rituximab in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), CVP (cyclophosphamide, vincristine, and prednisone), or FM (fludarabine and mitoxantrone) indicates that rituximab-CHOP (R-CHOP) and rituximab-FM (R-FM) may yield an improved time-to-treatment failure compared with rituximab-CVP, but also that R-FM is associated with greater toxicity.[8] Whether bendamustine plus rituximab offers an alternative to R-CHOP is unclear, although some data suggest that rituximab-bendamustine is associated with quality-of-life benefits and an improved toxicity profile.[9,10] It is also unclear if chemotherapy is required in all patients, with data from Phase II and III trials suggesting that single-agent rituximab may also represent an alternative approach, albeit with a relatively short progression-free survival (PFS) of only 20–30 months.[11,12] This is in contrast to the 3.5–7.4 years median PFS reported for patients on rituximab maintenance for 1–5 years.[12] The probability of long-term survival following single-agent rituximab therapy is 87–89%, as observed in the SAKK35/03, PRIMA, and Nordic trials, respectively.[7,11]

There is also evidence supporting the combination use of rituximab with the immunomodulatory agent, lenalidomide,[13] in patients with untreated indolent lymphoma. In an open-label Phase II study, patients with FL receiving rituximab plus lenalidomide (R2) achieved a response rate of 98%, a complete response (CR) rate of 87%, and >80% remained progression-free at 5 years.[13] These data supported the subsequent initiation of the Phase II randomised R2-trial SAKK 35/10, in which rituximab was administered alone or in combination with lenalidomide to patients with untreated FL (n=77, each).[14] At Week 23, overall response rates (ORR) were 82% in the combination arm, compared with 61% in the monotherapy arm (p=0.002), and with CR rates of 36% and 25%, respectively. An ongoing international study[15] comparing first-line R2 versus rituximab-chemotherapy in >1,000 patients will provide further insight into the benefits of the R2 combination also in maintenance therapy.

New therapeutic agents are undergoing evaluation in patients with relapsing or rituximab-refractory FL (Figure 1). The GADOLIN study in patients with rituximab-refractory FL assessed the combination of bendamustine with obinutuzumab followed by obinutuzumab maintenance therapy versus bendamustine alone without any maintenance.[16] Patients receiving the combination had improved PFS compared with those receiving bendamustine alone (median PFS not reached versus 14.9 months, p=0.0001) and there was evidence of benefits associated with obinutuzumab maintenance therapy compared with no maintenance therapy. Other new agents currently being studied in patients with relapsing or refractory disease are the PIK3-delta inhibitor, idelalisib, which has shown very high response rates in patients with refractory FL.[17] Later trials of combinations of idelalisib with rituximab or bendamustine plus rituximab were halted due to toxicities.[18] Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor that has shown robust clinical activity when combined with rituximab in treatment-naïve patients.[19] The BCL2 inhibitor, venetoclax, could be of particular interest in FL patients because of their over-expression of the BCL2 antiapoptotic protein.[20] Checkpoint inhibitors such as the humanised anti-monoclonal antibodies, pidilizumab and nivolumab (PD1 inhibitors), and durvalumab (a PD-L1 inhibitor), represent another exciting class of immunotherapy undergoing evaluation in patients with FL.

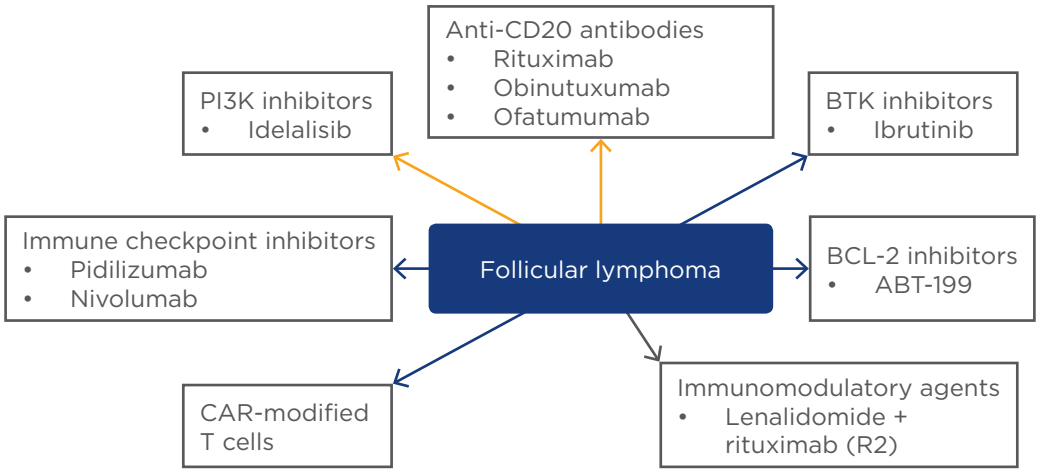

Figure 1: Novel and upcoming non-cytotoxic treatments* for patients with follicular lymphoma.
*Rituximab is approved as a single agent and in combination with chemotherapy for initial and maintenance treatment of follicular CD20-positive non-Hodgkin's lymphoma. Idelalisib is approved for relapsed/refractory follicular lymphoma. All other agents listed in Figure 1 are investigational.
BTK: Bruton's tyrosine kinase; CAR: chimeric antigen receptor; BCL-2: B cell lymphoma-2.

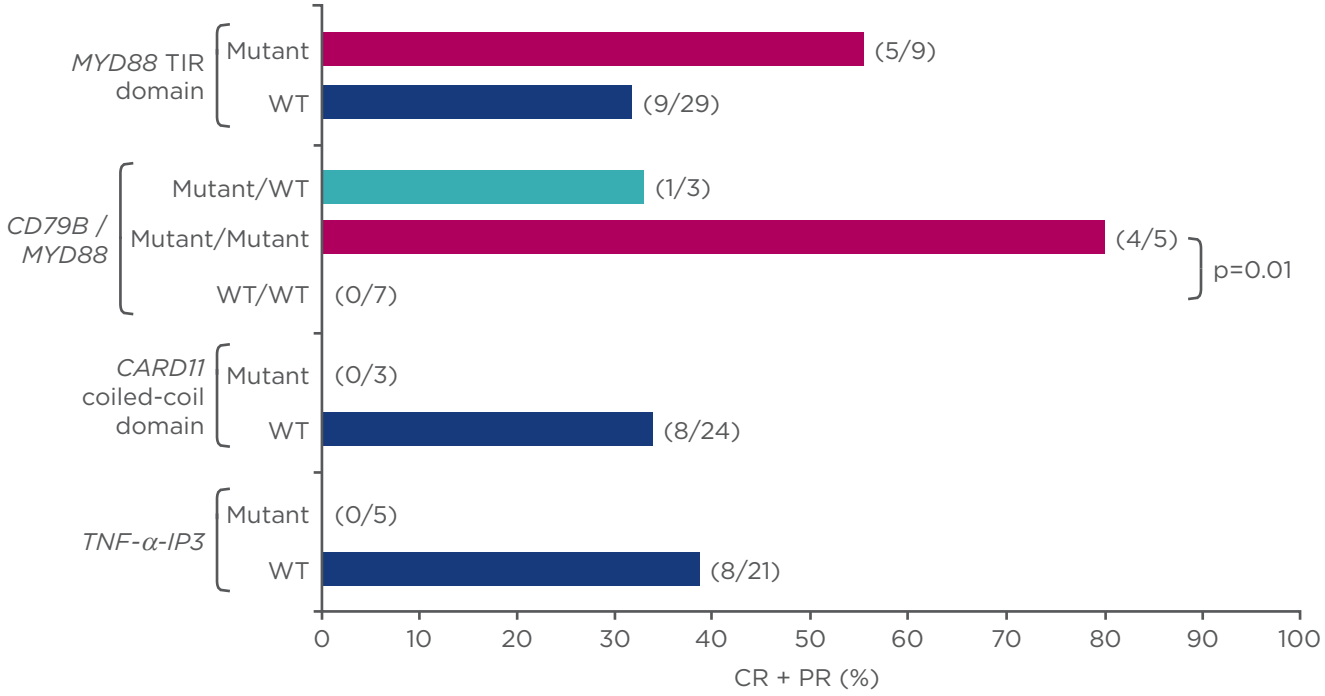

Figure 2: Ibrutinib shows improved survival benefit in patients with activated B cell like versus germinal centre B cell like diffuse large B cell lymphoma.[26]
*All other: 12/35, 10/32, 14/30, respectively. p-value versus other: 1.00, 0.057, 0.031, respectively.
CR: complete response; PR: partial response; TNF-α-IP3: tumour necrosis factor alpha-induced protein 3; WT: wild-type.

Further development of these interesting new agents would be facilitated by an early surrogate marker of PFS. In the current clinical trial environment, most first-line treatments will have a long remission period and patients can frequently have subsequent repeated relapses. The Follicular Lymphoma Analysis of Surrogate Hypotheses (FLASH) group has assessed individual patient data from 11 randomised clinical trials and identified the presence of a CR at 30 months (CR30) as a surrogacy candidate for PFS.[21] CR30 captures the effects of both induction and maintenance

treatments, and is supported by clinical data indicating that durable CR is associated with prolonged PFS.[22] Incorporation of markers such as CR30 into future clinical trial design may permit a reduction in overall clinical trial duration.

Novel Insights into Diffuse Large B Cell Lymphoma Diagnosis and Clinical Implications

Professor Georg Lenz

Existing diagnostic procedures for DLBCL are well established. A lymph node or extranodal biopsy is followed by morphological and immunohistochemistry (IHC) characterisation. IHC can also be employed for disease subtyping, fluorescent *in situ* hybridisation analysis for identification of translocations, and computed tomography or positron emission tomography (PET), and potentially bone marrow biopsy, for clinical staging. Within this diagnostic process, defining the DLBCL disease subtype is important because of the wide spectrum of heterogeneous disease. It is possible to distinguish a number of morphologic variants (centroblastic, immunoblastic, anaplastic large B cell, plasmablastic, etc.) each with specific morphology and pathology, and there is also wide heterogeneity with regard to disease manifestation (nodal DLBCL versus mediastinal, central nervous system [CNS], or testicular lymphomas) and treatment response.

Gene expression profiling (GEP) can help in resolving DLBCL heterogeneity by reliably and reproducibly distinguishing the activated B cell like (ABC) subtype (35% of DLBCLs); the germinal centre B cell like (GCB) subtype (40%); and the primary mediastinal B cell lymphoma subtype.[23] Importantly however, 15–20% of all DLBCLs cannot be classified according to GEP and are considered to represent a mixture of different lymphomas. Unfortunately, the clinical use of GEP is limited by the requirement for fresh frozen biopsies and therefore IHC algorithms have also been evaluated for clinical applicability. The use of IHC for disease subtyping is also subject to practical limitations, most notably the inability to identify the 15–20% of patients who are unclassifiable through GEP. IHC disease subtyping therefore identifies patients only as GCB or non-GCB.[24] Another approach to disease subtyping is the NanoString GEP assay, which is compatible with fresh frozen tissue and may therefore have utility within the clinical setting. Overall, disease subtyping is important because the DLBCL molecular subtypes rely of different oncogenic pathways: disease subtyping can therefore facilitate the rational use of targeted therapies.

The nuclear factor (NF)-κB pathway is one such oncogenic pathway that is utilised differently between ABCs and GCBs. NF-κB is a transcription factor family that is normally inactivated by inhibitory proteins but upon stimulation causes the release of NF-κB into the nucleus, resulting in cell proliferation and inhibition of apoptosis. In tumour cells, the NF-κB pathway can be constitutively activated and pre-clinical data suggest that the ABC subtype may be particularly reliant on this this oncogenic pathway.[25] Ibrutinib and lenalidomide are inhibitors of the NF-κB pathway and both have shown activity in patients with ABC DLBCL. Ibrutinib is a high specific inhibitor of BTK, which plays an important role in activating the NF-κB pathway. It has shown better response rates in patients with ABC compared with GCB (37% versus 5%, p=0.0106), which also translated into a trend towards improved OS in the ABC group (median OS: 10.32 months in ABC versus 3.35 months in GCB, p=0.056).[26] Ibrutinib responses appear to be dependent on the presence of specific NF-κB pathway mutations within *CD79B*, *MYD88*, the *CARD11* coiled coil domain, and TNF-α-IP3 (Figure 2). Lenalidomide also shows preferential activity in ABC compared with GCB DLBCL. A retrospective analysis has shown higher response rates in non-GCB patients compared with GCB patients with relapsed/refractory DLBCL receiving lenalidomide, resulting in significantly improved PFS in the non-GCB group.[27] Further evidence comes from a retrospective analysis of a Phase II study of R-CHOP alone or with lenalidomide in patients with DLBCL. Among patients receiving R-CHOP alone, OS was significantly better among GCB patients compared with non-GCB patients; however, in the combination therapy arm there was no difference in OS between GCB and non-GCB groups, suggesting a lenalidomide-driven improvement in OS in the non-GCB population.[28] Further data on these agents will come from the ongoing Phase III studies ROBUST[29] and PHOENIX,[30] which will assess the combination use of ibrutinib or lenalidomide with R-CHOP in patients with non-GCB DLBCL.

Although patients with GCB typically respond well to R-CHOP, there is also a need for additional targeted treatment options for these patients. *In vitro* evidence suggests that the PI3 kinase (PI3K) pathway may represent a rational drug target for GCB DLBCL.[31] Expression levels of the phosphatase and tensin homolog (PTEN) protein, which normally blocks the PI3K pathway and therefore acts as a tumour suppressor, are lower in GCB compared with non-GCB patients; the addition of PTEN induces toxicity in PTEN-deficient GCB DLBCL cell lines and inhibition of PI3K results in toxicity in PTEN-deficient models.

For the foreseeable future, R-CHOP is likely to remain the standard treatment choice in DLBCL; however, improved understanding of the oncogenic pathways in each molecular subtype may precipitate a movement towards increased use of targeted therapies. ABCs and GCBs are clearly different tumours characterised by different GEPs and different genetic abnormalities. The ability to distinguish ABC and GCB in the clinical setting will be the first step towards specific treatment approaches for each molecular subtype.

Post-Induction Treatment in Diffuse Large B Cell Lymphoma: Current Data and Perspectives

Professor Catherine Thieblemont

In patients with DLBCL, first-line R-CHOP therapy results in a clinical cure in approximately 60% of treated patients while the remaining 40% typically relapse within 2 years. Survival in patients who relapse is poor: those with late relapse (~half) have a median survival of approximately 5 years and those with early relapse (i.e. within 1 year, ~half) typically of less than 6 months.[32] Based on these data, it is clear that DLBCL is a 'one-shot' cancer with poor prognosis following relapse. Effective management approaches for preventing relapse are therefore required, raising two important questions: 'what are the specific characteristics of relapsed patients?' and 'how can these patients be effectively treated?' Patients with refractory disease can be identified based on the clinical features of the disease (the site of involvement, e.g. CNS disease and the IPI); their immunophenotypic characteristics (such as Ki67); biological characteristics (such as *MYC* or the double hit); gene expression signatures (GCB/ABC, microenvironment, stromal signatures); and their treatment response based on PET analysis.[33]

For patients who will relapse, the R-CHOP induction regimen is clearly insufficient and additional treatment is required. Various Phase III studies have examined replacement of CHOP with intensified chemotherapy regimens such as etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (CHO[E]P or EPOCH) or doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP), or the use of high-dose therapy plus autologous stem cell transplantation (ASCT) as consolidation.[34] Studies examining intensified chemotherapy regimens have generally not yielded improved survival rates compared with CHOP.[31] Alternatively, replacement of rituximab by another antibody such as ofatumumab or obinutuzumab may represent another approach to mitigating relapse. A third approach has therefore been pursued which involves an additional drug treatment to the R-CHOP regimen, either during the induction treatment phase or as a maintenance therapy. The rationale for the maintenance therapy approach is to prevent post-remission relapse in patients with a first complete remission after induction therapy. This approach has been successfully adopted with new agents in the treatment of other cancers such as multiple myeloma, where lenalidomide maintenance therapy following ASCT has been shown to significantly prolong OS.[35]

The assessment of minimal residual disease (MRD) based on circulating tumour DNA may be one approach to evaluating the benefit of maintenance therapy. Only one study has reported that after three cycles of first-line therapy, the presence of circulating tumour DNA is associated with reduced PFS and OS. Strategies to decrease MRD using maintenance therapy may therefore delay or prevent relapse.[36] While studies suggest no benefit associated with rituximab or everolimus maintenance therapy in DLBCL,[37,38] data in support of lenalidomide maintenance therapy are more promising. Notably, a Phase II study in chemosensitive patients with relapsed DLBCL receiving lenalidomide maintenance therapy after a complete or partial response to rituximab-based salvage therapy indicated a favourable survival advantage.[39] Of the 41 patients enrolled in this study, 30 had DLBCL and 11 had transformed DLBCL. The 1-year PFS was 74% and 1-year OS was 84%. Encouragingly, 6 of the 16 patients with a

partial response after salvage therapy achieved a CR during lenalidomide maintenance. In this study, lenalidomide had a predictable toxicity profile; 22% of patients developed Grade 3/4 neutropenia. Lenalidomide is also currently under evaluation as first-line maintenance therapy in the randomised Phase III REMARC study in patients with DLBCL and response following R-CHOP induction.[40]

In conclusion, maintenance therapy approaches may help to reduce or prevent relapse and improve treatment outcomes for patients with DLBCL. Evaluation of MRD may help to adapt this treatment approach specifically for DLBCL patients and studies are ongoing in this regard. Data from the REMARC study of maintenance lenalidomide after R-CHOP in elderly patients are also eagerly awaited.

New Frontiers in Immuno-oncology

Professor Pier Luigi Zinzani

Novel immunotherapeutic agents represent exciting new treatment modalities for relapsed/refractory NHL. At the forefront of research are a number of different strategies that utilise the immune system to promote tumour destruction. The pleiotropic pathway modifier, CC-122, exhibits both tumouricidal and anti-angiogenic effects *in vitro* (Figure 3), and shows excellent immunomodulatory activity compared with lenalidomide.[41] Whereas lenalidomide appears to be more effective in the ABC subtype, preliminary *in vitro* data suggest that CC-122 is active in both ABC and GCB subtypes, implying therapeutic potential in a wider cross-section of patients.[42] This agent is currently undergoing evaluation in patients with NHL, both as a single agent and also in combination with rituximab, ibrutinib, or obinutuzumab. Studies examining CC-122 in triple therapy regimens include combinations with rituximab plus a mammalian target of rapamycin inhibitor, or rituximab plus a BTK inhibitor.

A number of new monoclonal antibody-drug conjugates (ADC) are also undergoing evaluation in patients with relapsed or refractory NHL. The ROMULUS study compared two ADCs, pinatuzumab vedotin (a CD22 ADC) and polatuzumab vedotin (a CD79b ADC), each in combination with rituximab, in 41 patients with relapsed or refractory FL.[43] Most patients in this study were heavily pre-treated and all had received prior rituximab: 43% of those in the pinatuzumab and 25% of those in the polatuzumab arms became rituximab refractory within 6 months. Neurotoxicity was reported in both treatment arms, with 50–60% of patients reporting peripheral neuropathy or peripheral sensory neuropathy. CRs were 40% and 10% in the polatuzumab and pinatuzumab arms, with ORR of 70% and 62%, respectively. On the basis of these results, ongoing Phase II studies are evaluating combinations of polatuzumab plus rituximab plus CHOP and pinatuzumab plus rituximab plus bendamustine.

Denintuzumab mafodotin is another ADC under evaluation in relapsed/refractory NHL. It has a similar mechanism of action to polatuzumab and pinatuzumab (an anti-CD19 monoclonal antibody), but with a different vinca alkaloid cytotoxic agent (monomethyl auristatin F instead of monomethyl auristatin E) which confers a different toxicity profile.[44] A Phase I study including 54 patients with primarily relapsed/refractory DLBCL evaluated 3-weekly (0.5–6 mg/kg every 3 weeks) and 6-weekly (3 mg/kg every 6 weeks) dose regimens of single-agent denintuzumab mafodotin. Ocular toxicity, including blurred vision, dry eye, fatigue, keratopathy, and photophobia were the main safety observations, and, similar to polatuzumab and pinatuzumab, there was little evidence of significant haematological toxicity. The ORR was 37% with the 3-weekly regimen and 44% with the 6-weekly regimen, with CRs achieved in 20% and 44% of patients, respectively. These data are considered preliminary and the ocular toxicity profile suggests further study of this agent is required.

Checkpoint inhibitors represent another exciting therapeutic approach to the treatment of aggressive NHL. Nivolumab has shown activity in patients with previously treated NHL and pembrolizumab in those with relapsed/refractory Hodgkin's lymphoma. A Phase II study of the PD-1 inhibitor pidilizumab reported ORR of 51% in patients with relapsed/refractory DLBCL; and a Phase I study of single-agent nivolumab response rates of 28%, 36%, and 40% in patients with relapsed/refractory indolent non-follicular B cell lymphoma (n=29), DLBCL (n=11), and FL (n=10), respectively.[44,45] Checkpoint inhibitors also appear to have an acceptable safety profile with no concerning haematological or stomatological toxicity, and no clear association between pneumonitis and prior therapies.[46]

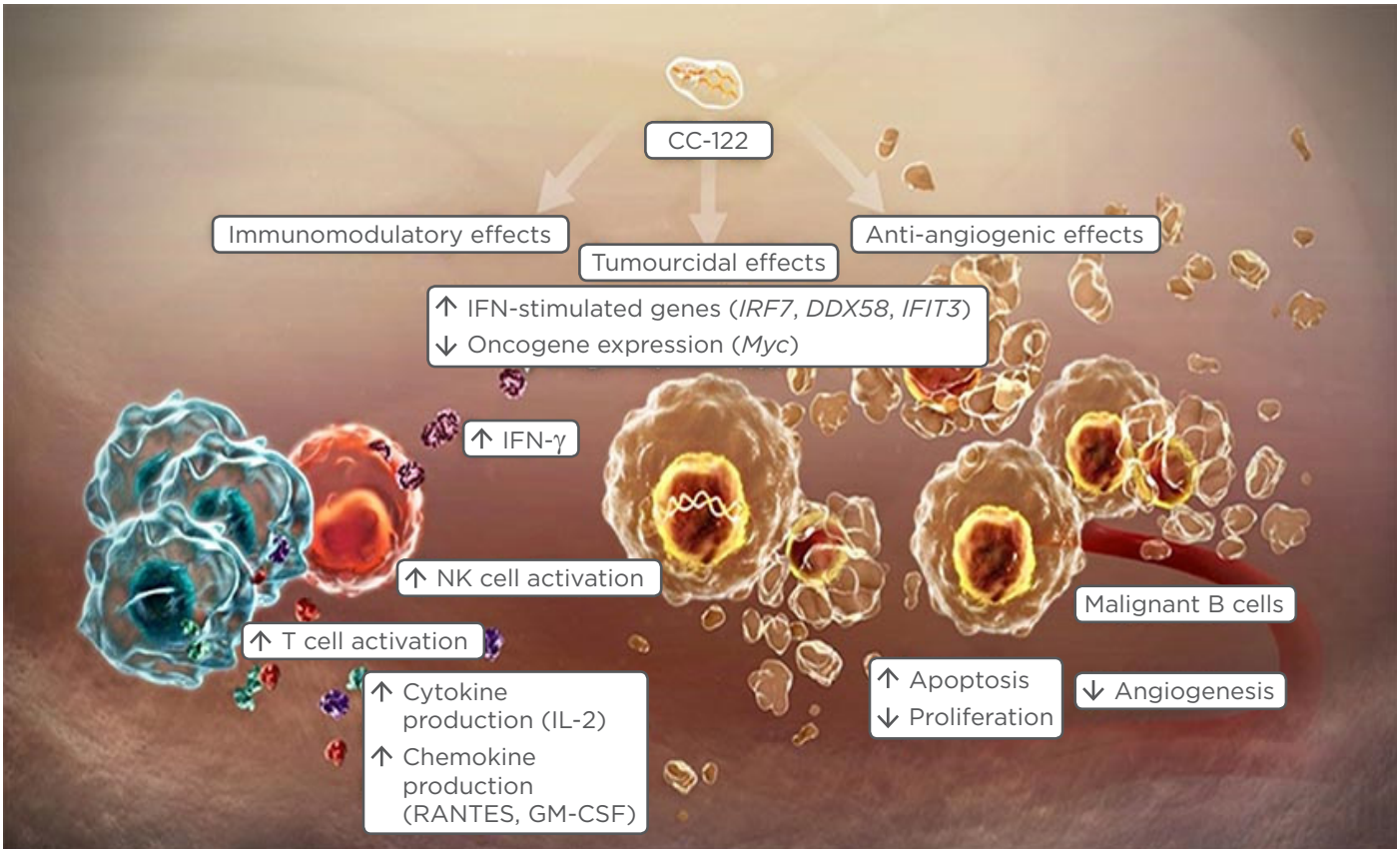

Figure 3: Mechanism of action of the pleiotropic pathway modifier, CC-122.
IFN-γ: interferon gamma; NK: natural killer; IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; RANTES: regulated on activation, normal T cell expressed and secreted.
Taken with permission from http://www.researchoncology.com/

Finally, durvalumab, a selective high-affinity human immunoglobulin G monoclonal antibody that blocks PD-L1,[47] is also beginning clinical evaluation in a Phase Ib/II2 study, both as a monotherapy and as a combination therapy in patients with lymphoma and chronic lymphocytic leukaemia.[48]

Chimeric antigen receptor (CAR)-modified T cells are known to be effective in treating relapsed and refractory acute and chronic lymphocytic leukaemia, and it has been hypothesised that CAR-modified T cells directed against CD19 may result in anti-tumour responses in patients with advanced CD19$^+$ B cell NHL.[49] Initial data from a Phase II study which enrolled patients with relapsed/refractory CD19$^+$ NHL suggest promising anti-tumour activity. Following a single intravenous infusion, ORR was 47% in patients with DLBCL and 73% in those with FL, with CRs achieved in 20% and 36% of patients, respectively.[49] The safety and anti-tumour activity of another CD19$^+$ targeted CAR-T cell, JCAR017, is also being evaluated in a Phase I study in patients with relapsed/refractory NHL.[50]

In conclusion, immunotherapy is advancing at a spectacular rate; its role in NHL continues to evolve and early phase clinical trial results are promising. New compounds with immunomodulatory activity, monoclonal antibodies, checkpoint inhibitors, and CAR-T cell therapy have yielded interesting preliminary data in DLBCL and FL. These new immunological approaches have the potential to improve treatment outcomes, and further clinical evaluation will help define their role in the treatment of patients with relapsed/refractory NHL.

REFERENCES

1. Solal-Céligny P et al. Follicular lymphoma international prognostic index. Blood. 2004;104(5):1258-65.

2. Federico M et al. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol. 2009;27(27):4555-62.

3. Pastore A et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111-22.

4. Ardeshna KM et al. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet. 2003;362(9383):516-22.

5. Ardeshna KM et al. Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: an open-label randomised phase 3 trial. Lancet Oncol. 2014;15(4):424-35.

6. Schulz H et al. Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2007; 99(9):706-14.

7. Salles G et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377(9759):42-51.

8. Federico M et al. R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. J Clin Oncol. 2013;31(12):1506-13.

9. Rummel MJ et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013; 381(9873):1203-10.

10. Burke JM et al. Differences in Quality of Life Between Bendamustine-Rituximab and R-CHOP/R-CVP in Patients With Previously Untreated Advanced Indolent Non-Hodgkin Lymphoma or Mantle Cell Lymphoma. Clin Lymphoma Myeloma Leuk. 2016;16(4):182-90.

11. Kimby E et al. Two courses of four weekly infusions of rituximab with or without interferon-α2a: final results from a randomized phase III study in symptomatic indolent B-cell lymphomas. Leuk Lymphoma. 2015;56(9):2598-607.

12. Taverna C et al. Rituximab Maintenance for a Maximum of 5 Years After Single-Agent Rituximab Induction in Follicular Lymphoma: Results of the Randomized Controlled Phase III Trial SAKK 35/03. J Clin Oncol. 2016;34(5):495-500.

13. Fowler NH et al. Safety and activity of lenalidomide and rituximab in untreated indolent lymphoma: an open-label, phase 2 trial. Lancet Oncol. 2014;15(12):1311-8.

14. Kimby E et al. Rituximab Plus Lenalidomide Improves the Complete Remission Rate in Comparison with Rituximab Monotherapy in Untreated Follicular Lymphoma Patients in Need of Therapy. Primary Endpoint Analysis of the Randomized Phase-2 Trial SAKK 35/10. Abstract 799. 56th American Society of Hematology, Annual Meeting and Exposition, San Francisco, California, USA, 6-9 December 2014.

15. Celgene Corporation. Combined Rituximab and Lenalidomide Treatment for Untreated Patients With Follicular Lymphoma (RELEVANCE). NCT01476787. Available at: https://clinicaltrials.gov/ct2/show/NCT01476787.

16. Sehn L et al. GADOLIN: Primary results from a phase III study of obinutuzumab plus bendamustine compared with bendamustine alone in patients with rituximab refractory indolent non Hodgkin lymphoma. Abstract LBA8502. ASCO Annual Meeting, Chicago, Illinois, USA, 29 May-2 June 2015.

17. Gopal AK et al. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med. 2014; 370(11):1008-18.

18. Salles GA et al. Idelalisib efficacy and safety in follicular lymphoma patients from a phase 2 study. Poster 346. ASCO Annual Meeting, Chicago, Illinois, USA, 29 May-2 June 2015.

19. Fowler N et al. Ibrutinib Plus Rituximab in Treatment-Naive Patients with Follicular Lymphoma: Results from a Multicenter, Phase 2 Study. Abstract 470. 57th American Society of Hematology, Annual Meeting and Exposition, Orlando, Florida, USA, 5-8 December 2015.

20. Gerecitano JF et al. A Phase 1 Study of Venetoclax (ABT-199 / GDC-0199) Monotherapy in Patients with Relapsed/Refractory Non-Hodgkin Lymphoma. Abstract 254. 57th American Society of Hematology, Annual Meeting and Exposition, Orlando, Florida, USA, 5-8 December 2015.

21. Sargent D et al. Evaluation of complete response rate at 30 months (CR30) as a surrogate for progression-free survival (PFS) in first-line follicular lymphoma (FL) studies: Results from the prospectively specified Follicular Lymphoma Analysis of Surrogacy Hypothesis (FLASH) analysis with individual patient data (IPD) of 3,837 patients (pts). Abstract 8504. ASCO Annual Meeting, Chicago, Illinois, USA, 29 May-2 June 2015.

22. Bachy E et al. Long-term follow-up of patients with newly diagnosed follicular lymphoma in the prerituximab era: effect of response quality on survival--A study from the groupe d'etude des lymphomes de l'adulte. J Clin Oncol. 2010;28(5): 822-9.

23. Nogai H et al. Pathogenesis of non-Hodgkin's lymphoma. J Clin Oncol. 2011; 29(14):1803-11.

24. Hans CP et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275-82.

25. Lam LT et al. Small molecule inhibitors of IkappaB kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. Clin Cancer Res. 2005;11(1): 28-40.

26. Wilson WH et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015;21(8):922-6.

27. Hernandez-Ilizaliturri FJ et al. Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer. 2011;117(22):5058-66.

28. Nowakowski GS et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-Cell lymphoma: a phase II study. J Clin Oncol. 2015;33(3):251-7.

29. Celgene Corporation. Efficacy and Safety Study of Lenalidomide Plus R-CHOP Chemotherapy Versus Placebo Plus R-CHOP Chemotherapy in Untreated ABC Type Diffuse Large B-cell Lymphoma (ROBUST). NCT02285062. Available at: https://clinicaltrials.gov/ct2/show/NCT02285062.

30. Janssen Research & Development, LLC. A Study of the Bruton's Tyrosine Kinase Inhibitor, PCI-32765 (Ibrutinib), in Combination With Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Patients

With Newly Diagnosed Non-Germinal Center B-Cell Subtype of Diffuse Large B-Cell Lymphoma. NCT01855750. Available at: https://clinicaltrials.gov/ct2/show/NCT01855750.

31. Pfeifer M et al. PTEN loss defines a PI3K/AKT pathway-dependent germinal center subtype of diffuse large B-cell lymphoma. Proc Natl Acad Sci U S A. 2013; 110(30):12420-5.

32. Coiffier B et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040-5.

33. Vaidya R, Witzig TE. Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era. Ann Oncol. 2014; 25(11):2124-33.

34. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood. 2015;25(1):22-32.

35. Attal M et al. Lenalidomide (LEN) maintenance (MNTC) after high dose melphalan and autologous stem cell transplant (ASCT) in multiple myeloma (MM): A meta analysis (MA) of overall survival (OS). Abstract 8001. ASCO 2016. Chicago, Illinois, USA, 3-7 June 2016.

36. Roschewski M et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol. 2015;16(5):541-9.

37. Jaeger U et al. Rituximab maintenance for patients with aggressive B-cell lymphoma in first remission: results of the randomized NHL13 trial. Haematologica. 2015;100(7):955-63.

38. Witzig E et al. PILLAR2: A randomized, double-blind, placebo-controlled, phase III study of adjuvant everolimus (EVE) in patients (pts) with poor risk diffuse large B cell lymphoma (DLBCL). Abstract 7506. ASCO 2016. Chicago, Illinois, USA, 3-7 June 2016.

39. Ferreri AJM et al. Lenalidomide maintenance significantly improves progression free survival (PFS) in patients with chemosensitive relapse of diffuse large B-cell lymphoma (DLBCL) who are not eligible for autologous stem cell transplantation (ASCT) or experienced relapse after transplantation: results of a multicentre phase II trial. Abstract 1547. 57th American Society of Hematology, Annual Meeting and Exposition, Orlando, Florida, USA, 5-8 December 2015.

40. The Lymphoma Academic Research Organisation. Study of Lenalidomide Maintenance Versus Placebo in Responding Elderly Patients With DLBCL and Treated With R-CHOP (REMARC). NCT01122472. Available at: https://clinicaltrials.gov/ct2/show/NCT01122472?term=NCT01122472&rank=1.

41. Nowakowski GS. Evolution: IMiDs to PPMs, revolution in DLBCL? Blood. 2015; 126(6):698-700.

42. Hagner PR et al. CC-122, a pleiotropic pathway modifier, mimics an interferon response and has antitumor activity in DLBCL. Blood. 2015;126(6):779-89.

43. Morschhauser F et al. Preliminary results of a phase II randomized study (ROMULUS) of polatuzumab vedotin (PoV) or pinatuzumab vedotin (PiV) plus rituximab (RTX) in patients (Pts) with relapsed/refractory (R/R) non-Hodgkin lymphoma (NHL). Abstract 8519. ASCO 2014, Chicago, Illinois, USA, May 20-June 3 2014.

44. Moskowitz CH et al. A Phase 1 Study of Denintuzumab Mafodotin (SGN-CD19A) in Relapsed/Refactory B-lineage Non-Hodgkin Lymphoma Blood. Abstract 182. American Society of Hematology, 57th Annual Meeting and Exposition, Orlando, Florida, USA, 5-8 December 2015.

45. Armand P et al. Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. J Clin Oncol. 2013;31(33): 4199-206.

46. Lesokhin AM et al. Preliminary Results of a Phase I Study of Nivolumab (BMS-936558) in Patients with Relapsed or Refractory Lymphoid Malignancies Blood. Abstract 291. 56th Annual Meeting and Exposition, San Francisco, California, 6-9 December 2014.

47. Segal NH et al. Safety and efficacy of MEDI4736, an anti-PD-L1 antibody, in patients from a squamous cell carcinoma of the head and neck (SCCHN) expansion cohort. Abstract 3011. ASCO 2015 Annual Meeting, Chicago, Illinois, USA, 29 May-2 June 2015.

48. Celgene Corporation. A Study to Determine Dose, Safety, and Efficacy of Durvalumab as Monotherapy and in Combination Therapy in Subjects With Lymphoma or Chronic Lymphocytic Leukemia (FUSION NHL 001). NCT02733042. Available at: https://clinicaltrials.gov/ct2/show/NCT02733042?term=NCT02733042&rank=1.

49. Schuster SJ et al. Sustained Remissions Following Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019) in Patients with Relapsed or Refractory CD19+ Lymphomas. Abstract 183. American Society of Hematology, 57th Annual Meeting and Exposition, Orlando, Florida, USA, 5-8 December 2015.

50. Juno Therapeutics, Inc. Study Evaluating the Safety and Pharmacokinetics of JCAR017 in B-cell Non-Hodgkin Lymphoma (NHL). NCT02631044. Available at: https://clinicaltrials.gov/ct2/show/NCT02631044?term=NCT02631044&rank=1.

NEW INSIGHTS IN BONE MARROW FAILURE

This satellite symposium took place on 9th June 2016, as a part of the 21st Congress of the European Hematology Association (EHA) 2016 in Copenhagen, Denmark

Chairperson

Gérard Socié[1]

Speakers

Austin Kulasekararaj,[2] Gérard Socié,[1] Alexander Röth[3]

1. Service d'Hématologie-Greffe de Moelle, Hôpital Saint-Louis, Paris, France

2. Department of Haematological Medicine, King's College Hospital NHS Foundation Trust and King's College London School of Medicine, London, UK

3. Department of Hematology, West German Cancer Center, University Hospital Essen, Essen, Germany

Disclosure: Prof Socié is a consultant for Alexion Pharmaceuticals. Dr Kulasekararaj has received funding and speaker's honoraria from Alexion, Celgene, Novartis, and Amgen. Dr Röth has received honoraria, has membership of an entity's board of directors and advisory committees, and has research funding from Alexion Pharmaceuticals.

Acknowledgements: Writing assistance was provided by Dr Joan Thomas of ApotheCom.

Support: The symposium and publication of this article was sponsored by Alexion Pharmaceuticals Inc. Authors received honoraria for preparation and delivery of their presentations. The views and opinions expressed are those of the authors and not necessarily Alexion Pharmaceuticals Inc.

Citation: EMJ Hematol. 2016;4[1]:47-54

MEETING SUMMARY

Several rare haematological diseases are linked to bone marrow failure (BMF). This symposium provided the latest scientific insights into the different pathophysiological mechanisms and clinical advances in the management of these conditions, with a specific focus on the clinical management of patients with paroxysmal nocturnal haemoglobinuria (PNH) in the context of aplastic anaemia (AA), and the pathophysiology, consequences, and identification of PNH in the context of BMF.

Prof Gérard Socié chaired the symposium and overviewed BMF. Dr Austin Kulasekararaj gave a presentation on new paradigms in BMF, followed by Prof Gérard Socié, who reviewed the diagnosis and management of AA. Dr Alexander Röth then discussed the diagnosis and management of PNH in the context of BMF. The symposium was concluded by a short question and answer session.

Overview of Bone Marrow Failure

Professor Gérard Socié

Several different diseases overlap within BMF syndromes and share the propensity to develop into myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) (Figure 1).[1] For any BMF or AA, especially in children or young adults, a congenital disorder should be ruled out during diagnosis. Patients with congenital BMF will not respond to immunosuppressive therapy (IST).

Anaemia is the most common clinical sign of BMF, others include neutropenia, monocytopenia, and thrombocytopenia. Blood analysis is important for differential diagnosis to detect abnormalities in neutrophils, platelets, blast cells, and other cells. During bone marrow analysis mast cells and plasma cells are frequently detected in patients with severe AA, increased erythroblasts frequently detected in PNH, and dyserythropoiesis in both.

Bone marrow aspirate and trephine biopsy are fundamental to a differential diagnosis. However, it is important to consider that ageing is also associated with reduced bone marrow cellularity.[2]

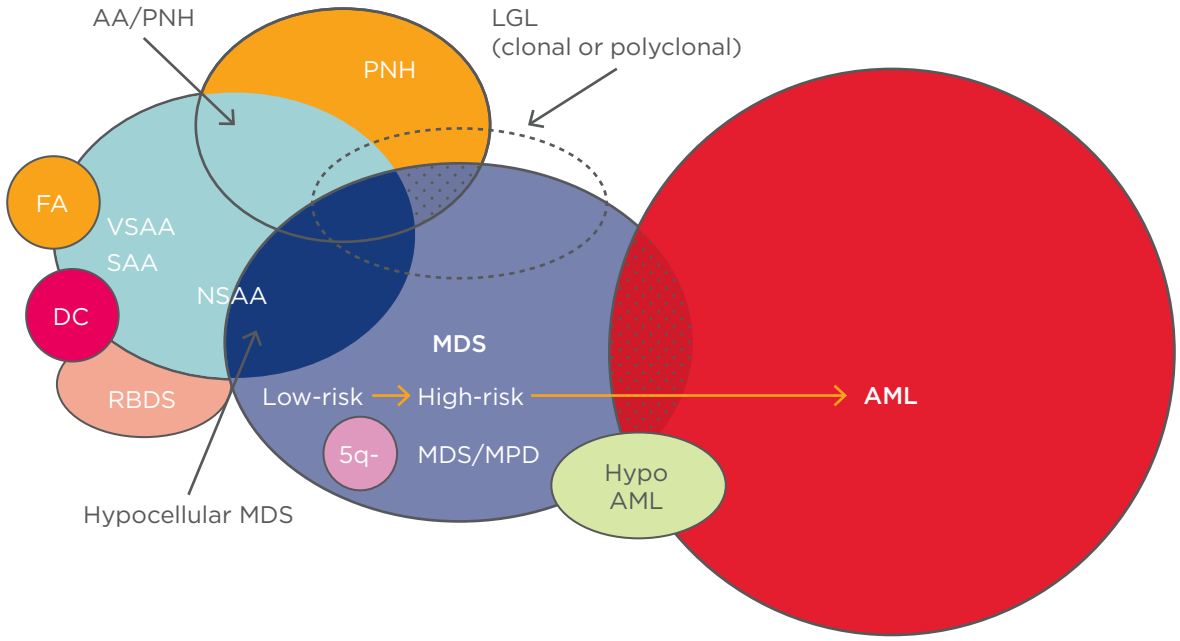

Figure 1: Overlapping entities in bone marrow failure syndromes.[1]
AA: aplastic anaemia; PNH: paroxysmal nocturnal haemoglobinuria; FA: Fanconi anaemia; VSAA: very severe aplastic anaemia; SAA: severe aplastic anaemia; NSAA: non-severe aplastic anaemia; DC: dyskeratosis congenita; RBDS: ribosomal dysgenesis syndrome; 5q-: q arm of chromosome 5 deletion; MDS: myelodysplastic syndrome; MPD: myeloproliferative disorder; LGL: large granular lymphocytic leukaemia; AML: acute myeloid leukaemia.

Theoretically, distinguishing between AA and hypoplastic MDS is relatively simple, as dysplastic neutrophils and, in particular, dysplastic megakaryocytes are key features of hypoplastic MDS but not of AA.[3] $CD34^+$ cells may also be used. Cytogenetic analysis plays a key role in diagnosis, as hypocellular bone marrow can lead to insufficient cellular metaphase. Fluorescence *in situ* hybridisation for chromosomes 5, 8, and 7 should be considered for differential diagnosis. However, an abnormal cytogenetic clone does not imply a diagnosis of MDS, particularly as trisomy 8 is frequently detected in patients who are otherwise typical for AA. Flow cytometry is a key diagnostic tool for PNH and will be discussed in depth later. Other diagnostic tools may include screening for viral hepatitis or autoimmune disease (although cases linked to these diseases are rare) and vitamin B12 dosing.

Two rare diseases to consider in children, and adolescents and young adults, are Fanconi anaemia, which can easily be discounted using a chromosomal breakage test, and dyskeratosis congenita. Over recent years, the number of patients with a diagnosis of dyskeratosis congenita has increased, even though the disease development presents a wide spectrum of clinical features. A family history of pulmonary fibrosis, unknown pulmonary complications, or non-alcohol-related cirrhosis should be investigated, and dyskeratosis congenita has a frequent association with mutations of the telomerase RNA compartment (*TERC*) or telomerase reverse transcriptase (*TERT*). Despite the variety of tests available, in practice differential diagnosis between MDS and severe AA/PNH is not necessarily easy.

New Paradigms in Bone Marrow Failure

Doctor Austin Kulasekararaj

AA is an immune-mediated non-malignant disease, and clonal haematopoiesis in AA is linked to the evolution of disorders such as PNH and MDS/AML, which are associated with acquired genetic abnormalities.[3] Recently, studies have identified inherited *GATA2* mutations in patients presenting with AA, MDS, and also paediatric neutropenia, but invariably with severe monocytopenia.[4]

AA is associated with $CD8^+$ autoreactive cytotoxic T cells which inhibit haematopoietic stem cells,

leading to apoptosis and BMF; a significant reduction in $CD4^+$ regulatory T cells, which are also non-functional and have an abnormal cytokine profile; and an expansion of $CD4^+$ T helper (Th) 1 and Th17 cells.[5-7] These anomalies can be identified by routine flow cytometry or cytokine profiling. Recent studies using newer techniques such as single cell mass cytometry (also called Cy-TOF) have identified aberrant subsets of T regulatory cells with pro-inflammatory properties in AA that predict poor response to IST.[8]

PNH in the context of BMF is characterised by progressive expansion of one or more PNH stem cell clones (immunological escape clones), which are deficient (partial or complete) in the expression of glycosylphosphatidylinositol (GPI)-anchored proteins.[9] Other examples of such escape clones include trisomy 8, which leads to expansion of Wilms' tumour 1-specific cytotoxic $CD8^+$ T cells,[10] uniparental disomy on chromosome 6p, the human leukocyte antigen (HLA) locus, and del 13q, which has been increasingly associated with BMF with PNH clones and responsive to IST, particularly in the context of AA rather than MDS.[11]

Several somatic mutations have been identified in a subgroup of patients with AA that can predict malignant transformation to MDS and monosomy 7.[12] In a study of 150 patients with AA and no morphological evidence of MDS, acquired somatic mutations were identified in approximately 20%.[12] *ASXL1* (8% of patients) and *DNMT3A* (5% of patients) were the most common mutations, while *BCOR* was present in 4% of patients. In this study, 41% of patients had <10% mutation clonal load.[12] In patients with AA with a disease duration of >6 months, these somatic mutations were associated with a 40% risk of transformation to MDS versus 4% risk without the mutations.[12] Similar findings have been reported by the National Institutes of Health (NIH), Cleveland, and Japanese groups.[13] The acquisition of these mutations increases with age, with each mutation demonstrating a different pattern of change over time.[13] Importantly, the identification of these mutations may allow prediction of response to IST.[13] Interestingly, presence of the *BCOR* mutation seems to predict a better response to IST.[12] Additionally, studies investigating the mutational landscape of PNH indicate a stepwise pattern of MDS-type mutations, with these mutations arising either as a sub-clone within the *PIG-A* mutant population or prior to *PIG-A* mutation.[14]

Telomere length/attrition is another important factor in transformation to MDS in AA.[12,15] Patients with AA and somatic mutations have been shown to have reduced telomere length. In a study of 13 patients with severe AA, patients who evolved to MDS and AML had marked telomere attrition that preceded the emergence of monosomy 7.[15] In a study of patients with refractory AA treated with eltrombopag (a small molecule agonist of the c-mpl [thrombopoietin] receptor), approximately 40% of patients showed an improved haematologic response; however, they also had an increased risk of clonal evolution to monosomy 7, with a shorter time to evolution.[16]

There are several different ways in which monosomy 7 can occur, including familial origin, myeloid neoplasia, following haematopoietic stem cell transplantation (HSCT), AA, and following treatment with granulocyte colony-stimulating factor.[17] In a recently reported Phase I/II study, patients with telomere diseases were treated with the synthetic steroid danazol 800 mg/day for 24 months, with the aim of attenuating accelerated telomere attrition. After just 3 months of treatment, almost all patients had a gain in telomere length and 79% had haematological response. Adverse effects included elevated liver enzymes (41%) and cramps (33%).[18]

Reports of spontaneous remission in patients with PNH have been described in the literature.[19,20] In the UK PNH Service, where 303 patients have been treated with eculizumab (a humanised monoclonal antibody to complement protein C5), five patients have stopped treatment due to spontaneous remission,[21] although the recent case report indicates that there is no recovery of normal haematopoiesis.[19]

A Phase III trial (A Prospective Randomized Multicenter Study Comparing Horse Antithymocyte Globuline [hATG] + Cyclosporine A [CsA] With or Without Eltrombopag as Front-line Therapy for Severe Aplastic Anemia Patients; RACE) is in progress to evaluate the efficacy and safety of eltrombopag versus placebo combined with IST.

Diagnosis and Management of Patients with Aplastic Anaemia

Professor Gérard Socié

AA is characterised by pancytopenia and persistent unexplained marrow hypoplasia. There is no

specific marker and the diagnosis is mainly consequent with the differential diagnosis of hypoplastic MDS.[22] Careful blood analysis, bone marrow aspirate, and bone marrow biopsy are all important aspects in correct diagnosis.[22] Differential diagnosis versus congenital disorders should always be considered, especially with regard to dyskeratosis congenita.[23] Finally, differentiation from MDS should be considered, as outlined earlier in this report.

Severe AA is defined as hypocellularity <30% and at least two criteria from: $<0.5\times10^9$/L polymorphonuclear neutrophils, $<20\times10^9$/L platelets, and $<20\times10^9$/L reticulocytes; very severe AA is defined as $<0.2\times10^9$/L polymorphonuclear neutrophils.[24] All patients with severe or very severe AA require treatment.

For patients <40 years of age with severe AA, HSCT is mandatory, as it has been shown to be effective with low treatment-related mortality. At a median follow-up of 73 months in 61 patients (mean age: 21 years) who underwent HSCT from an HLA-matched sibling donor after irradiation-based conditioning with cyclophosphamide and ATG, 6-year overall survival was 87% (95% confidence interval: 78–97).[25] Only one patient developed secondary malignancy. Osteonecrosis (observed in 10 patients) remains a concern in this disease.[25]

In patients >40 years of age or without a sibling donor, IST with ATG plus cyclosporine is an effective alternative to HSCT and improves blood counts and survival. A Phase III randomised trial comparing hATG and rabbit ATG (rATG) in 60 patients with severe AA showed that hATG was superior to rATG as a first-line treatment for severe AA. At 6-month follow-up, the haematologic response was 68% for patients treated with hATG versus 37% with rATG ($p<0.001$). Overall survival at Day 800 was 86% in the hATG group versus 68% in the rATG group ($p=0.009$). Transplant-free survival was 52% for rATG versus 76% for hATG ($p=0.002$).[26] Similar results were demonstrated in a Phase II study conducted in 35 patients with AA treated with rATG and compared with 105 age and disease severity-matched patients from the European Blood and Marrow Transplant (EBMT) registry.[27] The ongoing RACE study (mentioned earlier) is also investigating hATG with or without eltrombopag as first-line therapy for severe AA.

In patients refractory to IST, the decision to transplant stem cells from unrelated donors often represents a dilemma for physicians. Evaluations of outcomes in patients <30 years of age without a sibling donor and refractory to IST demonstrate that survival after unrelated HSCT for severe AA has improved significantly over the past 15 years, due to better HLA matching and improved conditioning regimens.[28] Similar improvements in survival rates have been shown in other studies in patients with severe AA and unrelated donors receiving fludarabine, cyclophosphamide, and ATG.[29]

For patients >30 years of age and refractory to IST and with no suitable unrelated donor, there are three options: second-line IST, androgens (20–30% response rate for each), or currently still an investigational drug, eltrombopag. Long-term follow-up of a cohort of 43 patients with severe AA treated with eltrombopag demonstrated durable tri and bi-lineage responses, with a 40% response rate at 3–4 months.[16]

In conclusion, in the diagnosis of AA, inherited AA and MDS must be excluded. First-line treatment remains IST or bone marrow transplant, according to the availability of a donor. In patients who are refractory to first-line therapy, HSCT from a matched unrelated donor may be used in patients <30 years of age, and in patients >30 years of age, eltrombopag, androgen, or second-line IST.

Diagnosis and Management of Paroxysmal Nocturnal Haemoglobinuria in the Context of Bone Marrow Failure

Doctor Alexander Röth

The classical clinical triad of PNH includes haemolytic anaemia, thrombophilia, and cytopenia. Haemoglobinuria is present in approximately one-third of patients at the time of diagnosis. All patients have some degree of BMF, from isolated thrombocytopenia to AA, which typically precedes PNH. Thromboembolic complications, usually involving the brain, liver, or abdomen, are the leading cause of morbidity and mortality in patients with PNH.[30-32] PNH originates from a somatic mutation in the *PIG-A* gene in a HSC, which is essential for biosynthesis of GPI-anchors for proteins, two of which (CD55 and CD59) are complement regulatory proteins. Selection and expansion of the mutant stem cell are both necessary for clinical development of PNH, which may explain the rarity of this disease.[33]

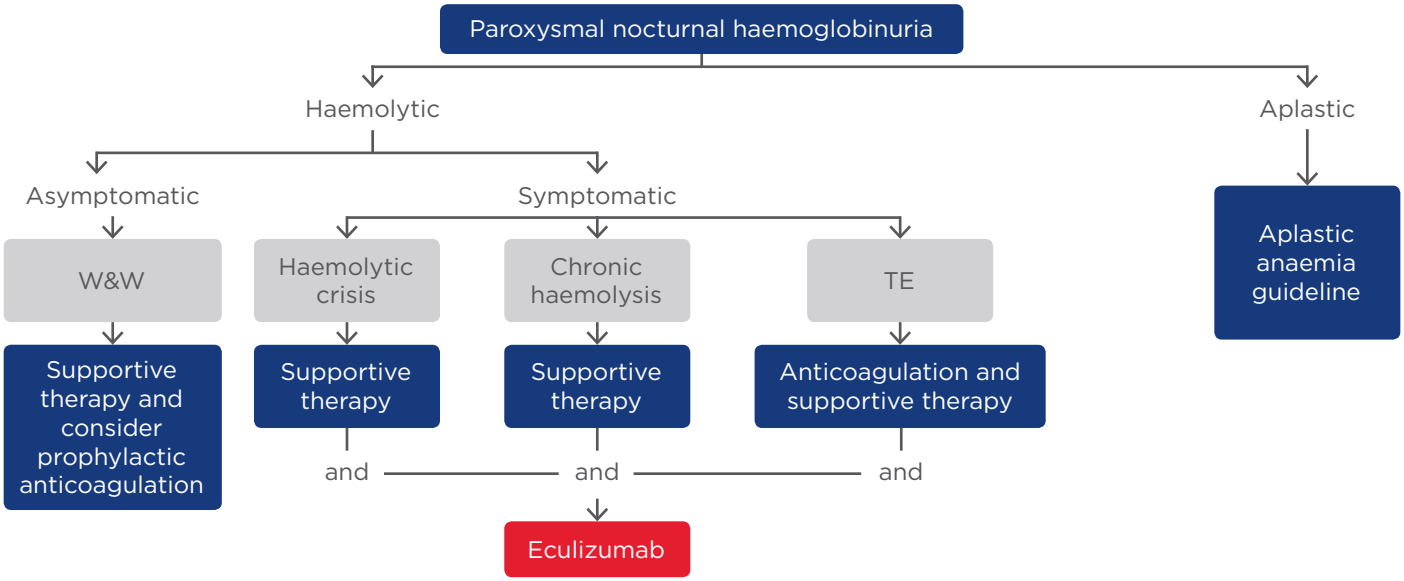

Figure 2: Proposed treatment algorithm for paroxysmal nocturnal haemoglobinuria.[61]
TE: thromboembolic event; W&W: wait and watch.

The absence of CD55 and CD59 on affected red blood cells leads to the formation of a membrane attack complex, which activates complement-mediated haemolysis and the release of free haemoglobin.[34] Large amounts of free haemoglobin causes depletion of nitric oxide. The resulting reduction of nitric oxide levels leads to the activation of haemostasis with thromboembolic complications and smooth muscle dystonias that occur in PNH, which manifest as abdominal pain, dysphagia, pulmonary and systemic hypertension, and erectile dysfunction caused by impaired regulation of smooth muscle contractions.[35]

Patients are typically diagnosed with PNH in their early 30s[36] and 5-year mortality is 35%, despite best supportive care.[19] Thrombosis occurs in 18–40% of patients with PNH and increases the risk of death 7-fold compared with patients without thrombosis.[19,37] Studies have reported a 21% incidence of a thrombotic event prior to PNH diagnosis.[19,38] Silent thromboembolic complications are also possible in PNH and may be severe. Evaluation of the entire blood vessel might be helpful for diagnosis and may be conducted using magnetic resonance angiography with the angiographic system for unlimited rolling fields of view (AngioSURF).[39]

Markers for risk of thrombosis include D-dimers and tissue factor microparticles, both of which may be elevated in patients with PNH.[40-43] Patients with thrombocytopenia also have an elevated risk of thromboembolism.[40] Data from the South Korean National PNH Registry identified several haemolysis and clinical symptoms associated with increased risk of thromboembolism.[37] Patients with elevated haemolysis (lactate dehydrogenase [LDH] levels ≥1.5-times the upper limit of normal [ULN] at diagnosis) were at significantly higher risk for thromboembolism than patients with LDH <1.5-times ULN (odds ratio: 7.0; p=0.013). The combination of LDH ≥1.5-times ULN with the clinical symptoms of abdominal pain, chest pain, dyspnoea, or haemoglobinuria was associated with a greater increased risk for thromboembolism than elevated haemolysis or clinical symptoms alone.[37]

Flow cytometry is the gold standard for diagnosis of PNH.[44] Detection of GPI-anchor proteins such as CD59 or CD55, or fluorescent aerolysin reagent (FLAER) on haematopoietic cells using monoclonal antibodies forms the basis of a specific PNH diagnostic test.[44,45] Identification of FLAER may be particularly useful as it selectively binds to the glycan core of GPI.[46] Patients at risk who should be tested for PNH include those with haemolysis (e.g. Coombs negative haemolytic anaemia, haemoglobinuria, or renal dysfunction), BMF (e.g. AA, MDS, or cytopenia), and patients with unexplained thrombosis.[44,47]

Supportive care for patients with PNH may include blood transfusion, folic acid and vitamin B12 supplementation, oral iron supplementation (if the patient is iron deficient), early treatment of

bacterial infections with antibiotics, hydration (in critical haemolysis), and anticoagulants (although these may be non-effective in many patients).[44,48-50] Supportive care however, does not affect disease progression and may be associated with additional adverse risk factors.

The monoclonal antibody eculizumab blocks the terminal complement cascade, eliminates the formation of the membrane attack complex, and prevents cell lysis.[51,52] In clinical trials, eculizumab improves survival and has demonstrated significant efficacy in reducing haemolysis, the need for blood transfusions, and the risk of thromboembolic complications versus controls. Patients treated with eculizumab also had reduced levels of fatigue, independent of haemoglobin levels, and a lower incidence of renal complications.[30,38,53-59] Eculizumab is indicated in adults and children for the treatment of patients with PNH and atypical haemolytic uraemic syndrome (aHUS).[29] In patients ≥40 kg, the recommended dose of eculizumab is 900 mg every 14±2 days.[30,60] Patients on eculizumab are susceptible to meningococcal infections, and vaccination with Meningococcal Group A, C, W135, Y conjugate vaccine (e.g. Menveo®) followed by Meningococcal Group B Vaccine (Bexsero®) should be considered.[58] Predictors of response to eculizumab include the degree of underlying BMF, concurrent inflammatory conditions such as bacterial and viral infections that can cause breakthrough haemolysis and autoimmune disease such as Crohn's disease, genetic factors such as missense C5 heterozygous mutation, and polymorphisms of CR1 that lead to a suboptimal response due to extravascular haemolysis. Figure 2 shows a proposed treatment algorithm for PNH; in the haemolytic setting, symptomatic patients can be treated with eculizumab in combination with supportive therapy.[61]

In a recently published case-report, a 64-year old female with PNH, thromboembolic complications, and subsequent transition to severe AA was treated with IST (hATG + cyclosporin A) in combination with eculizumab. No reduced ATG efficacy or severe adverse events were observed. Re-occurrence of PNH symptoms was prevented, while T cell depletion was similar to non-eculizumab-treated patients, with partial remission being evident by Day 83.[62]

In conclusion, PNH is a chronic, life-threatening disease associated with chronic complement-mediated haemolysis. Thrombosis is multifactorial, may occur unexpectedly, and is the primary cause of death in patients with PNH. The gold standard test for PNH is high-sensitivity flow cytometry performed on peripheral blood. Terminal complement inhibition with eculizumab has become the standard of care for symptomatic PNH.

REFERENCES

1. Young NS et al. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006;15;108(8):2509-19.

2. Ogawa T et al. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. Mech Ageing Dev. 2000;117(1-3):57-68.

3. Barrett J et al. Myelodysplastic syndrome and aplastic anemia: distinct entities or diseases linked by a common pathophysiology? Semin Hematol. 2000; 37(1):15-29.

4. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016. [Epub ahead of print].

5. Marsh J. 'Aplastic anaemia and pure red cell aplasia'. Warrell D et al. (eds.) Oxford Textbook of Medicine. 2013. Oxford University Press.

6. Solomou EE et al. Deficient CD4+ CD25+ FOXP3+ T regulatory cells in acquired aplastic anemia. Blood. 2007;110(5): 1603-6.

7. Kordasti S et al. Functional characterization of CD4+ T cells in aplastic anemia. Blood. 2012;119(9):2033-43.

8. Kordasti S. In press.

9. Araten DJ et al. Dynamics of hematopoiesis in paroxysmal nocturnal hemoglobinuria (PNH): no evidence for intrinsic growth advantage of PNH clones. Leukemia. 2002;16(11):2243-8.

10. Sloand EM et al. Preferential suppression of trisomy 8 compared with normal hematopoietic cell growth by autologous lymphocytes in patients with trisomy 8 myelodysplastic syndrome. Blood. 2005;106(3):841-51.

11. Hosokawa K et al. Favorable outcome of patients who have 13q deletion: a suggestion for revision of the WHO 'MDS-U' designation. Haematologica. 2012; 97(12):1845-9.

12. Kulasekararaj AG et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. Blood. 2014; 124(17):2698-704.

13. Yoshizato T et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. N Engl J Med. 2015;373(1):35-47.

14. Shen W et al. Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria. J Clin Invest. 2014;124(10):4529-38.

15. Dumitriu B et al. Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. Blood. 2015;125(4):706-9.

16. Desmond R et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. Blood. 2014;123(12):1818-25.

17. Sloand EM et al. Granulocyte colony-stimulating factor preferentially stimulates proliferation of monosomy 7 cells bearing the isoform IV receptor. Proc Natl Acad

Sci U S A. 2006;103(39):14483-8.

18. Townsley DM et al. Danazol Treatment for Telomere Diseases. N Engl J Med. 2016;374(20):1922-31.

19. Hillmen P et al. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995;333(19):1253-8.

20. Babushok DV et al. Clonal Replacement Underlies Spontaneous Remission in Paroxysmal Nocturnal Haemoglobinuria. Br J Haematol. 2016. [Epub ahead of print].

21. Kulasekararaj AG. Personal communication.

22. Killick SB et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol. 2016; 172(2):187-207.

23. Savage SA, Bertuch AA. The genetics and clinical manifestations of telomere biology disorders. Genet Med. 2010;12(12):753-64.

24. Camitta BM et al. Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. Blood. 1976;48(1):63-70.

25. Konopacki J et al. Long-term follow up after allogeneic stem cell transplantation in patients with severe aplastic anemia after cyclophosphamide plus antithymocyte globulin conditioning. Haematologica. 2012;97(5):710-6.

26. Scheinberg P et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med. 2011; 365(5):430-8.

27. Marsh JC et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. Blood. 2012;119(23):5391-6.

28. Maury S et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica. 2007;92(5):589-96.

29. Bacigalupo A et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA Working Party. Haematologica. 2010;95(6):976-82.

30. Soliris®. Summary of Product Characteristics. Available at: https://www.medicines.org.uk/emc/medicine/19966. Last accessed: 20 July 2016.

31. Brodsky RA. Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. Blood Rev. 2008;22(2): 65-74.

32. Röth A. Paroxysmale nächtliche Hämoglobinurie. Dtsch Arztebl. 2007 104(4):192-7.

33. Inoue N et al. Molecular genetics of paroxysmal nocturnal hemoglobinuria. Int J Hematol. 2003;77(2):107-12.

34. Rosse WF. New insights into paroxysmal nocturnal hemoglobinuria. Curr Opin Hematol. 2001;8(2):61-7.

35. Rother RP et al. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA. 2005;293(13):1 653-62.

36. Moyo VM et al. Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays. Br J Haematol. 2004;126(1):133-8.

37. Lee JW et al. Clinical signs and symptoms associated with increased risk for thrombosis in patients with paroxysmal nocturnal hemoglobinuria from a Korean Registry. Int J Hematol. 2013;97(6):749-57.

38. Hillmen P et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. Blood. 2007;110(12):4123-8.

39. Ruehm SG et al. [Whole-body MRA on a rolling table platform (AngioSURF)]. Rofo. 2000;172(8):670-4.

40. Socie G. Terminal complement inhibitor eculizumab improves complement-mediated platelet consumption and thrombocytopenia in patients with paroxysmal nocturnal hemoglobinuria (PNH). Abstract 4030. 51st American Society of Hematology Annual Meeting, New Orleans, Louisiana, USA, 5-8 December 2009.

41. Hill A et al. Recent developments in the understanding and management of paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2007;137(3):181-92.

42. Watt SG et al. Purpura fulminans in paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2007;137(4):271.

43. Helley D et al. Evaluation of hemostasis and endothelial function in patients with paroxysmal nocturnal hemoglobinuria receiving eculizumab. Haematologica. 2010;95(4):574-81.

44. Parker C et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005;106(12): 3699-709.

45. Richards SJ et al. Application of flow cytometry to the diagnosis of paroxysmal nocturnal hemoglobinuria. Cytometry. 2000;42(4):223-33.

46. Brodsky RA et al. Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. Am J Clin Pathol. 2000;114(3):459-66.

47. Sharma VR. Paroxysmal nocturnal hemoglobinuria: pathogenesis, testing, and diagnosis. Clin Adv Hematol Oncol. 2013;11 Suppl 13(9):2-8.

48. Marotta S et al. Hematopoietic stem cell transplantation for aplastic anemia and paroxysmal nocturnal hemoglobinuria: current evidence and recommendations. Expert Rev Hematol. 2014;7(6):775-89.

49. Santarone S et al. Hematopoietic stem cell transplantation for paroxysmal nocturnal hemoglobinuria: long-term results of a retrospective study on behalf of the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Haematologica. 2010; 5(6):983-8.

50. Bhatia S et al. Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. Blood. 2007;110(10):3784-92.

51. Rosse WF et al. Immune-mediated hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2004:48-62.

52. DeZern AE, Brodsky RA. Paroxysmal nocturnal hemoglobinuria: a complement-mediated hemolytic anemia. Hematol Oncol Clin North Am. 2015;29(3):479-94.

53. Hillmen P et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006; 355(12):1233-43.

54. Schubert J et al. Eculizumab, a terminal complement inhibitor, improves anaemia in patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol. 2008;142(2):263-72.

55. Brodsky RA et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood. 2008;111(4): 1840-7.

56. Hillmen P et al. Long-term effect of the complement inhibitor eculizumab on kidney function in patients with paroxysmal nocturnal hemoglobinuria. Am J Hematol. 2010;85(8):553-9.

57. de Latour RP et al. Paroxysmal nocturnal hemoglobinuria: natural history of disease subcategories. Blood. 2008; 112(8):3099-106.

58. Loschi M et al. Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: a treatment versus no-treatment study. Am J Hematol. 2016; 91(4):366-70.

59. Kelly RJ et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. Blood. 2011;117(25):6786-92.

60. Röth A, Dührsen U. Treatment of paroxysmal nocturnal hemoglobinuria in the era of eculizumab. Eur J Haematol. 2011;87(6):473-9.

61. Schubert J. Paroxysmale nächtliche

Hämoglobinurie (PNH). 2015. Available at: https://www.onkopedia.com/de/onkopedia/guidelines/paroxysmale-naechtliche-haemoglobinurie-pnh/@@view/html/index.html. Last accessed: 20 July 2016.

62. Alashkar F et al. Horse anti-thymocyte globulin and eculizumab as concomitant therapeutic approach in an aplastic paroxysmal nocturnal hemoglobinuria patient: go or no-go? Eur J Haematol. 2016. [Epub ahead of print].

NAVIGATING THE CHANGING MULTIPLE MYELOMA TREATMENT LANDSCAPE

This symposium took place on 9th June 2016, as a part of the 21st Congress of the European Hematology Association (EHA) 2016 in Copenhagen, Denmark

Paul Richardson,[1] Roman Hájek,[2] Antonio Palumbo,[3,4] Shaji Kumar,[5] Katarina Luptakova[4]

1. Harvard Medical School and Dana-Farber Cancer Institute, Boston, Massachusetts, USA
2. University Hospital Ostrava and University of Ostrava, Ostrava, Czech Republic
3. University of Turin, Turin, Italy
4. Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited
5. Mayo Clinic, Rochester, Minnesota, USA

Disclosure: Prof Richardson has received honoraria for scientific advisory board engagements from Celgene, Takeda Oncology, Novartis, Bristol-Myers Squibb, Genmab and Janssen, as well as research grants from Celgene and Takeda Oncology. Prof Hájek has received honoraria from Amgen, Janssen, Celgene, Takeda, and Bristol-Myers Squibb, as well as research grants from Amgen, Janssen, Celgene, and Takeda. Dr Palumbo has received honoraria from Amgen, Bristol-Myers Squibb, Genmab, Celgene, Janssen, Takeda, Onyx, Novartis, and Sanofi, as well as honoraria for consulting from Amgen, Bristol-Myers Squibb, Genmab, Celgene, Janssen, Takeda, and Onyx, and has been an employee of Takeda Oncology as of July 1st 2016. Prof Kumar has received honoraria for consulting from Takeda, Celgene, AbbVie, Janssen, Sanofi, SkylineDx, GlycoMimetics, NOXXON, and Kesios Therapeutics, as well as research grants from Takeda, Celgene, Novartis, AbbVie, Janssen, and Sanofi.
Acknowledgements: Writing assistance was provided by Mia Cahill BSc, ApotheCom.
Support: The publication of this article was funded by Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. The views and opinions expressed are those of the authors and not necessarily Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited.
Citation: EMJ Hematol. 2016;4[1]:55-65.

MEETING SUMMARY

The treatment landscape for patients with multiple myeloma (MM) is constantly evolving. Over the past decade, the introduction of novel agents including proteasome inhibitors (PI) and immunomodulatory agents has led to notable changes in therapeutic strategy and significant improvements in survival. Understanding this landscape and what this means in terms of translating clinical trials to everyday practice is essential.

Prof Paul Richardson opened the symposia with an introduction to currently available agents and recent developments in MM, and highlighted the importance of how we think about current studies. Prof Roman Hájek explored clonal evolution, how it can be prevented in the context of relapsed disease, and the evidence from clinical trials supporting the use of combination therapy. Dr Antonio Palumbo addressed the concept of continuous therapy in MM and where the field is at present. Prof Shaji Kumar described the early phase development of ixazomib. Prof Paul Richardson presented the results from the TOURMALINE-MM1 trial.

Exploring Recent Developments in Multiple Myeloma Therapeutics

Professor Paul Richardson

Prof Richardson opened the symposia by highlighting the array of agents currently available and the recent developments in therapies to treat MM (Figure 1). While the increasing range of effective therapeutic agents is very encouraging and promises to further improve patient outcomes, it adds to the complexity of clinical treatment decisions and clinicians therefore need to carefully examine the evidence base in guiding their decisions. The danger of cross-trial comparison was highlighted by the difference in response rates (RRs) reported in several trials studying the same drug combination in the same disease setting.[1,2]

Cross-trial comparisons therefore should be approached with caution and it is important to remember that outcomes depend on the patient population and study design which can lead to possible bias and choice of endpoints. Baseline patient characteristics, including cytogenetic risk, International Staging System (ISS) stage, age, renal function, refractory status, and prior therapy, will all influence outcomes and ideally patients baseline characteristics in these parameters should be well-balanced between treatment arms.[3-8]

Study design should be taken into account when comparing the outcomes of clinical trials; single-arm trials do not adequately characterise time-to-event endpoints (such as overall survival [OS], time-to-progression and progression-free survival [PFS]); therefore, randomised trials are necessary to evaluate these endpoints to account for the variability in the natural history of cancer.[9] Open-label trials can lead to assessment bias for PFS.[10] Blinding is preferred and is particularly important when patient or investigator assessments are included as components of the progression endpoint.[9]

Potential sources of bias can arise from the comparison between older studies that used modified European Society for Blood and Marrow Transplantation (EBMT) criteria, which would penalise high rates of complete response (CR) as reappearance of a positive immunofixation electrophoresis would be counted as progression from CR[11,12] while more recent trials use international uniform response criteria.[12,13] Additionally, open-label studies are at risk of asymmetrical censoring (attrition bias), especially early in the study, which can affect the Kaplan–Meier curve.[10] Double-blind, placebo controlled studies, on the other hand, are less vulnerable to bias.

While the ideal main endpoint would be OS, surrogate endpoints (e.g. PFS, RR, and rate of minimal residual disease [MRD]) are commonly used. The depth of response, including MRD, has been shown to correlate with PFS and OS.[14-16] Interestingly, outcomes can vary within categories of response; as an example patients who sustain a partial response have longer survival compared with patients achieving and then losing CR[17] and patients who improve their response to near-CR (nCR) post-transplant have longer OS compared with patients achieving nCR before transplant.[18] Arguably, sustained response may be more important than rapid achievement of deep response.

When interpreting clinical trials, the efficacy (OS, median PFS, hazard ratio [HR] for progression or death, and overall response rate [ORR]), patient population (how many prior lines of therapy), study design, primary endpoints, and eligibility criteria driving the patient study population should be considered. Additionally, when choosing a treatment regimen for an individual patient, the toxicity profile and real-world outcomes need to be considered (such as route of administration, the number of clinic visits, dosing schedule, administration time, whether premedication and pre-hydration are required, and what can be achieved in terms of convenience) (Table 1).

Preventing Clonal Evolution in Multiple Myeloma: Evidence from Clinical Trials

Professor Roman Hájek

Introducing the concepts of intraclonal heterogeneity and clonal evolution

MM is a complex disease that arises from an initial 'hit' that causes premalignant proliferation of plasma cells derived from post-germinal-centre B cells. The natural course of disease evolves from these early stages to monoclonal gammopathy of undetermined significance, progressing to smouldering myeloma, through to active myeloma, and eventually to plasma cell leukaemia, the most aggressive form of disease.[19,20]

In the initial stages of disease, primary genetic events include immunoglobulin heavy locus

(IGH) translocations and hyperdiploidy. Transition throughout different states is thought to involve increasing genetic instability with the acquisition of secondary genetic events, such as copy number abnormalities, DNA hypomethylation, and acquired mutations.[20]

At the time of diagnosis, multiple genetically distinct subclones are present and evolve over time due to selective pressure imposed by treatment and factors in the microenvironment which can result in disease progression and resistance to treatment.[21-25] Myeloma can exhibit either: no heterogeneity (all MM cells are the same), intraclonal heterogeneity (different MM clones that share features), and interclonal heterogeneity (different MM clones that do not share features).[25] In a proof-of-concept report, a patient with MM and multiple sites of extramedullary disease was followed serially with biopsies taken from multiple tumour sites. Different mutations were detected in samples from different biopsy sites in the same patient which outlines the concept of spatially divergent clonal evolution.[26]

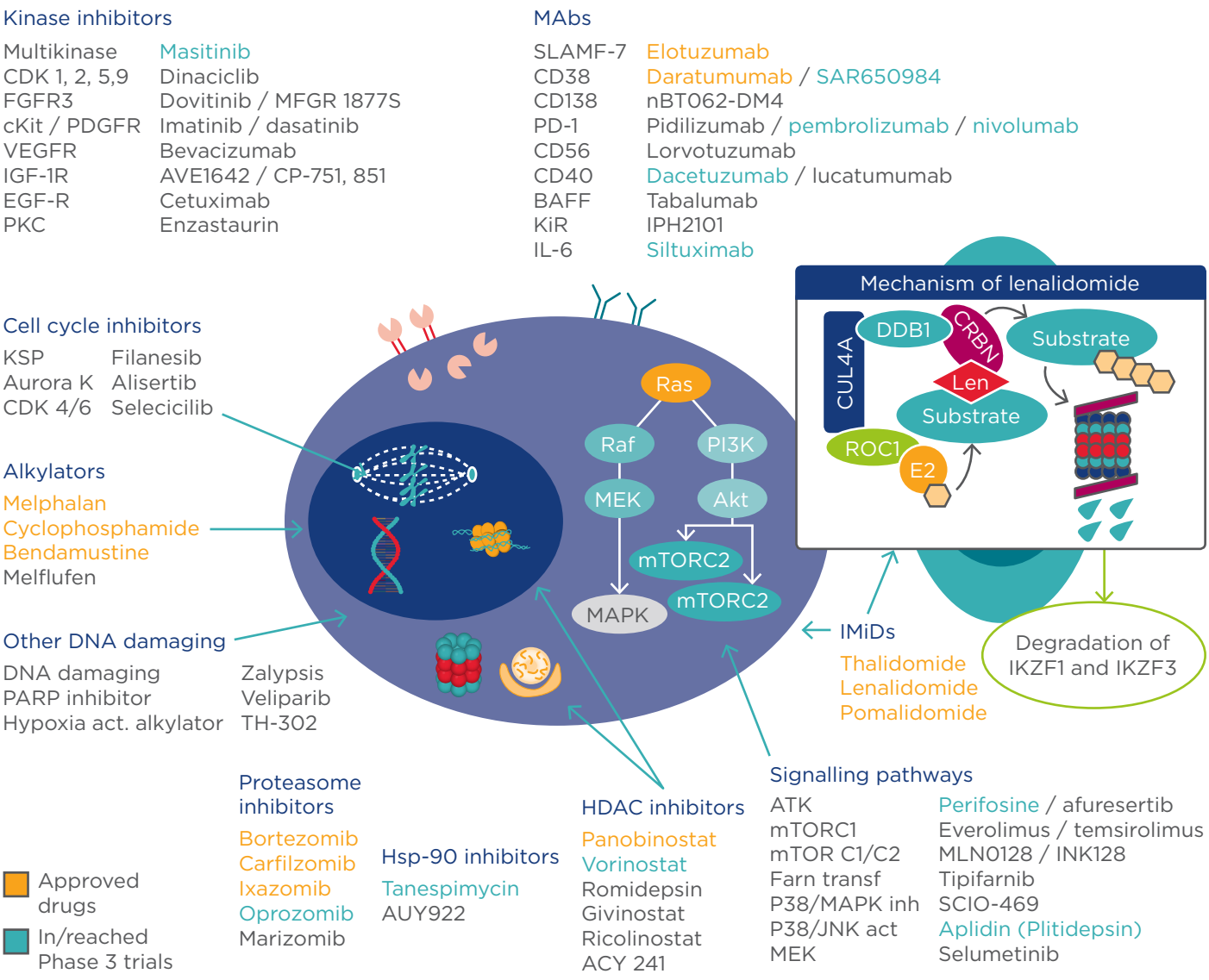

Figure 1: The changing treatment landscape in multiple myeloma.

IMiDs: immunomodulatory drugs; MAbs: monocolonal antibodies; PARP: poly A ribose polymerases; HDAC: histone deacetylase; Hsp-90: heat shock protein 90; IL: interleukin; FGFR3: fibroblast growth factor receptor 3; PDGFR: platelet-derived growth factor receptor; VEGFR: vascular endothelial growth factor receptor; IGF: insulin-like growth factor; EGF: epidermal growth factor; PD-1: programmed cell death protein 1; BAFF: B cell activating factor; KSP: kinesin spindle protein; MAPK: mitogen-activated protein kinase; MTORC: mammalian target of rapamycin complex.

Adapted from Ocio EM et al.[79] and Fink EC, Ebert BL.[80]

Table 1a: Phase III trials in relapsed/refractory multiple myeloma. Study overview and patient eligibility and real-world considerations.[29-34]

Clinical trial (Treatment arm)	Patient population	Study design	Primary endpoint	Patient eligibility					Route of administration	Clinic visits (18 cycles)	Dosing schedule	Premedication? Prehydration? Administration time?
				IMiD-refractory	PI-refractory	Primary refractory	Measurable by FLC only	CrCl cut-off (mL/min)				
PANORAMA-1 (Bortezomib + dexamethasone and panobinostat versus placebo)	1–3 prior lines of therapy	Placebo-controlled double-blind	PFS	Yes	No V-refractory	No	No	60	Oral	48	Days 1, 3, 5, 8, 10, 12 of 21-day cycle	No No NA
ASPIRE (Lenalidomide + dexamethasone ± carfilzomib)	1–3 prior lines of therapy	Open-label	PFS	No R-refractory	No	No	No	50	IV	96	Days 1, 2, 8, 9, 15, 16 of 28-day cycle	No Yes 10 minutes
ELOQUENT-2 (Lenalidomide + dexamethasone ± elotuzumab)	1–3 prior lines of therapy	Open-label	PFS, ORR	No R-refractory	Yes	Yes	No	30	IV	44	Days 1, 8, 15, 22 of 28-day cycles 1 and 2 then Days 1 and 15	Yes NR Up to 5 hours
TOURMALINE-MM1 (Lenalidomide + dexamethasone and ixazomib versus placebo)	1–3 prior lines of therapy	Placebo-controlled double-blind	PFS	No R-refractory	No	Yes	Yes	30	Oral	18	Days 1, 8, 15 of 28-day cycle	No No NA
CASTOR (Bortezomib + dexamethasone ± daratumumab)	≥1 prior line of therapy	Open-label	Patients with PFS (%)	Yes	No	No	NR	NR	IV	28	Weeks 1–8: weekly Weeks 9–24: every 2 weeks Then every 4 weeks	Yes No First infusion: ≥6.5 hours Subsequent: ≥3.5 hours
POLLUX (Lenalidomide + dexamethasone ± daratumumab)	≥1 prior line of therapy	Open-label	PFS	No R-refractory	Yes	No	Yes	30				

Table 1b: Phase III trials in relapsed/refractory multiple myeloma. Efficacy and key toxicities.

Clinical trial	Treatment arms	Median PFS (months)	HR for progression or death	ORR	Key toxicities of clinical relevance/considerations
PANORAMA-1	Bortezomib + dexamethasone and panobinostat versus placebo	12 vs. 8	0.63 p<0.0001	61% vs. 55%	Haemorrhage, hepatotoxicity, diarrhoea, cardiac toxicities, myelosuppression, infections
ASPIRE	Lenalidomide + dexamethasone ± carfilzomib	26 vs. 18	0.69 p=0.0001	87% vs. 67%	Cardiac toxicities, acute renal failure, TLS, pulmonary toxicity, pulmonary hypertension, dyspnoea, HTN, VTE, infusion reactions, thrombocytopenia, hepatic toxicity and hepatic failure, TTP/HUS, PRES
ELOQUENT-2	Lenalidomide + dexamethasone ± elotuzumab	19 vs. 15	0.70 p<0.001	79% vs. 66%	Infusion reactions, Infections, second primary malignancies, hepatotoxicity, interference with determination of CR
TOURMALINE-MM1	Lenalidomide + dexamethasone and ixazomib versus placebo	21 vs. 15	0.74 p=0.01	78% vs. 72%	Thrombocytopenia, GI toxicities, peripheral neuropathy, peripheral oedema, cutaneous reactions, hepatotoxicity
CASTOR	Bortezomib + dexamethasone ± daratumumab	NR vs. 7	0.39 p<0.0001	83% vs. 63%	Infusion reactions, interference with serological testing (causes false positive Coombs test) and with determination of CR*
POLLUX	Lenalidomide + dexamethasone ± daratumumab	NR vs. 18	0.37 p<0.0001	93% vs. 76%	

*Based on single-agent studies

IMiD: immunomodulatory drugs; PI: proteasome inhibitors; HR: hazard ratio; ORR: overall response rate; FLC: free light-chain; CrCl: creatinine clearance rate; PFS: progression-free survival; R/V-refractory: lenalidomide/bortozemib refractory; IV: intravenous; N/A: not applicable NR: not reported; HTN: hypertension; TTP: thrombotic thrombocytopenic purpura; HUS: haemolytic-uremic syndrome; PRES: posterior reversible encephalopathy syndrome; VTE: venous thromboembolism; CR: complete response; TLS: tumour lysis syndrome; GI: gastrointestinal.

What are the clinical implications of clonal evolution?

The clinical implications of clonal evolution can be simplified into three key points: 1) Rationale for earlier therapy when there is less genetic instability and a lower number of clones such as treating high-risk smouldering myeloma;[27] 2) There is generally no place for monotherapy, targeted therapy, and personalised therapy at the beginning of treatment in both newly diagnosed MM (NDMM) and relapsed/refractory patients, on the contrary combination therapy is needed; 3) However, residual disease may be possibly an important place for the use of targeted therapy.[22,25]

What are our current strategic approaches?

There are three main therapeutic strategic approaches to address clonal heterogeneity in MM:[10,11]

- Combining agents with different mechanisms of action (MoAs)
- Sequencing multiple different MoAs
- Prolonging duration of therapy

Combining several drugs with different MoAs has a proven place in preventing clonal evolution, as demonstrated by several clinical trials both in newly diagnosed and in the relapsed setting[28-38] that compared doublet versus triplet regimens. All of the reviewed trials demonstrated the superior outcomes seen with triplet over doublet

therapies. Furthermore, a meta-analysis of triplet versus doublet regimens in relapsed MM recently demonstrated a clear benefit for triplet therapy in RRs and PFS.[39] Among the patients with high-risk cytogenetics, improved outcomes with triplet therapy are particularly notable in TOURMALINE-MM1, where the addition of ixazomib to the lenalidomide-dexamethasone backbone improved median PFS among patients with high-risk cytogenetics; 9.7 months to 21.4 months (HR: 0.54, p=0.02).[30] Similarly, in ELOQUENT-2 the addition of elotuzumab to lenalidomide-dexamethasone backbone resulted in improvement in PFS among patients with high-risk cytogenetics (HR: 0.53).[31] In newly diagnosed patients, trials comparing triplet and doublet regimens have proven benefits for both PFS and OS outcomes, with impressive results from SWOG S0777, where the combination of bortezomib-lenalidomide-dexamethasone is compared to lenalidomide-dexamethasone. The combination of bortezomib-lenalidomide-dexamethasone, when compared to lenalidomide and dexamethasone, was associated with an improvement of PFS (median PFS: 43 versus 30 months respectively; HR: 0.712; p=0.0018) and OS (median OS: 75 versus 64 months, respectively, HR: 0.709; p=0.025).[37]

A sequential or alternating MoA schedule was recently investigated by Mateos et al.[40] The trial compared a sequential scheme of nine cycles of bortezomib plus melphalan and prednisone (VMP) and then nine cycles of lenalidomide plus low-dose dexamethasone (Rd) or an alternating scheme of VMP and Rd in newly diagnosed transplant-ineligible patients. Compared with treatment with VMP alone or Rd alone reported historically, both alternating and sequential schedules gave a greater RR as well as longer PFS.

In summary, MM is a complex disease, with multiple genetically distinct subclones present at diagnosis.[21-24] Intraclonal heterogeneity has implications for disease progression and resistance to therapy through clonal evolution.[25] Combining agents with different MoAs may have a synergistic effect and result in a deeper response by targeting all coexisting disease subclones.[22,25,41] Sequential use of agents with different MoAs also shows promise in improving outcomes for patients with MM.[40,42]

Continuous Therapy: Are We There Yet?

Doctor Antonio Palumbo

The progression of myeloma involves increasing genetic and epigenetic abnormalities; and the number of present mutations is increased at each relapse. While patients in the early phase of the disease can expect a 67% likelihood of being progression-free at 5 years, patients who are refractory to prior therapies have a median PFS of around 5 months.[43,44] Complete remission in response to therapy is more likely in NDMM than in relapsed/refractory MM.[30] Furthermore, with each subsequent relapse up to 40% will not proceed for various reasons to receive subsequent therapy. This is changing the clinical paradigm, where the aim should be to prolong the early phases of disease, while shifting the goals of care to consider early palliative care in the late stages.

Continuous therapy has been developed to prolong the early, sensitive phase of the disease. Although continuous therapy has cost implications, meta-analyses of thalidomide, lenalidomide, and bortezomib maintenance trials show a clear advantage for PFS and OS for maintenance therapy.[45]

While there may be a theoretical concern about therapy pressure leading to the new treatment-resistant clones, the results of clinical studies to date suggest that continuous treatment does not encourage the development of resistant tumours. On the contrary, when the tumour is not controlled, there is a higher risk of clonal evolution, whereas treatment delays the occurrence of mutations.[25] In a pooled analysis of three Phase III trials, an improvement of PFS1, PFS2, and OS was demonstrated for continuous therapy compared with fixed duration therapy, while the duration of second PFS was 15 months with both approaches.

Continuous therapy versus fixed-duration therapy makes a significant difference even in the subgroup of patients who achieved CR in terms of PFS (5-year PFS: 42% and 21%, respectively) and OS (5-year OS: 80% and 54%, respectively).[46] The reason for the survival benefit is likely because residual disease is present but undetected, even in patients who appear to have CR.[47,48]

The question of how long maintenance therapy should be continued has yet to be definitively answered, but tumour reduction can still be seen after 18 months of treatment.[49] If possible,

treatment should continue until progression. However, continuous therapy must be well tolerated; fatigue, diarrhoea, or other adverse events (AEs) are likely to impact heavily on patient quality of life over 5 years. Toxicities associated with therapies such as bortezomib or lenalidomide, like neutropenia or neuropathy,[50-52] will need to be managed or reduced. Early data on ixazomib single agent used in maintenance showed limited peripheral neuropathy and haematologic toxicity which opens the possibilities to examine the use of this PI in the maintenance setting. In fact, there are two ongoing large Phase III studies examining ixazomib for maintenance (TOURMALINE-MM3 and MM4, NCT02181413, and NCT02312258, respectively).

Another PI, carfilzomib, is also being studied in maintenance (FORTE study).[53] Maintenance therapy with monoclonal antibodies such as elotuzumab or daratumumab are also being investigated.[54]

The Ixazomib Development Journey Ixazomib for Myeloma: Where Could it Fit in?

Professor Shaji Kumar

PIs have played a very important role in all stages of myeloma therapy over the past 15 years. PI-based combinations are standard initial therapy regimens, and a meta-analysis has demonstrated improved survival outcomes.[55] They are critical for patients with high-risk cytogenetics[56] and renal failure,[57,58] and have roles in both consolidation and maintenance approaches.[49,57] There are some challenges we face with current PIs; most notably, peripheral neuropathy with bortezomib,[59,60] potential vascular side effects with carfilzomib,[61] and need for parenteral administration with both bortezomib and carfilzomib.[62,63] In this context, ixazomib was developed as an oral PI. Preclinically, it was shown to be efficacious against a variety of cell lines as well as xenograft models.[64]

In clinical trials, ixazomib has shown efficacy as a single agent or in combination.[65-68] As a single-agent in Phase I studies of twice-weekly and once-weekly dosing in heavily pretreated MM patients with relapsed/refractory disease, ixazomib achieved ORRs of 15%[69] and 27%[70] (at maximum tolerated dose), respectively. Its pharmacokinetic properties were not affected by body surface area, enabling fixed dosing of 4 mg (given at Days 1, 8, and 15 of a 28-day cycle), with a reduced dose of 3 mg for patients with severe renal insufficiency or moderate/severe hepatic impairment.[71-75]

While ixazomib induced a response as a single agent in some patients in early phase clinical trials, combination with dexamethasone improves efficacy. When combined with dexamethasone on an as-needed basis, in patients with relapsed disease who were not refractory to bortezomib, an ORR of about 30%[65] was achieved; and in combination with dexamethasone at two different doses, 4 mg and 5.5 mg, ORRs of 31% (95% confidence interval [CI]: 17–49) and 51% (95% CI: 37–71) were achieved, respectively.[65] Overall, patients in the ixazomib 5.5 mg treatment arm had more dose reductions, treatment delays, and a higher frequency of AEs (54% of patients in the 5.5 mg arm experienced Grade 3+ AEs compared to 26% in the 4 mg arm) than patients in the 4 mg treatment arm.[65]

In the newly diagnosed setting, a Phase I/II study of weekly ixazomib in combination with lenalidomide and dexamethasone demonstrated an ORR of 90%, with 27% of patients achieving CR or stringent CR (sCR)[66] after 12 cycles. In a similar study with twice-weekly ixazomib dosing, 26% of patients achieved CR, including 19% with sCR.[67] When combined with cyclophosphamide and dexamethasone in newly diagnosed patients, the ORR was 77% (95% CI: 63–88). Grade 3 or higher AEs were seen in 73% of patients and most commonly included cytopenias, fatigue, and gastrointestinal side effects.[1]

Ixazomib's side effects are generally manageable and toxicities are well managed with dose reduction and supportive care.[69,70,75] No cumulative toxicities have been seen within the timeframe of the clinical trials. The most common toxicities include cytopenias (potentially a class effect of PIs), gastrointestinal side effects, and fatigue.[69,70,75] Peripheral neuropathy, a significant side effect of bortezomib, does not appear to be very common in single-agent or combination therapy with ixazomib, and when present is generally low grade (Grade 1–2) with infrequent Grade 3 toxicity. Most patients recovered with dose modification.

Ixazomib has been shown to be effective with manageable side effects and, as an oral drug, is potentially more convenient. It is currently being studied in combination with alkylators such as melphalan and cyclophosphamide,

immunomodulatory drugs, histone deacetylase inhibitors, and monoclonal antibodies. Future directions aim to explore suitability with other novel drugs and alternating dosing schedules.

The Phase III TOURMALINE-MM1 Study

Professor Paul Richardson

As has been discussed in this symposium review, PIs form the backbone of therapy[29,44,67,76,77] but the critical point is the shift in treatment patterns towards extended therapy. In that context, acceptable side effect profiles are key to enable patients to continue therapy for prolonged periods of time.[77] Preclinical studies of ixazomib and lenalidomide have indicated synergy[64] and early-phase studies of ixazomib-Rd in NDMM appeared to be promising;[66,67] providing a rationale for the Phase III TOURMALINE-MM1 study.[30]

The TOURMALINE-MM1 study was the first Phase III study of an all-oral triplet regimen incorporating an oral PI and an immunomodulatory agent in relapsed/refractory myeloma patients.[30] After the Phase I development of ixazomib defined an optimal dose and schedule,[68,69] combination studies in the newly diagnosed setting appeared to support the use of Ixazomib in combination with lenalidomide.[65]

Study design

The global, double-blind, placebo-controlled study enrolled 722 patients with relapsed/refractory MM who had received 1–3 prior lines of therapy. Patients in the investigational arm (n=360) received ixazomib 4 mg on Days 1, 8, and 15, lenalidomide 25 mg on Days 1–21 and dexamethasone 40 mg on Days 1, 8, 15, and 22 (IRd). Patients in the control arm (n=362) received placebo, lenalidomide, and dexamethasone (placebo-Rd).[30]

Key inclusion criteria included confirmed diagnosis of MM, measurable disease by serum protein electrophoresis, urine protein electrophoresis or free light-chain assay, 1–3 prior lines of therapy, relapsed and/or refractory disease and creatinine clearance ≥30 mL/min. Patients who were refractory to previous PI or lenalidomide-based treatment and those with peripheral neuropathy higher than Grade 1 with pain or Grade 2 or more were excluded.[30]

Patient demographics were well balanced across both groups, including median age, ISS stage, prior lines of therapy, PI exposure, type of prior regimen, and cytogenetics.

Key efficacy data

The median PFS (primary endpoint) at the final PFS analysis was 20.6 months in the IRd arm and 14.7 months in the placebo-Rd arm (p=0.012, HR: 0.74). While not statistically powered for PFS subgroup analysis, PFS benefit was consistent across key pre-specified patient subgroups, including those with poor prognosis, such as patients with high-risk cytogenetic abnormalities (defined as del(17p), t(4;14) or t(14;16)), multiple prior lines of therapy and high disease burden. The median PFS data indicate that an ixazomib regimen might offer improved prognosis for patients with high-risk cytogenetics by lengthening PFS to a value that is similar to that among patients with standard-risk cytogenetics. Among the patients with high-risk cytogenetics, median PFS for IRd and placebo-Rd was 21.4 months and 9.7 months, respectively (HR: 0.543). There was an 11-month median PFS benefit for patients with del(17p) and a 7-month median PFS benefit for patients with t(4;14) translocation. Median OS was not reached in either arm. IRd improved RRs versus placebo-Rd, and deepening responses were observed with ongoing treatment.[30]

Key safety data

In terms of tolerability, the median relative dose intensity was 97.4% and 98.8% for ixazomib versus placebo. There was a higher frequency of Grade ≥3 AEs with IRd versus placebo-Rd, primarily due to thrombocytopenia. Serious AEs were marginally higher in the placebo group (49% in placebo-Rd versus 47% in IRd). In general, the difference in AEs was not pronounced; although, one AE, rash, was more prominent in the treatment group, from clinical experience, this is generally manageable.[30] Importantly, patient-reported quality of life (as measured by EORTC-QLQ-C30) was maintained when ixazomib was added to the lenalidomide/dexamethasone doublet regimen.[30,78]

Overall, ixazomib, when combined with Rd for patients with relapsed or refractory MM and compared with placebo-Rd, was associated with a significant and clinically meaningful improvement in PFS, improved time-to-progression and RRs. While not powered for subgroup analysis ixazomib regimen improved PFS in high-risk patients that

was similar to that seen in the overall patient population and standard-risk patients. Limited additional toxicities were seen with ixazomib; and importantly, rates of peripheral neuropathy, cardiac, or renal AEs were low, making long-term treatment feasible.[30] Ixazomib has since been approved in the USA in combination with lenalidomide and dexamethasone for the treatment of patients with MM who have received at least one prior therapy.[74]

REFERENCES

1. Richardson PG et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood. 2010;116(5):679-86.

2. Durie B et al. Bortezomib, Lenalidomide and Dexamethasone Vs. Lenalidomide and Dexamethasone in Patients (Pts) with Previously Untreated Multiple Myeloma without an Intent for Immediate Autologous Stem Cell Transplant (ASCT): Results of the Randomized Phase III Trial SWOG S0777. Abstract 25. American Society of Hematology Annual Meeting, San Diego, California, USA, 5-8 December 2015.

3. Kumar SK et al. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia. 2014;28(5):1122-8.

4. Palumbo A et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. J Clin Oncol. 2015;33(26): 2863-9.

5. Ludwig H et al. Survival and years of life lost in different age cohorts of patients with multiple myeloma. J Clin Oncol. 2010; 28(9):1599-605.

6. Gonsalves WI et al. Improvement in renal function and its impact on survival in patients with newly diagnosed multiple myeloma. Blood Cancer J. 2015;5:e296.

7. Majithia N et al. Outcomes of primary refractory multiple myeloma and the impact of novel therapies. Am J Hematol. 2015;90(11):981-5.

8. Kumar SK et al. Clinical course of patients with relapsed multiple myeloma. Mayo Clin Proc. 2004;79(7):867-74.

9. FDA. Guidance for Industry: Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics. 2007. Available at: http://www.fda.gov/downloads/Drugs/.../Guidances/ucm071590.pdf. Last accessed: 20 July 2016.

10. Freidlin B et al. Proposal for the use of progression-free survival in unblinded randomized trials. J Clin Oncol. 2007;25(15):2122-6.

11. Bladé J et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol. 1998;102(5):1115-23.

12. Durie BG et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20(9):1467-73.

13. Rajkumar SV et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood. 2011;117(18): 4691-5.

14. Gay F et al. Complete response correlates with long-term progression-free and overall survival in elderly myeloma treated with novel agents: Analysis of 1175 patients. Blood. 2011;117(11):3025-31.

15. Rawstron AC et al. Minimal residual disease in myeloma by flow cytometry: Independent prediction of survival benefit per log reduction. Blood. 2015; 125(12):1932-5.

16. Martinez-Lopez J et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood. 2014;123(20):3073-9.

17. Hoering A et al. Complete remission in multiple myeloma examined as time-dependent variable in terms of both onset and duration in Total Therapy protocols. Blood. 2009;114(7):1299-305.

18. Lahuerta JJ et al. Influence of pre- and post-transplantation responses on outcome of patients with multiple myeloma: Sequential improvement of response and achievement of complete response are associated with longer survival. J Clin Oncol. 2008;26(35): 5775-82.

19. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364(11): 1046-60.

20. Morgan GJ et al. The genetic architecture of multiple myeloma. Nat Rev Cancer. 2012;12(5):335-48.

21. Bahlis NJ. Darwinian evolution and tiding clones in multiple myeloma. Blood. 2012;120(5):927-8.

22. Keats JJ et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012;120(5):1067-76.

23. Bianchi G, Ghobrial IM. Biological and Clinical Implications of Clonal Heterogeneity and Clonal Evolution in Multiple Myeloma. Curr Cancer Ther Rev. 2014;10(2):70-9.

24. Bolli N et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun. 2014; 5:2997.

25. Brioli A et al. The impact of intra-clonal heterogeneity on the treatment of multiple myeloma. Br J Haematol. 2014; 165(4):441-54.

26. Raab MS et al. Spatially divergent clonal evolution in multiple myeloma: overcoming resistance to BRAF inhibition. Blood. 2016;127(17):2155-7.

27. Mateos MV et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. N Engl J Med. 2013; 369(5):438-47.

28. Garderet L et al. Superiority of the triple combination of bortezomib-thalidomide-dexamethasone over the dual combination of thalidomide-dexamethasone in patients with multiple myeloma progressing or relapsing after autologous transplantation: the MMVAR/IFM 2005-04 Randomized Phase III Trial from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2012;30(20):2475-82.

29. Stewart AK et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med. 2015;372(2):142-52.

30. Moreau P et al. Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. N Engl J Med. 2016; 374(17):1621-34.

31. Lonial S et al. Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. N Engl J Med. 2015;373(7):621-31.

32. Palumbo A et al. Phase III randomized controlled study of daratumumab, bortezomib, and dexamethasone (DVd) versus bortezomib and dexamethasone (Vd) in patients (pts) with relapsed or refractory multiple myeloma (RRMM). Abstract LBA4. ASCO Annual Meeting, 3-7 June 2016.

33. Dimopoulos M et al. An open-label, randomised Phase 3 study of daratumumab, lenalidomide and dexamethasone (DRD) versus lenalidomide and dexamethasone (RD) in relapsed or refractory multiple myeloma (RRMM): POLLUX. Abstract LB2238. EHA

Annual Meeting, 9-12 June 2016.

34. San-Miguel JF et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol. 2014;15(11):1195-206.

35. San Miguel JF et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9):906-17.

36. Mateos MV et al. Bortezomib plus melphalan and prednisone compared with melphalan and prednisone in previously untreated multiple myeloma: updated follow-up and impact of subsequent therapy in the phase III VISTA trial. J Clin Oncol. 2010;28(13):2259-66.

37. San Miguel JF et al. Persistent overall survival benefit and no increased risk of second malignancies with bortezomib-melphalan-prednisone versus melphalan-prednisone in patients with previously untreated multiple myeloma. J Clin Oncol. 2013;31(4):448-55.

38. Liang M et al. Utility of Hepatocyte Growth Factor as a Biomarker for Early Diagnosis of Pulmonary Artery Hypertension. Mol Diagn Ther. 2016. [Epub ahead of print].

39. Nooka AK, et al. Efficacy and safety of triplet versus doublet salvage therapies among relapsed myeloma patients: Meta-analysis of phase 3 randomized controlled trials. Abstract 8020. ASCO Annual Meeting. 3-7 June 2016.

40. Mateos MV et al. Sequential vs alternating administration of VMP and Rd in elderly patients with newly diagnosed MM. Blood. 2016;127(4):420-5.

41. Hideshima T et al. Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. Mol Cancer Ther. 2011;10(11):2034-42.

42. Palumbo A et al. Continuous Therapy Versus Fixed Duration of Therapy in Patients With Newly Diagnosed Multiple Myeloma. J Clin Oncol. 2015;33(30): 3459-66.

43. Usmani SZ et al. Improvement in long-term outcomes with successive Total Therapy trials for multiple myeloma: are patients now being cured? Leukemia. 2013;27(1):226-32.

44. Kumar SK et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. Leukemia. 2012;26(1):149-57.

45. Ludwig H et al. IMWG consensus on maintenance therapy in multiple myeloma. Blood. 2012;119(13):3003-15.

46. Arnott C et al. Survival outcomes in severe congenital versus non-congenital pulmonary hypertension. Heart Asia. 2016; 8(1):3-7.

47. Paiva B et al. New criteria for response assessment: Role of minimal residual disease in multiple myeloma. Blood. 2015; 125(20):3059-68.

48. Moreau P et al. Prospective Evaluation of MRI and PET-CT at Diagnosis and before Maintenance Therapy in Symptomatic Patients with Multiple Myeloma Included in the IFM/DFCI 2009 Trial. Blood. 2014; 124:3359.

49. Gambella M et al. Minimal Residual Disease Monitoring During Maintenance In Multiple Myeloma Patients. Blood. 2013; 122:3126.

50. Palumbo A et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol. 2010;28(34):5101-9.

51. Palumbo A et al. Autologous transplantation and maintenance therapy in multiple myeloma. N Engl J Med. 2014; 371(10):895-905.

52. Kumar S et al. Long-Term Ixazomib Maintenance Is Tolerable and Improves Depth of Response Following Ixazomib-Lenalidomide-Dexamethasone Induction in Patients (Pts) with Previously Untreated Multiple Myeloma (MM): Phase 2 Study Results. Abstract 82. ASH Annual Meeting, 6-9 December 2014.

53. Silvio Aime. Evaluation of the Safety and the Efficacy of Carfilzomib Combined With Cyclophosphamide and Dexamethasone (CCyd) or Lenalidomide and Dex (CRd) Followed by ASCT or 12 Cycles of Carf Combined With Dex and Len for Patients Eligible for ASCT With Newly Diagnosed Multiple Myeloma. (FORTE). NCT02203643. https://clinicaltrials.gov/ct2/show/NCT02203643.

54. Intergroupe Francophone du Myelome. A Study to Evaluate Daratumumab in Transplant Eligible Participants With Previously Untreated Multiple Myeloma (Cassiopeia). NCT02541383. https://clinicaltrials.gov/ct2/show/NCT02541383.

55. Sonneveld P et al. Bortezomib-based versus nonbortezomib-based induction treatment before autologous stem-cell transplantation in patients with previously untreated multiple myeloma: a meta-analysis of phase III randomized, controlled trials. J Clin Oncol. 2013;31(26):3279-87.

56. Sonneveld P et al. Treatment of multiple myeloma with high-risk cytogenetics: A consensus of the International Myeloma Working Group. Blood. 2016;127(24):2955-62.

57. Dimopoulos MA et al. Bortezomib-based triplets are associated with a high probability of dialysis independence and rapid renal recovery in newly diagnosed myeloma patients with severe renal failure or those requiring dialysis. Am J Hematol. 2016;91(5):499-502.

58. Sonneveld P et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: Results of the randomized phase III HOVON-65/ GMMG-HD4 trial. J Clin Oncol. 2012;30(24):2946-55.

59. Bringhen S et al. Efficacy and safety of once-weekly bortezomib in multiple myeloma patients. Blood. 2010; 116(23):4745-53.

60. Moreau P et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: A randomised, phase 3, non-inferiority study. Lancet Oncol. 2011; 12(5):431-40.

61. Chari A, Hajje D. Case series discussion of cardiac and vascular events following carfilzomib treatment: Possible mechanism, screening, and monitoring. BMC Cancer. 2014;14:915.

62. EMA. Velcade: EPAR- European Medicines Agency. 2016. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000539/WC500048471.pdf. Last accessed: 20 July 2016.

63. Europa. Kyprolis, INN-carfilzomib. 2016. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003790/WC500197692.pdf. Last accessed: 20 July 2016.

64. Chauhan D et al. In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res. 2011;17(16):5311-21.

65. Kumar SK et al. Phase 2 trial of ixazomib in patients with relapsed multiple myeloma not refractory to bortezomib. Blood Cancer J. 2015;5:e338.

66. Kumar SK et al. Safety and tolerability of ixazomib, an oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma: an open-label phase 1/2 study. Lancet Oncol. 2014;15(13):1503-12.

67. Richardson PG et al. Twice-Weekly Oral MLN9708 (Ixazomib Citrate), An Investigational Proteasome Inhibitor, In Combination With Lenalidomide (Len) and Dexamethasone (Dex) In Patients (Pts) With Newly Diagnosed Multiple Myeloma (MM): Final Phase 1 Results and Phase 2 Data. Abstract 535. ASH Annual Meeting, 7-10 December 2013.

68. Dimopoulos MA et al. Randomized

Phase 2 Study of the All-Oral Combination of Investigational Proteasome Inhibitor (PI) Ixazomib Plus Cyclophosphamide and Low-Dose Dexamethasone (ICd) in Patients (Pts) with Newly Diagnosed Multiple Myeloma (NDMM) Who Are Transplant-Ineligible (NCT02046070). Abstract 26. ASH Annual Meeting, 5-8 December 2015.

69. Richardson PG et al. Phase 1 study of twice-weekly ixazomib, an oral proteasome inhibitor, in relapsed/refractory multiple myeloma patients. Blood. 2014;124(7):1038-46.

70. Kumar SK et al. Phase 1 study of weekly dosing with the investigational oral proteasome inhibitor ixazomib in relapsed/refractory multiple myeloma. Blood. 2014;124(7):1047-55.

71. Gupta N et al. Pharmacokinetics and safety of ixazomib plus lenalidomide-dexamethasone in Asian patients with relapsed/refractory myeloma: a phase 1 study. J Hematol Oncol. 2015;8:103.

72. Gupta N et al. A pharmacokinetics and safety phase 1/1b study of oral ixazomib in patients with multiple myeloma and severe renal impairment or end-stage renal disease requiring haemodialysis. Br J Haematol. 2016. [Epub ahead of print].

73. Gupta N et al. Pharmacokinetics of ixazomib, an oral proteasome inhibitor, in solid tumour patients with moderate or severe hepatic impairment. Br J Clin Pharmacol. 2016. [Epub ahead of print].

74. Gupta N et al. Switching from body surface area-based to fixed dosing for the investigational proteasome inhibitor ixazomib: A population pharmacokinetic analysis. Br J Clin Pharmacol. 2015;79(5):789-800.

75. FDA. Ninlaro Prescribing Information. 2015. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208462lbl.pdf. Last accessed: 20 July 2016.

76. Lacy MQ et al. Phase 1/2 trial of ixazomib, cyclophosphamide, and dexamethasone for newly diagnosed multiple myeloma (NDMM). Abstract 8002. ASCO Annual Meeting, 3-7 June 2016.

77. Dimopoulos MA et al. Current treatment landscape for relapsed and/or refractory multiple myeloma. Nat Rev Clin Oncol. 2015;12(1):42-54.

78. Moreau P et al. Ixazomib, an Investigational Oral Proteasome Inhibitor (PI), in Combination with Lenalidomide and Dexamethasone (IRd), Significantly Extends Progression-Free Survival (PFS) for Patients (Pts) with Relapsed and/or Refractory Multiple Myeloma (RRMM): The Phase 3 Tourmaline-MM1 Study (NCT01564537). Abstract 727. ASH Annual Meeting, 5-8 December 2015.

79. Ocio EM et al. New drugs and novel mechanism of action in multiple myeloma in 2013: A report from the International Myeloma Working Group (IMWG). Leukemia. 2014;28(3):525-42.

80. Fink EC, Ebert BL. The novel mechanism of lenalidominde activity. Blood. 2015; 126(21):2366-9.

EVIDENCE-BASED TRANSFUSION MEDICINE FOR COAGULOPATHY OF CHRONIC LIVER DISEASE

***Pratima Chowdary**

KD Haemophilia Centre and Thrombosis Unit, Royal Free Hospital, London, UK
**Correspondence to p.chowdary@nhs.net*

Patients with chronic liver disease variably exhibit abnormalities of coagulation related to decreased synthesis of coagulation proteins. This results in prolongation of routine coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (APTT). In addition, patients suffer from thombocytopenia of variable severity and the mechanisms underlying this decreased platelet count are multifactorial, including decreased production of thrombopoietin (TPO). These abnormalities of coagulation are often equated with an excess risk of bleeding and patients are often treated in the context of active bleeding, or peri-procedurally in the presence of abnormalities. Commonly used haemostatic replacement therapies include fresh frozen plasma (FFP), prothrombin complex concentrates (PCC), cryoprecipitate, and/or platelets. The antifibrinolytic agent tranexamic acid along with TPO receptor agonists have been used in restricted clinical circumstances, the latter to increase platelet counts.

Practicing clinicians are faced with two questions in the management of this coagulopathy: the first is when to treat, and the second is what to use. The evidence base for products that are available for use is less contentious. FFP has been used for many decades in this context and multiple studies have demonstrated that the response to treatment is related to volume, i.e. a higher dose of FFP results in a better correction of PT with higher factor levels.[1] Similarly, PCC have been used with complete correction of the international normalised ratio seen in the majority of the patients, unlike the situation seen with the use of FFP.[2] PCC have been demonstrated to be efficacious and there does not appear to be an obvious excess of thrombotic events. Platelet transfusions are commonly prescribed for bleeding patients and peri-procedurally in the context of moderate-to-severe thombocytopenia. There is ongoing debate regarding transfusion thresholds and values used in liver disease, as they are based on evidence gathered from other areas, principally oncology patients undergoing intense chemotherapy and stem cell transplantation. TPO agonists have also been used peri-procedurally in the context of clinical trials and one of them (eltrombopag) is licensed for use in the context of interferon-based treatment for hepatitis C infection, where thrombocytopenia limits treatment options. TPO agonists have been associated with an unexpectedly high risk of thrombosis, in particular portal vein thrombosis, and caution needs to be exercised when used in routine clinical practice.[3]

Whilst the effects of the various products are well documented, there is much debate concerning the appropriate transfusion thresholds. Laboratory and clinical data show dysregulated coagulation with an increased risk of thrombosis; this is concurrently associated with a diminished reserve and a potential for excess risk of bleeding.[4-6] Conventionally, the PT, APTT, fibrinogen, and platelet count have been used to define transfusion thresholds. There is increasing evidence that use of viscoelastic tests of whole blood (TEG®, ROTEM®) may decrease the number of patients requiring treatment.[7,8] A recent study evaluating two different tests for transfusion demonstrated markedly decreased requirements for transfusion when a TEG-based strategy was instituted. Prospective studies with PT as an endpoint demonstrate that PCC are more effective than FFP, and with the latter the effectiveness is related to the volume infused with sub-therapeutic doses being in common usage.[1,2] Thresholds for platelet transfusion are similarly variable and in a prospective study of eltrombopag and platelet transfusion that aimed for a target of 50,000 per mm^3, bleeding complications were seen in approximately one-fifth of the patients.[3]

The dilemma relates to appropriate transfusion thresholds. Pragmatically, this is dictated by individual clinicians' perception of risk versus benefit

in a given patient for a given procedure, and indeed a national audit of transfusion practices in cirrhosis in the UK reflects this anxiety and ambiguity.[9] There is an urgent need for observational studies correlating abnormal coagulation parameters to bleeding outcomes and interventional studies to redefine the transfusion thresholds for procedures and haemostatic products.

REFERENCES

1. Youssef WI et al. Role of fresh frozen plasma infusion in correction of coagulopathy of chronic liver disease: A dual phase study. Am J Gastroenterol. 2003;98(6):1391-4.

2. Mannucci PM et al. Correction of abnormal coagulation in chronic liver disease by combined use of fresh-frozen plasma and prothrombin complex concentrates. Lancet. 1976;2(7985):542-5.

3. Afdhal NH et al. Eltrombopag before procedures in patients with cirrhosis and thrombocytopenia. N Engl J Med. 2012;367(8):716-24.

4. Monroe DM, Hoffman M. The coagulation cascade in cirrhosis. Clin Liver Dis. 2009;13(1):1-9.

5. Tripodi A et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology. 2005;41(3):553-8.

6. Gilmore IT et al. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: An audit by the British Society of Gastroenterology and the Royal College of Physicians of London. Gut. 1995;36(3):437-41.

7. Massicotte L et al. Classical notions of coagulation revisited in relation with blood losses, transfusion rate for 700 consecutive liver transplantations. Semin Thromb Hemost. 2015;41(5):538-46.

8. Venon WD et al. Usefulness of thromboelastometry in predicting the risk of bleeding in cirrhotics who undergo invasive procedures. Eur J Gastroenterol Hepatol. 2015;27(11):1313-9.

9. Desborough MJ et al. Patterns of blood component use in cirrhosis: A nationwide study. Liver Int. 2016;36(4):522-9.

MANAGEMENT OF SUPERFICIAL VEIN THROMBOSIS

***Benilde Cosmi**

Department of Angiology & Blood Coagulation, Saint Orsola-Malpighi University Hospital, Bologna, Italy

**Correspondence to benilde.cosmi@unibo.it*

Superficial vein thrombosis (SVT), indicating thrombosis of the superficial vein system, is less well-studied than deep vein thrombosis (DVT) because it has been considered a minor, self-limiting disease, easily diagnosed on clinical grounds, and deserving only symptomatic relief. The most frequently affected area of the superficial vein system are the lower limbs, especially the long and short saphenous vein, mostly in relation to varicose veins. The incidence of SVT of the lower limbs is estimated to be approximately 0.64 out of 1,000 per year in primary care, which is a lower incidence than that of venous thromboembolism (VTE), which is estimated to be 1 out of 1,000 per year. Lower limb SVT shares the same risk factors as DVT, such as advanced age, obesity, active cancer, previous thromboembolic episodes, pregnancy, oral contraceptives, hormone replacement therapy, recent surgery, and auto-immune diseases (particularly Behçet's and Buerger's diseases). It can propagate into the deep veins and have a complicated course with pulmonary embolism. Clinical diagnosis may not be accurate and presently ultrasonography is indicated for both confirmation and evaluation of SVT extension and exclusion of concomitant DVT. No validated diagnostic algorithms are available for SVT of the lower limbs. As a result, the approach to SVT diagnosis in clinical routine is variable and dependent on local sources; unlike DVT diagnosis, objective testing may still be considered neither mandatory nor urgent. Studies conducted on D-dimer diagnostic accuracy for SVT are few, with a high false negative rate. Treatment aims are symptom relief and prevention of VTE in relation to the thrombotic burden. SVT of the long saphenous vein within 3 cm of the sapheno-femoral junction (SFJ) is considered equivalent to DVT and thus deserving therapeutic anticoagulation. Less severe forms of SVT of the lower limbs not involving the SFJ have been included in randomised clinical trials of surgery, compression hosiery, non-steroidal anti-inflammatory agents, unfractionated heparin, and low molecular weight heparins (LMWH) with inconclusive results. The largest randomised clinical trial available, including 3,004 patients

with lower limb SVT not involving SFJ, showed that fondaparinux 2.5 mg once daily for 6 weeks is more effective than placebo in reducing the risk of the composite endpoint of death from any cause and symptomatic VTE (0.9% versus 5.9%). The second largest study, STEFLUX, in 664 patients showed that LMWH, parnaparin, at 8,500 UI aXa (anti-factor Xa activity) once daily for 10 days followed by 6,400 UI aXa once daily for 20 days is effective and safe for the treatment of lower limb SVT. Inherited thrombophilic alterations have a similar frequency in SVTs when compared with DVT, however there are no data to indicate that the presence of thrombophilia should alter the management or influence rates of SVT recurrence or progression. No data are available on the best treatment of Trousseau's syndrome, which is a recurrent and migratory pattern of inflammation of superficial veins in cancer. No studies are available either on the optimal treatment of cancer-associated SVT or screening for an occult malignancy after SVT. Pregnant women have usually been excluded from studies of SVT treatment, but pregnancy is a risk factor for both VTE and SVT. Unfractionated heparin and LMWH do not cross the placenta and are the agents of choice for treatment in pregnancy. Further studies are needed to define the optimal management strategies of SVT of the lower limbs and other possible SVT sites, such as the upper limbs, abdominal or thoracic wall, penis, or neck.

GBT440, A NOVEL SICKLE HAEMOGLOBIN POLYMERISATION INHIBITOR, INCREASES HAEMOGLOBIN OXYGEN AFFINITY AND RESULTS IN A RAPID IMPROVEMENT IN HAEMOLYSIS AND ANAEMIA

Paul Telfer,[1] *Josh Lehrer-Graiwer,[2] Margaret Tonda,[2] Eleanor Ramos[2]

1. Barts Health NHS and Queen Mary, University of London, London, UK
2. Global Blood Therapeutics, South San Francisco, California, USA
**Correspondence to jlehrer@globalbloodtx.com*

GBT440 is a novel, small molecule haemoglobin oxygen affinity modifier. *In vitro* data has shown that by increasing sickle haemoglobin (HbS) oxygen affinity, GBT440 can delay HbS polymerisation and inhibit red blood cell sickling. By acting on the fundamental pathophysiologic mechanism in sickle cell disease (SCD), GBT440 has the potential to inhibit polymerisation, decrease red blood cell damage, and prevent the downstream clinical complications caused by haemolysis and vaso-occlusion. The GBT440-001 study was designed with the objective of understanding pharmacokinetics, pharmacodynamics, and safety, as well as to determine the effect on haemolysis and irreversibly sickled cells.

GBT440-001 is a randomised, placebo-controlled, double-blind, single and multiple ascending dose study evaluating GBT440 in both healthy subjects and patients with SCD. Data presented at EHA included 46 SCD patients at three dose levels including 6 patients who completed 700 mg/day for 90 days, 12 patients who completed 700 mg/day for 28 days, 10 patients who completed 500 mg/day for 28 days, 6 patients who completed 1,000 mg/day (administered as 500 mg twice a day for 28 days), and 12 patients who received placebo.

The 90-day results with GBT440 700 mg/day (n=6) showed a durable reduction in haemolysis from baseline to Day 90, as evidenced by a rapid and sustained reduction in bilirubin starting as early as Day 4 and continuing through Day 90 (median decrease of >35% compared with an increase of approximately 20% with placebo). A median 1.1 g/dL increase in haemoglobin was observed with

GBT440 treatment compared with 0.2 g/dL decrease with placebo. A median decrease of approximately 20% in reticulocyte count, compared with an approximately 20% increase with placebo, suggested that the observed increase in haemoglobin is due to a decrease in haemolysis. A sustained reduction in irreversibly sickled cells was observed, with a median decrease of approximately 70% within 90 days compared to an increase of approximately 15% with placebo. Results from cohorts dosed for 28 days showed similar trends.

GBT440 was well-tolerated in all 28 and 90-day cohorts. There have been no drug-related serious or severe adverse events. The majority of adverse events were Grade 1 or 2 in severity and appeared in similar if not higher frequency in the placebo arms as compared to GBT440-treated arms. This data was presented in a moderated poster session at EHA, as well as in an educational session on novel SCD therapeutics. The results generated great interest among haematologists and investigators because of the large unmet need in SCD and the paucity of experimental drugs that directly target the underlying mechanism of disease and therefore have the potential to chronically control symptoms and disease progression. Many SCD investigators expressed interest in participating in future studies with this compound. These results suggest that GBT440 is rapidly and durably inhibiting HbS polymerisation *in vivo* at well-tolerated doses, and that a meaningful reduction in haemolysis and anaemia is achieved. Given that the mechanism of action of GBT440 addresses the underlying pathophysiology of sickling, these results suggest the potential of GBT440 to modify the course of this devastating disease over time. Future clinical studies to investigate GBT440's potential clinical benefit in longer duration studies are being planned.

ROLE OF THE CALRETICULIN GENE AND ITS DEREGULATION IN MYELOPROLIFERATIVE NEOPLASMS

***Thorsten Klampfl**

Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK
**Correspondence to tk443@cam.ac.uk*

Polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF) are the three classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). More than 95% of PV patients and ~50% of ET and PMF patients have mutations in the *JAK2* gene. Around 5% of patients with ET or PMF carry mutations in the thrombopoietin receptor gene *MPL*. In December 2013, two research groups reported mutations in the calreticulin gene (*CALR)* in ~25% of ET and 35% of PMF patients.[1,2] Mutations in *JAK2, MPL,* or *CALR* were found to be mutually exclusive. ET and PMF patients who did not have mutations in any of the three genes were referred to as 'triple-negative'.[1] The two groups identified 36[1] and 19[2] different types of *CALR* mutations, two of which were common: a 52 base pair deletion (Type 1; c.1099_1150del) and a 5 base pair insertion (Type 2; c.1154_1155insTTGTC). These accounted for 53%[1]/45%[2] and 32%[1]/41%[2] of all the *CALR* mutations, respectively. In recent reports, non-Type 1/2 *CALR* mutations have been categorised together with Type 1 or 2 into 'Type 1-like' and 'Type 2-like' mutations.[3,4] All mutations in *CALR* led to a shift of the reading frame and the expression of a novel, common C-terminal peptide in the CALR protein.[1,2] *CALR* mutations were described as early events in the clonal development of the disease[1,2] and were detected in a range of haematopoietic stem and progenitor cell (HSPC) fractions from patients.[2]

In ET, *CALR* mutations were associated with significantly lower age, lower haemoglobin levels, lower white blood cell counts, and higher platelet levels compared with *JAK2* mutations.[1,2,5-7] When stratifying ET patients according to genotype

(mutant *JAK2, MPL* or *CALR*, or triple-negative), there was neither a significant difference in overall survival nor in the incidence of disease progression to myelofibrosis or acute myeloid leukaemia based on statistical models correcting for age.[5-7] *CALR* mutant ET patients had a significantly lower incidence of thrombosis compared to patients with the *JAK2* mutation.[6] A stratification of ET patients according to Type 1-like and Type 2-like *CALR* mutations showed in one study that particularly Type 2-like mutations were associated with a lower incidence of thrombosis,[3] while no differences were observed in another study.[8] Patients with *CALR* Type 1-like mutations had a significantly higher incidence of myelofibrotic transformation compared to both, patients with *JAK2* or *CALR* Type 2-like mutations.[3]

In PMF, patients with *CALR* mutations were significantly younger, had a higher platelet count,[1,9,10] and a lower leukocyte count[1,9] than those with *JAK2* mutations. They were less likely to develop anaemia, thrombocytopenia, or leukocytosis.[9] In contrast to ET, *CALR* mutations patients had a significantly better age-corrected overall survival than patients with *JAK2* mutations or triple-negative patients.[9,10] This difference is predominantly attributable to Type 1-like rather than Type 2-like mutations.[4] In multivariable models correcting for age and variables of the International Prostate Symptom Score (IPSS), or Dynamic International Prognostic Scoring System for myelofibrosis (IPSS), or the Dynamic IPSS plus the mutational status had an independent prognostic effect.[9,10] *CALR* mutant patients had a significantly lower age-adjusted cumulative incidence of thrombosis than *JAK2* mutant patients with PMF.[9]

Biochemical studies in cell lines revealed that mutant *CALR* binds to the thrombopoietin receptor MPL and activates downstream signalling.[11-13] In a retroviral mouse model, mice transplanted with mutant *CALR*-expressing HSPCs developed an MPN-like disease phenotype showing elevated platelet counts and progression to myelofibrosis.[14] While in the stem cell compartment only *CALR* Type 1 mutant cells had a competitive advantage over wildtype cells, both Type 1 and Type 2 *CALR* mutations were associated with elevated numbers of megakaryocytes and platelets.[14] Expression of MPL was required for the development of the disease phenotype in mice.[14]

In conclusion, mutations in *CALR* are novel markers in MPN that are strongly associated with thrombocytosis in patients. Activated MPL signalling is a central requirement for the disease pathogenesis in a mutant *CALR* context. Genotyping for *CALR* mutations aids diagnosis, and may have a role in prognosis and treatment decisions, although so far only retrospective studies have been published.

REFERENCES

1. Klampfl T et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25): 2379-90.

2. Nangalia J et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25): 2391-405.

3. Pietra D et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia. 2016;30(2):431-8.

4. Tefferi A et al. The prognostic advantage of calreticulin mutations in myelofibrosis might be confined to Type 1 or Type 1-like CALR variants. Blood. 2014;124(15):2465-6.

5. Rotunno G et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. Blood. 2014;123(10):1552-5.

6. Rumi E et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544-51.

7. Tefferi A et al. Calreticulin mutations and long-term survival in essential thrombocythemia. Leukemia. 2014;28(12):2300-3.

8. Tefferi A et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: A collaborative study of 1027 patients. Am J Hematol. 2014;89(8):E121-4.

9. Rumi E et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Blood. 2014;124(7):1062-9.

10. Tefferi A et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472-7.

11. Araki M et al. Activation of the thrombopoietin receptor by mutant calreticulin in CALR-mutant myeloproliferative neoplasms. Blood. 2016;127(10):1307-16.

12. Chachoua I et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. Blood. 2016;127(10):1325-35.

13. Abdelfattah N et al. Physical Interaction Between Mutant Calreticulin and the Thrombopoietin Receptor Is Required for Hematopoietic Transformation. Blood. 2015;126(23):LBA-4.

14. Marty C et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. Blood. 2016;127(10):1317-24.

FAMILY-DIRECTED CORD BLOOD BANKING FOR SICKLE CELL DISEASE: A 20-YEAR EXPERIENCE ON BEHALF OF EUROCORD-MONACORD AND THE INTERNATIONAL SICKLE CELL DISEASE OBSERVATORY

***Hanadi Rafii,[1,2] Françoise Bernaudin,[3] Marina Cavazzana,[4] Valérie Vanneaux,[5] Audrey Cras,[5] Valérie Gauthereau,[6] Aurélie Stanislas,[4] Hélène Rouard,[7] Christèle Ferry,[8] Chantal Kenzey,[1,2] Barbara Cappelli,[2] Annalisa Ruggeri,[1,2] Mariane De Montalembert,[9] Claire Rieux,[10] Mathieu Kuentz,[11] Robert Girot,[12] Jérôme Larghero,[5] Eliane Gluckman[1,2]**

1. Eurocord - Monacord, Hôpital Saint-Louis, AP-HP, Paris, France
2. Eurocord - Monacord, Centre Scientifique de Monaco, Monaco, Monaco
3. Centre de référence de la Drépanocytose, Hôpital Intercommunal de Créteil, AP-HP, Creteil, France
4. Département de Biothérapie, Hôpital Necker, AP-HP, Paris, France
5. Unité de Thérapie Cellulaire, Centre d'Investigation Clinique en Biothérapies, Hôpital Saint-Louis, AP-HP, Paris, France
6. Fédération Parisienne Pour le Dépistage et la Prévention des Handicaps de l'Enfant (FPDPHE), Hôpital Necker, AP-HP Paris, France
7. Unité d'Ingénierie et de Thérapie Cellulaire, EFS Ile de France, Créteil, France
8. Service de Greffe de Moelle, Hôpital Saint-Louis, AP-HP, Paris, France
9. Service de Pédiatrie, Hôpital Necker, AP-HP, Paris, France
10. Unité d'Hémovigilance, site Henri-Mondor, EFS Ile de, Paris, France
11. Service d'Hématologie, Hôpital Henri-Mondor, AP-HP, Créteil, France
12. Service d'hématologie biologique, Hôpital Tenon, AP-HP, Paris, France
**Correspondence to hanadi.rafii-elayoubi@aphp.fr*

BACKGROUND

Cord blood transplantation (CBT) from a related family member is an effective therapy for patients with sickle cell disease (SCD) resulting in encouraging outcomes with similar or superior survival to adult donor transplant. Efforts to implement family-directed umbilical cord blood (UCB) banking have been developed in the past two decades for siblings requiring stem cell transplantation. Public UCB banks are faced with the challenge regarding the units to be stored, be discarded, or used for other endeavours such as research.

AIM OF THE STUDY

We report here our 20-year experience in family-directed UCB banking for SCD. The aim of the study is to review the characteristics of the UCB units collected between 1995 and 2014 and to evaluate their take-up rate.

METHODS

Families were eligible if they had a child with SCD and were expecting the birth of a sibling. Participation was voluntary and free of charge. All mothers underwent a panel of serologic screening tests. UCB units were collected in remote sites. Collections were processed and stored in two public banks. UCB testing included viral serology, bacterial cultures, and cell counts. Human leukocyte antigen typing of the UCB was not routinely performed unless requested by the physician caring for the affected child.

RESULTS

A total of 338 directed UCB units were collected and stored for 302 families, including 4 mothers with twins and 28 with two to three births. All collections were for an existing affected sibling. Potential recipients had a median age of 6 years (range: 11 months–15 years) at time of UCB harvest. Fifteen families (5%) had more than one affected child.

All UCBs were negative for human immunodeficiency virus and 64 UCBs (30%) had positive hepatitis B surface antigen and/or anti-hepatitis B core antigen with negative hepatitis B surface antigen. Median UCB volume at cryopreservation was 91 mL (range: 23–196) with a unit volume lower than 40 mL in only 9% of the cases (29 units). Median total nucleated cell count at cryopreservation was $8.6x10^8$ (range: $0.7–75x10^8$). The median collected CD34+ and colony forming units of granulocytes-monocytes colonies cell counts were $2.5x10^6$ (range: $0.14–61x10^6$) and $3.4x10^5$ (range: $0.15–63x10^5$), respectively.

The haemoglobin status of the UCB units was assessed through the neonatal screening. Data were available for 298 UCBs (88%), including 105 (31%) haemoglobin AA, 152 (45%) haemoglobin AS, 36 (11%) haemoglobin SS, and 5 (<1%) other types of abnormal haemoglobin.

Out of 338 banked UCB units 28 were released for CBT either alone (n=24) or in combination with the bone marrow of the same donor (CBT + bone marrow transplant, n=4), reflecting a utilisation rate of 8% over 20 years. Infused cells had a median total nucleated cell count of $3.7x10^8$ (range: $0.2–10.4x10^8$) and median CD34+ of $1.7x10^6$ (range: $0.2–38.4x10^6$). Post-transplant data was available for 25 patients: all of them have stable engraftment of donor cells and are alive, free of SCD.

CONCLUSION

Our data showed that family-directed UCB banking is feasible and yields good quality cord blood units for sibling transplantation. However, the number of CBTs performed is disappointing despite the good results of sibling transplantation in SCD. Therefore, one must think about the cost-effectiveness of this approach when a human leukocyte antigen identical sibling donor is available.

REAL-WORLD EXPERIENCE OF IBRUTINIB IN MORE THAN 700 PATIENTS WITH MANTLE CELL LYMPHOMA: DATA FROM A GLOBAL NAMED PATIENT PROGRAMME

David Tucker,[1] *Simon Rule[2]

1. Clinical Research Fellow, Department of Haematology, Derriford Hospital, Plymouth, UK
2. Consultant Haematologist, Derriford Hospital, Plymouth Hospitals NHS Trust, Plymouth, UK
**Correspondence to simon.rule@plymouth.ac.uk*

Mantle cell lymphoma (MCL) is an uncommon but aggressive form of B cell non-Hodgkin lymphoma. It often presents at an advanced stage and with a median survival of 3–5 years and is generally regarded as incurable.[1,2] Ibrutinib, an orally activeinhibitor of Bruton's tyrosine kinase, has demonstrated impressive safety and efficacy in patients with relapsed/refractory MCL within several large clinical trials.[3,4] Prior to licensing, the global named patient program (NPP) gave access to ibrutinib for eligible patients with relapsed/refractory MCL in a large number of countries.

Using real-world data from the ibrutinib MCL NPP, the aim of this study was to investigate whether treatment benefits reported in randomised clinical trials were reflected in clinical practice. Data was collected from the NPP on the ordering and reordering of ibrutinib in order to estimate patient time-on-treatment and therefore to provide a

conservative approximation of progression-free survival using Kaplan–Meier and Cox proportional hazard regression analysis.

In total, 715 patients from 26 countries were included in this analysis. The median age was 70 years, and 76.1% were male. After 12 months, 52.3% (95% confidence interval: 43.5–60.4%) remained on treatment. This estimate is similar to the 12-month time-on-treatment rate observed in the Phase III RAY study of ibrutinib for relapsed/refractory MCL.[5] Moreover, Kaplan–Meier curves for time on treatment for the global MCL NPP population and the RAY study population were not statistically different. An exploration of time-on-treatment using multivariate analysis revealed that the timing of MCL diagnosis was the only independently significant variable, with time-on-treatment being longer in patients diagnosed with MCL within the last 2 years. In total, 168 patients (23.5%) discontinued treatment during the observation period with the most common reasons being death (10.8%), disease progression (7.3%), and adverse events (1.3%).

Although these data are based on physician declarations and are therefore unmonitored, this analysis provided a conservative proxy for progression-free survival which is similar to that observed in the RAY study. This suggests that the impressive safety and efficacy results observed in clinical trials of ibrutinib for MCL are reproducible in real-world clinical practice.

REFERENCES

1. Zhou Y et al. Incidence trends of mantle cell lymphoma in the United States between 1992 and 2004. Cancer. 2008;113(4):791-8.

2. Morton LM et al. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006;107(1):265-76.

3. Tucker DL, Rule SA. Ibrutinib for mantle cell lymphoma. Future Oncol. 2016;12(4):477-91.

4. Herrera AF, Jacobsen ED. Ibrutinib for the treatment of mantle cell lymphoma. Clin Cancer Res. 2014;20(21):5365-71.

5. Dreyling M et al. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: An international, randomised, open-label, phase 3 study. Lancet. 2016;387(10020):770-8.

THE SPECTRUM OF LATE EFFECTS AFTER CANCER: LESSONS FROM PAEDIATRIC ONCOLOGY AND THE VALUE OF INTERNATIONAL COLLABORATION

***Rod Skinner**

Department of Paediatric and Adolescent Haematology and Oncology, Great North Children's Hospital, Newcastle, UK
**Correspondence to roderick.skinner@newcastle.ac.uk*

This presentation described the occurrence of late side effects (commonly described as 'late effects') in childhood cancer survivors (CCS), and how paediatric oncology healthcare professionals and researchers across the world have worked together to reduce or eliminate their impact on the long-term health of survivors.

The long-term overall survival of childhood cancer or leukaemia is now at 80%, but this modern-day success story has created an ever-increasing number of long-term survivors, many of whom have a high-risk of late effects of their cancer and especially of its treatment. Large cohort studies have shown that 15–30 years after diagnosis of their initial malignancy, approximately 60–75% of CCS suffer from at least one late effect, 25–45% from at least two late effects, and 25–40% from a severe, life-threatening, or disabling late effect. The range of late effects observed in CCS is very wide. Most are due to damage to the function, or structure, of organs, organ systems, or tissues; for example: cardiovascular, gonadal/reproductive, neurological/sensory, renal/urinary tract, and respiratory toxicities. Some late effects lead to impaired quality of life or neurocognitive

function, whilst others have more general consequences such as the development of the metabolic syndrome, accelerated ageing (frailty), or secondary malignancies.

Late effects have many different causes, most commonly chemotherapy drugs, radiotherapy, and major surgical procedures. In some children, particularly those with brain tumours, the site and nature of the primary tumour may affect the risk of developing late effects and determine their consequences. Genetic susceptibility may also play an important role, for example genetic polymorphisms may modify the risk of late effects by influencing drug pharmacokinetics and pharmacodynamics, whilst cancer predisposition syndromes can increase the risk of second or subsequent malignancies. By their very nature, most late effects are persistent, causing chronic medical, psychological, or social problems. Nevertheless, late effects vary greatly in their timing of onset, with some, such as chemotherapy induced renal toxicity, initially presenting during or soon after treatment, whilst others, for example secondary malignancies, may occur decades later. Some late effects appear to be largely or entirely due to one cause, for example sensorineural hearing loss due to platinum drugs, whilst others are complex and multifactorial, such as cardiac damage which may be due to anthracycline drugs or radiotherapy to a field, including the heart, or both. The impact of late effects is also very variable, ranging from easily correctable subclinical organ dysfunction, such as compensated hypothyroidism, to life-threatening consequences, such as secondary malignancies, or even late (but still very premature) mortality.

The risks of late effects have led to the development of long-term follow-up (LTFU) for CCS, seeking to manage existing late effects, undertake surveillance to allow earlier detection or ideally prevention of potentially severe late effects, and provide information for CCS about their current and future late effects risks and what steps they can take to optimise their health. Alongside this development of clinical LTFU care, several large cohorts of CCS have been constructed in North America (for example, the Childhood Cancer Survivor Study) and especially in Europe (such as the British Childhood Cancer Survivor Study). These cohorts have allowed detailed epidemiological study of the nature and outcomes of late effects in CCS and have identified which survivors are at highest risk due to personal, genetic (in some cases), disease, and treatment-related factors. This can allow development and evaluation of risk stratification approaches to guide surveillance for clinically important late effects. Ongoing research is needed to inform optimal management which may comprise both medical treatment of incipient or existing late effects and pharmacological and/or lifestyle interventions to reduce the risk of future effects.

International collaboration also continues to play a vital role in efforts to improve the longevity and quality of life of CCS. PanCareSurFup (Childhood and Adolescent Cancer Survivor Care and Follow-Up Studies) is a landmark Pan-European study investigating the risk factors for cardiotoxicity, second malignancies, and premature mortality in unprecedented detail. Furthermore, PanCareSurFup is developing evidence-based clinical practice guidelines for models of LTFU care, transition to age-appropriate care in adolescence and young adulthood, and health promotion which are all important components of LTFU. Reassuringly, large-scale international randomised clinical trials are now including late effects as important study outcomes in an effort to reduce long-term toxicities of curative treatment.

CHRONIC LYMHOCYTIC LEUKAEMIA: TREATMENT OF THE ELDERLY

***Barbara Eichhorst**

German Chronic Lymphocytic Leukemia Study Group (GCLLSG), Department I of Internal Medicine, University Hospital of Cologne, Cologne, Germany
**Correspondence to Barbara.eichhorst@uk-koeln.de*

Chronic lymphocytic leukaemia (CLL) mainly affects elderly patients.[1] With the increasing comorbidity burden and frailty in elderly patients, evaluation of functional status before treatment initiation is essential. So far, there is no ideal tool to measure the comorbidity burden, but geriatric assessment for example, with the G8 score[2] before treatment initiation, is strongly recommended.[3,4] While more intensive combination regimens based on chemotherapy plus antibody, such as the combination of fludarabine, cyclophosphamide, and rituximab (FCR), are standard in younger and/or physically fit patients, with concomitant diseases and physical fitness playing a major role in the selection of treatment for the elderly who do not tolerate such regimens well.[5] Dose reductions or milder chemoimmunotherapy regimens are well tolerated in the elderly and yield promising results.[6,7] The combination of chlorambucil plus a CD20 antibody has become the new standard first-line therapy in many countries because it yields long progression-free survival (PFS) rates of 15–27 months.[8-10] Data from the CLL11 study presented at European Hematology Association (EHA) Congress 2016 showed that achieving a negative measure of minimal residual disease in peripheral blood or bone marrow by polymerase chain reaction was associated with significantly longer PFS among elder patients being treated with chlorambucil plus rituximab or obinutuzumab.[11]

With the approval of new oral drugs that inhibit kinases attached to the B cell receptor, better treatment options which are well tolerated are now available for relapsed CLL patients[12,13] as well as front-line therapy.[14] A randomised Phase III study in elderly CLL patients showed a clear superiority of the Bruton's tyrosine kinase inhibitor ibrutinib over chlorambucil alone for PFS and overall survival.[14] However, even in a front-line setting minimal residual disease negativity is rarely achieved with the new substances. With these continuously administered substances, drug interactions and compliance have to be considered, particularly in elderly patients. A retrospective analysis presented at EHA 2016 showed that a different side effect profile has to be considered with each new compound, as for example atrial fibrillation, which may occur in elderly patients receiving ibrutinib.[15] Current studies are investigating the bcl2 inhibitor venetoclax in front-line therapy of elderly. The combination with obinutuzumab was shown to be safe in a run-in phase study.[16] These as well as many other data presented at EHA 2016 regarding kinase inhibitors show that the selection of optimal treatment for elderly CLL patients remains challenging.

REFERENCES

1. Howlader N NA et al. SEER Cancer Statistics Review, 1975-2011, National Cancer Institute, Bethesda, Maryland, USA. 2014. Available at: http://seer.cancer.gov/archive/csr/1975_2011/. Last accessed: 23 June 2016.

2. Hamaker ME et al. Frailty screening methods for predicting outcome of a comprehensive geriatric assessment in elderly patients with cancer: A systematic review. Lancet Oncol. 2012;13(10);e437-44.

3. Goede V et al.; German CLL Study Group. Interactions between comorbidity and treatment of chronic lymphocytic leukemia: Results of the German CLL Study Group. Haematologica. 2014; 99(6):1095-100.

4. Hamaker ME et al. The relevance of a geriatric assessment for elderly patients with a haematological malignancy--a systematic review. Leuk Res. 2014;38(3):275-83.

5. Shvidel L et al. Conventional dose fludarabine-based regimens are effective but have excessive toxicity in elderly patients with refractory chronic lymphocytic leukemia. Leuk Lymphoma. 2003;44(11):1947-50.

6. Dartigeas C et al. Evaluating abbreviated induction with fludarabine, cyclophosphamide, and dose-dense rituximab in elderly patients with chronic lymphocytic leukemia. Leuk lymphoma. 2015;28:1-7.

7. Michallet A-S et al. Rituximab in combination with bendamustine or chlorambucil for the treatment of chronic lymphocytic leukaemia: Primary results from the randomised phase IIIb MABLE study. Leuk Lymphoma. 2015;56(Suppl 1):149.

8. Goede V et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Eng J Med. 2014;

370(12):1101-10.

9. Hillmen P et al. Chlorambucil plus ofatumumab versus chlorambucil alone in previously untreated patients with chronic lymphocytic leukaemia (COMPLEMENT 1): A randomised, multicentre, open-label phase 3 trial. Lancet. 2015;385(9980):1873-83.

10. Goede V et al. Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: Updated results of the CLL11 study. Leukemia. 2015;29(7):1602-4.

11. Ritgen M et al. Quantitative mrd is prognostic for progression-free and overall survival in elderly patients receiving chlorambucil alone or with obinutuzumab/rituximab: A prospective analysis of the GCLLSG CLL11 study. Abstract S428. EHA Learning Center, 9-12 June 2016.

12. Furman RR et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Eng J Med. 2014;370(11):997-1007.

13. Byrd JC et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood. 2015;125(16):2497-506.

14. Burger JA et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med. 2015;373(25): 2425-37.

15. Thompson P et al. Atrial fibrillation in CLl patients treated with ibruitnib: An international retrospective study. Abstract P224. EHA Learning Center, 9-11 June 2016.

16. Fischer K et al. Results of the Safety Run-in Phase of CLL14 (BO25323): A prospective, open-label, multicenter randomized Phase III trial to compare the efficacy and safety of obinutuzumab and venetoclax (GDC0199/ ABT199) with obinutuzumab and chlorambucil in patients with previously untreated CLL and coexisting medical conditions. Blood. 2015;126(23):496.

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EDITOR'S PICK

Direct oral anticoagulants (DOAC) provide clinicians with a valuable method for the prevention and treatment of thrombotic events. However, DOAC also expose patients to the risk of bleeding or major bleeding, which can lead to major adverse events as a result of their inappropriate use. This article by Vornicu et al. highlights the various aspects that require consideration in order to minimise the incidence of these adverse events and points to the approaches available to ensure this can be done effectively. The article also provides important practical guidance in specific situations where DOAC are vulnerable to mismanagement.

MINIMISATION OF BLEEDING RISKS DUE TO DIRECT ORAL ANTICOAGULANTS

Ovidiu Vornicu,[1] Anne-Sophie Larock,[2] Jonathan Douxfils,[3] *François Mullier,[3,4] Virginie Dubois,[1] Jean-Michel Dogné,[3] Maximilien Gourdin,[1] Sarah Lessire,[1,3] Anne-Sophie Dincq[1]

1. Université catholique de Louvain, CHU UCL Namur, Department of Anesthesiology, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute of Life Sciences (NARILIS), Yvoir, Belgium
2. Université catholique de Louvain, CHU UCL Namur, Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), Yvoir, Belgium
3. Université de Namur, Department of Pharmacy, Namur Thrombosis and Hemostasis Center Namur (NTHC), Research Institute for Life Sciences (NARILIS), Namur, Belgium
4. Université catholique de Louvain, CHU UCL Namur, Hematology Laboratory, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), Yvoir, Belgium
**Correspondence to mullierfrancois@gmail.com*

Disclosure: The authors have declared no conflicts of interest. Sarah Lessire and Anne-Sophie Dincq contributed equally to the work.
Received: 29.02.16 **Accepted:** 14.06.16
Citation: EMJ Hematol. 2016;4[1]:78-90.

ABSTRACT

Direct oral anticoagulants (DOAC) are used in several indications for the prevention and treatment of thrombotic events. As highlighted by data from clinical trials and case studies, all DOAC carry the risk of bleeding despite careful selection and patient management. Previous publications have demonstrated the limited knowledge of many physicians concerning the indications for, and correct management of, these anticoagulants. Health institutions should develop risk minimisation strategies and educational materials to prevent major adverse events related to DOAC administration. Major bleeding events are reported in clinical practice and specific antidotes are emerging from Phase III trials. Some antidotes are licensed but their high cost might limit routine use. We therefore illustrate approaches and tools that can help physicians prescribe DOAC appropriately. We focus on screening for modifiable bleeding risk factors and adapting doses according to the individual benefit-risk profile. We also provide recommendations on managing a missed dose, switching, bridging, and resumption.

Keywords: Prevention, bleeding, direct oral anticoagulants (DOAC).

INTRODUCTION

Bleeding events have been reported in all the major clinical trials comparing direct oral anticoagulants (DOAC) with other anticoagulants despite regular monitoring of adverse events, strong medication adherence, and careful patient selection.[1-4]

Large randomised trials comparing bleeding risks of different DOAC are not available as DOAC have always been compared with warfarin, low molecular weight heparin (LMWH), and antiplatelet agents. When DOAC were prescribed at prophylactic doses in orthopaedic surgery, rates of bleeding were similar to LMWHs.[5] When DOAC were prescribed for patients with non-valvular atrial fibrillation (NVAF) or venous thromboembolism (VTE), a recent meta-analysis showed a lower rate of fatal bleeding and case-fatality of major bleeding (MB) events for DOAC than with warfarin.[6] Another meta-analysis showed that patients with a creatinine clearance (CrCl) between 50–80 mL/min who received DOAC had significantly fewer MB events than those receiving vitamin K antagonists (VKA). For patients with CrCl <50 mL/min, the difference in MB was not statistically significant, but using indirect comparisons, apixaban was associated with significantly fewer MB episodes than other DOAC.[7]

Post-marketing surveillance registries provide real-world data on the safety and efficacy of DOAC. A retrospective cohort study of Medicare beneficiaries with NVAF found a higher incidence of MB with dabigatran than with warfarin, regardless of anatomical site.[8] A smaller Italian cohort study revealed a safe profile of either 110 mg or 150 mg dabigatran twice daily (bid), regarding fatal bleeding in patients at high risk of haemorrhage and thromboembolism.[9] The Dresden New Oral Anticoagulants (NOAC) registry found that the routine safety profile of dabigatran etexilate (DE) was no worse than that reported in the RE-LY trial even if selection bias might exist.[10] The Danish national registry found a higher bleeding rate in previous VKA users than VKA-naïve patients. This observation may reflect patient selection and 'drug switching' practices.[11] Concerning rivaroxaban, a large observational study showed that the MB rate was generally consistent with registration trial results and that fatal bleeds were rare.[12] The MB rate with rivaroxaban from the Dresden NOAC registry was lower than that for VKA.[13] However, the ROCKET AF trial identified age, sex, diastolic blood pressure, prior gastrointestinal bleeding, prior aspirin use, and anaemia as clinically relevant factors associated with MB risk with rivaroxaban and VKA.[14] This review aims to discuss strategies to prevent DOAC-related bleeding in normal clinical practice.

HOW TO PREVENT MAJOR BLEEDING

Various aspects of the management of patients treated with DOAC should be highlighted to reduce the incidence of MB.

Appropriate Use of Direct Oral Anticoagulants

Off-label use or misuse

The off-label use or misuse of DOAC means use outside of approved indication, at an inappropriate dose, or an inappropriate choice of DOAC according to patient characteristics. Inappropriate use is frequent (occurring in 8–49% of patients) and may cause infra or supra-therapeutic anticoagulation carrying risks of thromboembolism or severe, even fatal bleeding (Table 1).[11,15-19] A recent publication indicated a significant risk for patients related to lack of physicians' knowledge about DOAC and highlighted the need for additional education and training.[20]

Renal function

Renal failure is the most common risk factor associated with bleeding in elderly patients, and should therefore be assessed before initiating DOAC and during treatment.[21] The updated European Heart Rhythm Association (EHRA) practical guide on the use of DOAC suggests that renal function should be checked every 6 months in patients >75–80 years (especially those on DE or edoxaban), and in frail patients.[22] The proposed recheck interval (in months) is the CrCl divided by 10, if the CrCl is <60 mL/min. More frequent checks are recommended in patients with conditions that might affect renal function. In clinical trials of DOAC for NVAF, drug eligibility and dosing were determined using the Cockcroft–Gault equation to estimate CrCl. The modification of diet in renal disease equation (used to estimate glomerular filtration rate) tends, at low estimate glomerular filtration rate values, to overestimate renal function compared with the Cockcroft–Gault equation.[23,24] This overestimation may lead to incorrect decisions about eligibility or dose.

Bioavailability

If DE capsules are opened, the bioavailability increases to 75% and the bleeding risk is greatly enhanced.[25] Therefore, DE should not be given via a gastrostomy or jejunostomy and capsules should not be opened or crushed before administration. Rivaroxaban and apixaban have similar bioavailability when administered in crushed form mixed with apple sauce or water through a nasogastric tube or gastrostomy.[26,27] To the best of our knowledge, there are currently no data available for edoxaban.

Table 1: Summary of pharmacokinetic properties, indication, and dose regimens of direct oral anticoagulants in the European Union.[29,35,46,54,62-83]

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Target	Factor IIa	Factor Xa	Factor Xa	Factor Xa
Prodrug	Yes	No	No	No
T_{max} (hours)	1.5–3.0	2.0–4.0	3.0–4.0	1.0–2.0
Distribution volume (L)	60–70	±50	23	107
Half-life (hours)	11: healthy individuals 12–13: elderly	5–9: healthy individuals 11–13: elderly	8–15: healthy individuals	10–14
Bioavailability	3–7% pH sensitive	2.5–10 mg dose: 80–100% (fasting/fed) 15–20 mg dose: 66% (fasting) ± 100% (fed)	±50%	62%
Protein binding	35%	>90%	87%	55%
Metabolism	Conjugation	CYP-dependent and independent mechanisms	CYP-dependent mechanisms	CYP-dependent (<5%) and independent mechanisms (<10%)
Active metabolites	Yes: glucuronide conjugates	No	No	Yes (<15%)
Elimination	80% renal	33% unchanged via the kidney	27% renal	50% renal
	20% bile (glucuronide conjugation)	66% metabolised in the liver into inactive metabolites eliminated via the kidney (50%) or the colon (50%)	73% through the liver while the residue is excreted by the hepatobiliary route	50% metabolism and biliary/intestinal excretion
Effects of food	T_{max} delayed; C_{max} & AUC unchanged	T_{max} delayed; C_{max} & AUC increased (15–20 mg)	T_{max} delayed; C_{max} & AUC unchanged	C_{max} increased, but minimal effect on total exposure
CYP substrate	No	CYP3A4, CYP2J2	CYP3A4	CYP3A4 (<5%)
P-gp substrate	DE: Yes	Yes	Yes	Yes
Venous thromboembolism Prophylaxis	**220 mg/day** (2 caps 110 mg qd) C_{max}: 71 (35–162) ng/mL (mean; 25–75 PCTL) C_{min}: 22 (13–36) ng/mL (mean; 25–75 PCTL) **150 mg/day** (2 caps 75 mg qd) → if CrCl 30–50 mL/min, if >75 years, if verapamil, amiodarone, and quinidine THR: 28–35 days TKR: 10 days	**10 mg/day** (1 tablet 10 mg qd) C_{max}: 125 (91–196) ng/mL (median; 5–95 PCTL) C_{min}: 9 (1–38) ng/mL (median; 5–95 PCTL) THR: 5 weeks TKR: 2 weeks	**5 mg/day** (1 tablet 2.5 mg bid) C_{max}: 77 (41–146) ng/mL (median; 5–95 PCTL) C_{min}: 51 (23– 109) ng/mL (median; 5–95 PCTL) THR: 32–38 days TKR: 10 days	× (EU)

Table 1 continued.

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Non-valvular atrial fibrillation*	**300 mg/day** (1 caps 150 mg bid) C_{max}: 175 (117-275) ng/mL (mean; 25–75 PCTL) C_{min}: 91 (61-143-200) ng/mL (mean; 25–75–90 PCTL) **220 mg/day** (1 caps 110 mg bid) → if >80 years or verapamil	**20 mg/day** (1 tablet 20 mg qd) C_{max}: 249 (184–343) ng/mL (mean; 5–95 PCTL) C_{min}: 44 (12–137) ng/mL (mean; 5–95 PCTL) **15 mg/day** (1 tablet 15 mg qd) → if CrCl between 15-49 mL/min C_{max}: 229 (178–313) ng/mL (mean: 5–95 PCTL) C_{min}: 57 (18–136) ng/mL (mean; 5–95 PCTL)	**10 mg/day** (1 tablet 5 mg bid) C_{max}: 171 (91–321) ng/mL (median; 5–95 PCTL) C_{min}: 103 (41–230) ng/mL (median; 5–95 PCTL) **5 mg/day** (1 tablet 2.5 mg bid) → If at least 2 of the following conditions: ≥80 years, ≤60 kg or serum creatinine ≥ 1.5 mg/dL; → Or if CrCl 15-29 mL/min C_{max}: 123 (69–221) ng/mL (median; 5–95 PCTL) C_{min}: 79 (34–162) ng/mL (median; 5–95 PCTL)	**60 mg/day** (1 tablet 60 mg qd) C_{max}: +/-170 (165–195) ng/mL (median; IQR) C_{min}: 36 (19–62) ng/mL (median; IQR) **30 mg/day** (1 tablet 30 mg qd) → if CrCl between 15–50 mL/min, ≤60 kg or concomitant use of dronedarone, erythromycin, ketoconazole, ciclosporin C_{min}: 27 (15–45) ng/mL (median; IQR)
Venous thromboembolism treatment	**300 mg/day** (1 caps 150 mg bid) after at least 5 days of parenteral anticoagulants C_{max}: 175 (117–275) ng/mL (mean; 25–75 PCTL) C_{min}: 60 (39–95–146) ng/mL (mean; 25–75–90 PCTL) **220 mg/day** (1 caps 110 mg bid) → if >80 years or verapamil	**Treatment phase:** **30 mg/day** (1 tablet 15 mg bid) for 21 days → followed by **20 mg/day** (1 tablet 20 mg qd) for 3–6 months C_{max}: 270 (189–419) ng/mL (mean; 5–95 PCTL) C_{min}: 26 (6–87) ng/mL (mean; 5–95 PCTL) **15 mg/day** (1 tablet 15 mg qd) → if CrCl between 15–49 mL/min and the risk of bleeding outweighs the risk of recurrent DVT or PE	**Treatment phase:** **20 mg/day** (2 tablet 5 mg bid) for 7 days C_{max}: 251 (111–572) ng/mL (median; 5–95 PCTL) C_{min}: 120 (41–335) ng/mL (median; 5–95 PCTL) → followed by **10 mg/day** (1 tablet 5 mg bid) for 3–6 months C_{max}: 132 (59–302) ng/mL (median; 5–95 PCTL) C_{min}: 63 (22–177) ng/mL (median; 5–95 PCTL) If high risk of recurrent DVT or PE: **5 mg/day** (1 tablet 2.5 mg bid) after 6 months treatment	**60 mg/day** (1 tablet 60 mg qd) after at least 5 days of parenteral anticoagulant treatment **30 mg/day** (1 tablet 30 mg qd) → if CrCl between 15–50 mL/min, BW ≤60 kg or concomitant use of dronedarone, erythromycin, ketoconazole, ciclosporin
Prevention of athero-thrombotic events after ACS with elevated cardiac biomarkers	×	**5 mg/day** (1 tablet 2.5 mg bid) with ASA (75–100 mg) or, ASA + clopidogrel (75 mg) or ticlopidine C_{max}: 46 (28–70) ng/mL (median; 5–95 PCTL) C_{min}: 17 (6–37) ng/mL (median; 5–95 PCTL)	×	×

T_{max}: time to reach peak concentration; C_{max}: peak concentration; AUC: area under the curve; CYP3A4: cytochrome P450 isozyme 3A4; P-gp: P-glycoprotein; BRCP: breast cancer resistance protein; caps: capsule; qd: once daily; bid: twice daily; CrCl: creatinine clearance; DVT: deep-vein thrombosis;

Table 1 continued.

PE: pulmonary embolism; THR: total hip replacement; TKR: total knee replacement; ASA: acetylsalicylic acid; ACS: acute coronary syndrome; C_{max} and C_{min}: peak and trough concentrations providing from the clinical trials; PCTL: percentiles; IQR: interquartile range; BW: body weight; DE: dabigatran etexilate. × = off-label; EU: European Union.

Patient body weight

There is evidence that anticoagulant clearance rates increase with body weight, however optimal dosing strategies for DOAC in obese patients remain unknown.[28] In the RE-LY study, dabigatran concentrations tended to increase with decreasing body weight.[28] Close clinical surveillance is recommended by several authorities because of limited clinical experience in these populations.[29,30]

Kubitza et al.[31] showed that rivaroxaban peak concentration (C_{max}) was increased by 24% in subjects weighing ≤50 kg while the area under the curve (AUC) was unaffected (difference was <25%) by body weight resulting in a small (15%) increase in prolongation of prothrombin time, which was not considered to be clinically relevant. Similarly to dabigatran, no dose adjustment is currently proposed by the European or American agencies.[32]

Regarding apixaban, a 30% and 20% increase in C_{max} and AUC, respectively, have been reported in patients weighing <50 kg.[33] Since body weight seems to have a modest effect on apixaban exposure, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recommend dose adjustment in patients weighing <60 kg (2.5 mg bid instead of 5 mg bid) in the presence of additional risk factors, namely age ≥80 years or serum creatinine >1.5 mg/dL.[34,35]

Dose adjustment in patients weighing <60 kg is also recommended for edoxaban (30 mg once daily since C_{max} and AUC in patients with low body weight (median 55 kg) were increased by 40% and 13%, respectively, compared with patients with high body weight (median 84 kg) in a population pharmacokinetic analysis of the ENGAGE AF-TIMI 48 study in NVAF.[36]

Patients with impaired hepatic function

The manufacturer's recommendations for DOAC in patients with impaired hepatic function are based on both Child–Pugh classification and liver-related exclusion criteria applied in clinical trials (elevated liver enzymes [aspartate transaminase/alanine transaminase] >2-times the upper limit of normal or total bilirubin ≥1.5-times the upper limit of normal).[37] The EMA contraindicates the use of dabigatran in patients with hepatic impairment or liver disease expected to have any impact on survival.[29]

In contrast to dabigatran, liver metabolism is an important route of elimination for FXa inhibitors. Approximately two-thirds of the administered rivaroxaban dose is metabolised by the liver via CYP3A4, 2J2, and CYP-independent mechanisms into inactive metabolites.[32] Apixaban undergoes liver metabolism mainly via CYP3A4/5, but other isoenzymes are also involved, while edoxaban is metabolised via carboxylesterase-1 hydrolysis, conjugation, or oxidation by CYP3A4/5 (<10%). Therefore, the EMA contraindicates their use in cases of hepatic disease associated with coagulopathy and clinically relevant bleeding risk. Apixaban and edoxaban are not recommended in patients with severe hepatic impairment, rivaroxaban is also contraindicated in cirrhotic patients with Child–Pugh B/C. Although one limited study showed a similar bleeding risk in Child–Pugh A/B cirrhosis patients treated with apixaban or rivaroxaban compared with traditional anticoagulants, further evaluations and larger studies are needed to better inform clinicians' decision-making.[38]

Drug interactions

In addition to these specific populations, DOAC also interact with P-glycoprotein substrates and CYP3A4 inhibitors, which may greatly increase their plasma concentrations, and hence, bleeding risks. Table 2 summarises drug-drug interactions reported in the literature as well as recommendations for dose adaptation and contraindications for the EMA and FDA prescribing guidelines.[22,39,40] As there is an increased risk of bleeding, DOAC should be used with caution if a concomitant use of antiplatelet agents is indicated[29,32,35] and non-steroidal anti-inflammatory drugs (NSAID) should be avoided if possible.[28,31,39] Concomitant use with

any other anticoagulant is strictly contraindicated, except during switching procedures from DOAC to VKA or when unfractionated heparin (UFH) is given at doses necessary to maintain an open central venous or arterial catheter.[29]

Screening for Bleeding Risk Factors

Non-modifiable and potentially reversible clinical features associated with higher bleeding risks must be carefully identified when initiating DOAC therapy and assessed regularly throughout treatment. Various risk stratification scores can help clinical decision-making.[41,42] The bleeding risk score HAS-BLED has shown better performance than the HEMORR(2)HAGES and ATRIA risk scores in predicting MB in anticoagulated AF patients.[41] A value ≥3 indicates a high risk for haemorrhage and suggests review and correction of modifiable risk factors (hypertension, renal/hepatic impairment, alcohol excess, concomitant use of non-steroidal anti-inflammatory drugs, antiplatelet agents, or selective serotonin reuptake inhibitors). The simple five-element ORBIT AF bleeding risk score (older age [>75 years], reduced haemoglobin/haematocrit history of anaemia, bleeding history, insufficient kidney function, and treatment with antiplatelet agents) had the best ability to predict MB in patients with NVAF compared with HAS-BLED and ATRIA risk scores.[43]

Table 2: Summary of drug-drug interactions with available recommendations for dose adaptation or contraindications by the competent authorities.[22,39,40,83]

		Dabigatran P-gp substrate	Rivaroxaban P-gp and CYP3A4/2J2 substrate	Apixaban P-gp and CYP3A4/5 substrate	Edoxaban P-gp substrate
Molecule	**Mechanism**				
Antiarrhythmics					
Dronedarone	P-gp and CYP3A4 inhibitor	AUC: +114% (400 mg: single dose)*	Minor effect (use with caution if CrCl 15-50 mL/min)†	No data yet	C_{max}: +46% AUC: +85%***
		AUC: +136% (400 mg: multiple doses)			
Quinidine	P-gp competition	AUC: +53% (1,000 mg: single dose)**	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet	AUC: +77% C_{max}: +85% (300 mg: multiple doses)
Verapamil	P-gp competition and weak CYP3A4 inhibitor	AUC: +18% (120 mg IR: single dose taken 2 hours after DE intake)**/***	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet	AUC and C_{max}: +53% (240 mg: multiple doses)
		AUC: +143% (120 mg IR: single dose, 1 hour before DE intake)**/***			
		C_{max}: +12% (120 mg IR: single dose taken 2 hours after DE intake)**/***			
		C_{max}: +179% (120 mg IR: single dose, 1 hour before DE intake)**/***			
Amiodarone	P-gp competition	AUC: +58% (600 mg: single dose)**	Minor effect (use with caution if CrCl 15–50 mL/min)	No clinically relevant effect	AUC: +40% C_{max}: +66% (400 mg: single dose)

Table 2 continued.

		Dabigatran P-gp substrate	Rivaroxaban P-gp and CYP3A4/2J2 substrate	Apixaban P-gp and CYP3A4/5 substrate	Edoxaban P-gp substrate
Molecule	**Mechanism**				
Diltiazem	P-gp and CYP3A4 inhibitor	No effect	Minor effect (use with caution if CrCl 15–50 mL/min)	AUC: +40%	No data yet
Antianginal/antihypertensive drugs					
Ranolazine	P-gp and CYP3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet	No data yet
Felodipine	P-gp and CYP3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet	No data yet
Anti-inflammatory					
Naproxen	P-gp competition	No data yet	AUC: +10% (500 mg)	C_{max}: +61% AUC: +55% (500 mg)	No effect, but pharmacod namically increased bleeding time
Antihypercholesterolemiant					
Atorvastatin	P-gp and CYP3A4 substrate	AUC: +18%	No effect	No PK data yet	No effect
Antimycotic					
Ketoconazole	P-gp and CYP3A4 inhibitor	AUC: +138% (400 mg: single dose)*	C_{max}: +72% (400 mg: single dose †	C_{max}: +62% (400 mg multiple doses)†	C_{max}: +89%*** (400 mg multiple doses)
		AUC: +153% (400 mg: multiple doses)	AUC: +158% (400 mg: single dose)†	AUC: +100% (400 mg multiple doses)†	AUC: +87% *** (400 mg multiple doses)
Itraconazole	P-gp and CYP3A4 inhibitor	No data yet*	No data yet, but similar results than ketoconazole are expected†	No data yet, but similar results than ketoconazole are expected†	No data yet
Voriconazole	P-gp and CYP3A4 inhibitor	No data yet			No data yet
Posaconazole	P-gp and CYP3A4 inhibitor	No data yet***	No data yet†		No data yet
Fluconazole	CYP3A4 inhibitor	No data yet - supposed no effect	C_{max}: +28% AUC: +42%	No data yet	No data yet
Antibacterial					
Clarithromycin	P-gp and CYP3A4 inhibitor	C_{max}: +49%		No data yet	No data yet
		AUC: +60%	AUC: +54% (500 mg multiple doses)		
Azithromycin	P-gp and CYP3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet	No data yet
Erythromycin	P-gp and CYP3A4 inhibitor	No data yet	AUC: +34% (500 mg multiple doses)	No data yet	C_{max}: +68%*** AUC: +85%***
Rifampicin	P-gp and CYP3A4 inducer	C_{max}: -65,5%† AUC: -67%† (600 mg: multiple doses)	AUC: -50%†	AUC: -54%†	AUC: -35% but with compensatory increase of active metabolite (avoid if possible)

Table 2 continued.

		Dabigatran P-gp substrate	Rivaroxaban P-gp and CYP3A4/2J2 substrate	Apixaban P-gp and CYP3A4/5 substrate	Edoxaban P-gp substrate
Molecule	**Mechanism**				
Protease inhibitors					
Ritonavir	P-gp and CYP 3A4 inhibitor	No data yet†	C_{max}: +55% (600 mg multiple doses)†	No PK data but strong increase†	No data yet†
			AUC: +153% (600 mg multiple doses)†		
Immunosuppressor					
Ciclosporine	P-gp competition	No data yet*	AUC: +50%	No data yet	AUC: +73%*** C_{max}: +74% (500 mg single dose)
Tacrolimus	P-gp competition	No data yet†	AUC: +50%	No data yet	No data yet

*Contraindicated by the European Medicines Agency (EMA).
**The EMA recommends dose reduction for dabigatran: from 220 mg qd to 150 mg qd in prevention of VTE in joint replacement.
***The EMA recommends dose reduction for dabigatran: from 150 mg bid to 110 mg bid) in patients with NVAF or VTE; and for edoxaban a dose reduction of 50% (30 mg qd) in patients with NVAF or VTE.
†Concomitant treatment with these drugs are not recommended by the EMA.
NVAF: non-valvular arterial fibrillation; VTE: venous thromboembolism; P-gp: p-glycoprotein; CYP: cytochrome; AUC: area under the curve; CrCl: creatinine clearance; PK: pharmacokinetics; C_{max}/C_{min}: peak and trough concentrations providing from the clinical trials; qd: once daily; bid: twice daily; DE: dabigatran etexilate.

Screening for injuries (e.g. recent intracranial haemorrhage) and conditions (e.g. colon cancer) that may lead to MB is essential before starting DOAC therapy.

Particular attention should be paid to renal protective strategies for patients on dabigatran and edoxaban. These patients should be informed about the impact of concurrent medications (e.g. NSAID) or comorbidities (e.g. dehydration) on their renal function, which could lead to an enhanced and prolonged anticoagulant effect.

Adapting Direct Oral Anticoagulant Dosage to Individual Benefit-Risk Ratios

Why and when?

Several criteria should be taken into account when considering drug monitoring: 1) intra and 2) inter-individual variability in plasma drug level, both justifying the identification of the optimal dose for the individual at the start of treatment, 3) variability and reproducibility of the testing method, 4) correlation between drug level and clinical outcome, and 5) the therapeutic value of drug monitoring.

DOAC demonstrate high intra and inter-individual variability depending on patients' age, renal and hepatic function, drug-drug interactions, and body weight.[44] While package inserts recommend dose adaptation according to the patient's characteristics, they do not give guidance for patients with multiple interfering factors in whom a targeted plasma level cannot be reached. The importance of targeted plasma levels is supported by analysis of large trials (RE-LY and the ENGAGE-AF TIMI 48), which showed that plasma concentrations of dabigatran and edoxaban were correlated with the incidence of MB and thrombotic events.[45-47] Although there are currently no published data for rivaroxaban and apixaban, data from the FDA Clinical Pharmacology and Biopharmaceutics Reviews (available at

http://www.fda.gov) clearly suggest an association between drug exposure and safety outcomes. Thus, patients may benefit from individualised dose tailoring.

In addition, specific situations require an assessment of the intensity of anticoagulation to prevent bleeding and other complications. These include the peri-procedural management of urgent surgery or elective procedures, before thrombolysis or percutaneous coronary intervention, and bridging therapy. Patients on dual antiplatelet therapy added to DOAC could also benefit from a reduced posology to prevent bleeding.[48]

How to handle and perform such measurements?

Compared to VKA, the effect of DOAC on clotting tests varies greatly depending on the time between the last dose and blood sampling (Table 1).[49] Therefore, the time of the last dose in relation to the blood sampling must be known in order to interpret the results of a particular test.

In ambulatory patients, regulatory documents only state thresholds associated with a risk of bleeding for dabigatran. The cut-offs proposed for apixaban, rivaroxaban, and edoxaban are based on the pharmacokinetic characteristics of these drugs where it is suggested that exceeding the highest percentile of a trough concentration carries a risk of bleeding. Regarding which test should be used, Table 3 summarises recommendations depending on the situation and the drug. While limitations have been mentioned for routine coagulation tests such as prothrombin time, activated partial thromboplastin time, and even thrombin time,[50,51] the aim of this comprehensive approach is to provide reliable options, even for laboratories where more specific tests are not available, to give physicians sufficient information to support their clinical decisions.

Dealing with Missed Doses

If a missed dose is noticed within 6 hours of the correct time for dabigatran/apixaban or within 12 hours for edoxaban/rivaroxaban, patients should take the forgotten dose immediately. Beyond these times, the dose should be skipped and the next scheduled dose should be taken.[22]

Table 3: Current indication, clinical need, and required assay characteristics for the measurement of direct oral anticoagulants.[49-51]

Indication	Emergent situation†	Information required	Characteristic of the assay	Assays recommended††
Bleeding	Yes	Identifying above on-therapy levels	• Sensitive (qualitative and quantitative) • Rapid turn-around time	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors) • Global coagulation tests (i.e. PT (rivaroxaban - edoxaban) - aPTT (dabigatran) or dRVVT)
Elective invasive procedure	No	Excluding on-therapy level and ensure safe procedure‡	• Sensitive (qualitative and quantitative) • Accurate	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors) • Thrombin time (dabigatran)
Urgent invasive procedure	Yes	Excluding on-therapy levels and ensure safe procedure‡	• Sensitive (qualitative and quantitative) • Accurate • Rapid turn-around time	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors) • Global coagulation tests (i.e. PT (rivaroxaban edoxaban) - aPTT (dabigatran) or dRVVT) • Thrombin time (dabigatran)
Thrombolysis	Yes	Exclude on-therapy drug level and ensure safe procedure	• Sensitive (qualitative and quantitative) • Accurate • Rapid turn-around time	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors) • Thrombin time (dabigatran)

Table 3 continued.

Indication	Emergent situation†	Information required	Characteristic of the assay	Assays recommended††
Overdose (without complication)	No	Detection of overdose and inform on period at risk of bleeding	• Sensitive (qualitative and quantitative) • Rapid turn-around time	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors) • Global coagulation tests (i.e. PT (rivaroxaban edoxaban) - aPTT (dabigatran) or dRVVT)
Factors interfering with pharmacokinetics (drug interactions, renal or hepatic impairment, genetic polymorphism)	No	Ensure on-therapy level and exclude too low or too high drug levels	• Accurate quantitative test	• Specific coagulation tests (i.e. dTT, ECA - dabigatran; chromogenic anti-Xa - direct FXa inhibitors)

†Emergency situations are those in which anticoagulant effects should be measured within 30 minutes.
††Assays recommended are presented from the more to the less suitable among those able to respond the clinical need.
‡The assessment will depend on the type of procedure.
aPTT: activated partial thromboplastin time; dRVVT: dilute Russell's Viper Venom Time; dTT: dilute thrombin time; ECA: ecarin chromogenic assays; PT: prothrombin time; FXa: factor Xa.

A double dose should never be taken to make up for a missed dose except during the first 21 days of rivaroxaban administration for VTE treatment, where two 15 mg tablets may be taken simultaneously if a dose is missed to ensure a total daily dose of 30 mg.[32]

Adherence to Switching Procedures

The correct timing of switching procedures is essential to avoid excessive anticoagulation due to additional effects and to reduce thromboembolic risk due to transitory low anticoagulation. This timing has been fixed for each DOAC taking into account the drug profile and trial protocols.[52] The Dresden NOAC registry reported that 25% of patients did not have an international normalised ratio (INR) measurement before switching from VKA to DE or rivaroxaban.[53]

Practically, when VKA are switched to DOAC, VKA should be discontinued and DOAC should be started as soon as the INR is ≤2 for dabigatran and apixaban, ≤2.5 for edoxaban, or ≤3 for rivaroxaban. The EMA recommends that for patients with a deep vein thrombosis, rivaroxaban should be initiated once the INR is ≤2.5.[22]

When DOAC are switched to VKA, DOAC should be administered concomitantly until the INR reaches an appropriate anticoagulation level. As DOAC can have an impact on the INR measurements, this should be taken just before the next scheduled administration of the DOAC and be rechecked 24 hours later. Close monitoring of the INR is recommended within the first month of switching. For edoxaban, the administration of a half dose is recommended when VKA is started.[22] For DE, the starting time of the VKA should be based on CrCl as follows: 3 days if CrCl is >50 mL/min, 2 days if CrCl is 30–50 mL/min, and 1 day if CrCl is 15–30 mL/min.[29,30]

When switching from DOAC to parenteral anticoagulants (LMWH or UFH), these should be started when the next DOAC dose is due. Inversely, DOAC should be started at the same time or up to 2 hours before the next parenteral anticoagulant dose. For intravenous UFH, DOAC should be started at the time of discontinuation of the infusion.[22,29]

The EMA recommends that edoxaban should be started 4 hours after stopping the UFH infusion.[54] The renal function should be assessed before switching from heparin to DOAC.

Adherence to Bridging Procedures

The peri-procedural interruption of DOAC treatment depends on the bleeding risk of the procedure and the patient's thromboembolic risk. Interruption may require bridging therapy

with LMWH or UFH, especially for patients with high thromboembolic risks ($CHADS_2$ score >4, recent stroke, VTE, or transient ischaemic attack within the past 3 months, hypercoagulable state) and in cases of severe renal impairment when interruption is >96 hours.[55] Recent data showed increased peri-procedural MB events in patients bridged with heparins after DOAC were stopped, with no decrease in the incidence of cardiovascular or thrombotic events compared with patients who were not bridged.[56-58] The BRIDGE-trial showed similar results concerning patients on warfarin.[59] The first prospective perioperative study including patients receiving dabigatran showed a safe profile in terms of haemorrhagic and thrombotic events without the use of LMWH bridging.[60] The use of LMWH bridging after antiplatelet therapy cessation in patients with coronary stents who underwent non-cardiac surgery, showed a worse ischaemic outcome at 30 days and a significant risk of bleeding compared with the group who did not stop antiplatelet therapy and had no bridging.[61] Therefore, arguments for bridging therapy should be balanced, as it may be harmful for some patients, and further studies are needed to validate the safety of bridging therapy in specific populations, for example, thrombophilic patients or patients with high $CHADS_2$ score (5–6).

Resuming DOAC after an invasive procedure should only be considered when haemostasis is achieved, usually 6–8 hours after procedures with a standard risk of bleeding or atraumatic spinal/epidural anaesthesia. For procedures with a high risk of bleeding, DOAC at full therapeutic dosage should be deferred until 48–72 hours after the invasive procedure. This interval is justified by the fact that specific antidotes are not available everywhere in case of postoperative bleeding or re-operation.[22] Furthermore, the high cost of these antidotes may severely limit their use in clinical practice. For cases of prolonged fasting, immobilisation, or patients with high thrombotic risk profile, short-term administration of reduced venous thromboprophylactic or an intermediate dose of LMWH can be considered before resuming DOAC.[22] Renal function should be assessed postoperatively when anticoagulants are resumed.

CONCLUSIONS

Despite the considerable advantages that DOAC bring to the field of anticoagulation, their inappropriate use can lead to a higher than expected risk of bleeding. Continuous education of healthcare practitioners and patients about dose adjustment and compliance guidance should be supported in all health institutions. Modifiable bleeding risk factors should be identified before initiation of DOAC and reviewed regularly. Individual benefit-risk may be improved in some clinical settings or in patients at risk of supra or infra-therapeutic plasma levels by altering the dose following coagulation monitoring. Protocols for switching, bridging, and resuming anticoagulants should be standardised and systems should be designed to optimise the management of patients treated with DOAC.

Acknowledgements

The authors thank Dr Elizabeth Wager for language editing and Prof Edith Collard for logistic support.

REFERENCES

1. Sholzberg M et al. Bleeding complications from the direct oral anticoagulants. BMC Hematol. 2015;15:18.

2. Southworth MR et al. Dabigatran and postmarketing reports of bleeding. N Engl J Med. 2013;368(14):1272-4.

3. Harenberg J et al. Interpretation of endpoints in a network meta-analysis of new oral anticoagulants following total hip or total knee replacement surgery. Thromb Haemost. 2012;108(5):903-12.

4. Huisman MV et al. Antithrombotic Treatment Patterns in Patients with Newly Diagnosed Nonvalvular Atrial Fibrillation: The GLORIA-AF Registry, Phase II. Am J Med. 2015;128(12):1306-13.e1.

5. Riva N et al. Major Bleeding and Case Fatality Rate with the Direct Oral Anticoagulants in Orthopedic Surgery: A Systematic Review and Meta-Analysis. Semin Thromb Hemost. 2016;42(1):42-54.

6. Chai-Adisaksopha C et al. Mortality outcomes in patients receiving direct oral anticoagulants: a systematic review and meta-analysis of randomized controlled trials. J Thromb Haemost. 2015;13(11): 2012-20.

7. Raccah BH et al. Major Bleeding and Hemorrhagic Stroke with Direct Oral Anticoagulants in Patients with Renal Failure: Systematic Review and Meta-Analysis of Randomized Trials. Chest. 2016: doi: 10.1016/j.chest.2015.12.029. [Epub ahead of print].

8. Hernandez I et al. Risk of bleeding with dabigatran in atrial fibrillation. JAMA

Intern Med. 2015;175(1):18-24.

9. Russo V et al. Efficacy and safety of dabigatran in a "real-life" population at high thromboembolic and hemorrhagic risk: data from MonaldiCare registry. Eur Rev Med Pharmacol Sci. 2015;19(20): 3961-7.

10. Beyer-Westendorf J et al. Effectiveness and safety of dabigatran therapy in daily-care patients with atrial fibrillation. Results from the Dresden NOAC Registry. Thromb Haemost. 2015;113(6):1247-57.

11. Sorensen R et al. Dabigatran use in Danish atrial fibrillation patients in 2011: a nationwide study. BMJ Open. 2013;3(5): doi: 10.1136/bmjopen-2013-002758.

12. Tamayo S et al. Characterizing major bleeding in patients with nonvalvular atrial fibrillation: a pharmacovigilance study of 27 467 patients taking rivaroxaban. Clin Cardiol. 2015;38(2):63-8.

13. Beyer-Westendorf J et al. Rates, management, and outcome of rivaroxaban bleeding in daily care: results from the Dresden NOAC registry. Blood. 2014;124(6):955-62.

14. Goodman SG et al. Factors associated with major bleeding events: insights from the ROCKET AF trial (rivaroxaban once-daily oral direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation). J Am Coll Cardiol. 2014;63(9):891-900.

15. Kirley K et al. National trends in oral anticoagulant use in the United States, 2007 to 2011. Circ Cardiovasc Qual Outcomes. 2012;5(5):615-21.

16. Lim SL, Maxwell E. An audit of dabigatran etexilate prescribing in Victoria. Med J Aust. 2013;198(6):314-5.

17. Larock AS et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother. 2014;48(10):1258-68.

18. Armbruster AL et al. Evaluation of dabigatran for appropriateness of use and bleeding events in a community hospital setting. Am Health Drug Benefits. 2014;7(7):376-84.

19. Tellor KB et al. Evaluation of the appropriateness of dosing, indication and safety of rivaroxaban in a community hospital. J Clin Pharm Ther. 2015;40(4):447-51.

20. Olaiya A et al. An observational study of direct oral anticoagulant awareness indicating inadequate recognition with potential for patient harm. J Thromb Haemost. 2016:doi: 10.1111/jth.13288. [Epub ahead of print].

21. Schulman S. New oral anticoagulant agents - general features and outcomes in subsets of patients. Thromb Haemost. 2014;111(4):575-82.

22. Heidbuchel H et al. Updated European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist anticoagulants in patients with non-valvular atrial fibrillation. Europace. 2015; 17(10):1467-507.

23. Hellden A et al. Renal function estimations and dose recommendations for dabigatran, gabapentin and valaciclovir: a data simulation study focused on the elderly. BMJ Open. 2013;3(4):doi: 10.1136/bmjopen-2013-002686.

24. Maccallum PK et al. Patient safety and estimation of renal function in patients prescribed new oral anticoagulants for stroke prevention in atrial fibrillation: a cross-sectional study. BMJ Open. 2013; 3(9):e003343.

25. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP) Assessment report. 2012. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000829/WC500131783.pdf. Last accessed: 18 February 2016.

26. Moore KT et al. Rivaroxaban crushed tablet suspension characteristics and relative bioavailability in healthy adults when administered orally or via nasogastric Tube. Clinical Pharmacology in Drug Development. 2014;3(4):321-32.

27. Song Y et al. Relative Bioavailability of Apixaban Solution or Crushed Tablet Formulations Administered by Mouth or Nasogastric Tube in Healthy Subjects. Clin Ther. 2015;37(8):1703-12.

28. Patel JP et al. Anticoagulating obese patients in the modern era. Br J Haematol. 2011;155(2):137-49.

29. European Medicines Agency. Summary of Product Characteristics: Pradaxa. 2015. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf. Last accessed: 18 February 2016.

30. Food and Drug Administration. Pradaxa: Full Prescribing Information. 2015. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/022512s028lbl.pdf. Last accessed: 18 February 2016.

31. Kubitza D et al. Body weight has limited influence on the safety, tolerability, pharmacokinetics, or pharmacodynamics of rivaroxaban (BAY 59-7939) in healthy subjects. J Clin Pharmacol. 2007;47(2): 218-26.

32. European Medicines Agency. Summary of Product Characteristics: Xarelto. 2015. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf. Last accessed: 18 February 2016.

33. Upreti VV et al. Effect of extremes of body weight on the pharmacokinetics, pharmacodynamics, safety and tolerability of apixaban in healthy subjects. British J Clin Pharmacol. 2013;76(6)908-16.

34. U.S. Food and Drug Administration. Eliquis: Highlights of Prescribing Information. 2015. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/202155s011lbl.pdf. Last accessed: 18 February 2016.

35. Salazar DE et al. Modelling and simulation of edoxaban exposure and response relationships in patients with atrial fibrillation. Thromb Haemost. 2012; 107(5):925-36.

36. Graff J, Harder S. Anticoagulant therapy with the oral direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban and the thrombin inhibitor dabigatran etexilate in patients with hepatic impairment. Clin Pharmacokinet. 2013;52(4):243-54.

37. Intagliata NM et al. Direct Oral Anticoagulants in Cirrhosis Patients Pose Similar Risks of Bleeding When Compared to Traditional Anticoagulation. Dig Dis Sci. 2016. [Epub ahead of print].

38. Nutescu E et al. Drug and dietary interactions of warfarin and novel oral anticoagulants: an update. J Thromb Thrombolysis. 2011;31(3):326-43.

39. Walenga JM, Adiguzel C. Drug and dietary interactions of the new and emerging oral anticoagulants. Int J Clin Pract. 2010;64(7):956-67.

40. European Medicines Agency. Summary of Product Characteristics: Eliquis. 2016. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf. Last accessed: 18 February 2016.

41. Apostolakis S et al. Performance of the HEMORR(2)HAGES, ATRIA, and HAS-BLED bleeding risk-prediction scores in patients with atrial fibrillation undergoing anticoagulation: the AMADEUS (evaluating the use of SR34006 compared to warfarin or acenocoumarol in patients with atrial fibrillation) study. J Am Coll Cardiol. 2012;60(9):861-7.

42. Roldan V et al. Predictive value of the HAS-BLED and ATRIA bleeding scores for the risk of serious bleeding in a "real-world" population with atrial fibrillation receiving anticoagulant therapy. Chest. 2013;143(1):179-84.

43. O'Brien EC et al. The ORBIT bleeding score: a simple bedside score to assess bleeding risk in atrial fibrillation. Eur Heart J. 2015;36(46):3258-64.

44. Douxfils J et al. Dose tailoring of dabigatran etexilate: obvious or excessive? Expert Opin Drug Saf. 2015;14(8):1283-9.

45. Reilly PA et al. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial

(Randomized Evaluation of Long-Term Anticoagulation Therapy). J Am Coll Cardiol. 2014;63(4):321-8.

46. Ruff CT et al. Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet. 2015;385(9984):2288-95.

47. Weitz JI et al. Randomised, parallel-group, multicentre, multinational phase 2 study comparing edoxaban, an oral factor Xa inhibitor, with warfarin for stroke prevention in patients with atrial fibrillation. Thromb Haemost. 2010;104(3): 633-41.

48. Lip GY et al. Management of antithrombotic therapy in atrial fibrillation patients presenting with acute coronary syndrome and/or undergoing percutaneous coronary or valve interventions: a joint consensus document of the European Society of Cardiology Working Group on Thrombosis, European Heart Rhythm Association (EHRA), European Association of Percutaneous Cardiovascular Interventions (EAPCI) and European Association of Acute Cardiac Care (ACCA) endorsed by the Heart Rhythm Society (HRS) and Asia-Pacific Heart Rhythm Society (APHRS). Eur Heart J. 2014;35(45):3155-79.

49. Douxfils J et al. Measurement of non-VKA oral anticoagulants versus classic ones: the appropriate use of hemostasis assays. Thromb J. 2014;12:24.

50. Lessire S et al. Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study. Thromb Res. 2015;136(3):693-6.

51. Douxfils J et al. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. Biomed Res Int. 2015:345138.

52. Guimaraes PO et al. Practical and clinical considerations in assessing patients with atrial fibrillation for switching to non-vitamin K antagonist oral anticoagulants in primary care. Int J Gen Med. 2015;8:283-91.

53. Beyer-Westendorf J et al. Safety of switching from vitamin K antagonists to dabigatran or rivaroxaban in daily care--results from the Dresden NOAC registry. Br J Clin Pharmacol. 2014;78(4):908-17.

54. European Medicines Agency. Summary of Product Characteristics: Lixiana. 2015. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002629/WC500189045.pdf. Last accessed: 18 February 2016.

55. Mar PL et al. Periprocedural management of anticoagulation in patients taking novel oral anticoagulants: Review of the literature and recommendations for specific populations and procedures. Int J Cardiol. 2016;202:578-85.

56. Beyer-Westendorf J et al. Peri-interventional management of novel oral anticoagulants in daily care: results from the prospective Dresden NOAC registry. Eur Heart J. 2014;35(28):1888-96.

57. Steinberg BA et al. Use and outcomes associated with bridging during anticoagulation interruptions in patients with atrial fibrillation: findings from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF). Circulation. 2015;131(5):488-94.

58. Douketis JD et al. Perioperative bridging anticoagulation during dabigatran or warfarin interruption among patients who had an elective surgery or procedure. Substudy of the RE-LY trial. Thromb Haemost. 2015;113(3):625-32.

59. Douketis JD et al. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. New Engl J Med. 2015;373(9):823-33.

60. Schulman S et al. Perioperative Management of Dabigatran: A Prospective Cohort Study. Circulation. 2015;132(3): 167-73.

61. Capodanno D et al. Impact of bridging with perioperative low-molecular-weight heparin on cardiac and bleeding outcomes of stented patients undergoing non-cardiac surgery. Thromb Haemost. 2015;114(2):423-31.

62. Bauersachs R et al; EINSTEIN Investigators. Oral rivaroxaban for symptomatic venous thromboembolism. New Eng J Med. 2010;363(26):2499-510.

63. Büller HR et al; EINSTEIN–PE Investigators et al. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. New Engl J Med. 2012;366(14): 1287-97.

64. Connolly SJ et al. Dabigatran versus warfarin in patients with atrial fibrillation. New Engl J Med. 2009;361(12):1139-51.

65. Eriksson BI et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. New Engl J Med. 2008; 358(26):2765-75.

66. Eriksson BI et al. Oral dabigatran versus enoxaparin for thromboprophylaxis after primary total hip arthroplasty (RE-NOVATE II*). A randomised, double-blind, non-inferiority trial. Thromb Haemost. 2011;105(4):721-9.

67. Eriksson BI et al. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: the RE-MODEL randomized trial. J Thromb Haemost. 2007;5(11): 2178-85.

68. Eriksson BI et al. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. Lancet. 2007; 370(9591):949-56.

69. Granger CB et al. Apixaban versus warfarin in patients with atrial fibrillation. New Engl J Med. 2011;365(11):981-92.

70. Kakkar AK et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. Lancet. 2008;372(9632): 31-9.

71. Lassen MR et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. New Engl J Med. 2008;358(26):2776-86.

72. Lassen MR et al. Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. New Engl J Med. 2010; 363(26):2487-98.

73. Lassen MR et al. Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. Lancet. 2010;375(9717):807-15.

74. Lassen MR et al. Apixaban or enoxaparin for thromboprophylaxis after knee replacement. New Engl J Med. 2009; 361(6):594-604.

75. Mega JL et al. Rivaroxaban versus placebo in patients with acute coronary syndromes (ATLAS ACS-TIMI 46): a randomised, double-blind, phase II trial. Lancet. 2009;374(9683):29-38.

76. Mega JL et al. Rivaroxaban in patients with a recent acute coronary syndrome. New Engl J Med. 2012;366(1):9-19.

77. Patel MR et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. New Engl J Med. 2011;365(10):883-91.

78. Turpie AG et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. Lancet. 2009;373(9676): 1673-80.

79. Giugliano RP et al. Edoxaban versus warfarin in patients with atrial fibrillation. New Engl J Med. 2013;369(22):2093-104.

80. Frost C et al. Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor Xa inhibitor, in healthy subjects. Br J Clin Pharmacol. 2013;76(5):776-86.

81. Mueck W et al. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet. 2014; 53(1):1-16.

82. Stangier J, Clemens A. Pharmacology, pharmacokinetics, and pharmacodynamics of dabigatran etexilate, an oral direct thrombin inhibitor. Clin Appl Thromb Hemost. 2009;15 Suppl 1:9S-16S.

83. Parasrampuria DA, Truitt KE. Pharmacokinetics and Pharmacodynamics of Edoxaban, a Non-Vitamin K Antagonist Oral Anticoagulant that Inhibits Clotting Factor Xa. Clin Pharmacokinet. 2015. [Epub ahead of print].

BRIDGING TO TRANSPLANT IN DIFFUSE LARGE B CELL LYMPHOMA

***Christian Gisselbrecht,[1] Eric Van Den Neste[2]**

1. Hôpital Saint Louis, Paris, France
2. Cliniques Universitaires UCL Saint-Luc, Brussels, Belgium
**Correspondence to christian.gisselbrecht@sls.aphp.fr*

Disclosure: The authors have declared no conflicts of interest.
Support: The publication of this article was supported by CTI and Servier. The views and opinions expressed are those of the authors and not necessarily of CTI and Servier.
Received: 28.04.16 **Accepted:** 01.07.16
Citation: EMJ Hematol. 2016;4[1]:91-99.

ABSTRACT

Non-Hodgkin lymphoma (NHL) is the eighth most common malignancy worldwide. Diffuse large B cell lymphoma (DLBCL) is the most frequent subtype, accounting for >30% of NHL cases. Advances in novel approaches in the last two decades, such as immunotherapy with rituximab, have achieved improvements in terms of overall and long-term survival rates. The current standard of care for the first-line treatment of DLBCL is chemotherapy with rituximab plus cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone; this regimen achieves complete and sustained remission in approximately 60% of patients. Nevertheless, DLBCL relapses in 30–40% of patients, of which 10% develop refractory disease. Recent findings have demonstrated that substantial responses could be achieved after second or third-line treatments with combined chemotherapy. Since 2012, the aza-anthracenedione, pixantrone, has been approved as a single agent for relapsed or refractory DLBCL. The drug could be a new option as a bridging therapy to consolidate autologous or allogeneic stem cell transplantation, which in turn, can deliver prolonged durations of remission. Numerous clinical studies are ongoing that aim to improve salvage rates, outcomes, and access to stem cell transplantations for relapsed or refractory DLBCL. The development of novel targeted therapies or chemotherapeutics, such as pixantrone, will help to salvage more patients and achieve further sustained and complete responses without compromising their quality of life.

Keywords: Non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma (DLBCL), refractory DLBCL, relapsed DLBCL, salvage therapy, pixantrone, stem cell transplantation (SCT).

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is the eighth most common malignancy worldwide,[1] with diffuse large B cell lymphoma (DLBCL) being its most frequent subtype. DLBCL accounts for over 30% of cases of NHL.[2] It is a rapidly growing and aggressive malignancy in which large B cells with high levels of mitotic activity spread into lymph nodes or other tissues outside the lymphatic system. DLBCL generally occurs in patients >50 years old, and is slightly more common in women.[3]

Over the last two decades, advances in novel therapeutic approaches, such as immunotherapy with rituximab, have achieved very good results in terms of overall and long-term survival.[4-6] The current standard of care for the first-line treatment of DLBCL is chemotherapy with rituximab plus cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone, yielding complete and sustained remission in ~60% of cases.[5]

Despite these good results, between 30% and 40% of patients relapse following first-line therapy and an additional 10% present the refractory disease.[6-8] As defined by the criteria of Cheson et al.,[9] relapsed DLBCL is characterised by the appearance of any new lesion after a complete response (CR), while refractory DLBCL is defined as failure of <50% of lesions to reduce in size following initial treatment. In these clinical settings, the standard therapeutic

option is to initiate high-dose therapy prior to either autologous or allogeneic stem cell transplantation (SCT). Patients who are ineligible for SCT or who fail after second-line therapy have a poor prognosis,[10] but recent findings have revealed that they could benefit from alternative salvage therapies.[11] Salvage therapies may also be used as a bridge to autologous or allogeneic SCT. The aim of this article is to provide an overview of advances and perspectives related to induction therapies as a bridge to transplantation in relapsed or refractory DLBCL (RR-DLBCL), as well as novel strategies in multiply relapsed DLBCL (MR-DLBCL).

MANAGEMENT OF RELAPSED OR REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA

Management of Refractory Diffuse Large B Cell Lymphoma in Patients Eligible for Stem Cell Transplantation

Salvage chemotherapy as a bridge to autologous SCT is the standard therapeutic option for relapsed DLBCL and is successful in 30–40% of patients.[12] High-risk, chemotherapy-sensitive patients with a low probability of success with autologous SCT may be oriented to allogeneic SCT. Rates of relapse and progression in high-risk patients are comparable for the two approaches, although allogeneic SCT is associated with higher rates of non-relapse mortality than autologous SCT.[13,14]

There are many salvage therapies available, mostly involving rituximab in combination with standard antineoplastic agents. The most frequently used combinations are as follows:[15]

- R-ICE: rituximab plus ifosfamide, carboplatin, and etoposide
- R-DHAP: rituximab plus cytosine, arabinoside, cisplatin, and dexamethasone
- R-GDP: rituximab plus gemcitabine, dexamethasone, and cisplatin
- R-ESHAP: rituximab plus etoposide, methylprednisolone, cytarabine, and cisplatin
- R-GemOx: rituximab plus gemcitabine and oxaliplatin

What is the Best Salvage Therapy?

The best chemotherapy regimens are those that provide the highest response rates with the most tolerable toxicity. There is still no clear evidence regarding the superiority of one regimen over the other. Two prospective randomised studies have compared available salvage therapies (CORAL [COllaborative trial in Relapsed Aggressive Lymphoma] and LY12) but have failed to detect any significant differences in clinical outcomes, as detailed below.

COllaborative trial in Relapsed Aggressive Lymphoma (CORAL) Study

In the multicentre Phase III CORAL study, 477 patients with CD20+ DLBCL during their first relapse or who had disease that was refractory to first-line therapy were randomly allocated (1:1) to three courses of R-ICE (243 patients) or three courses of R-DHAP (234 patients). In both groups, treatment was followed by high-dose chemotherapy with carmustine, etoposide, cytarabine, and melphalan (BEAM), and then autologous SCT.[16,17] Response rates were 64% (95% confidence interval [CI]: 56–70%), and 63% (95% CI: 55–69%), respectively. Overall, 50% of patients were able to proceed to autologous SCT, mainly due to an insufficient response to the second-line therapy. There was no significant difference between the two rituximab (R-ICE and R-DHAP) regimens in terms of 3-year event-free survival (EFS) or overall survival (OS).

In the subpopulation that underwent autologous SCT, 122 patients received 1-year maintenance treatment with rituximab and 120 patients were assigned to observation only.[16] At 4 years, no difference in EFS was observed between the rituximab maintenance group and the control group (52% versus 53%, respectively), although there was a 15% attributable risk of serious adverse events in the active therapy group. Rituximab is therefore not recommended as a maintenance therapy after autologous SCT.

In the subpopulation that failed to proceed to autologous SCT, 13 patients died and 6 withdrew consent.[18] The remaining 203 patients were candidates for third-line chemotherapy, for which they had an overall response rate (ORR) of 39%, including 27% CR/unconfirmed complete response (CRu) and 12% partial response (PR) (Figure 1). Of these, 32% (n=64) of patients subsequently underwent autologous SCT (n=56) or allogeneic SCT (n=8). The authors concluded that, while third-line salvage chemotherapy for DLBCL can lead to a clinical response, with the chance for transplantation and long-term survival, the rates are still relatively low and there is an urgent need for new drugs.

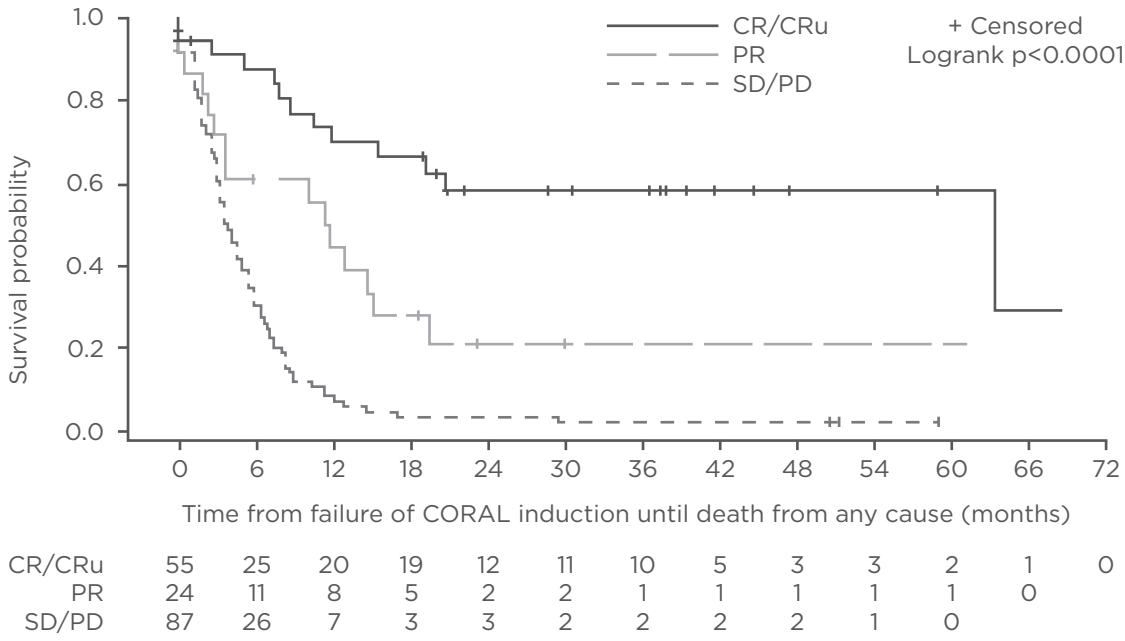

Figure 1: Overall survival (months) of 116 patients from time-to-treatment failure of CORAL induction according to the response to the third-line regimen.[18]

DLBCL: diffuse large B cell lymphoma; SD/PD: stable disease/progressive disease; PR: partial remission; CR: complete response; CRu: unconfirmed complete response; CORAL: COllaborative trial in Relapsed Aggressive Lymphoma study.

LY12 Study

The LY12 Phase III study was conducted by the National Cancer Institute of Canada in patients with RR-DLBCL. The aim was to compare two salvage therapies R-DHAP (n=310) and R-GDP (n=309),[19,20] followed by autologous SCT, which was performed in 49% and 52% of cases, respectively. Four-year survival rates were comparable, with EFS rates of 26% and 26%, and OS of 39% and 39%, respectively. Notably, patients in the R-GDP treatment arm seemed to benefit from lower toxicity and higher scores for quality of life.

Other Studies

Other agents are being explored as bridging therapies in RR-DLBCL. As an example, in an open-label Phase II study, the combination of everolimus (an mTOR inhibitor) and rituximab were evaluated in 24 heavily pre-treated patients with RR-DLBCL.[21] The ORR was 38% with three patients achieving CR and six patients with PR. Two of the patients with a CR were able to use this regimen as a bridging therapy and consequently underwent allogeneic SCT. After a follow-up period of 19 months, both patients were alive and disease-free.

Recently, the German High Grade Non-Hodgkin Lymphoma Study Group conducted a Phase II study in 84 patients with relapsed and refractory aggressive NHL to evaluate rituximab as an addition to graft-versus-host-disease prophylaxis following SCT.[22] After myeloablative conditioning, all patients underwent allogeneic SCT, after which they were randomly allocated to receive rituximab or placebo (1:1). The results demonstrated that the addition of rituximab did not affect the incidence of graft-versus-host-disease or OS.

Other authors have also explored the addition of a new monoclonal antibody to a chemotherapy regimen. For example, a multicentre Phase II trial investigated the safety and efficacy of the anti-CD^{20} monoclonal antibody, ofatumumab, combined with ICE or DHAP as a second-line therapy in 61 patients with B cell lymphomas, including RR-DLBCL.[23] The ORR was 61% for a CR of 37% (stem cell mobilisation was successful in 43 out of 45 patients). In the subsequent randomised comparison with rituximab, there was no difference between the two arms in terms of relative risk or progression-free survival (PFS).[24]

Can we Predict the Outcomes for Patients with Refractory Diffuse Large B Cell Lymphoma?

The poor outcomes obtained in patients with RR-DLBCL indicate that there remains a substantial

unmet medical need. A recent multi-cohort study, SCHOLAR-1 (Retrospective NHL Research) in patients with refractory DLBCL reported homogeneous outcomes with response rates of between 20% and 30% and a median OS of approximately 6 months.[25] Some prognostic factors have been identified for salvage therapy in RR-DLBCL. In the CORAL study, after BEAM and autologous SCT, 3-year EFS and OS were not significantly different but the outcomes were dependent on prior rituximab use, relapse within the first 12 months, and secondary age-adjusted International Prognostic Index (IPI) (Figure 1).[16] Poorer outcomes in patients with early relapse after autologous SCT (<12 months) are consistent with those of the PARMA trial and other earlier studies.[12,26,27]

As previously stated, rituximab-naïve patients from the CORAL study had higher response rates and 3-year EFS than patients previously exposed to rituximab (response rates: 83% versus 51%, p<0.001; and 3-year EFS, 47% versus 21%, p<0.001, respectively).[16] These results are in accordance with data from a retrospective study on R-ESHAP in patients with or without previous exposure to rituximab.[28] As many patients develop disease that is refractory to rituximab, the available evidence suggests that its role in salvage therapy should be reconsidered. This challenge should also be overcome by the development of new chemotherapy combinations and novel agents.[29] Finally, relapsed patients appear to have a higher OS than refractory patients.[30] A low-risk age-adjusted IPI at relapse was also associated with higher PFS and OS rates.[31]

Management of Refractory Diffuse Large B Cell Lymphoma in Patients Not Eligible for High-Dose Therapy and Autologous Stem Cell Transplantations

A substantial proportion of patients are not eligible for high-dose chemotherapy followed by autologous SCT. This may result from advanced age or comorbidities, as they are refractory to second-line treatment, or because they express a wish not to undergo the treatment. Patients who are ineligible for high-dose chemotherapy followed by autologous SCT as described in the bone marrow transplant guidelines have distinctly lower survival rates.[10,32,33] Treatment options comprise enrolment in Phase I or II clinical trials, palliative care with radiotherapy, rituximab therapy, and optimal supportive care.[34]

Management of Multiply Relapsed Diffuse Large B Cell Lymphoma

The standard of care for patients experiencing a second relapse is not clearly established and prognosis is extremely poor.[35] Third-line chemotherapy may be attempted in chemosensitive patients, with the objective of achieving sufficient response to initiate allogeneic SCT. Allogeneic SCT appears to be the main option in MR-DLBCL, in the event that a histocompatible donor is available for a patient. This option offers two main advantages, namely the infusion of tumour-free stem cells and the graft-versus-lymphoma effect.[36-38]

In a retrospective study of the GITMO (Gruppo Italiano Trapianto di Midollo Osseo) database, 165 patients who underwent autologous SCT relapsed and were subsequently treated with allogeneic SCT.[39] The results showed an ORR of 49%, with 43% of patients achieving CR and a further 5% obtaining PR. On the other hand, myeloablative conditioning with high-dose chemotherapy can generate higher transplant-related morbidity and non-relapse mortality. Thus, non-myeloablative or reduced-intensity conditioning approaches have been and continue to be evaluated.[40-44]

Chemotherapy can also be effective in MR-DLBCL. The CORAL study investigators conducted a retrospective analysis of patients failing second-line therapy (R-ICE or R-DHAP) and who could not proceed to autologous SCT (n=203).[11] Third-line therapy included ICE (19%), DHAP (18%), gemcitabine-containing (14%), and miscellaneous regimens (32%), with or without rituximab. ORRs were lower than those of second-line therapies in the intent-to-treat analysis (39% versus 63%), but still acted as a bridging therapy in 32% of patients who underwent high-dose chemotherapy followed by autologous SCT (n=56), or high-dose chemotherapy followed by allogeneic SCT (n=8). In this third-line setting all patients were ineligible for (allogeneic) SCT, and a non-negligible proportion of this cohort benefited from autologous SCT.

In the subgroup of patients who were able to undergo a transplant, the median OS was 11.1 months, with a 1-year OS of 42%, compared with a median OS of 3.3 months (1-year OS, 16%) in patients who did not undergo transplantation (p<0.0001). OS was influenced by secondary age-adjusted IPI at the point of failure.

Table 1: Overall survival according to prognostic factors.[18]

Parameter	n	Median OS (months)	Range (months)	1-year OS (%)	95% CI (lower–upper)	p-value
Total population	193	4.4	3.4–5.9	23.0	16.8–29.8	-
Tertiary IPI						
0–2	63	10.3	5.9–12.8	41.3	29.1–53.0	<0.0001
>2	52	3.2	2.6–4.2	6.4	1.7–15.7	-
Third-line immunotherapy						
Yes	56	5.6	3.4–10.6	33.2	20.3–46.7	0.42
No	116	5.4	3.4–6.9	21.3	13.7–30.0	-
Response to third-line regimen						
CR/CRu	55	63.6	15.5–NA	70.0	50.5–83.1	<0.0001
PR	24	11.7	2.6–15.2	44.4	22.2–64.6	-
SD/PD	87	3.7	3.2–5.0	8.3	3.6–15.3	-
Transplantation						
Yes	64	11.1	8.3–19.5	41.6	26.7–55.8	<0.0001
No	129	3.3	2.7–4.2	16.3	10.3–23.5	-
Transplantation type						
Autologous SCT	56	11.5	8.5–NA	43.1	26.9–58.3	0.37
Allogeneic SCT	8	7.9	1.3-NA	33.3	4.6–67.6	-

SCT: stem cell transplantation; CI: confidence interval; CRu: complete response undetermined; IPI: International Prognosis Index; NA: not available; OS: overall survival; SD/PD: stable disease/progressive disease; PR: partial remission.

However, the type of third-line regimen did not affect the outcomes, nor did the type of SCT (autologous or allogeneic). In a multivariate analysis, the IPI at relapse and SCT independently predicted OS (hazard ratio [HR]: 2.41 and 0.38, respectively) (Table 1).

Overall, it appears that prolonged remission can still be achieved in an acceptable proportion of MR-DLBCL patients, with effective salvage regimens acting as bridging therapies to enable SCT. However, an improvement in salvage rates is a crucial requirement that needs to be addressed; the development of novel therapeutic agents will improve outcomes that allow more patients to be treated with SCT.

Pixantrone in Multiply Relapsed B Cell Non-Hodgkin syndrome

Pixantrone dimaleate is a novel aza-anthracenedione with unique structural and physiochemical properties,[45,46] whose effects on DNA damage induction and cell death appear to be different from those of doxorubicin and anthracyclines. Pixantrone impairs mitotic fidelity, resulting in aberrant mitosis. The mechanism of action and efficacy of pixantrone seem to be independent of p53 status and influenced by checkpoint kinase 1 inhibition.[47-49]

The development of pixantrone was initiated to address severe cardiotoxicity issues related to the anthracyclines. As pixantrone lacks an iron-binding site, it has less potential to produce reactive oxygen species and does not form toxic drug-metal complexes. Pixantrone has also been demonstrated to be selective for Type II topoisomerase in stabilising enzyme-DNA complexes, which has also been hypothesised to explain the attenuated cardiotoxicity.[50] These advantages, along with less alcohol metabolite formation in cardiac tissue, account for the limited cardiac toxicity potential. Thus with pixantrone, there are no dose restrictions or warnings related to prior anthracycline use. However, as stated in the summary of product characteristics, patients with prior cumulative doses of doxorubicin or equivalent exceeding 450 mg/m^2 should receive careful risk versus benefit consideration before receiving treatment with pixantrone.[45]

Table 2: Response rates until the end of study in patients with aggressive B cell non-Hodgkin lymphoma receiving their third or fourth-line therapy.[53]

	Pixantrone	Comparator	p-value
Patients with aggressive B cell non-Hodgkin lymphoma with histology determined by central review (n=78)			
Number of patients	39	39	-
CR (%)	7 (17.9)	0 (0.0)	0.012
CR or CRu (%)	9 (23.1)	2 (5.1)	0.047
ORR (%)	17 (43.6)	5 (12.8)	0.005
Patients with aggressive B cell non-Hodgkin lymphoma with histology determined by central review who had previously received rituximab (n=38)			
Number of patients	20	18	-
CR or CRu (%)	6 (30.0)	1 (5.6)	0.093
ORR (%)	9 (45.0)	2 (11.1)	0.033

CR: complete response; CRu: unconfirmed complete response; ORR: overall response rate.
p-value versus comparator (Fisher's exact test).

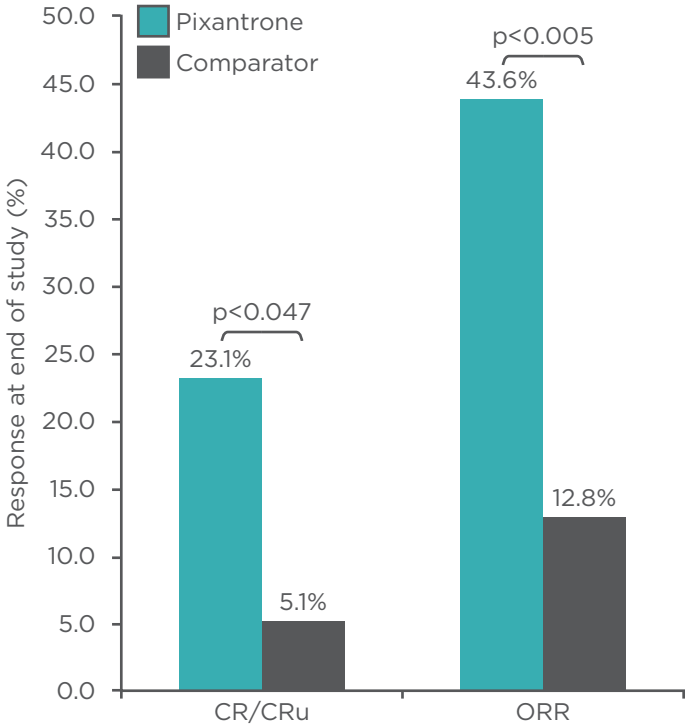

Figure 2: Post-hoc analysis: Response at end of study in patients with aggressive B cell non-Hodgkin lymphoma (determined by central review) receiving their third or fourth-line of therapy.[53]
CR: complete response; CRu: unconfirmed complete response; ORR: overall response rate.
p-value versus comparator (Fisher's exact test).

Pixantrone (Pixuvri®, CTI BioPharma Corp.) has a conditional European marketing approval for monotherapy in adults with RR or MR aggressive B cell NHL. This authorisation was based on the results of the Phase III PIX301 study. This open-label, randomised, controlled, multicentre, single-agent, Phase III trial evaluated the efficacy of pixantrone in the treatment of patients with relapsed, aggressive NHL after more than two combination chemotherapy regimens.[51,52] Pixantrone was therefore assessed in the setting of third-line therapy and beyond. A total of 140 patients were randomised (1:1) into two treatment arms: pixantrone (on Days 1, 8, and 15 of 28-day cycles) or a comparator (at the physician's discretion) for up to six cycles of treatment. The primary endpoint was independently assessed CR and CRu. Approximately half of the patients had previously received rituximab therapy.

In the intention-to-treat population, the primary endpoint CR/CRu rate at the end of study was 24.3% (median duration 9.6 months) and 7.1% (median duration 4.0 months) for the pixantrone and the comparator arm, respectively (p=0.009).[52] The ORR at the end of the study reached 37.1% versus 14.3% (p=0.001). Most importantly, pixantrone achieved CR/CRu in patients that had PR, stable disease, or progressive disease from prior intensive salvage therapies. Overall, 82% (14 out of 17) of the pixantrone CR/CRu had a suboptimal response to these prior therapies and yet went on to achieve a CR with pixantrone. Median PFS survival was longer in the pixantrone arm (5.3 months; 95% CI: 2.3–6.2) than in the comparator arm (2.6 months; 95% CI: 1.9–3.5;

p=0.005; HR: 0.6 [95% CI: 0.42–0.86]). Similar results were observed in the subpopulation of patients with DLBCL (4.6 months; 95% CI: 2.3–6.5 versus 2.1 months; 95% CI: 1.8–3.2; p<0.001; HR: 0.47 [95% CI: 0.30–0.71]).

In a *post hoc* analysis of the trial, the population with a histologically confirmed diagnosis after central review was subdivided according to previous rituximab use and whether they received the study treatment as a third or fourth-line.[53] In this population, when it was used in the third or fourth-line, pixantrone monotherapy was more effective than comparator in terms of response (CR: 23.1% versus 5.1%, p=0.047; ORR: 43.6% versus 12.8%, p=0.005; Table 2, Figure 2). These results were found to be consistent in patients who had previously received rituximab. Moreover, the observation of a 45.0% ORR with pixantrone in those patients versus 43.6% in the whole population (Table 2) suggests that treatment with pixantrone may have more potential as a bridge to transplant.

The most common Grade 3 or 4 adverse events for pixantrone in the entire study were cytopenias (uncomplicated, non-cumulative neutropenias, leukopenias, or thrombocytopenias), with an incidence of febrile neutropenias in 7.4% of cases for pixantrone versus 3.0% for comparator agents.[52] No high-grade treatment-emergent alopecia, mucosal inflammation, or opportunistic infections were reported for pixantrone and the incidence of severe infections was low.[45]

Following these promising results, a larger scale randomised, active-controlled, multicentre Phase III trial (PIX306 study) was initiated and is currently recruiting participants.[54,55] This trial aims to enrol 260 patients with RR B cell NHL or follicular Grade 3 lymphoma who previously received at least one rituximab-containing multi-agent therapy regimen and are not eligible for SCT. In the study protocol, patients are randomised (1:1) to pixantrone and rituximab combination or gemcitabine plus rituximab for up to six cycles of treatment. The primary endpoint is PFS; secondary endpoints comprise OS, CR rate, ORR, and safety outcomes.

The National Institute for Health and Care Excellence (NICE) published a final guidance document on February 26th 2014, on pixantrone monotherapy in RR or MR B cell NHL.[56] Key clinical evidence from PIX301 allowed NICE to appraise pixantrone under the single technology appraisal process, which concluded that the available results demonstrated that pixantrone can be a therapeutic option in RR or MR B cell NHL in the third or fourth-line settings. Cost-effectiveness assessments revealed that pixantrone was a cost-effective therapeutic option with an incremental cost-effectiveness ratio of £22,000 per quality-adjusted life year gained. Thus, pixantrone is currently licensed under the indications cited above; further clinical studies will evaluate its benefits in other therapeutic situations alone or in combination therapies.

IS PIXANTRONE A CANDIDATE FOR BRIDGING TO AUTOLOGOUS STEM CELL TRANSPLANTATION?

The trial evidence with pixantrone indicates that this agent can induce CR in patients with relapsed aggressive NHL.[52,53] Clinical responses have even been observed in patients with disease that was refractory to standard salvage chemotherapy. The response appears to be sufficiently strong to hypothesise that mobilisation of stem cells may be an option. Although this has not been demonstrated in the clinical trial setting, this finding implies that bridging to autologous SCT may be feasible with pixantrone and should be explored further.

FUTURE PERSPECTIVES AND CONCLUSION

Patients failing after second-line therapy for DLBCL suffer from an overall poor prognosis. Nevertheless, recent findings have demonstrated that substantial responses could be achieved after second or third-line treatments with combined chemotherapy. The novel agent, aza-anthracenedione pixantrone as a third-line approach or beyond, has demonstrated efficacy in the same patient setting. Thus, these therapies might be successfully used as a bridge to consolidation of autologous or allogeneic SCT, which in turn can deliver prolonged remission durations. Numerous clinical studies are being conducted to improve salvage rates and outcomes of RR-DLBCL. The development of novel chemotherapeutic agents or targeted therapies will certainly help to salvage more patients and achieve further sustained CRs without compromising the quality of life of the patient.

REFERENCES

1. International Agency for Research on Cancer, GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, IARC, Lyon, France, 2013. Available at: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Last accessed: 1 July 2016.

2. Jaffe ES et al. Classification of lymphoid neoplasms: The microscope as a tool for disease discovery. Blood. 2008; 112(12):4384-99.

3. Smith A et al. Incidence of haematological malignancy by sub-type: A report from the Haematological Malignancy Research Network. Br J Cancer. 2011;105(11):1684-92.

4. Matsuki E, Younes A. Checkpoint Inhibitors and other immune therapies for Hodgkin and non-Hodgkin lymphoma. Curr Treat Options Oncol. 2016;17(6):31.

5. Coleman M et al. Role of rituximab and rituximab biosimilars in diffuse large B-cell lymphoma. Clin Lymphoma Myeloma Leuk. 2016;16(4):175-81.

6. Coiffier B et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: A study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. Sep 23 2010;116(12):2040-5.

7. Pfreundschuh M et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol. 2006;7(5):379-91.

8. Habermann TM et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006; 24(19):3121-7.

9. Cheson BD et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25(5):579-86.

10. Feugier P et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2005;23(18):4117-26.

11. Van Den Neste E et al. Diffuse Large B-Cell Lymphoma (DLBCL) Patients Failing Second-Line R-DHAP Or R-ICE Chemotherapy included In the Coral Study. Abstract 764. 55th ASH Annual Meeting and Exposition. 7–10 December 2013.

12. Philip T et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;333(23): 1540-5.

13. Doocey RT et al. Allogeneic haematopoietic stem-cell transplantation for relapsed and refractory aggressive histology non-Hodgkin lymphoma. Br J Haematol. 2005;131(2):223-30.

14. Lazarus HM et al. A comparison of HLA-identical sibling allogeneic versus autologous transplantation for diffuse large B cell lymphoma: A report from the CIBMTR. Biol Blood Marrow Transplant. 2010;16(1):35-45.

15. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood. 2015;125:22-32.

16. Gisselbrecht C et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 28(27): 4184-90.

17. Gisselbrecht C et al. Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20(+) diffuse large B-cell lymphoma: Final analysis of the collaborative trial in relapsed aggressive lymphoma. J Clin Oncol. 2012; 30(36):4462-9.

18. Van Den Neste E et al. Outcome of patients with relapsed diffuse large B-cell lymphoma who fail second-line salvage regimens in the International CORAL study. Bone Marrow Transplant. 2016; 51(1):51-7.

19. Crump M et al. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. J Clin Oncol. 2014;32(31): 3490-6.

20. Kuruvilla J et al. Salvage chemotherapy and autologous stem cell transplantation for transformed indolent lymphoma: a subset analysis of NCIC CTG LY12. Blood. 2015;126(6):733-8.

21. Barnes JA et al. Everolimus in combination with rituximab induces complete responses in heavily pretreated diffuse large B-cell lymphoma. Haematologica. 2013;98(4):615-9.

22. Glass B et al. Rituximab after lymphoma-directed conditioning and allogeneic stem-cell transplantation for relapsed and refractory aggressive non-Hodgkin lymphoma (DSHNHL R3): An open-label, randomised, Phase 2 trial. Lancet Oncol. 2014;15(7):757-66.

23. Matasar MJ et al. Ofatumumab in combination with ICE or DHAP chemotherapy in relapsed or refractory intermediate grade B-cell lymphoma. Blood. 2013;122(4):499-506.

24. van Imhoff GW et al. Ofatumumab versus rituximab salvage chemoimmunotherapy in relapsed or refractory diffuse large B-cell lymphoma: The Orcharrd Study (OMB110928). Abstract 630. 56th ASH Annual Meeting and Exposition. 6–9 December 2014.

25. Crump M et al. Outcomes in refractory aggressive diffuse large b-cell lymphoma (DLBCL): Results from the international SCHOLAR-1 study. Abstract 7516. ASCO Annual Meeting. 3–7 June 2016.

26. Vellenga E et al. Rituximab improves the treatment results of DHAP-VIM-DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: A prospective randomized HOVON trial. Blood. 2008; 111(2):537-43.

27. Guglielmi C et al. Time to relapse has prognostic value in patients with aggressive lymphoma enrolled onto the Parma trial. J Clin Oncol. 1998;16(10): 3264-9.

28. Martin A et al. R-ESHAP as salvage therapy for patients with relapsed or refractory diffuse large B-cell lymphoma: the influence of prior exposure to rituximab on outcome. A GEL/TAMO study. Haematologica. 2008;93(12): 1829-36.

29. Gisselbrecht C. Ofatumumab in diffuse large B cell lymphoma? Blood. 2013;122(4):469-70.

30. Kewalramani T et al. Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. Blood. 2004;103(10):3684-8.

31. Hamlin PA et al. Age-adjusted International Prognostic Index predicts autologous stem cell transplantation outcome for patients with relapsed or primary refractory diffuse large B-cell lymphoma. Blood. 2003;102(6):1989-96.

32. Thieblemont C, Coiffier B. Lymphoma in older patients. J Clin Oncol. 2007; 25(14):1916-23.

33. Jabbour E et al. Outcome of elderly patients with aggressive Non-Hodgkin's lymphoma refractory to or relapsing after first-line CHOP or CHOP-like chemotherapy: A low probability of cure. Leuk Lymphoma. 2004;45(7):1391-4.

34. Murthy V et al. Efficacy of palliative low-dose involved-field radiation therapy in advanced lymphoma: A phase II study. Clin Lymphoma Myeloma. 2008;8(4): 241-5.

35. Vose JM et al. Progressive disease

after high-dose therapy and autologous transplantation for lymphoid malignancy: clinical course and patient follow-up. Blood. 1992;80(8):2142-8.

36. Rezvani AR et al. Non-myeloablative allogeneic haematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: A multicentre experience. Br J Haematol. 2008;143(3): 395-403.

37. van Besien KW et al. Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect. Bone marrow transplantation. Bone Marrow Transplant. 1997;19(10):977-82.

38. Bishop MR et al. Clinical evidence of a graft-versus-lymphoma effect against relapsed diffuse large B-cell lymphoma after allogeneic hematopoietic stem-cell transplantation. Ann Oncol. 2008; 19(11):1935-40.

39. Rigacci L et al. Allogeneic hematopoietic stem cell transplantation in patients with diffuse large B cell lymphoma relapsed after autologous stem cell transplantation: A GITMO study. Ann Hematol. 2012 Jun;91(6):931-9.

40. Sirvent A et al. Low nonrelapse mortality and prolonged long-term survival after reduced-intensity allogeneic stem cell transplantation for relapsed or refractory diffuse large B cell lymphoma: Report of the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. Biol Blood Marrow Transplant. 2010;16(1): 78-85.

41. Thomson KJ et al. Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2009;27(3):426-32.

42. van Kampen RJ et al. Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: An analysis of the European Group for Blood and Marrow Transplantation Registry. J Clin Oncol. 2011;29(10):1342-8.

43. Bacher U et al. Conditioning regimens for allotransplants for diffuse large B-cell lymphoma: Myeloablative or reduced intensity? Blood. 2012;120(20):4256-62.

44. Hamadani M et al. Impact of pretransplantation conditioning regimens on outcomes of allogeneic transplantation for chemotherapy-unresponsive diffuse large B cell lymphoma and grade III follicular lymphoma. Biol Blood Marrow Transplant. 2013;19(5):746-53.

45. European Medicines Agency. Pixuvri - Summary of Product Characteristics. 2013. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002055/WC500127968.pdf. Last accessed: 1 July 2016.

46. Adnan N et al. DNA binding by pixantrone. Org Biomol Chem. 2010; 8(23):5359-66.

47. Beeharry N et al. Pixantrone induces cell death through mitotic perturbations and subsequent aberrant cell divisions. Cancer Biol Ther. 2015;16(9):1397-406.

48. Pettengell R, Kaur J. Pixantrone dimaleate for treating non-Hodgkin's lymphoma. Expert Opinion on Orphan Drugs. 2015;3(6):747-57.

49. Results presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 2013, Boston.

50. Hasinoff BB et al. Cellular mechanisms of the cytotoxicity of the anticancer drug elesclomol and its complex with Cu(II). Biochem Pharmacol. 2015;93(3):266-76.

51. Engert A et al. EXTEND PIX301: A phase III randomized trial of pixantrone versus other chemotherapeutic agents as third-line monotherapy in patients with relapsed, aggressive non-Hodgkin's lymphoma. Clin Lymphoma Myeloma. 2006;7(2):152-4.

52. Pettengell R et al. Pixantrone dimaleate versus other chemotherapeutic agents as a single-agent salvage treatment in patients with relapsed or refractory aggressive non-Hodgkin lymphoma: A phase 3, multicentre, open-label, randomised trial. Lancet Oncol. 2012;13(7):696-706.

53. Pettengell R et al. Monotherapy with pixantrone in histologically confirmed relapsed or refractory aggressive B-cell non-Hodgkin lymphoma: Post-hoc analyses from a phase III trial. Br J Haematol. 2016. [Epub ahead of print].

54. U.S. National Institutes of Health. Comparison of Pixantrone + Rituximab with Gemcitabine + Rituximab in Patients with Aggressive B-cell Non-Hodgkin Lymphoma or Follicular Grade 3 Lymphoma Who Have Relapsed After Therapy and Are Not Eligible for Stem Cell Transplant (PIX-R). NCT01321541. Available at: http://clinicaltrials.gov/ct2/show/NCT01321541.

55. Belada D et al. Pixantrone-rituximab versus gemcitabine-rituximab in relapsed/refractory aggressive non-Hodgkin lymphoma. Future Oncol. 2016 [Epub ahead of print].

56. National Institute for Health and Care Excellence Technology appraisal guidance TA306. Pixantrone monotherapy for treating multiply relapsed or refractory aggressive non-Hodgkin's B-cell lymphoma. Available at: https://www.nice.org.uk/Guidance/ta306. Last accessed: 1 July 2016.

THROMBOCYTOPENIA CAUSED BY INHERITED HAEMATOPOIETIC TRANSCRIPTION FACTOR MUTATION: CLINICAL PHENOTYPES AND DIAGNOSTIC CONSIDERATIONS

David J. Rabbolini, Christopher M. Ward, *William S. Stevenson

Department of Haematology and Transfusion Medicine, Royal North Shore Hospital; Northern Blood Research Centre, Kolling Institute of Medical Research, University of Sydney, Sydney, Australia
**Correspondence to william.stevenson@sydney.edu.au*

Disclosure: The authors have declared no conflicts of interest.
Received: 10.03.16 **Accepted:** 24.06.16
Citation: EMJ Hematol. 2016;4[1]:100-109.

ABSTRACT

Inherited thrombocytopenias comprise a heterogeneous group of blood disorders with abnormalities in genes related to glycoproteins and adhesion molecules, signalling pathways, cytoskeletal components, granule formation, and transcription factor complexes. Recent improvements in sequencing technology have increased the number of transcription factor mutations that have been implicated as causative for these platelet disorders. Mutations in *RUNX1, GATA1, GFI1B, FLI1*, and *ETV6* share common features, including a variable bleeding history often associated with abnormal but non-specific changes in platelet morphology and platelet function testing. The phenotype of the underlying platelet disorder is often variable despite mutations in the same transcription factor, suggesting that the site of mutation and the protein domain that is perturbed is an important determinant of the clinical syndrome. Importantly, some of these transcription factor mutations are associated with other physical abnormalities, including an increased risk of acute leukaemia as well as solid organ malignancies. Genetic diagnosis of these disorders allows rational medical management to prevent bleeding, as well as providing an opportunity for family screening in order to reduce disease burden.

Keywords: Transcription factors, inherited thrombocytopenia, megakaryopoiesis, next-generation sequencing.

INTRODUCTION

Platelets mediate primary haemostasis after tissue trauma. Abnormalities in the number or function of platelets may predispose to spontaneous mucosal bleeding, or may precipitate problematic bleeding at the time of surgery. The correct diagnosis of platelet disorders provides an opportunity to prevent bleeding episodes and allows optimisation of haemostatic function for surgery. Isolated thrombocytopenia is a relatively common abnormality detected on full blood count analysis with most cases resulting from an increase in consumption of platelets in the circulation or a failure of adequate production from the bone marrow. Common causes include autoimmune or drug-induced consumption, sepsis, immunodeficiency syndromes, or bone marrow suppression from malignancy. Inherited causes of thrombocytopenia are caused by a range of genetic defects and are important to correctly identify; isolated thrombocytopenia has been misdiagnosed as immune thrombocytopenia in a number of clinical cohort studies with subsequent inappropriate treatment using corticosteroid therapy and splenectomy.[1,2] Traditional laboratory investigation of inherited causes of thrombocytopenia includes assessment of blood film morphology, functional aggregation studies, and flow cytometry.[3] In recent years, an increasing use of genetic technologies with DNA sequencing has allowed better molecular characterisation of platelet disorders and provided

a platform for the discovery of new genetic defects that are causative of platelet-related bleeding.

THE ROLE OF TRANSCRIPTION FACTORS IN MEGAKARYOPOIESIS AND PLATELET PRODUCTION

Megakaryopoiesis and platelet production occur through a series of co-ordinated differentiation and maturation events that are orchestrated by cytokines and tightly regulated by networks of haematopoietic transcription factors.[4,5] Different transcription factors have been identified to act at various stages of haematopoiesis,[4] and in the megakaryocyte lineage a complex of key transcription factors (*GATA2, LYL1, TAL1, ERG, FLI1, RUNX1, LMO2*) expressed by haematopoietic stem cells is considered to initiate the development programme.[5] These 'prime' sets of lineage-specific genes[4,5] such as *GP1BA* and *GP1BB*, are uniquely expressed in platelets and provide haemostatic potential. The principal components of this complex are the GATA-binding transcription factors (GATA1 and GATA2), E twenty-six (ETS) factors (FLI1 and GABP-α), and the transcription activator-like helix-loop-helix factors that regulate target genes and interact with other factors.[4]

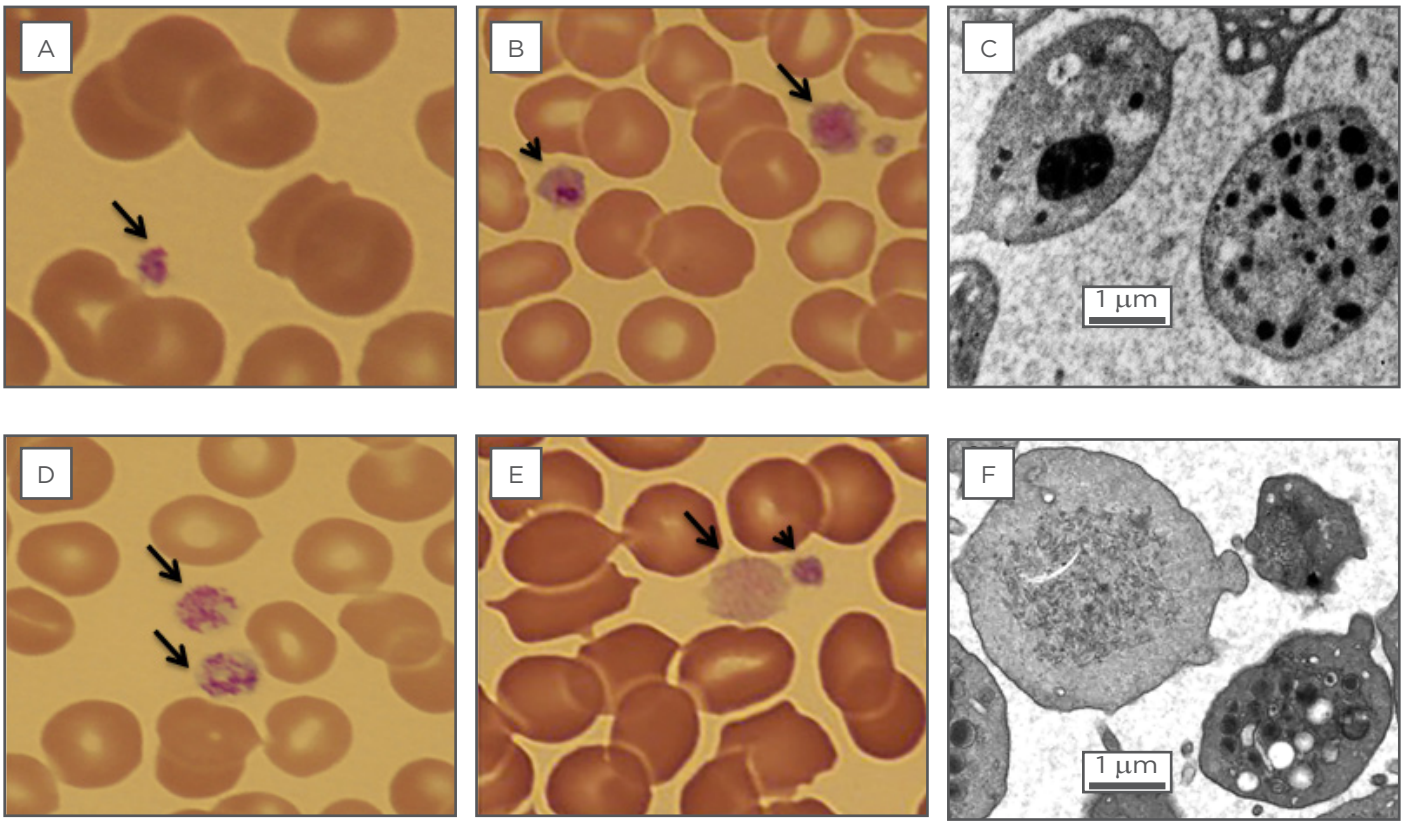

Figure 1: Platelet abnormalities associated with haematopoietic transcription factor mutation.
A) Photomicrograph of a blood film (100x) from an individual with a *RUNX1* C-terminal transactivation domain mutation. A normal sized platelet with normal granulation is seen (arrow).
B) Photomicrograph of a blood film (100x) from an individual with the *FLI1 R324W* mutation. Two large abnormal platelets are seen (arrow and arrow head). One of these platelets (arrow head) appears to contain a giant granule.
C) Transmission electron micrograph from an individual with the *FLI1 R324W* mutation showing a platelet with a giant fused α-granule and a platelet with normal α-granule distribution.
D) Photomicrograph of a blood film (100x) from an individual with the *GFI1B C168F* mutation. Two large platelets with normal granulation are seen (arrows).
E) Photomicrograph of a blood film (100x) from an individual with the *GFI1B H294fs* mutation. A Large abnormal hypogranular platelet is present (arrow) as well as a normogranular platelet (arrow head).
F) Transmission electron micrograph from an individual with the *GFI1B H294fs* mutation showing both an agranular platelet and a platelet with normal α-granule content.

Runt-Related Transcription Factor 1

The core binding factor (CBF) is a haematopoietic transcription complex that is composed of α (CBF-α) and β (CBF-β) subunits. RUNX1 is one of three possible α subunits (RUNX1, RUNX2, or RUNX3) that binds to DNA via a conserved 128-amino-acid runt homology domain (RHD). CBF-β does not bind DNA directly but binds the RHD and stabilises the complex.[6] RUNX1 acts on numerous target genes and proteins that are important in megakaryocytic maturation (for example *MYH10*), and platelet formation (*MPL*, *MYL9*, *MYH9*, *TUBB1/2*) and function (protein kinase C-theta and integrin αIIbβ3).[7-10]

Table 1: Genetic and clinical features of transcription factor mutations.

Gene references	Platelet disorder OMIM entry	Inheritance	Target megakaryocytic and platelet genes	Characterised mutations	Platelet phenotype	Associated features
RUNX1 [11, 12, 13, 15, 16, 18, 19, 20]	Familial platelet disorder and predisposition to acute myeloid leukaemia (601399).	AD	*MYH10, MPL, MYL9, TUBB1/2*	R201Q, K83E, R135fs, Y260X, A107P, T148fs, A129E	**Symptoms:** Mild-to-moderate bleeding usually present from childhood (variable). **Blood film**: Mild-to-moderate thrombocytopenia. (normal sized platelets) **LTA**: Reduced response to several platelet agonists (ADP, epinephrine, AA, collagen, TRAP). **Flow cytometry:** Deficiency of platelet dense granules with reduced uptake and release of mepacrine.	Increased risk of MDS/AML.
GATA1 (XLT) [27, 28, 29, 30, 31]	X-linked thrombocytopenia with or without dyserythropoietic anaemia (300367)	X-linked recessive	*GP1BA, GP1BB, PF4, MPL, NFE2*	V205M, G208R, G208S, D218G, D218Y	**Symptoms:** Moderate-to-severe bleeding from infancy. **Blood film:** Moderate-to-severe macrothrombocytopenia. (pale platelets: reduced platelet α-granules)	Red cell anisopoikilocytosis and anaemia. Bilateral cryptochordism (V205M and G208R).
GATA1 (XLTT) [33]	X-linked thrombocytopenia with thalassaemia (314050).	X-linked recessive		R216Q	**Symptoms:** Mild-to-moderate bleeding tendency (from childhood) **Blood film:** Mild-to-moderate macrothrombocytopenia. (paleplatelets: reduced platelet α-granules) **LTA:** Absent platelet shape change in response to collagen. Normal platelet aggregation in response to collagen and ADP. Normal agglutination with ristocetin. **Other:** Abnormal clot retraction.	Unbalanced α:β haemoglobin chain production resembling mild β-thalassaemia. Mild haemolytic anaemia. Splenomegaly
GFI1B [41, 42,43]	GFI1B-related thrombocytopenia /Bleeding disorder, platelet-type, 17 (187900).	AD	*GFI1B, TGFBR3*	G272fs H294fs Q287*	**Symptoms:** Moderate bleeding severity (variable). **Blood film:** Mild-to-moderate macrothrombocytopenia. (heterogeneous reduction of platelet α-granules) **LTA:** Variably reduced response to numerous platelet agonists (ADP, AA, epinephrine, TRAP), most marked to collagen. **Other:** CD34 expression by megakaryocytes and platelets.	Red cell anisocytosis. Bone marrow: dysplastic megakaryocytes, emperipolesis, mild myelofibrosis.

Gene references	Platelet disorder OMIM entry	Inheritance	Target megakaryocytic and platelet genes	Characterised mutations	Platelet phenotype	Associated features
FLI1 [46, 47, 48]	Paris-Trousseau thrombocytopenia (188025/600588).	AD	*MYH10, GP6, GP9, ITGA2B*	N331fs R337W Y343C	**Blood film:** Variable mild thrombocytopenia. **LTA:** Platelet dense granule secretion defect.	Alopecia
		AR		R324W	**Symptoms:** Moderate bleeding. **Blood film:** Moderate thrombocytopenia. **LTA:** Absent collagen induced platelet aggregation. **Electron microscopy:** Giant α-granules in 1-5% of platelets.	
		De novo[a]			**Blood film:** Moderate-to-severe neonatal macrothrombocytopenia. Counts may improve over months to years. **Other:** Platelet dysfunction with prolonged bleeding times that persist after normalisation of platelet counts. **Electron microscopy:** Platelets contain giant fused α-granules and/or are deficient in dense granules.	Intellectual disability, altered facial structure, heart defects.
ETV6 [54, 55, 56]	Thrombocytopenia 5 (616216).	AD	*PF4, MMP3*	P214L, L349P, R369Q, N385fs, R399C, R418G	**Blood film:** Moderate thrombocytopenia. **LTA:** Reduced responses to platelet agonists ADP and AA. **Flow cytometry:** Normal platelet glycoprotein expression.	Increased MCV. Predisposition to solid tumours and haematological malignancy, particularly ALL.
EVI1 [57, 58, 59, 60]	Radioulnar synostosis with amegakaryocytic thrombocytopenia 2 (616738).	Uncertain	*AP-1, TGF-β*	T756A, H751R, R750W	**Blood film:** Moderate-to-severe thrombocytopenia.	Anaemia. Fusion of the radius and ulnar bones. Progressive bone marrow failure.

AA: arachidonic acid; AD: autosomal dominant; ALL: acute lymphoblastic leukaemia; AR: autosomal recessive; LTA: light transmission aggregometry; MCV: mean cell volume; MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia; OMIM: Online Mendelian Inheritance in Man; TRAP: thrombin activating peptide; ADP: adenosine diphosphate.

[a] Familial cases are rare with most individuals having *de novo* isolated 7-16 Mb deletions. Occasional individuals with AD inheritance have been reported.[49]

¶ Mutations of *RUNX1* selected from OMIM. Over 27 mutations in 36 pedigrees have been identified.[65]

Germ-line mutation of *RUNX1* on chromosome 21q22.12 is associated with an autosomal dominant disorder called familial platelet disorder with propensity to acute myeloid leukaemia (FPD/AML).[11-13] FPD/AML is characterised by dysmegakaryopoiesis, thrombocytopenia with normal sized platelets (Figure 1), a platelet function defect, and a propensity to develop myelodysplasia or acute leukaemia.[8,9,11-16] Mutations causing FPD/AML commonly affect the RHD and less commonly the C-terminal transactivation domain.[17] Affected individuals have a variable mucosal bleeding tendency from childhood ranging from asymptomatic to moderate.[17-19] Most individuals with FPD/AML have mild-to-moderate thrombocytopenia, however normal platelet

counts have been documented in a number of family pedigrees and do not exclude the presence of a significant *RUNX1* mutation.[18,19] Similarly, the platelet functional defect detected by light transmission aggregometry may be variable and has included reduced responses to collagen, adenosine diphosphate (ADP), arachidonic acid (AA), and epinephrine (Table 1). These changes are attributed to a storage pool deficiency and in some cases resemble an 'aspirin' like defect.[8,11-13,15,16,20] A recent review of 41 carriers with *RUNX1* alterations in nine unrelated French families described platelet dense granule release defects in 10 patients, regardless of the type of *RUNX1* mutation, suggesting a link between this defect and FPD/AML.[18] Further qualitative platelet dysfunction is caused by impaired integrin αIIbβ3 activation.[8,20]

The evolution to leukaemia appears partly determined by the functional impact of the specific *RUNX1* mutation.[14,16] Mutations of either the RHD or C-terminal domain that alter the ability of *RUNX1* to bind DNA but retain an ability to heterodimerise with CBF-β, generate a dominant negative phenotype that interferes with the function of the wild-type allele.[21] These dominant negative acting mutations are associated with a higher risk of leukaemic transformation (over 40% in some cases) compared with mutations that induce haploinsufficiency by reducing levels of wild-type *RUNX1* with resultant deregulation of key target genes, causing genomic instability and the potential to acquire additional somatic mutations, sometimes affecting the second *RUNX1* allele.[14,17] An ability to predict the phenotype based on the mechanism of action of the *RUNX1* variant is therefore desirable and not possible without utilising diagnostic sequencing platforms.

Moreover, although the detection of a dense granule release defect in the presence of an autosomal dominant thrombocytopenia with normal platelet size may raise the suspicion of FPD/AML, the variable bleeding tendency, mild thrombocytopenia, and absence of morphological platelet abnormalities on the peripheral blood film (Figure 1) make the diagnosis of *RUNX1* mutations (solely by phenotypic testing) very difficult without a high index of clinical suspicion based on family history. Optimal management of affected individuals and their families is largely based on expert opinion.[22] Recommendations include: referral to a specialist team consisting of a physician and genetic counsellor, initial blood tests comprising full blood count and full human-leukocyte antigen typing of the patient and their first degree relatives (in the event that a bone marrow transplant is required in the future), bone marrow biopsy (to detect occult malignancy), and subsequent regular full blood count assessment and biannual haematological review.[22]

Globin Transcription Factor 1

The GATA1 transcription factor is encoded by *GATA1* on chromosome Xp11.[23] and is expressed in erythroid cells, megakaryocytes, mast cells, and eosinophils.[23] During megakaryopoiesis, GATA1 assists in lineage commitment of 'primed' genes and, in concert with GATA2 and other transcription factors, including FLI1 and GABPA, regulates the activation of megakaryocyte and platelet specific genes such as *GP1BA*, *GP1BB*, *PF4*, *MPL*, and *NFE2*.[4,24]

GATA1 has two important functional domains, the N terminal domain that mediates transcriptional activation and the C terminal zinc finger domain. The zinc finger domain is required for an interaction with its transcriptional co-factor, the friend of GATA1 (FOG1), and also influences GATA1 binding affinity to DNA at specific genomic sites.[25,26] The location of GATA1 mutations within these protein domains has been correlated to clinical phenotypes. To date, all known mutations resulting in GATA1-related cytopenias affect the amino terminal zinc finger domain of GATA1. Five variants (*V205M*, *G208R*, *G208S*, *D218G*, and *D218Y*) have been reported to affect the FOG1 binding face of the amino terminal zinc finger.[27-31] These decrease the GATA1 to FOG1 interaction but preserve GATA1 to DNA binding ability and cause X-linked thrombocytopenia (XLT).[25] The severity of the clinical phenotype is dependent on the degree of the GATA to FOG1 interaction disruption caused by the mutation.[31] Individuals with *V205M*, *G208R*, and *D218Y* have the most severe phenotype characterised by mucocutaneous bleeding from infancy, macrothrombocytopenia (some platelets displaying reduced granulation on the blood film), red cell anisopoikilocytosis, and severe anaemia requiring transfusions (Table 1). Interestingly, two male half siblings and an unrelated male child with *V205M* and *G208R*, respectively, had cryptorchidism.[27,28] A milder phenotype was described in men from two unrelated families carrying *G208S* and *D218G* mutations.[29,30] In these cases, the phenotype was characterised by profound bleeding, macrothrombocytopenia, and mild dyserythropoiesis without anaemia. X-linked thrombocytopenia with thalassaemia (XLTT) is

caused by mutations affecting the DNA binding face of the amino terminal zinc finger of *GATA1* resulting in decreased *GATA1* binding to DNA.[32] A single variant, R216Q, has been reported in multiple families causing XLTT. This mutation causes milder anaemia and macrothrombocytopenia than those individuals with XLT.[33] In addition, a distinguishing feature is unbalanced α:β globin chain synthesis with the production of abnormal α3:β1 and α4 haemoglobin molecules, creating a phenotype resembling mild β-thalassaemia.[25,33] Notably, platelets in XLTT have reduced α granules, giving the appearance of grey platelets on the blood film (Table 1).[33]

Growth Factor-Independent 1B

Growth factor-independent 1B (GFI1B) is a member of the GFI zinc-finger transcriptional repressor family. Its expression in differentiated cells is restricted to erythroid and megakaryocytic cells where it plays an essential role in development.[34,35] GFI1B contains an N-terminal repressor Snail/Gfi1 (SNAG) domain, which binds protein complexes that mediate transcriptional change, and six C-terminal zinc fingers that demonstrate sequence specific DNA binding.[36] It is predicted that zinc fingers 3, 4, and 5 directly bind to DNA, while the functions of zinc fingers 1, 2, and 6 are not known.[37] GFI1B regulates megakaryocytic genes controlled by other lineage-restricted regulators, such as GATA1, FLI1, SCL1, TEL/ETV6, RUNX1, MKL2, and SRF, and binds to its own promoter, auto-regulating its expression.[38,39]

Ten inherited mutations of GFI1B associated with thrombocytopenia have been documented.[40-42] Seven patients with non-synonymous single-nucleotide polymorphisms were detected by analysis of the *GFI1B* locus in 529 exome-sequenced cases enrolled by the BRIDGE consortium.[40] The best characterised mutations, G272fs, *H294fs*, and *Q287**, disrupt the fifth DNA-binding zinc finger and produce a bleeding disorder with an autosomal dominant pattern of inheritance (Table 1).[41-43] Mild bleeding symptoms were reported in the proband who carried the G272fs mutation.[43] Individuals with the *H294fs* mutation experience variable bleeding; some experience significant bleeding including urological bleeding (requiring nephrectomy) and intracranial bleeding, while other members of the family report only bleeding following surgery.[41] Laboratory features for both families include macrothrombocytopenia and platelets with heterogeneous granulation with some platelets appearing relatively agranular (Figure 1).[41,43,44] Notably, a platelet function defect was demonstrated by light transmission aggregometry in the three individuals tested carrying the *H294fs* mutation. All three individuals had absent responses to collagen and variably reduced responses to platelet agonists ADP, AA, epinephrine, and thrombin receptor-activating peptide. Similarly, individuals with the *Q287** mutation have moderate thrombocytopenia (range: 42–116x10^9/L) with moderate-to-severe bleeding symptomatology on bleeding scores.[42] Platelets were agranular on blood film examination and pleomorphic megakaryocytes were found on bone marrow examination in association with mild myelofibrosis.[42] Functionally, the G272fs, *H294fs*, and *Q287** mutations de-repress transcription of GFI1B-related target genes and inhibit wild-type GFI1B transcriptional activity in a dominant-negative manner.[41-43] These transcriptional changes cause deficiency of key haemostatic proteins with marked reductions in platelet fibrinogen and P-selectin, and an increase in CD34 expression.[42,43] Preliminary data from a mutation predicted to disrupt the first non-DNA binding zinc finger of GFI1B demonstrate phenotypic variability for abnormalities in this transcription factor. The *C168F* mutation has been reported by two groups[40,45] and alters the conformation of the first zinc finger but does not disrupt transcription to the same degree as mutations altering the fifth zinc finger. The affected individuals appear to have a mild clinical phenotype with moderate thrombocytopenia (range: 78–121x10^9/L) but no bleeding history, and platelets with normal granulation.[45] It is likely these individuals would not be diagnosed with traditional diagnostic platelet studies, but correct classification may be important as their mild phenotype may predict for reduced medical intervention at the time of surgery.

Friend Leukaemia Integration 1

Friend leukaemia integration 1 (FLI1) is a member of the ETS family of transcription factors and is predominantly expressed in vascular and haematopoietic tissue.[46] Point mutation and small deletion in the ETS DNA-binding domain of FLI1 are reported as causative of a bleeding disorder with abnormal platelet function (Table 1). Three families with autosomal dominant inheritance of a bleeding disorder associated with FLI1 mutation were identified in the UK GAPP study.[46] These individuals had a mild bleeding disorder with evidence

of defective secretion of dense granules on laboratory testing.[47] A further two siblings were reported in an Australian cohort[48] that had a bleeding disorder with a recessive pattern of inheritance. These two siblings had moderate mucosal bleeding symptomatology, moderate macrothrombocytopenia, and prominent abnormalities in collagen induced platelet aggregation (Figure 1) (Table 1). All reported point mutations appear to perturb transcription at the recognised FLI1 target *GP6* gene with subtle differences in phenotype, probably reflecting changes in the site of the described mutations in the ETS DNA-binding domain. Protein modelling predicts the autosomal dominant mutations alter the third α-helix that directly binds DNA, whereas the recessive mutation, *R324W*, is positioned outside the third α-helix and may alter the protein conformation of FLI1.[48]

Hemizygous deletion of the entire FLI1 locus is also suggested to be responsible for the platelet defect associated with Jacobsen syndrome. Jacobsen syndrome is a multisystem disease characterised by developmental delay, short stature, congenital heart disease, and dysmorphic facial features.[48] It is due to a variably-sized deletion of the terminal end of chromosome 11q. Approximately 90% of individuals with Jacobsen syndrome have a platelet-related bleeding disorder called Paris-Trousseau thrombocytopenia.[49] Patients with Paris-Trousseau thrombocytopenia typically have neonatal thrombocytopenia that may improve with age, however platelet function remains abnormal throughout life. Paris-Trousseau platelets are variable in size and 15% of circulating platelets contain abnormal, large fused α-granules that are characteristic of this disorder.[50] Various genes in the deleted region have been proposed to be causative of the various organ pathologies with three lines of evidence, implicating FLI1 as responsible for the platelet defect. Firstly, small terminal deletions of 11q, not involving the FLI1 locus, are rarely reported to be associated with a partial Jacobsen syndrome and these individuals do not exhibit platelet pathology.[51] *In vitro* studies of megakaryocytes derived from individuals with Paris-Trousseau thrombocytopenia suggest overexpression of FLI1 may rescue some features of the defect[52] and finally, the characteristic giant fused α-granules present in Paris-Trousseau thrombocytopenia have also been demonstrated in the two individuals with the *R324W* mutation of the DNA-binding domain of FLI1 (Figure 1).[48]

A mild bleeding disorder with thrombocytopenia and decreased α-granules is caused by decreased binding of FLI1 and RUNX1 to the 5' untranslated region of their transcriptional target gene, *ANKRD26*. This transcription factor binding defect leads to a failure of normal transcriptional silencing of the transcript during megakaryocyte development with subsequent abnormal cytokine signalling, causing thrombocytopenia and an observed predisposition to leukaemia.[53]

ETS Variant 6

The transcriptional repressor, ETS variant 6 (ETV6), is also a member of the ETS family of transcription factors. Somatic mutations in ETV6 are recognised in haematological malignancy and deletion of the transcription factor in mice causes failure of haematopoiesis within the bone marrow.[54] Individuals with point mutations and small deletions in the ETS DNA-binding domain and the adjacent central domain of ETV6 have moderate thrombocytopenia with normal platelet volume that is inherited in an autosomal dominant manner.[55-57] Some of these family members have bleeding symptomatology and at least one family had features of altered α-granule production with morphological evidence of granule elongation.[55-57] Other haematological parameters are unaffected with the exception of an elevated mean corpuscular volume reported in some families.[55] A non-specific platelet functional defect with reduced responses to agonists ADP and AA was seen in one family harbouring a *P214L* mutation, but was not observed in another family with the *R418G* mutation (Table 1).[55] These described mutations act in a dominant-negative fashion to decrease transcriptional repression at the promoters of target genes *PF4* and *MMP3* as measured by luciferase reporter assays.[55] The described mutant ETV6 proteins also appear to dimerise with wild-type protein and promote aberrant cellular localisation of the transcription factor to the cytoplasm. Importantly, individuals in many of these families demonstrate an increased incidence of haematological malignancies and solid tumours, including colorectal cancer. There was a marked predominance of acute lymphoblastic leukaemia described with 10 cases reported in the eight unrelated families identified with germline ETV6 mutation.[55-57]

Ecotropic Viral Integration Site 1

The *MECOM* gene encodes EVI1 and MDS-EVI1 proteins from different transcription start sites. EVI1 is a transcriptional regulator with multiple zinc fingers that is normally expressed in haemopoietic tissue and is associated with a poor prognosis when over-expressed or mutated in myeloid leukaemia. Three distinct missense mutations predicted to disrupt the DNA binding function of the 8th zinc finger domain of EVI1 have been described in three unrelated children.[58] These children were born with moderate-to-severe thrombocytopenia associated with fusion of the radius and ulnar. Two of the children displayed concomitant anaemia and developed progressive bone marrow failure that required haematopoietic stem cell transplantation. Deletion of the entire *MECOM* locus and mutations in the homeodomain transcription factor, *HOXA11*, have been associated with very similar phenotypes.[59-61]

DIAGNOSTIC STRATEGIES

Diagnosis of inherited thrombocytopenia and platelet function disorders are hindered by lack of consensus regarding a standardised approach to testing, as well as diagnostic criteria for the conditions themselves.[62] This has prompted international groups and societies to draft guidelines[63,64] suggesting a logical step-wise approach using clinical symptoms and signs together with combinations of predominantly phenotypic screening and discriminatory assays to diagnose these conditions.[62] Genetic testing in these algorithms is generally recommended as a third-tier investigation.[64] Transcription factors interact with each other and multiple target genes in a combinatorial manner,[4,5] therefore mutation of a single transcription factor may disrupt the expression of multiple genes that are important in megakaryocyte maturation. The result, in many cases, is a complex platelet phenotype that is further influenced by the site of the mutation. Hence, it is not surprising that, despite multiple phenotypic tests that interrogate only isolated structural elements and/or functional pathways in platelets, a diagnosis is not achieved. An argument could be made that DNA-based diagnosis be prioritised in the diagnostic algorithm aiming for a 'one-stop one-step' diagnosis for patients. Sequencing in this context may provide a definitive diagnosis as well as indicate the likely impact of the mutation, based on the location of the mutation within a specific protein domain. Accurate diagnosis of these disorders may avoid the misdiagnosis of immune thrombocytopenia as well as allowing other members of the family to be screened to minimise bleeding events and identify inherited risk of oncogenesis.

Acknowledgements

Transmission electron microscopy was performed with the assistance of the scientific staff at the University of New South Wales and the University of Sydney Centres for Microscopy and Microanalysis.

REFERENCES

1. Bader-Meunier B et al. Misdiagnosis of chronic thrombocytopenia in childhood. J Pediatr Hematol Oncol. 2003;25(7): 548-52.

2. Gohda F et al. Identification of inherited macrothrombocytopenias based on mean platelet volume among patients diagnosed with idiopathic thrombocytopenia. Thromb Res. 2007;119(6):741-6.

3. Harrison P et al. Guidelines for the laboratory investigation of heritable disorders of platelet function. Br J Haematol. 2011;155(1):30-44.

4. Pimkin M et al. Divergent functions of hematopoietic transcription factors in lineage priming and differentiation during erythro-megakaryopoiesis. Genome Res. 2014;24(12):1932-44.

5. Wilson NK et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. Cell Stem Cell. 2010;7(4):532-44.

6. Cohen MM Jr. Perspectives on RUNX genes: an update. Am J Med Genet A. 2009; 149a(12):2629-46.

7. Heller PG et al. Low Mpl receptor expression in a pedigree with familial platelet disorder with predisposition to acute myelogenous leukemia and a novel AML1 mutation. Blood. 2005;105(12): 4664-70.

8. Sun L et al. Association of CBFA2 mutation with decreased platelet PKC-theta and impaired receptor-mediated activation of GPIIb-IIIa and pleckstrin phosphorylation: proteins regulated by CBFA2 play a role in GPIIb-IIIa activation. Blood. 2004;103(3):948-54.

9. Bluteau D et al. Dysmegakaryopoiesis of FPD/AML pedigrees with constitutional RUNX1 mutations is linked to myosin II deregulated expression. Blood. 2012; 120(13):2708-18.

10. Antony-Debre I et al. MYH10 protein expression in platelets as a biomarker of RUNX1 and FLI1 alterations. Blood. 2012; 120(13):2719-22.

11. Ho CY et al. Linkage of a familial platelet disorder with a propensity to

develop myeloid malignancies to human chromosome 21q22.1-22.2. Blood. 1996; 87(12):5218-24.

12. Dowton SB et al. Studies of a familial platelet disorder. Blood. 1985;65(3): 557-63.

13. Song WJ et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999; 23(2):166-75.

14. Antony-Debre I et al. Level of RUNX1 activity is critical for leukemic predisposition but not for thrombocytopenia. Blood. 2015;125(6): 930-40.

15. Buijs A et al. A novel CBFA2 single-nucleotide mutation in familial platelet disorder with propensity to develop myeloid malignancies. Blood. 2001;98(9): 2856-8.

16. Beri-Dexheimer M et al. Clinical phenotype of germline RUNX1 haploinsufficiency: from point mutations to large genomic deletions. Eur J Hum Genet. 2008;16(8):1014-8.

17. Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. Haematologica. 2011;96(10):1536-42.

18. Latger-Cannard V et al. Haematological spectrum and genotype-phenotype correlations in nine unrelated families with RUNX1 mutations from the French network on inherited platelet disorders. Orphanet J Rare Dis. 2016;11:49.

19. Owen CJ et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008; 112(12):4639-45.

20. Gerrard JM, McNicol A. Platelet storage pool deficiency, leukemia, and myelodysplastic syndromes. Leuk Lymphoma. 1992;8(4-5):277-81.

21. Michaud J et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. Blood. 2002;99(4): 1364-72.

22. Churpek JE et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. Leuk Lymphoma. 2013;54(1):28-35.

23. Harigae H. GATA transcription factors and hematological diseases. Tohoku J Exp Med. 2006;210(1):1-9.

24. Vyas P et al. Consequences of GATA-1 deficiency in megakaryocytes and platelets. Blood. 1999;93(9):2867-75.

25. Ciovacco WA et al. Human phenotypes associated with GATA-1 mutations. Gene. 2008;427(1-2):1-6.

26. Tsang AP et al. FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. Cell. 1997; 90(1):109-19.

27. Nichols KE et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat Genet. 2000;24(3): 266-70.

28. Del Vecchio GC et al. Dyserythropoietic anemia and thrombocytopenia due to a novel mutation in GATA-1. Acta Haematol. 2005;114(2):113-6.

29. Mehaffey MG et al. X-linked thrombocytopenia caused by a novel mutation of GATA-1. Blood. 2001;98(9): 2681-8.

30. Freson K. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. Blood. 2001;98(1): 85-92.

31. Freson K et al. Different substitutions at residue D218 of the X-linked transcription factor GATA1 lead to altered clinical severity of macrothrombocytopenia and anemia and are associated with variable skewed X inactivation. Hum Mol Genet. 2002;11(2):147-52.

32. Yu C et al. X-linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA-1 affecting DNA binding rather than FOG-1 interaction. Blood. 2002;100(6):2040-5.

33. Balduini CL et al. Effects of the R216Q mutation of GATA-1 on erythropoiesis and megakaryocytopoiesis. Thromb Haemost. 2004;91(1):129-40.

34. Doerks T et al. Systematic identification of novel protein domain families associated with nuclear functions. Genome Res. 2002;12(1):47-56.

35. Vassen L et al. Gfi1b:green fluorescent protein knock-in mice reveal a dynamic expression pattern of Gfi1b during hematopoiesis that is largely complementary to Gfi1. Blood. 2007;109(6): 2356-64.

36. Grimes HL et al. The Gfi-1 proto-oncoprotein contains a novel transcriptional repressor domain, SNAG, and inhibits G1 arrest induced by interleukin-2 withdrawal. Mol Cell Biol. 1996; 16(11):6263-72.

37. Zweidler-Mckay PA et al. Gfi-1 encodes a nuclear zinc finger protein that binds DNA and functions as a transcriptional repressor. Mol Cell Biol. 1996;16(8): 4024-34.

38. Laurent B et al. Gfi-1B promoter remains associated with active chromatin marks throughout erythroid differentiation of human primary progenitor cells. Stem Cells. 2009;27(9):2153-62.

39. Foudi A et al. Distinct, strict requirements for Gfi-1b in adult bone marrow red cell and platelet generation. J Exp Med. 2014;211(5):909-27.

40. Chen L et al. Transcriptional diversity during lineage commitment of human blood progenitors. Science. 2014;345(6204) :1251033.

41. Stevenson WS et al. GFI1B mutation causes a bleeding disorder with abnormal platelet function. J Thromb Haemost. 2013; 11(11):2039-47.

42. Monteferrario D et al. A dominant-negative GFI1B mutation in the gray platelet syndrome. N Engl J Med. 2014;370(3): 245-53.

43. Kitamura K et al. Functional characterization of a novel GFI1B mutation causing congenital macrothrombocytopenia. J Thromb Haemost. 2016. [Epub ahead of print].

44. Stevenson WS et al. Platelets are not all gray in GFI1B disease. Clin Genet. 2015; 87(3):299.

45. Rabbolini DJ et al. The GFI1B c.503G>T mutation in the first zinc finger domain predicts a milder bleeding phenotype than the GFI1B c.880-881insC mutation. J Thromb Haemost. 2015;13416-7.

46. Hart A et al. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. Immunity. 2000;13(2):167-77.

47. Stockley J et al. Enrichment of FLI1 and RUNX1 mutations in families with excessive bleeding and platelet dense granule secretion defects. Blood. 2013;122(25): 4090-3.

48. Stevenson WS et al. Paris-Trousseau thrombocytopenia is phenocopied by the autosomal recessive inheritance of a DNA-binding domain mutation in FLI1. Blood. 2015;126(17):2027-30.

49. Favier R et al. Jacobsen syndrome: Advances in our knowledge of phenotype and genotype. Am J Med Genet C Semin Med Genet. 2015;169(3):239-50.

50. Grossfeld PD et al. The 11q terminal deletion disorder: a prospective study of 110 cases. Am J Med Genet A. 2004;129A(1): 51-61.

51. Breton-Gorius J et al. A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha-granules and chromosome 11 deletion at 11q23. Blood. 1995;85(7): 1805-14.

52. Bernaciak J et al. Clinical and molecular-cytogenetic evaluation of a family with partial Jacobsen syndrome without thrombocytopenia caused by an approximately 5 Mb deletion del(11) (q24.3). Am J Med Genet A. 2008;146A(19): 2449-54.

53. Bluteau D et al. Thrombocytopenia-associated mutations in the

ANKRD26 regulatory region induce MAPK hyperactivation. J Clin Invest. 2014;124(2):580-91.

54. Wang LC et al. The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. Genes Dev. 1998;12(15): 2392-402.

55. Noetzli L et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47(5):535-8.

56. Zhang MY et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015; 47(2):180-5.

57. Topka S et al. Germline ETV6 Mutations Confer Susceptibility to Acute Lymphoblastic Leukemia and Thrombocytopenia. PLoS Genet. 2015;11(6): e1005262.

58. Niihori T et al. Mutations in MECOM, Encoding Oncoprotein EVI1, Cause Radioulnar Synostosis with Amegakaryocytic Thrombocytopenia. Am J Hum Genet. 2015;97(6):848-54.

59. Thompson AA, Nguyen LT. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with HOXA11 mutation. Nature genetics. 2000; 26(4):397-8.

60. Thompson AA et al. Congenital thrombocytopenia and radio-ulnar synostosis: a new familial syndrome. Br J Haematol. 2001;113(4):866-70.

61. Nielsen M et al. Deletion of the 3q26 region including the EVI1 and MDS1 genes in a neonate with congenital thrombocytopenia and subsequent aplastic anaemia. J Med Genet. 2012;49(9): 598-600.

62. Gresele P et al. Diagnosis of suspected inherited platelet function disorders: results of a worldwide survey. J Thromb Haemost. 2014;12(9):1562-9.

63. Balduini CL et al. Inherited thrombocytopenias: a proposed diagnostic algorithm from the Italian Gruppo di Studio delle Piastrine. Haematologica. 2003;88(5):582-92.

64. Gresele P; Subcommittee on Platelet Physiology of the International Society on Thrombosis and Hemostasis. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. J Thromb Haemost. 2015;13(2):314-22.

65. Heller PG, Glembotzky AC. Familial platelet disorder with predisposition to acute myelogenous leukemia. Atlas Genet Cytogenet Oncol Haematol. 2013;17(2): 138-43.

THE RELATIVE CONTRIBUTIONS OF GERMLINE VARIATION, EPIMUTATION, AND SOMATIC MUTATION TO PAEDIATRIC LEUKAEMIA PREDISPOSITION

***Todd E. Druley[1,2]**

1. Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, Missouri, USA
2. Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA
**Correspondence to druley_t@wustl.edu*

Disclosure: The author has declared no conflicts of interest.
Received: 22.03.16 **Accepted:** 08.06.16
Citation: EMJ Hematol. 2016;4[1]:110-116.

ABSTRACT

The next-generation sequencing era has repeatedly demonstrated that the amount of acquired somatic mutations in paediatric cancers can rarely account for the total incidence of any cancer subtype. In addition, many cancer-related mutations can be found in healthy individuals. These findings strongly suggest that additional genetic or epigenetic variation is required for malignant transformation, particularly in children who have significantly less environmental exposure and resulting genetic damage. Current studies now suggest that 3–33% of paediatric cancer patients have a predisposition to cancer. These germline genetic or epigenetic changes are frequently found in molecular mechanisms regulating normal human development which have long informed our understanding of developmental biology. Blockade of development is a mechanism of transformation consistent with the higher number of immature cancer cell types in paediatric patients. Thus, while nearly every cancer is a combination of germline variation and somatic mutation, the relative contribution to tumourigenesis in paediatrics is weighted toward germline changes. This review will explore how paediatric predisposition to leukaemia is influenced by germline genetic and epigenetic variability of variable penetrance. Improved understanding of these critical developmental mechanisms will lead to improved surveillance and perhaps guide a new class of therapeutics aimed at promoting normal differentiation rather than widespread cytotoxicity.

Keywords: Paediatric, cancer, predisposition, epimutation, genetics.

INTRODUCTION

The next-generation sequencing era has facilitated exciting progress in understanding the varied aetiologies of cancer on a genomic level. This success has primarily come through a model of tumour/normal sequencing from the same individual, where the normal sequence is simply used to subtract germline variation from cancer and identify cancer-specific mutations. In children, however, these cancer-specific mutations are much less common,[1] and very rarely does one find the 2–8 deleterious mutations thought to be necessary to transform a healthy cell to cancer.[2] In fact, the paediatric leukaemia with the highest mortality rate (>50%), infant leukaemia, has the fewest somatic mutations of any sequenced cancer with an average of 1.3 non-synonymous mutations per genome.[3] With respect to somatic mutations, this makes perfect sense given the rarity of paediatric cancers, fewer cell divisions, and decades less of environmental exposures compared with adults. Unlike adult cancers, where 5–10% have an identifiable genetic predisposition[4,5] and appear primarily driven by acquired somatic mutation, mutations are mainly in mechanisms regulating cellular maintenance, division, and DNA repair.[2] In contrast, the factors contributing to paediatric tumourigenesis are more likely to affect genes regulating age-specific mechanisms of differentiation and cell fate determination.[6-9]

Most paediatric cancer sequencing studies have focussed on identifying somatic coding mutations

in leukaemia. The inability to account for paediatric cancer solely via somatic mutation suggests that additional contributory information may lie in the non-coding or inherited genome or epigenome. In this regard, a Swedish epidemiology study analysing >10 million individuals examined the risk of acute lymphocytic leukaemia (ALL) in full, non-twin siblings of children with the disease, and found an increased odds ratio of 7.08,[10] consistent with a familial contribution to paediatric ALL. The variable penetrance of familial leukaemias suggests that these variants do not act by dominant modes of inheritance. If we accept that malignant transformation requires 2–8 'mutations',[2] then children must possess a combination of 2–8 (dependent upon effect size) inherited or acquired, genetic or epigenetic alterations that facilitate transformation. This review will focus on the relative contributions of germline variation, epimutations, and acquired somatic mutation to the aetiology of paediatric leukaemia, where more is known about the roles of these three types of variability. In simplistic terms, we can break this model down into an age-dependent schematic (Figure 1) and explore the interplay between these groups of genetic and epigenetic variability.

GERMLINE VARIABILITY

If paediatric cancer were solely a disease of somatic mutation, the relative lack of somatic mutations in paediatric cancer would suggest the scant mutations found would have greater effect sizes, reducing the need for the additional mutations seen in adult cancers. Yet, when introduced into model systems and expressed at physiologic levels, the majority of these somatic changes fail to induce cancers that phenocopy the paediatric disease.[11-13] This further suggests that somatic mutation alone is insufficient to explain the incidence of most paediatric malignancies, and that children's cancer evolves by mechanisms distinct from adult cancer. Multiple studies suggest that anywhere from 3–33% of children with cancer have a predisposition toward malignancy,[4,14,15] which presumably does not drive transformation but rather lowers the threshold for transformation. Indeed, many paediatric cancers appear to be derived from defects in normal developmental programmes,[16] consistent with the majority of paediatric cancers arising from immature cell types (e.g. the 'blastomas', pre-cursor B cell ALL, germ cell tumours, etc.). This is in contrast to adults, who more commonly possess tumours derived from more terminally differentiated cell types (e.g. carcinomas) that have accumulated enough damage to become irresponsive to appropriate cell signalling.

Cancer Predisposition Syndromes

It has long been recognised that several genetic syndromes, which include a variety of pathognomonic clinical and phenotypic features, increase the risk of paediatric leukaemia and other cancers. Yet germline variation alone appears insufficient for malignant transformation. Individuals with Li–Fraumeni syndrome, *BRCA1/2* variants, hereditary non-polyposis colorectal cancer, familial adenomatous polyposis, or other single cancer-related genetic syndromes typically do not develop cancer until the third decade of life, after enough cell divisions and the accumulation of additional genetic mutations.[17,18] While individually rare, there are numerous paediatric cancer predisposition syndromes that are typically divided into functional classes: aneuploidy-associated predisposition, hereditary transcription factor syndromes, DNA instability syndromes, bone marrow failure syndromes, and differentiation defects. While each is briefly mentioned below, they have been reviewed in detail elsewhere.[19]

Aneuploidy-associated predisposition

One of the earliest recognised cancer predisposing conditions, Down's syndrome (DS) is the archetypal aneuploidy-associated leukaemia. Children with DS have a 10 to 20-fold increase in leukaemia risk. The increase for acute megakaryocytic leukaemia (French-American-British classification M7) is even higher at a nearly 500-fold increased risk, such that when acute megakaryocytic leukaemia is seen in children without DS, there is often mosaicism or other anomalies in chromosome 21.[20] There is a profound association to leukaemia in DS and somatic mutations in the X-linked gene, *GATA1*, generally in exons two or three, which leads to a clinically variable phenotype ranging from self-limited neonatal transient myeloproliferative disorder to fatal leukaemias in childhood. There is also growing evidence for cohesin mutations as leukaemia modulators,[21] which are the topics of current investigation.

DNA instability syndromes

This group is well known due to the inability to repair double-stranded DNA breaks, leading to

abundant somatic mutation. Well known instability syndromes are Li–Fraumeni and Bloom syndrome, ataxia-telangiectasia, and Fanconi anaemia, but there are other less common syndromes as well.[17,19,22-25]

Bone marrow failure syndromes

These are well-studied disorders that often result in myeloid, rather than lymphoid, leukaemia and consist of severe congenital neutropenia, dyskeratosis congenita, Diamond–Blackfan anaemia, thrombocytopenia and absent radii syndrome, Shwachman–Diamond syndrome, and congenital amegakaryocytic thrombocytopenia.[17,19,22,26-28]

Differentiation defects

With respect to leukaemia, juvenile myelomonocytic leukaemia is most commonly secondary to *RAS* mutations in neurofibromatosis Type 1 or *PTPN11* mutations in Noonan syndrome,[29,30] but a similar phenotype and acute myeloid leukaemia (AML) incidence has recently been described in individuals with germline *CBL* variants and developmental anomalies.[31]

Birth Defects

Within the past 10 years, there has been a growing body of epidemiologic literature recognising that children with birth defects (variably defined) have an increased risk of developing cancer. An examination of >3 million children in the Texas Birth Defect Registry found that children with birth defects harboured an incident rate ratio of 1.4 for developing leukaemia.[32] While children with chromosomal birth defects have a greater risk of leukaemia (mainly due to DS), children with non-chromosomal birth defects are more likely to develop lymphomas, neuroblastoma, germ cell tumours, and central nervous system malignancies.[33] Hypospadias, cleft lip, and hydrocephalus had no effect on cancer risk, but cleft palate, microcephaly, and renal or cardiac defects increased the risk of cancer nearly 3-fold.[34] Recently, the AGORA database in the Netherlands has collected data on 3,747 children with birth defects, of whom 905 have childhood cancer. To date, the study has found that nearly 30% of these children develop ALL, the most common cancer in the registry, and another 17% have lymphoma.[35] The fact that these defects in normal human differentiation carry an increased risk of childhood cancer further strengthens the link between abnormal development and cancer. Future studies should move beyond large-scale epidemiological surveys and focus on how the disrupted mechanisms resulting in congenital anomalies contribute to overgrowth and malignant transformation.

Inherited, Functional, and Single Gene Variants

In addition to syndromes and birth defects with clear phenotypic effects, there is growing evidence for cancer predisposition as a result of inherited germline variants in >60 different genes, without other signs or symptoms. As one would expect, this is the fastest growing category of genetic variation associated with paediatric leukaemia.

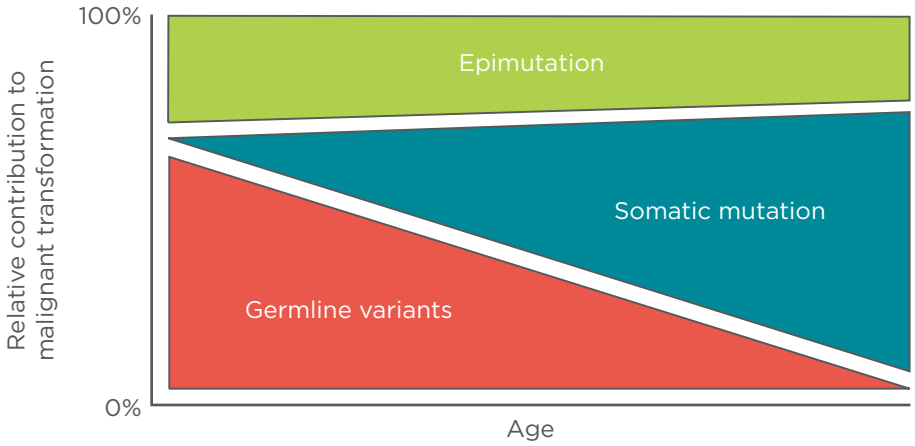

Figure 1: The relative contributions of germline variation, somatic mutation, and epimutation to malignant transformation as a function of age.

In this model, early childhood leukaemias depend on multigenic variability and mutation, and therefore may not show strong familial inheritance patterns as would be observed in a Mendelian disease.

Genome-wide association studies have identified heritable variation in *IKZF1*, *ARID5B*, *CEBPE*,[36] and *CDKN2A*[37] and larger regions of disequilibrium associated with paediatric *de novo*[38,39] or *ETV6-RUNX1*-associated ALL.[40]

More recently, leukaemia predisposition (mainly to B cell ALL) has been associated with inherited single gene defects in transcription factors that skew normal haematopoietic differentiation For example, *PAX5*, required for normal B cell maturation, demonstrates heritable variants (mainly *G547A*) in multiple related individuals. These are transmitted in an autosomal dominant fashion with variable penetrance and predispose to lymphoblastic transformation due to B cell differentiation defects.[41-43] *ETV6* is another transcription factor required for nuclear localisation of transcriptional machinery, and is part of the most common translocation observed in paediatric leukaemia (*ETV6-RUNX1*). Exome and genome sequencing studies of paediatric pre-B cell ALL have identified hypomorphic germline variants that are defective in nuclear localisation, leading to aberrant target gene expression and a predisposition to B cell ALL that is typically accompanied by familial thrombocytopenia.[44-46] More recently, *PRDM9*, important for regulating meiotic recombination events, was shown to be enriched for deleterious germline variants in multiple children with pre-B cell ALL.[47] For AML, a condition of growing interestis familial platelet disorder with predisposition to AML secondary to hypomorphic germline *RUNX1* variants that skew normal haematopoiesis in association with variable degrees ofthrombocytopenia and platelet dysfunction.[14,19] However, germline *RUNX1* variants have also been associated with ALL and thrombocytopenia[48] and in patients with acquired mutations in *ASXL1*.[49]

Given that the germline variants appear to only establish a pre-leukaemic state, actual cases of leukaemia are almost certainly going to include a combination of multiple germline variants along with somatic mutations. For example, while infant leukaemia has the fewest non-synonymous point mutations per genome of any cancer sequenced to date, and over 75% of these cases harbour *MLL*-rearrangements, germline exomes from these children found a significant enrichment for rare, non-synonymous germline variants. In particular, bi-allelic variation in the pan-cancer tumour suppressor gene and histone methyltransferase, *MLL3 (KMT2C)*, was a common feature.[50,51] This pattern of inherited multi-allelic, multi-gene variation establishing a pre-cancerous state is likely to be increasingly recognised for many types of paediatric cancer that show a paucity of acquired mutation.

EPIMUTATIONS

With the realisation that inherited or acquired sequence changes cannot fully account for the incidence of paediatric cancer, the field has begun looking at other sources of variation. Research has shown epigenetic variability to be more diverse and complex than initially imagined. There is a qualitative difference in the nature of epimutation in paediatric versus adult cancer. Because many inherited variants or somatic mutations in developmental pathways interrupt gene regulation necessary for cell fate specification,[52] there is a strong correlation between gene silencing as either the cause or the effect of other genetic events in paediatric cancer.[16] In adults, somatic mutations are found in quite different epigenetic regulators (e.g. *DNMT3A*, *IDH1/2* in adult AML) that are rarely variant or mutated in paediatrics. The true burden of epimutation as a function of age is not well described. The speculation is that there is probably a slightly bigger contribution from epimutation in paediatric leukaemia compared to adults due to the involvement of developmental mechanisms, which is depicted in Figure 1.

Secondary to Germline Variation or Somatic Mutation

Epimutations that occur secondary to germline variation or somatic mutation significantly overlap with the inherited transcription factor predisposition syndromes described above and have been reviewed for paediatric ALL elsewhere.[53] Many of the most commonly described somatic mutations in paediatric leukaemias are found in epigenetic regulators: histone methyltransferases (*MLL1/KMT2A*, *MLL2/KMT2B*, *EZH2*), demethylases (*TET2*, *KDM6A*), polycomb repressor proteins (*ASXL2*) and histone acetyltransferases (*CREBBP*, *EP300*), and DNA methyltransferases and their regulators (*DNMT3A*, *IDH1/2*) among others.[54,55] Epigenetic silencing of these genes may also occur due to environmental influences[55,56] which may partially explain why the incidence of paediatric cancer has been steadily rising for the past 40 years.[57] While the largest germline analysis to date of paediatric cancer patients finds the most common variant genes to be canonical autosomal dominant cancer drivers, *TP53*, *APC*, and *BRCA2*,

the largest absolute number of variants were in the category including epigenetic modulators such as *ARID5B*, *EP300*, *TET2*, *ATRX*, and *SETD2* among others, and 51% of these patients had leukaemia.[15] Without appropriate gene expression during different embryonic or developmental stages, the barrier to malignant transformation is apparently lowered and uncontrolled proliferation results as the affected cells attempt to bypass whatever developmental blockade is encountered.

Inherited (Causal)

The actual heritability of epimutations is a topic of some debate as epigenetic signals are generally thought to be 'erased' after fertilisation and gradually re-established during embryogenesis and development.[52,58] Mendelian inheritance of single nucleotide or copy number variants alter DNA methylation in a manner that inappropriately silences (tumour suppressors) or activates (oncogenes) gene expression as an initiating event in tumourigenesis.[52,58] This mechanism was reported in a family with chronic lymphocytic leukaemia due to a rare variant promoting silencing of *DAPK1*.[59] However, irrespective of an identified sequence change, the same DNA methylation-mediated silencing of *MLH1* and *MSH2* in all tissues from affected individuals in pedigrees with Lynch syndrome,[60] suggesting that heritable epimutations may arise from multiple mechanisms and result in cancer predisposition. While many studies have evaluated DNA methylation in various paediatric leukaemias for diagnostic or prognostic value, the contribution of inherited epimutations in paediatric leukaemia is not well characterised.

ACQUIRED SOMATIC MUTATION

Paediatric cancer is marked by a high prevalence of canonical and cryptic translocations, but nearly all data point toward additional environmental, genetic, or epigenetic events having an additive effect leading to malignancy. In this model, we can envision a continuum where the risk of malignancy is dependent upon the abundance, the cumulative effect size of multiple genetic (germline and somatic), and epigenetic lesions. If this is the case, then these translocations should occur stochastically in the general population without resulting in malignancy due to the lack of the additive events. While many somatic mutations identified in cancer can occasionally be found in the germline of healthy individuals, prior searches to identify the true population-based incidence of translocations such as *ETV6-RUNX1*[61] and *MLL*-rearrangements[62] have been highly controversial and no clear consensus on this topic exists. Alternatively, an aggregation of individually low penetrance variation where multiple variants are aggregated within a single individual and confer a greater additive effect size could account for the gap between the 8.5% of paediatric cancer patients with germline variation in known cancer genes.[15] Additionally, 29% of paediatric cancer survivors (compared to only 5% of adult cancer survivors) meet criteria for predisposition when incorporating additional factors such as family history, cancer type, and comorbidities.[4]

CLINICAL APPLICATION

Longitudinal surveillance in sporadic and familial breast and colorectal cancer has markedly improved outcomes since implementation.[63] Currently, no recommendations exist for systematic paediatric leukaemia screening, which is more likely due to the heterogeneity of the disease as opposed to the prevalence. When considering children with cancer predisposition, it is not always evident who should actually be screened or what combination of genetic (and epigenetic) lesions should be considered disease-related. For those that have clear phenotypic anomalies associated with a canonical syndrome or a strong family history, identifying at-risk individuals is straightforward even if the incidence of cancer is quite variable. In addition to who should be screened, an even more difficult question concerns which modality to use for screening. Genome sequencing, exome sequencing, methylation arrays, hybridisation-based methylomes, and bisulphite-treated genomes are all potentially informative. While the cost of genomic technology is gradually decreasing, these modalities are currently still too costly for governments, insurance companies, or individuals to offer for population-based screening. In addition, a majority of the data generated is often difficult, if not impossible, to interpret because we simply do not understand enough of the intricate interplay between these sources of genetic and epigenetic variability, giving these tests a high sensitivity, but a poor specificity.

In addition to screening, the invocation of congenital sequence or epigenetic variation that disrupts normal developmental mechanisms raises therapeutic concerns that are unique to

paediatric cancer. While targeting developmental mechanisms may eliminate the leukaemia, there can be broad consequences to disrupting an essential developmental mechanism that often results in late-onset toxicities and long-term morbidities involving growth, cognition, fertility, and perhaps an even greater increased risk of secondary malignancies compared to patients without predisposition.[64] An example of this is seen in murine models of Gorlin syndrome where inhibition of sonic hedgehog reduced rates of medulloblastoma but resulted in various defects in other systems.[65] Thus, when it comes to predisposition toward paediatric leukaemia, even 'targeted' therapeutics may have untoward collateral damage if the primary goal of treatment is cytotoxicity.

However, if paediatric leukaemias are in fact the result of developmental blockades in normal haematopoiesis, perhaps an alternative strategy for leukaemia treatment can be extrapolated from acute promyelocytic leukaemia where the PML-RARα translocation arrests maturation of promyelocytes. The introduction of all-trans retinoic acid therapy allows the majority of promyelocytes to overcome this blockade and mature to myelocytes.[66] This change in the acute promyelocytic leukaemia treatment approach, so-called 'differentiation therapy', has significantly reduced the need for cytotoxic therapy, resulting in improved outcomes. By identifying and overcoming blockades in normal haematopoietic development incurred in other paediatric leukaemias due to germline genetic or epigenetic variation, additional difficult-to-treat subtypes of paediatric leukaemia may enjoy improved outcomes with less long-term morbidity.

SUMMARY

Paediatric leukaemia is increasingly recognised to be the result of pre-existing predisposition. The landscape of germline variation, somatic mutation, and epimutation differs substantially from that seen in adults. The difference is not necessarily in the total amount of genetic or epigenetic damage inherent between the two groups, but in the source of that damage. Children are more likely to harbour germline variation that alters epigenetic regulation of developmental mechanisms, which are primarily active only in childhood. Identifying these mechanisms and their relative influence on paediatric leukaemogenesis will improve clinical screening protocols, which will hopefully occur in parallel with continued reductions in the cost of sequencing and a concomitant augmentation of our ability to accurately interpret the results. In addition, improved characterisation of these developmental pathways may inform novel therapeutics that depend less upon cytotoxicity and more on differentiation therapy to enable these malignancies to achieve their desired cell fate.

REFERENCES

1. Radtke I et al. Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. Proc Natl Acad Sci U S A. 2009;106(31):12944-9.

2. Vogelstein B et al. Cancer genome landscapes. Science. 2013;339(6127): 1546-58.

3. Andersson AK et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330-7.

4. Knapke S et al. Hereditary cancer risk assessment in a pediatric oncology follow-up clinic. Pediatr Blood Cancer. 2012;58(1): 85-9.

5. Garber JE, Offit K. Hereditary cancer predisposition syndromes. J Clin Oncol. 2005;23(2):276-92.

6. Stubbs MC, Armstrong SA. Therapeutic implications of leukemia stem cell development. Clin Cancer Res. 2007; 13(12):3439-42.

7. Sell S. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol. 2004;51(1):1-28.

8. Scotting PJ et al. Chilhood solid tumours: A developmental disorder. Nat Rev Cancer. 2005;5(6):481-8.

9. Tartaglia M et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. Blood. 2004;104(2): 307-13.

10. Hemminki K, Jiang Y. Risks among siblings and twins for childhood acute lymphoid leukaemia: Results from the Swedish Family-Cancer Database. Leukemia. 2002;16(2):297-8.

11. Montes R et al. Enforced expression of MLL-AF4 fusion in cord blood CD34+ cells enhances the hematopoietic repopulating cell function and clonogenic potential but is not sufficient to initiate leukemia. Blood. 2011;117(18):4746-58.

12. Bueno C et al. FLT3 activation cooperates with MLL-AF4 fusion protein to abrogate the hematopoietic specification of human ESCs. Blood. 2013;121(19): 3867-78, S1-3.

13. Bursen A et al. The AF4.MLL fusion protein is capable of inducing ALL in mice without requirement of MLL.AF4. Blood. 2010;115(17):3570-9.

14. Kratz CP et al. Genetic predisposition to acute lymphoblastic leukemia: Overview on behalf of the I-BFM ALL Host Genetic Variation Working Group. Eur J Med Genet. 2016;59(3):111-5.

15. Zhang J et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med. 2015;373(24):2336-46.

16. Federico S et al. Childhood cancer and developmental biology. Cancer Dev. 2011; 94:1-13.

17. Malkin D et al. Predisposition to

pediatric and hematologic cancers: a moving target. Am Soc Clin Oncol Educ Book. 2014:e44-55.

18. Vasen HFA et al. Clinical management of hereditary colorectal cancer syndromes. Nat Rev Gastroenterol Hepatol. 2015;12(2):88-97.

19. Seif AE. Pediatric leukemia predisposition syndromes: Clues to understanding leukemogenesis. Cancer Genet. 2011;204(5):227-44.

20. Mateos MK et al Down syndrome and leukemia: Insights into leukemogenesis and translational targets. Transl Pediatr. 2015; 4(2):76-92.

21. Yoshida K et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013; 45(11):1293-9.

22. Alter BP. Fanconi anemia and the development of leukemia. Best Pract Res Clin Haematol. 2014;27(3-4):214-21.

23. Arora H et al. Bloom syndrome. Int J Dermatol. 2014;53(7):798-802.

24. Bielorai B et al. Acute lymphoblastic leukemia in early childhood as the presenting sign of ataxia-telangiectasia variant. Pediatr Hematol Oncol. 2013; 30(6):574-82.

25. Boultwood J. Ataxia telangiectasia gene mutations in leukaemia and lymphoma. J Clin Pathol. 2001;54(7): 512-6.

26. Druley TE. Commentary. Clin Chem. 2013; 59(1):50-1.

27. Heerema NA et al. Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: a report from the Children's Cancer Group. Leukemia. 2004; 18(5):939-47.

28. Stieglitz E, Loh ML. Genetic predispositions to childhood leukemia. Ther Adv Hematol. 2013;4(4):270-90.

29. Lauchle JO et al. Inherited predispositions and hyperactive Ras in myeloid leukemogenesis. Pediatr Blood Cancer. 2006;46(5):579-85.

30. Strullu M et al. Juvenile myelomonocytic leukaemia and Noonan syndrome. J Med Genet. 2014;51(10): 689-97.

31. Niemeyer CM et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010; 42:794-800.

32. Carozza SE et al. Are children with birth defects at higher risk of childhood cancers? Am J Epidemiol. 2012;175(12): 1217-24.

33. Fisher PG et al. Cancer in children with nonchromosomal birth defects. J Pediatr. 2012;160(6):978-83.

34. Botto LD et al. Cancer risk in children and adolescents with birth defects: A population-based cohort study. PLoS One. 2013;8(7):e69077.

35. van Rooij IA et al. AGORA, a data- and biobank for birth defects and childhood cancer. Birth Defects Res A Clin Mol Teratol. 2016. [Epub ahead of print].

36. Papaemmanuil E et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet. 2009;41(9):1006-10.

37. Sherborne AL et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet. 2010;42(6):492-4.

38. Moriyama T et al. Inherited genetic variation in childhood acute lymphoblastic leukemia. Blood. 2015;125(26):3988-95.

39. Migliorini G et al. Variation at 10p12.2 and 10p14 influences risk of childhood B-cell acute lymphoblastic leukemia and phenotype. Blood. 2013;122(19):3298-307.

40. Ellinghaus E et al. Identification of germline susceptibility loci in ETV6-RUNX1-rearranged childhood acute lymphoblastic leukemia. Leukemia. 2012;26(5):902-9.

41. Hyde RK, Liu PP. Germline PAX5 mutations and B cell leukemia. Nat. Genet. 2013;45(10):1104-5.

42. Shah S et al. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet. 2013;45(10):1226-31.

43. Auer F et al. Inherited susceptibility to pre B-ALL caused by germline transmission of PAX5 c.547G>A. Leukemia. 2014;28(5):1136-8.

44. Zhang MY et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015; 47(2):180-5.

45. Noetzli L et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47(5):535-8.

46. Topka S et al. Germline ETV6 mutations confer susceptibility to acute lymphoblastic leukemia and thrombocytopenia. PLoS Genet. 2015;11(6): e1005262.

47. Hussin J et al. Rare allelic forms of PRDM9 associated with childhood leukemogenesis. Genome Res. 2013;23(3): 419-30.

48. Linden T et al. Childhood B-cell precursor acute lymphoblastic leukaemia in a patient with familial thrombocytopenia and RUNX1 mutation. Br J Haematol. 2010;151(5):528-30.

49. Prebet T et al. Concomitant germ-line RUNX1 and acquired ASXL1 mutations in a T-cell acute lymphoblastic leukemia. Eur J Haematol. 2013;91:277-9.

50. Valentine MC et al. Excess congenital non-synonymous variation in leukemia-associated genes in MLL-infant leukemia: a Children's Oncology Group report. Leukemia. 2014;28:1235-41.

51. Lawrence MS et al. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. 2014;505(7484): 495-501.

52. Hesson LB et al. Epimutations and cancer predisposition: importance and mechanisms. Curr Opin Genet Dev. 2010; 20(3):290-8.

53. Burke MJ, Bhatla T. Epigenetic modifications in pediatric acute lymphoblastic leukemia. Front Pediatr. 2014;2:42.

54. Liang DC et al. Cooperating gene mutations in childhood acute myeloid leukemia with special reference on mutations of ASXL1, TET2, IDH1, IDH2, and DNMT3A. Blood. 2013;121:2988-95.

55. Yiu TT, Li W. Pediatric cancer epigenome and the influence of folate. Epigenomics. 2015;7(6):961-73.

56. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007;8(4):253-62.

57. Howlader N et al. SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. Bethesda, MD. Available at: http://seer.cancer.gov/csr/1975_2012/. Last accessed: 6 April 2016.

58. Dobrovic A, Kristensen LS. DNA methylation, epimutations and cancer predisposition. Int J Biochem Cell Biol. 2009;41(1):34-9.

59. Raval A et al. Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. Cell. 2007; 129(5):879-90.

60. Hitchins MP. Inheritance of epigenetic aberrations (constitutional epimutations) in cancer susceptibility. Adv Genet. 2010; 70:201-43.

61. Mori H et al. Chromosome translocations and covert leukemic clones are generated during normal fetal development. Proc Natl Acad Sci U S A. 2002;99(12): 8242-7.

62. Uckun FM et al. Clinical significance of MLL-AF4 fusion transcript expression in the absence of a cytogenetically detectable t(4;11)(q21;q23) chromosomal translocation. Blood. 1998;92(30):810-21.

63. Clegg LX et al. Cancer survival among US whites and minorities. Arch Intern Med. 2002;162(7):1985.

64. Oeffinger KC et al. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med. 2006;355(15): 1572-82.

65. Gorlick R et al. Initial testing (stage 1) of lapatinib by the pediatric preclinical testing program. Pediatr Blood Cancer. 2009; 53(4):594-8.

66. Grimwade D et al. Acute promyelocytic leukemia: a paradigm for differentiation therapy. Cancer Treat Res. 2010;145: 219-35.

CURRENT MANAGEMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA: EMERGING INSIGHTS AND OUTSTANDING QUESTIONS

***Xavier Thomas, Caroline Le Jeune**

Hematology Department, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Pierre Bénite, France
**Correspondence to xavier.thomas@chu-lyon.fr*

Disclosure: The authors have declared no conflicts of interest.
Received: 21.03.16 **Accepted:** 28.06.16
Citation: EMJ Hematol. 2016;4[1]:117-128.

ABSTRACT

Less than 50% of patients with adult acute lymphoblastic leukaemia (ALL) experience long-term survival and for those adults >60 years old, long-term survival rates are only 10%. However, significant advances have been reported over the last decade. Both the efficacy of chemotherapy and the safety of transplants have improved. Improved outcomes have been seen in younger adults treated with paediatric-inspired chemotherapy regimens. Minimal residual disease has been identified as an independent predictor of relapse risk and is currently widely used for risk-adapted treatment. Newly developed targeted therapies have been developed to improve treatment outcomes. Tyrosine kinase inhibitors (TKI) have become an integral part of front-line therapy for Philadelphia (Ph) chromosome positive ALL. Ph-positive ALL serves as the first example of truly targeted treatment, although the choice of the most effective TKI is not yet settled. The last few years have also seen a surge in immune therapies for B cell lineage ALL. The success of the anti-CD20 monoclonal antibody rituximab provided proof-of-principle for exploiting the immune system therapeutically. Novel immune therapies recruit (bispecific T cell engager) or modify (chimeric antigen receptor T cells) the patient's own T cells to fight leukaemic cells. These new approaches led us to predict that ALL therapy might be based heavily on non-chemotherapeutic approaches in the near future. The role of allogeneic stem cell transplantation is also increasingly called into question. Herein, we review the background and development of these distinct treatments, and assess the current clinical knowledge of their efficacy and safety.

Keywords: Acute lymphoblastic leukaemia (ALL), treatment, targeted therapy, allogeneic stem cell transplantation (SCT), prognosis.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is a malignant neoplasm of the lymphocyte precursor cells. ALL is characterised by aberrations in proliferation and differentiation of lymphoblasts, leading to failure of normal immune response and decreased haematopoiesis. It represents a heterogeneous group with distinct morphologic, cytogenetic, and molecular groupings. It is a clonal disease that can be separated by immunophenotyping into a B lineage ALL group (about 75%) and a T cell lineage ALL group (about 25%) and their subtypes according to the stage of maturation/differentiation. Standard cytogenetics, fluorescence *in situ* hybridisation, and reverse transcriptase polymerase chain reaction (RT-PCR) allow the detection of chromosomal translocations and the corresponding gene rearrangement. The identification of these rearrangements has provided critical insights into leukaemogenesis and is currently central to risk stratification. Gene expression profiling, novel molecular techniques, and next-generation sequencing can also recognise newly defined ALL entities.[1,2]

ALL remains a major therapeutic challenge in adults. In this review, we cover the clinical and biological characteristics, pathophysiology, and therapy for ALL. The evidence for minimal residual disease (MRD) is discussed as well as novel

molecular targets and newly developed therapies, such as new immunotherapeutic approaches.

TREATMENT PRINCIPLES

Most therapeutic advances in adult ALL have arisen from adaptation of ALL treatment in children. Historically, induction therapy for adult ALL has been built around a 'backbone' of vincristine and prednisone. Standard induction regimens can be labelled as four-drug or five-drug (vincristine, prednisone, anthracycline, cyclophosphamide, and L-asparaginase) regimens.[1] Modifications in the chemotherapeutic schedule could concern the type and timing of corticosteroids, the addition of other drugs during induction, intensification of anthracycline dose, or introduction of high-dose cytarabine to provide efficient prophylaxis of central nervous system (CNS) relapse. However, these approaches do not appear to be superior to conventional treatment, and it remains uncertain as to which subgroups would benefit in terms of leukaemia-free survival (LFS) following such modifications.

The goal of induction chemotherapy is to achieve a morphologic clinical remission (CR), or better still, a complete molecular response. With current regimens, the CR rate reaches 80–90%. Long-term survival reaches 80–90% with chemotherapy only in Burkitt-like ALL, 60–75% with chemotherapy alone in standard-risk B cell lineage ALL, >50% with chemotherapy and allogeneic stem cell transplantation (SCT) in high-risk Philadelphia (Ph)-negative B cell lineage ALL, and 50–60% in Ph-positive ALL. In T cell lineage ALL, long-term survival is observed in 60–70% with chemotherapy alone in the thymic subtype, in about 50% with chemotherapy and allogeneic SCT in the mature T subtype, and in 30–40% with chemotherapy and allogeneic SCT in the early T precursor ALL subtype.[3] Over the past decades, survival in ALL has improved in all age groups except for those >60 years old.[4] Using elderly-specific protocols with less intensive therapy, a CR rate of about 71% has been demonstrated. Early death rate was 15% and the median overall survival (OS) was 33 months.[5]

Salvage chemotherapy results are comparatively poor in the setting of refractory/relapsed ALL.[6,7] Only 30–40% of adults achieve a second CR and 10–20% infurther salvages. The median OS ranges from 4.5–8.4 months and 5-year survival rates are just 7–10%. At this stage, only allogeneic SCT offers a chance of long-term survival, however few patients can be bridged to this treatment.[6,7]

RISK STRATIFICATION

Standard Prognostic Factors

Standard prognostic factors (age, white blood cell count, immunophenotyping, cytogenetics, and genetic aberrations) are identified at the time of diagnosis.[8] Patients without any risk factors are considered as standard-risk patients and are treated with chemotherapy courses only. Patients displaying one or more risk factors are classified as high-risk patients and are candidates for allogeneic SCT in first CR. Treatment decision making will soon be refined in accordance to risk stratification based on genetics at diagnosis and MRD after induction therapy.

Genetics of Acute Lymphoblastic Leukaemia

Multiclonality at diagnosis is common in ALL. The ALL genome is not static but evolves over time. The advent of microarrays and sequencing has demonstrated acquisition of new deletions and mutations and the loss of diagnosis-specific lesions at the time of relapse, but with preservation of key alterations.[9] In the majority of cases, diagnosis and relapse clones arise from a common pre-leukaemic clone that has acquired genetic alterations required to established frank leukaemia. Many relapse-acquired lesions are enriched in specific pathways, including B cell development (*IKZF1*), tumour suppression (*TP53*), *RAS* signalling, chromatin modification (*CREBBP*, *SETD2*), and drug metabolism (*NT5C2*). Several alterations are known to confer resistance to specific chemotherapy agents and glucocorticoids.[10] Initial therapy eradicates all subclones apart from those that survive to propagate relapse and acquire additional mutations that facilitate resistance to therapy. Recent studies have implicated epigenetic deregulation in leukaemogenesis and treatment failure in ALL.[11] This involves mutations in genes that regulate the epigenome. These findings are of clinical relevance since the introduction of drugs that target histone readers (bromodomain inhibitors) and histone modifiers (histone demethylase and histone deacetylase inhibitors).

Multiple susceptibility loci associated with ALL risk have been recently identified. The most reproducible associations have been in genes that are also targets of somatic genetic alteration in ALL: *IKZF1, ARID5B, CEBPE,* and *CDKN2A*.[12]

Minimal Residual Disease

MRD is the detection of residual leukaemic cells detectable as leukaemia-specific aberrant immunophenotypes by flow cytometry, leukaemia-specific rearranged immunoglobulin, or T cell receptor sequences by quantitative RT-PCR, or detection of fusion genes associated with chromosomal abnormalities. The detection limit is 10^{-3}–10^{-5}. Measurement of MRD has significantly improved risk stratification and helped guide the intensification of therapy. MRD overrides all of the pre-therapeutic risk factors and is currently regarded as the most important prognostic factor for survival and a major component of a personalised treatment algorithm.[13] MRD levels have been evaluated at various early time-points using either flow cytometry or immunoglobulin/T cell receptor gene amplification. For example in a French study, MRD was studied 6 weeks (MRD1) and 12 weeks (MRD2) after initiation of induction.[13] MRD1 and MRD2 levels were strongly correlated. Patients with molecular response after induction chemotherapy had a significantly better outcome than those with a persistently positive MRD.[13] MRD1 response allowed researchers to significantly discriminate high-risk versus standard-risk patients amongst those defined by their early morphological response. Therefore, patients achieving molecular remission are now considered as standard-risk patients, while those with a positive MRD are defined as high-risk patients. Persistence of a positive MRD after induction is an indication for allogeneic SCT in first morphological remission. Patients achieving MRD negativity after induction will only receive consolidation and maintenance chemotherapy. Of these patients, 20–30% will relapse because of loss of sensitivity, evolution of leukaemic subclones, or extramedullary localisation of the disease.

PAEDIATRIC-INSPIRED THERAPIES

Lessons have been drawn from the management of ALL in adolescents and young adults; studies in this patient subgroup have demonstrated improved survival for patients treated with paediatric rather than adult protocols.[14] Reasons explaining this difference include differences in protocol designs with higher doses of drugs, early and more frequent CNS prophylaxis, and dexamethasone instead of prednisone; biological differences; different practice patterns; and social factors such as support systems and compliance.

Paediatric treatment protocols have thus been applied to adult patients, providing increased drug intensity at several stages of treatment including higher cumulative doses of corticosteroids, vincristine, and L-asparaginase. L-asparaginase is an integral component of therapy for ALL. The ability to identify patients with inadequate asparaginase activity is of great value in clinical decision making and has the potential to improve clinical outcomes. Serum asparaginase activity levels are the best and most reliable indicators of asparaginase activity. Trough asparaginase activity levels ≥0.1 IU/mL appears to be a safe target to ensure therapeutic benefit.[15] Screening for silent inactivation (development of asparaginase antibodies and asparaginase inactivity without the development of overt or recognised allergy symptoms) should be considered in all patients undergoing therapy for ALL with asparaginase. Survival rates at 5 years ranged from 67–78%, and compared favourably to rates between 34% and 41% observed with the former protocols.[16] These emerging data showing substantially improved outcomes for younger adults question the role of allogeneic SCT in the upfront setting.

STEM CELL TRANSPLANTATION

Place of Transplantation in Acute Lymphoblastic Leukaemia

Allogeneic SCT in the first CR remains the standard of care for adults with high-risk ALL. In a recent meta-analysis, the 5-year OS was 49.9% for patients with a donor versus 42.7% for those without.[17] However, graft-related toxicity after myeloablative conditioning remains an important issue and consideration should be given to the use of less intensive conditioning regimens. The 6-month and 2-year non-relapse mortality rate was 7.3% and 35.8% versus 2.0% and 13.6% in high-risk patients with donor and patients with no donor, respectively.[18] In standard-risk patients, the 6-month and 2-year non-relapse mortality was 3.4% and 19.4% versus 1.2% and 6.9%, respectively. It is well established that relapse probability is higher in patients with no acute graft-versus-host disease (GvHD) as compared with patients with Grade I–III acute GvHD.[19]

Reduced-Intensity Conditioning in Acute Lymphoblastic Leukaemia

Allogeneic SCT after reduced-intensity conditioning have been shown to be feasible in adult ALL.[20]

Results appeared significantly better when performed in first CR as compared with second or further CR. No differences were found in terms of LFS when compared with allogeneic SCT after myeloablative conditioning.[21,22] Allogeneic SCT after reduced-intensity conditioning with cord blood showed a 2-year OS of 50%, a 2-year non-relapse mortality of 27%, and a 2-year relapse rate of 36%.[23]

Haploidentical and Cord Blood Transplantations

Allogeneic SCT from haploidentical donors has rapidly developed over the past few years.[24] A higher probability of finding a suitable haploidentical donor has been demonstrated as compared with suitable umbilical cord blood. The other advantages are a shorter time-to-transplant and a lower cost.[25] A large comparative study between unrelated cord blood and unmanipulated haploidentical SCT suggested that results are likely comparable, indicating that both transplant strategies are suitable for patients lacking a human leukocyte antigen (HLA) matched donor or when transplantation cannot be delayed.[26] However, a lower treatment-related mortality was observed after haploidentical SCT (10% versus 23%), while relapse rate was identical (about 30%).

TARGETED THERAPIES AND IMMUNOTHERAPY

Monoclonal antibodies represent a new approach for the treatment of B cell lineage ALL.[27] B lineage blast cells express a variety of specific antigens, such as CD19, CD20, and CD22. Unconjugated monoclonal antibodies have demonstrated efficacy in ALL. Anti-CD20 monoclonal antibody rituximab has improved the outcome of patients with Burkitt leukaemia/lymphoma.[28,29] With repeated short cycles of intensive chemotherapy combined with rituximab, the 5-year OS of these patients increased from 60% to >80%.[29] In precursor B ALL, Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, [also known by its trade name, adriamycin®], and dexamethasone) plus rituximab gave results significantly better than those observed with chemotherapy alone.[30] These findings were confirmed by a German group[31] and more recently by the randomised study from a French group showing a significant advantage with chemotherapy combined with rituximab with a 2-year OS of 71% versus 52% and a 2-year event-free survival (EFS) of 65% versus 52%.[32] Adding rituximab to standard chemotherapy should therefore be the standard of care for these patients, although optimal dose schedule remains to be determined. Combination of chemotherapy with other anti-CD20 agents has also been tested. Ofatumumab combined with Hyper-CVAD showed 97% of CR and 67% of MRD-negativity after induction and a 2-year OS of 87%.[33] Monoclonal antibodies directed against CD22, such as inotuzumab ozogamicin or epratuzumab are being evaluated as potential therapeutic agents for ALL. Inotuzumab ozogamicin, which links an anti-CD22 antibody to the chemotherapeutic agent, calicheamicin, showed a CR rate of 66% in relapsed/refractory ALL patients. Among remitters, 78% achieved a complete molecular response.[34,35] In a Phase III study comparing inotuzumab to standard chemotherapy, complete response was obtained in 81% versus 33%, respectively.[36] MRD-negativity in remitters was observed in 78% of cases versus 28%. In the mini-Hyper-CVD-inotuzumab ± rituximab, the overall response rate was 97% with MRD-negativity in 100% of cases and the 2-year OS was 64%.[37] Venous occlusive disease did not exceed 10%. An even more sophisticated antibody design is that of blinatumomab, a bispecific T cell engaging (BiTE®) antibody, which directly recruits effector T cells to augment the anti-leukaemic effect. Blinatumomab contains the variable domains of a CD19 antibody and a CD3 antibody, which are joined by a non-immunogenic linker. On binding to CD19, cytotoxic T cells become activated and induce cell death via the pore-forming perforin system. In MRD-positive ALL, blinatumomab facilitated a switch to MRD-negativity in 80% of cases.[38] In adult patients with refractory/relapsed ALL, the response rate was 43% and the MRD response rate in remitters was 82%.[39] The most frequently reported adverse events were Grade 3/4 reversible neurological events (17–19%).[38,40] Monoclonal antibody development could however, be challenged by the novel approach based on chimeric antigen receptor (CAR) T cells targeting CD19, which has already been tested with encouraging first results.[41] In relapsed/refractory patients, a response was obtained in 93% of cases, and the 1-year relapse-free survival was 55%.[42] Immune toxicity after infusion correlates with disease burden. Treatment may consist of short courses of steroids and interleukin-6R antagonism via tociluzumab.[43] A maximum tolerated dose ($1x10^6$/kg) was defined beyond which toxicity was frequently unacceptable.[44] MRD-

negativity was reached in 50–83% of cases and a large proportion of patients went on to receive allogeneic SCT after CAR T cells.[44,45] CAR T cells targeting CD22 or CD19/CD22 are under investigation.

SPECIFIC SUBTYPES

T Cell Lineage Acute Lymphoblastic Leukaemia

Genetic and epigenetic reprogramming events, which transform T cell precursors into malignant T-ALL lymphoblasts, have been extensively characterised over the past decade. T lineage ALL are distributed into different subtypes according to maturation stage: thymic (56%), early-T (23%), and mature-T (21%). A correlation has been reported between maturation stage and outcome with the best outcome for thymic T cell ALL (OS: 60–70%) compared with early (33%) and mature-T phenotypes (22%).[46,47] T cell lineage ALL is caused by an accumulation of more than 10 genomic lesions.[48] T-ALL leukaemic cells commonly have rearrangements which dysregulate oncogenes, including *HOX11, HOX11L2, LYL1, TAL1*, and *MLL* genes.[49] T-ALL exhibits a very high frequency of homozygous deletion of *CDKN2A/CDKN2B* and activating mutations of *NOTCH1*. Genome-wide analyses have also identified deletions dysregulating *LMO2*, amplification of *MYB*, amplification associated with the *NUP214-ABL1* rearrangement, fusion of *SET* to *NUP214*, and deletion and mutation of *BCL11B, FBXW7, PHF6, PTEN, PTPN2*, and *WT1*. Additional genetic defects are shared among the different genetic subclasses and activate oncogenic signalling cascades. The IL7R/JAK1/3-STAT5 axis is an important oncogenic pathway in T cell ALL which represents about 28% of mutant cases.[50] There is a strong association between JAK1/JAK3/IL7R and epigenetic mutations. *IL7R* mutations result in ligand-independent receptor activation.[51] Two types of JAK3 mutations are observed in T-ALL: *JAK3* mutants that signal through *JAK1* and *JAK3* mutants that are *JAK1* independent. The former are sensitive to JAK1 inhibition, such as tofacitinib or ruxolitinib.

In an attempt to start integrating molecular findings into the clinic, a French group has implemented a *NOTCH1/FBXW7/RAS/PTEN*-based risk classification, in which patients with mutated *NOTCH1* or *FBXW7* who present with a wild-type *RAS* and *PTEN* are considered low-risk, whereas all other adult T-ALL are classified as high-risk.[52] Additional inclusion of poor prognostic genetic markers, such as *DNMT3A, IDH1*, and *IDH2*, should be considered to further improve this risk stratification approach.

Recent studies have characterised early T cell precursor (ETP) ALL that lacks expression of several T cell markers and exhibits aberrant expression of myeloid stem cell markers.[53] Recurring mutations have been described in various pathways: haematopoietic development, RAS and cytokine receptor signalling, and chromatin-modifying genes. Consecutively, ETP ALL belongs to the group of neoplasms that arise in progenitors that retain multilineage potential and possibly biphenotypic and bilineal ALL. The involvement of JAK-STAT and PRC2 pathways in this subtype of ALL suggests that JAK inhibition and/or chromatin-modifying agents may be therapeutically useful in ETP ALL.

Leukaemia patients with T lineage ALL are still treated by high-dose multiagent chemotherapy, potentially followed by allogeneic SCT. With regard to paediatric regimens, clinical studies have examined the dose intensification of non-myelosuppressive drugs. The use of dexamethasone, a higher total dose of methotrexate, or a higher total dose of L-asparaginase have been shown to improve outcomes of adult patients with T-lineage ALL.[54] However, about 40% of these patients relapse, wing to acquired therapy resistance, and have a poor prognosis with high mortality rates. Following a better understanding of T cell pathology, several new therapeutic options are forthcoming, including the purine analogue nelarabine[55] and the purine nucleoside phosphorylase forodesine.[56]

Gamma-secretase, which participates in the release of the NOTCH1 intracellular domain before it translocates to the nucleus, is a potential therapeutic target. Gamma-secretase inhibitors were shown to enhance the apoptotic effect induced by chemotherapeutic agents[57] or dexamethasone.[58] Alternative strategies include specific NOTCH1-inhibitory antibodies and stapled peptides that target the NOTCH1 transcriptional complex.[59]

The *NUP214-ABL1* fusion is mainly present in T lineage ALL expressing *HOX11* or *HOX11L2*. It recently appeared that imatinib mesylate could potentially be efficacious in these cases. Mammalian target of rapamycin (mTOR) acts as

a nutrient sensor and regulator of translation in encoding proteins involved in regulating the G1 to S phase transition. Rapamycin and the second-generation mTOR inhibitors (temserolimus, everolimus, deforolimus) form potentially synergistic combinations with doxorubicin and methotrexate and might modulate glucocorticoidv resistance in T-ALL.[60]

The aberrant activation of JAK/STAT signalling in ETP ALL lymphoblasts suggests the JAK1/2 inhibitor ruxolitinib and the JAK3 inhibitor tofacitinib may be efficacious therapeutic agents in this setting.[61] Bcl2 is another attractive target for therapy in immature subtypes of T cell lineage ALL.[62]

Philadelphia Chromosome Positive Acute Lymphoblastic Leukaemia

The Ph chromosome [t(9;22) (q34;q11)] is the most frequent cytogenetic abnormality in human leukaemia. It produces a fusion gene on chromosome 22, namely *BCR-ABL*. Patients with Ph-ALL represent approximately 25% of adult B lineage ALL patients. The management of adult Ph-positive ALL has been recently reviewed.[63]

Imatinib mesylate, a tyrosine kinase inhibitor (TKI) that targets BCR-ABL, is now an integral component of therapy for Ph-ALL. CR can be obtained in almost 95% of cases. The current consensus is that imatinib improves patient outcomes compared with historical control patients treated with chemotherapy alone. Efficacy analyses based on *BCR-ABL* transcript levels showed a clear advantage of the simultaneous over the alternating schedule.[64] It also appeared important to maintain imatinib dose intensity during the initial phase of treatment.[65] Furthermore, an induction regimen combining reduced-intensity chemotherapy and imatinib was recently validated in a randomised study in which it was compared with a standard imatinib/chemotherapy treatment.[66] The rate of molecular remission increased from 5% to >50%, and the 5-year survival to ≥50%. The number of patients able to receive SCT, and the outcome of SCT, has improved.[66] However, imatinib is ineffective at preventing or treating CNS involvement.[67] Faster and deeper molecular responses can be achieved with second-generation TKIs (dasatinib and nilotinib), median MRD was lower and a better post-SCT outcome was observed.[68] Dasatinib plus chemotherapy achieved CR in 96% of older patients with Ph-ALL.[69] EFS was 41% at 3 years. The combination of nilotinib with high-dose cytotoxic drugs achieved high cumulative complete molecular remission and haematologic relapse-free survival rates.[70] Ponatinib, a third-generation TKI targeting the *T315I* mutation which is resistant to imatinib and second-generation TKIs, is currently in development.[71] The combination of chemotherapy (hyper-CVAD) with ponatinib is effective in achieving early sustained remissions with major molecular response in 95% of patients.[72] The 2-year EFS rate was 81%. OS was similar with or without controlling for allogeneic SCT. Ponatinib was given at 45 mg daily for 14 days during induction and continuously at 30 mg thereafter until the achievement of complete molecular remission, and then at 15 mg daily indefinitely thereafter. New strategies, including dosing titration of ponatinib and optimised control of vascular risk factors, might further improve outcomes.

Allogeneic SCT in first CR remains the standard of care for adult Ph-ALL.[66,73] Reducing BCR-ABL transcript levels has resulted in a lower pre-SCT leukaemia burden. Allogeneic SCT with myeloablative conditioning regimen overcomes MRD prior to SCT in some but not all studies. It is superior to allogeneic SCT after non-myeloablative conditioning regimen if MRD is not considered, but may be equivalent in patients with complete molecular response. Non-myeloablative allogeneic SCT approaches are therefore promising in patients with Ph-ALL.[20] Treatment-related toxicity has been reported in 20–30% of cases with high rates of chronic GvHD.[23,74] Prophylactic TKI given after engraftment may improve outcomes by preventing a resurgence of the leukaemic clone.[75] However, the optimal duration of this treatment has not yet been established. In MRD-positive patients, imatinib at 400 mg/day has been shown to prevent relapse and to achieve molecular remission in 52% of cases after 1.5 months of treatment.[76] However, imatinib is sometimes poorly tolerated after allogeneic SCT and should either be discontinued or the dose reduced.[77] The median duration of negative MRD in patients with post-transplant imatinib administration has been reported as 6 months when imatinib was administered upon detecting MRD after allogeneic SCT compared with 12 months when imatinib was given as soon as possible after allogeneic SCT.[78] Reappearance of *BCR-ABL1* transcripts early after SCT identifies a small subset of patients who do

not benefit sufficiently from imatinib, and in whom alternative approaches should be explored.[79] Alternative therapies could be considered in patients who remain positive for *BCR-ABL* transcripts more than 2 months after starting imatinib therapy following transplant. Blinatumomab has shown promising results in patients with high-risk ALL.[38] Activity has been demonstrated in Ph-ALL with *T315I* mutation.[80] CR was achieved in 36% of all cases and in 40% of patients with mutation *T315I*. Preliminary results indicate that treatment with blinatumomab is able to convert MRD-positive ALL into a MRD-negative status. Eighty-eight percent of patients achieved a complete MRD response.[80]

Alternative donors may be considered for patients lacking a matched related or matched unrelated donor. Haploidentical SCT represents an encouraging treatment option.[81] The incidence of non-relapse mortality was similar between the patients who received HLA-matched donor cells and those who received haploidentical donor cells. The incidence of cytomegalovirus infection was, however, significantly higher in the latter group. Haploidentical SCT reduced the relapse rate. The status of umbilical cord blood transplantation in adults with Ph-ALL is not well established. Recent analyses showed that MRD-positivity before umbilical cord blood transplantation was associated with increased relapses.[82] Autologous SCT remains a possible therapeutic option when MRD is not present before the procedure.[66] Results of autologous SCT have significantly improved in the era of TKIs,[83,84] however there is no consensus on how best to use TKIs after autologous SCT.

BCR-ABL-LIKE Acute Lymphoblastic Leukaemia

Leukaemic cells from many patients with B cell lineage ALL lack known chromosomal alterations. A new entity of high-risk B cell precursor ALL has been recently described, namely 'Ph-like' ALL or '*BCR-ABL1*-like' ALL which is defined by a more similar gene signature than Ph-ALL without BCR-ABL translocation. This subtype represents up to 25% of adolescent and young adult ALL and is associated with a poor outcome, but incidence does not increase with age.[85] Comparative genomic hybridisation arrays and molecular cytogenetics are necessary for the diagnosis. However, validation of a robust gene expression classifier is still warranted for a routine clinical use.[86] More than 80% of Ph-like ALL cases have abnormalities in genes involved in B cell development, such as *IKZF1* deletions which facilitate leukaemia transformation by inducing constitutive kinase activation and signalling through the activation of ABL1 and/or JAK/STAT pathways,[87] and *CLRF2* overexpression and tyrosine kinase activating rearrangements involving *ABL1*, *JAK2, PDGFRB,* and several other genes.[88] The gene expression profile of *IKZF1*-mutated, *BCR-ABL1*-negative ALL is enriched for haematopoietic stem cell genes and exhibits decreased expression of B cell receptor signalling and differentiation genes.[88] Preclinical results in Ph-like ALL have suggested a potential role of a targeted therapeutic strategy according to the molecular profile of leukaemia cells.[89,90] Recently, several cases have been reported with responses to TKIs (imatinib or dasatinib).[91,92] Fifty percent of Ph-like ALL shows activation of JAK-STAT and PI3K/mTOR pathways and should also be sensitive to JAK2 (ruxolitinib) and mTOR inhibitors.[89,90,93]

Mixed-Lineage Leukaemia-Rearranged Acute Lymphoblastic Leukaemia

ALL carrying a chromosomal translocation involving the mixed-lineage leukaemia (*MLL*) gene on chromosome segment 11q23 or displaying a *MLL*-rearranged ALL in their molecular biology have a particularly poor prognosis. ALL with *MLL* translocations can be separated from conventional ALL. Immunophenotypic differences include a lack of the early lymphocyte antigen CD10, expression of proteoglycan NG2, and the propensity to co-express myeloid antigens. *MLL* shows a gene expression profile markedly different from that of conventional ALL.[94] These include genes expressed in early B cells (*MME, CD24, CD22, DNTT*), genes required for appropriate B cell development (*TCF3, TCF4, POU2AF1, LIG4*), and genes correlated with B precursor ALL (*SMARCA4*). Genes encoding for adhesion molecules are relatively over-expressed in *MLL*-rearranged ALL (*LGALS1, ANXAI, ANXA2, CD44, SPN*). Genes that are expressed in progenitors (*PROML1, FLT3, LMO2*), myeloid-specific genes (*CCNA1, SER-PINB1, CAPG, RNASE3*), and natural killer cell-associated gene (*NKG2D*) are also highly expressed in *MLL*-rearranged ALL. As *MLL* normally regulates the expression of *HOX* genes, its role in leukaemogenesis may include altered patterns of *HOX* gene expression.

Patients with *MLL*-rearranged ALL are highly resistant to steroids and L-asparaginase, but sensitive to nucleoside analogue drugs such as cytarabine, cladribine, and clofarabine,[95] suggesting

'acute myeloid leukaemia-like' chemotherapy courses may be efficacious in this type of leukaemia. Allogeneic SCT is still considered to play an important role as a consolidation therapy. However, patients achieving MRD negativity pre-SCT had better outcomes than those with persistent MRD pre or post-SCT.[96] MLL fusion proteins aberrantly recruit epigenetic regulatory proteins, including histone deacetylases, histone methyltransferases, bromodomain-containing proteins, and transcription elongation factors to mediate chromatin remodelling and regulate tumourigenic gene expression programmes. Histone deacetylases inhibitors, such as panobinostat, and cyclin-dependent kinase inhibitors were potent inducers of apoptosis in MLL-rearranged cells.[97] Combined MEK and VEGFR-2 inhibition strengthened the reduction in MLL-rearranged leukaemia cell survival by blocking the Akt/mTOR and MAPK pathways simultaneously.[98] Inhibitors of the protein methyltransferase DOT1L have also shown efficacy as single-agents and synergistically with other chemotherapeutics and hypomethylating agents.[99] AMPK inhibition, including endothelial nitric oxide synthetase and BCL-2 inhibition, was shown to synergistically enhance the antiproliferative effects of chemotherapy in *MLL*-rearranged cell lines.[100] Combining all-trans retinoic acid with cytarabine has also shown a synergistic anti-leukaemic effect in *MLL*-rearranged-positive cells.[101] FLT3 expression identifies *MLL-AF4*$^{+}$ ALL patients at very high-risk of treatment failure, emphasising the potential value for FLT3 inhibitors.[102] These findings highlight a new therapeutic approach to potentially overcome the resistance of *MLL*-rearranged ALL to conventional chemotherapies.

CONCLUSIONS AND PERSPECTIVES

Due to risk-stratified treatment, more optimised treatment protocols, and improved supportive care, the 5-year EFS on contemporary treatment protocols in adult ALL is approaching 50%. Treatment is relatively well structured with the development of risk-adapted therapies and paediatric-like protocols and by standardisation and monitoring of MRD. Advances in the management of adult ALL including the identification of novel sub-entities, the evaluation of MRD, and the use of new targeted therapies has progressively led to a more personalised treatment approach in adult ALL.

Genomic analysis represents a major advance for identifying the genetic basis of leukaemogenesis and disease relapse in ALL. Sensitive detection of mutations during therapy is important to track progression and adjust therapy. Relapse usually arises from a minor clone unless relapse-enriched mutations are already present. Early detection of minor clones may guide prediction of relapse. Genomic analysis has been especially important for T cell ALL and raised new challenges, such as to better understand how T cell oncogenes and tumour suppressors co-operate to drive overt disease; to determine the exact order in which genetic lesions are acquired during initiation, progression, and maintenance of the disease; to determine the exact cell of origin for T cell transformation, and the molecular mechanism that regulates pre-leukaemic stem cell activity of thymic precursors; and to determine the oncogenic contributions of deregulated microRNAs, long non-coding RNAs, enhancer activities, chromatin remodelling, and epigenetic changes in the setting of malignant T cell transformation.

Molecular targets can be identified in the vast majority of patients with adult ALL. MRD is currently the main prognostic factor. MRD methodology and terminology need standardisation. MRD can be identified at different times in the disease and potentially allow risk stratification. However, MRD relevance at any time-point is dependent on specific prior therapy and possibly cannot be extrapolated from one protocol to another.

Further studies need to address how new drugs should be combined with up-front chemotherapy and how this can be reduced to minimise side effects without jeopardising clinical outcome. Harmonising the backbone of chemotherapeutic protocols of different study groups should also be beneficial for performing clinical trials with new drugs in rare subsets of patients and accelerate the development of those drugs in co-operation with pharmaceutical companies.

Advances have been particularly prevalent over the last few years in Ph-ALL. Less chemotherapy intensity in induction prior to SCT was shown not to be inferior, while initial dose intensity of TKI was demonstrated as important. Second and third-generation TKIs improved results in terms of MRD, but there are no prospective randomised trials on outcome. Overall, SCT is still superior to no SCT, but subsets of patients (perhaps likely defined by MRD) may do well long-term

without allogeneic SCT. Furthermore, it has been suggested that a lower number of patients with Ph-ALL need allogeneic SCT when treated with second-generation TKIs. After molecular response achievement, autologous SCT or chemotherapy plus TKI yielded similar results to those seen following allogeneic SCT. Further questions in Ph-ALL should focus on the reduction of allogeneic SCT indications and whether a chemo-free strategy could become a reality with the incorporation of other targeted therapies, such as monoclonal antibodies and immunotherapy.

Allogeneic SCT still remains a major issue in the treatment of adult ALL. There are recent trends showing an increased use of reduced-intensity conditioning and alternative donors. Issues to be investigated with reduced-intensity conditioning include the optimal chemotherapy to be applied prior to reduced-intensity conditioning, the optimal type of conditioning regimen to be used, and how to improve quality of life. Designing maintenance strategies after reduced-intensity conditioning allogeneic SCT may further improve the outcome. The role of alternative donors is yet to be established. MRD and molecular marker analyses should in time modify therapeutic strategies.

New monoclonal antibodies and immunotherapy tools are currently changing the landscape of ALL therapy. However, many questions remain: challenges with CAR T cells including definition of the optimal CAR design and effector cell population; how to modulate immunotoxicity without losing efficacy; defining the optimal placement of CAR therapy either as bridge to SCT or alternative to SCT; defining the optimal therapeutic strategy with administration in relapse or after remission achievement; and defining when it should be given in relation to chemotherapy. Another major issue with such treatments is the cost and the complexity of manufacture.

Over the coming years, the treatment of adult ALL will certainly change from disease-type to molecular-target type and from risk-stratified treatment schedules to more personalised therapies. More specific therapies and new immunotherapy-based approaches are the most promising advances for improving prognosis and reducing treatment-related morbidity.

REFERENCES

1. Al Ustwani O et al. Clinical updates in adult acute lymphoblastic leukemia. Crit Rev Oncol Hematol. 2016;99:189-99.

2. Hoelzer D. Personalized medicine in adult acute lymphoblastic leukemia. Haematologica. 2015;100(7):855-8.

3. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol. 2011;29(5):532-43.

4. Pulte D et al. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. Blood. 2009;113(7):1408-11.

5. Gökbuget N. How I treat older patients with ALL. Blood. 2013;122(8):1366-75.

6. Tavernier E et al. Outcome of treatment after first relapse in adults with acute lymphoblastic leukemia initially treated by the LALA-94 trial. Leukemia. 2007; 21(9):1907-14.

7. Gökbuget N et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. Blood. 2012; 120(10):2032-41.

8. Inaba H et al. Acute lymphoblastic leukaemia. Lancet. 2013;381(9881): 1943-55.

9. Mullighan CG et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 2008; 322(5906):1377-80.

10. Mullighan CG et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature. 2011;471(7337):235-9.

11. Figueroa ME et al. Integrated genetic and epigenetic analysis of childhood acute lymphoblastic leukemia. J Clin Invest. 2013;123(7):3099-111.

12. Yang JJ et al. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. Nat Genet. 2011; 43(3):237-41.

13. Beldjord K et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014;123(24):3739-49.

14. Boissel N et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. J Clin Oncol. 2003; 21(5):774-80.

15. Van der Sluis IM et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. Haematologica. 2016;101(3):279-85.

16. Huguet F et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009;27(6):911-8.

17. Gupta V et al.; Acute Leukemia Stem Cell Transplantation Trialists' Collaborative Group. Allogeneic, but not autologous, hematopoietic cell transplantation improves survival only among younger adults with acute lymphoblastic leukemia in first remission: an individual patient data meta-analysis. Blood. 2013;121(2):339-50.

18. Goldstone AH et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/ maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood. 2008;111(4):1827-33.

19. Pulsipher MA et al. The addition of sirolimus to tacrolimus/methotrexate GVHD prophylaxis in children with ALL: a phase 3 Children's Oncology Group/ Pediatric Blood and Marrow Transplant Consortium trial. Blood. 2014;123(13): 2017-25.

20. Mohty M et al. Reduced intensity conditioning allogeneic stem cell transplantation for adult patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation Haematologica. 2008; 93(2):303-6.

21. Mohty M et al. Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. Blood. 2010; 116(22):4439-43.

22. Marks DI et al. The outcome of full-intensity and reduced-intensity conditioning matched sibling or unrelated donor transplantation in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia in first and second complete remission. Blood. 2010; 116(3):366-74.

23. Bachanova V et al. Prolonged survival in adults with acute lymphoblastic leukemia after reduced-intensity conditioning with cord blood or sibling donor transplantation.Blood. 2009; 113(13):2902-5.

24. Passweg JR et al. Hematopoietic SCT in Europe 2013: recent trends in the use of alternative donors showing more haploidentical donors but fewer cord blood transplants. Bone Marrow Transplant. 2015;50(4):476-82.

25. Kekre N, Antin JH. Hematopoietic stem cell transplantation donor sources in the 21st century: choosing the ideal donor when a perfect match does not exist. Blood. 2014;124(3):334-43.

26. Ruggeri A et al. Comparison of outcomes after unrelated cord blood and unmanipulated haploidentical stem cell transplantation in adults with acute leukemia. Leukemia. 2015;29(9):1891-900.

27. Le Jeune C, Thomas X. Antibody-based therapies in B-cell lineage acute lymphoblastic leukaemia. Eur J Haematol. 2015;94(2):99-108.

28. Thomas DA et al. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. Cancer. 2006;106(7):1569-80.

29. Hoelzer D et al. Improved outcome of adult Burkitt lymphoma/leukemia with rituximab and chemotherapy: report of a large prospective multicenter trial. Blood. 2014;124(26):3870-9.

30. Thomas DA et al. Long-term outcome after hyper-CVAD and imatinib (IM) for de novo or minimally treated Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-ALL). J Clin Oncol. 2010;28(Suppl 15):6506.

31. Hoelzer D et al. Immunochemotherapy with rituximab improves molecular CR rate and outcome in CD20+ B-lineage standard and high risk patients; results of 263 CD20+ patients studied prospectively in GMALL study 07/2003. Blood. 2010;116(21):170.

32. Maury S et al. Addition of rituximab improves the outcome of adult patients with CD20-positive, Ph-negative, B-cell precursor acute lymphoblastic leukemia (BCP-ALL): results of the randomized Graall-R 2005 study. Blood. 2015;126(23):1.

33. Sasaki K et al. Phase II study of the frontline Hyper-CVAD in combination with ofatumumab for adult patients (pts) with CD-20 positive acute lymphoblastic leukemia (ALL). Blood. 2015;126(23):1295.

34. Kantarjian H et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. Lancet Oncol. 2012;13(4):403-11.

35. Kantarjian H et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphoblastic leukemia. Cancer. 2013;119(15):2728-36.

36. Kantarjian HM et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016. [Epub ahead of print].

37. Jabbour E et al. Frontline inotuzumab ozogamicin in combination with low-intensity chemotherapy (mini-hyper-CVD) for older patients with acute lymphoblastic leukemia (ALL). 83. American Society of Hematology 57th Annual Meeting & Exposition, 5th–8th December, 2015.

38. Topp MS et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011;29(18):2493-8.

39. Topp MS et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicenter, single-arm, phase 2 study. Lancet Oncol. 2015;16(1):57-66.

40. Topp MS et al. Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. J Clin Oncol. 2014;32(36):4134-40.

41. Brentjens RJ et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013;5(177):177ra38.

42. Maude SL et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16): 1507-17.

43. Maude SL et al. Managing cytokine release syndrome associated with novel T cell-engaging therapies. Cancer J. 2014; 20(2):119-22.

44. Lee DW et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385(9967):517-28.

45. Davila ML et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014;6(224):224ra25.

46. Vitale A et al. Adult T-cell acute lymphoblastic leukemia: Biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMEMA LAL 0496 protocol. Blood. 2006;107(2):473-9.

47. Marks DI et al. T-cell acute lymphoblastic leukemia in adults: Clinical features, immunophenotype, cytogenetics, and outcome from the large randomized prospective trial UKALL XII/ ECOG 2993. Blood. 2009;114(25):5136-45.

48. De Keersmaecker K et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013;45(2):186-90.

49. Graux C et al. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia. 2006;20(9): 1496-510.

50. Vicente C et al. Targeted sequencing identifies associations between IL7R-JAK mutations and epigenetic modulators in T-cell acute lymphoblastic leukemia. Haematologica. 2015;100(10):1301-10.

51. Degryse S, Cools J. JAK kinase inhibitors for the treatment of acute lymphoblastic leukemia. J Hematol Oncol. 2015;8:91.

52. Trinquand A et al. Toward a NOTCH1/ FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. J Clin Oncol. 2013; 31(34):4333-42.

53. Coustan-Smith E et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10(2):147-56.

54. Kako S et al. Meta-analysis and meta-regression analysis to compare the outcomes of chemotherapy for T- and B-lineage acute lymphoblastic leukemia (ALL): the use of dexamethasone, L-asparaginase, and/or methotrexate may improve the outcome of T-lineage ALL. Ann Hematol. 2016;95(1):87-92.

55. DeAngelo DJ et al. Nelarabine induces

complete remissions in adults with relapsed or refractory T-lineage acute lymphoblastic leukemia or lymphoblastic lymphoma: Cancer and Leukemia Group B study 19801. Blood. 2007;109(12): 5136-42.

56. Gandhi V et al. A proof-of-principle pharmacokinetic, pharmacodynamic, and clinical study with purine nucleoside phosphorylase inhibitor immucillin-H (BCX-1777, forodesine). Blood. 2005; 106(13):4253-60.

57. Yoon SO et al. Gamma secretase inhibitors enhance vincristine-induced apoptosis in T-cell in a NOTCH-independent manner. Apoptosis. 2014; 19(11):1616-26.

58. Samon JB et al. Preclinical analysis of the gamma-secretase inhibitor PF-03084014 in combination with glucocorticoids in T-cell acute lymphoblastic leukemia. Mol Cancer Ther. 2012;11(7):1565-75.

59. Wu Y et al. Therapeutic antibody targeting of individual Notch receptors. Nature. 2010;464(7291):1052-7.

60. Teachey DT et al. mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. Blood. 2008;112(5):2020-3.

61. Maude SL et al. Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. Blood. 2015;125(11):1759-67.

62. Peirs S et al. ABT-199 mediated inhibition of BCL-2 as a novel therapeutic strategy in T-cell acute lymphoblastic leukemia. Blood. 2014;124(25):3738-47.

63. Chiaretti S, Foà R. Management of adult Ph-positive acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015:406-13.

64. Wassmann B et al. Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia-positive acute lymphoblastic leukemia (Ph^+ ALL). Blood. 2006;108(5):1469-77.

65. Lim SN et al. Long-term follow-up of imatinib plus combination chemotherapy in patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Am J Hematol. 2015;90(11):1013-20.

66. Chalandon Y et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood. 2015;125(24):3711-9.

67. Leis JF et al. Central nervous system failure in patients with chronic myelogenous leukemia lymphoid blast crisis and Philadelphia chromosome positive acute lymphoblastic leukemia treated with imatinib (STI-571). Leuk Lymphoma. 2004;45(4):695-8.

68. Foà R et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2011; 118(25):6521-8.

69. Rousselot P et al. Dasatinib (Sprycel®) and low intensity chemotherapy for first-line treatment in patients with de novo Philadelphia positive ALL aged 55 and over : final results of the EWAL-Ph-01 study. 666. 54th ASH Annual Meeting and Exposition, 8th–12th December, 2012.

70. Kim DY et al. Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. Blood. 2015;126(6):746-56.

71. Cortes JE et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013; 369(19):1783-96.

72. Jabbour E et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: a single-centre, phase 2 study. Lancet Oncol. 2015;16(15): 1547-55.

73. Fielding AK, Goldstone AH. Allogeneic haematopoietic stem cell transplant in Philadelphia-positive acute lymphoblastic leukaemia. Bone Marrow Transplant. 2008; 41(5):447-53.

74. Ram R et al. Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high risk acute lymphoblasticleukemia. Haematologica. 2011;96(8):1113-20.

75. Carpenter PA et al. Prophylactic administration of imatinib after hematopoietic cell transplantation for high-risk Philadelphia chromosome-positive leukemia. Blood. 2007;109(7): 2791-3.

76. Wassmann B et al. Early molecular response to post-transplantation imatinib determines outcome in MRD+ Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood. 2005;106(2): 458-63.

77. Wassmann B et al. Up-front versus minimal residual disease triggered imatinib after stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukaemia: interim results of a randomized phase III GMALL study. Bone Marrow Transplant. 2009;43(Suppl 1):S48.

78. Nishiwaki S et al. Impact of post-transplant imatinib administration on Philadelphia chromosome-positive acute lymphoblastic leukaemia. Anticancer Res. 2010;30(6):2415-8.

79. Pfeifer H et al. Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for BCR-ABL1-positive acute lymphoblastic leukemia. Leukemia. 2013;27(6):1254-62.

80. Martinelli G et al. Complete molecular and hematologic response in adult patients with relapsed/refractory (R/R) Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia (ALL) following treatment with blinatumomab: results from a phase 2 single-arm, multicenter study (ALCANTARA). Blood. 2015;126(23):679.

81. Gao L et al. Favorable outcome of haploidentical hematopoietic stem cell transplantation in Philadelphia chromosome-positive acute lymphoblastic leukemia: a multicentre study in Southwest China. J Hematol Oncol. 2015;8:90.

82. Tucunduva L et al. Impact of minimal residual disease on outcomes after umbilical cord blood transplantation for adults with Philadelphia-positive acute lymphoblastic leukaemia: an analysis on behalf of Eurocord, Cord Blood Committee and the Acute Leukaemia working party of the European group for Blood and Marrow Transplantation. Br J Haematol. 2014;166(5):749-57.

83. Giebel S et al. Improving results of autologous stem cell transplantation for Philadelphia-positive acute lymphoblastic leukaemia in the era of tyrosine kinase inhibitors: a report from the Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation. Eur J Cancer. 2014;50(2): 411-7.

84. Böhm A et al. Stable non-transforming minimal residual disease in Philadelphia chromosome positive acute lymphoblastic leukemia after autologous transplantation: origin from neoplastic yet 'pre-leukemic' stem cells? Leuk Lymphoma. 2011;52(5):842-8.

85. Herold T et al. Ph-like acute lymphoblastic leukemia in older adults. N Engl J Med. 2014;371(23):2235.

86. Harvey RC et al. Development and validation of a highly sensitive and specific gene expression classifier to prospectively screen and identify B-precursor acute lymphoblastic leukemia (ALL) patients with a Philadelphia chromosome-like ("Ph-like" or "BCR-ABL1-like") signature for therapeutic targeting and clinical intervention. Blood. 2013;122(21):826.

87. Roberts KG et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22(2):153-66.

88. Mullighan CG et al. Deletion of IKZF1 and prognostic in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5): 470-80.

89. Maude SL et al. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-

like acute lymphoblastic leukemia. Blood. 2012;120(17):3510-8.

90. Eyre T et al. Episomal amplification of NUP214-ABL1 fusion gene in B-cell acute lymphoblastic leukemia. Blood. 2012; 120(22):4441-3.

91. Lengline E et al. Successful tyrosine kinase inhibitor therapy in a refractory B-cell precursor acute lymphoblastic leukemia with EBF1-PDGFRB fusion. Haematologica. 2013;98(11):e146-8.

92. Masuzawa A et al. Poor responses to tyrosine kinase inhibitors in a child with precursor B-cell acute lymphoblastic leukemia with SNX2-ABL1 chimeric transcript. Eur J Haematol.2014;92(3):263-7.

93. Roberts KG et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005-15.

94. Armstrong SA et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30(1):41-7.

95. Stumpel DJ et al. The potential of clofarabine in MLL-rearranged infant acute lymphoblastic leukaemia. Eur J Cancer. 2015;51(14):2008-21.

96. Sutton R et al. Persistent MRD before and after allogeneic BMT predicts relapse in children with acute lymphoblastic leukaemia. Br J Haematol. 2015;168(3):395-404.

97. Baker A et al. The CDK9 inhibitor dinaciclib exerts potent apoptotic and antitumor effects in preclinical models of MLL-rearranged acute myeloid leukemia. Cancer Res. 2016;76(5):1158-69.

98. Kampen KR et al. Insights in dynamic kinome reprogramming as a consequence of MEK inhibition in MLL-rearranged AML. Leukemia. 2014;28(3):589-99.

99. Klaus CR et al. DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. J Pharmacol ExpTher. 2014;350(3):646-56.

100. Accordi B et al. AMPK inhibition enhances apoptosis in MLL-rearranged pediatric B-acute lymphoblastic leukemia cells. Leukemia. 2013;27(5):1019-27.

101. Sakamoto K et al. Sensitivity of MLL-rearranged AML cells to all-trans retinoic acid is associated with the level of H3K4me2 in the RARα promotor region. Blood Cancer J. 2014;4:e205.

102. Chillón MC et al. Prognostic significance of FLT3 mutational status and expression levels in MLL-AF4+ and MLL-germline acute lymphoblastic leukemia. Leukemia. 2012;26(11):2360-6.

MANAGEMENT OF CHILDREN WITH SICKLE CELL DISEASE IN EUROPE: CURRENT SITUATION AND FUTURE PERSPECTIVES

***Raffaella Colombatti, Laura Sainati**

Veneto Region Reference Center for the Diagnosis and Management of Sickle Cell Disease in Childhood, Clinic of Pediatric Hematology Oncology, Department of Child and Maternal Health, Azienda Ospedaliera-University of Padova, Padova, Italy
**Correspondence to rcolombatti@gmail.com*

Disclosure: The authors have declared no conflicts of interest.
Received: 22.02.16 **Accepted:** 11.05.16
Citation: EMJ Hematol. 2016;4[1]:129-135.

ABSTRACT

Sickle cell disease (SCD) is the most common haemoglobinopathy worldwide and its frequency has steadily increased in Europe in the past decades. SCD is a complex multisystem disorder characterised by chronic haemolytic anaemia, vaso-occlusive crisis, and vasculopathy. Clinical manifestations can be very different, ranging from mild haemolysis to life-threatening acute clinical complications and chronic disabilities. This review will explore service delivery across Europe to children with SCD, reporting on the available minimum standards of care and future perspectives.

Keywords: Sickle cell disease (SCD), childhood, management, Europe.

INTRODUCTION

Sickle cell disease (SCD) is one of the most common severe monogenic disorders worldwide, with an average of 300,000 children born annually with sickle syndromes.[1] SCD was initially endemic in areas with prevalent malarial disease (Africa, the Mediterranean, Southern Asia). In Europe, it was present only in Greece and Southern Italy,[2] but historic and recent migration movements have increased the frequency of SCD in areas where it was previously uncommon. In Europe, SCD has become the paradigm of immigration haematology.[3] Although considered a rare disease because of its lower frequency in the 28 countries of the European Union (EU), SCD is the most prevalent genetic disease in France[4] and the UK,[5] and its frequency is steadily rising in many other countries in Northern, Central, and Southern Europe.[3,6-10] The World Health Organization (WHO) and the United Nations (UN) recognised inherited haemoglobin (Hb) disorders as a global public health problem in 2006[11] and 2008,[12] respectively, and to face this issue the WHO called upon national health systems "to design, implement, and reinforce …comprehensive national integrated programs for the prevention and management of SCD."[11] Following this invitation, several countries havedeveloped a specific response to meet the needs of patients with SCD, but the recent migrant flow from the Middle East and Africa has further increased the number of patients with SCD in many European countries, subsequently increasing the urgency to fill the gaps in specialised healthcare delivery to this vulnerable group of patients.

SCD is a chronic and complex multisystem disorder requiring comprehensive care that includes screening, prevention, health education, and management of acute and chronic complications.[13,14] Poor service organisation and episodic healthcare lead to higher rates of acute events and chronic complications with increased burden on hospital structures and higher costs for health systems.[15]

The European Hematology Association (EHA) recently published a consensus document, a 'Roadmap for Hematology Research in Europe'[16] with the aim of describing the major achievements in diagnosis and treatment of blood disorders in

Europe and identifying the greatest unmet clinical and scientific needs. For SCD, the necessity to improve treatment strategies for both acute and chronic complications was identified as a key area of intervention for optimising patient care, in order to positively affect patient health and quality of life, and to reduce hospitalisation length, patient disability, and cost of care.

Table 1: Genetic variants leading to sickle cell disease.

HbS/S
HbS/β^0 thalassaemia
HbS/β^+ thalassaemia
HbS/O Arab
HbS/D Punjab
HbS/C Harlem
HbC/S Antilles
HbS/Quebec-CHORI
HbS/C
HbA/S Oman

Table 2: Important clinical manifestations of sickle cell disease during childhood and adolescence.[14]

Acute manifestations
Bacterial sepsis or meningitis*
Recurrent vaso-occlusive pain (dactylitis, musculoskeletal, or abdominal pain)
Splenic sequestration*
Aplastic crisis*
Acute chest syndrome*
Stroke*
Priapism haematuria, including papillary necrosis
Chronic manifestations
Anaemia
Jaundice
Splenomegaly
Functional asplenia
Cardiomegaly and functional murmurs
Hyposthenuria and enuresis
Proteinuria
Gallstones (cholelithiasis)
Delayed growth and sexual maturation
Restrictive lung disease*
Pulmonary hypertension*
Avascular necrosis
Proliferative retinopathy
Leg ulcers
Transfusional haemosiderosis*

*Potential cause of mortality

This review will assess the management of children with SCD in European countries by exploring the current situation in the delivery of minimum standards of care, health service availability, and future perspectives.

SICKLE CELL DISEASE CLASSIFICATION AND CLINICAL MANIFESTATIONS

The term SCD is used to describe all the different genotypes that cause the characteristic clinical syndrome.[13] Its most frequent variant (sickle cell anaemia or homozygous SS disease) is caused by a single amino acid substitution at the sixth residue of the β-globin subunit (p.Glu6Val), which results in the production of the characteristic sickle Hb. Several double heterozygous forms also give rise to the clinical picture of SCD, the most common being the double heterozygous Sβ-thalassaemia° and SCD. The double heterozygous Sβ-thalassaemia° (S mutation coupled with a thalassaemia β° mutation) is the most severe form with a clinical picture similar to homozygous SS disease, while the double heterozygous SCD (in which the Hb composition is approximately 50% HbS and 50% HbC) and the Sβ-thalassaemia+ (a condition that presents a minimal amount of HbA) display intermediate severity. The genetic variants leading to SCD, including the less common ones, are shown in Table 1.

The mutated Hb leads to changes in the shape and behaviour of red blood cells resulting in haemolytic anaemia, vaso-occlusion, and vasculopathy, which are the hallmarks of SCD pathophysiology. Other factors such as hypercoagulability, inflammation, and hypoxia-reperfusion are also involved in the organ damage of SCD.

Despite being a monogenic disorder, SCD presents with extreme phenotypic variability. Some patients remain virtually asymptomatic, while others suffer repeated acute events requiring hospital admissions and chronic organ damage with increasing disabilities. The reasons for this variability have not been completely clarified, although a percentage of HbF and co-inheritance of α-thalassaemia and glucose 6-phosphate deficiency can have a role.[13,17-19] The most common clinical manifestations occurring in children with SCD are shown in Table 2.

MANAGEMENT OF SICKLE CELL DISEASE IN CHILDHOOD AND ACCESS TO MINIMUM STANDARDS OF CARE

In recent decades, advances in basic research and clinical investigation have produced a marked decrease in morbidity and mortality during early childhood.[20-23] Comprehensive care including newborn screening, prophylactic penicillin, effective vaccinations (against pneumococcus, haemophilus, and annual influenza), stroke prevention programmes with transcranial Doppler (TCD) screening, chronic transfusion for children at risk of stroke, and disease-modifying treatments such as hydroxyurea (HU) have largely contributed to these results. This has virtually eliminated mortality from infections and stroke in British, French, and Belgian cohorts in the past decade.[20-23] In contrast, mortality from sepsis and stroke is still an issue in areas where neonatal screening and stroke prevention programmes have been implemented more recently.[24,25] The benefit of the previously described interventions (comprehensive care, newborn screening, antibiotic prophylaxis and vaccination, TCD screening, and HU) have been proven and they are considered as minimum standards of care for patients with SCD in several national European guidelines. In fact, the first action undertaken in many countries to address SCD has been the creation of a national network or group of experts for SCD and the development of national guidelines tailored to each country's healthcare system characteristics and treatment availability.[5,26-30] Such guidelines can be accessed online by paediatricians and paediatric haematologists, and address the management of acute complications (such as fever, vaso-occlusive painful crisis, acute chest syndrome, splenic sequestration) and chronic organ damage (pulmonary hypertension, kidney disease, cognitive impairment, etc.), detailing clinical pathways for accurate diagnosis and treatment.

The delivery of specialised, multidisciplinary, comprehensive care to a socially vulnerable population like the one constituted by patients with SCD in Europe, who are mainly first or second-generation immigrants, was challenging.[31]

Health operators in all countries were faced with language, cultural, and social barriers that in many cases were overcome by developing patient-centred services focussed on health education, with multilingual leaflets or booklets also available online (as in the case of the ones developed in France: www.rosfed.fr). A co-ordinated system of care between specialised centres, smaller hospitals, and primary care physicians is present in some areas and is useful to facilitate appropriate access to healthcare. Wherever implemented, comprehensive medical strategies, intended as global and holistic care, have reduced emergency and inpatient admissions while improving adherence to treatment both in the UK and Italy.[31,32]

NEWBORN SCREENING

Newborn screening allows early identification of affected patients, introduction of penicillin prophylaxis from 2 months of age, and reduction of mortality from infection, while allowing prompt enrolment in comprehensive care programmes. Universal newborn screening is available in the UK, Netherlands, several cities in Belgium, and Spain.[5,7,33-36] Targeted screening for high-risk populations according to ethnic origin is available in France.[37] Pilot newborn screening projects have been undertaken in Ireland, Italy, and Germany,[8,38-43] but a national screening programmeis yet to be developed in these countries. Antenatal screening in the framework of anational programme for the prevention of haemoglobinopathies is present in Greece.[2] Several obstacles or limits have been described in the implementation of the screening programmes;[44-48] first and foremost the lack of funding from health authorities.[8,40] Ethnic and racial issues due to the heritage of the second world war have also slowed the development of newborn screening or hampered the communication of test results to carriers in the Netherlands and Germany.[29,44] Failure to identify affected patients in selective newborn screening due to misinterpretation of patient origin has also been described and raises the need for increased attention.[44] After screening results are available, the management of patients and families varies across the countries, from being invited to genetic counselling[45] to being notified and taken into paediatric SCD reference centres.[4,5,8] Global comprehensive care after newborn screening is generally seen as the gold standard.

PENICILLIN PROPHYLAXIS AND VACCINATIONS

Penicillin prophylaxis and vaccination coverage have dramatically reduced the incidence of sepsis and mortality from infections in patients with

SCD, not only in research studies but also in clinical care settings.[20,31] The best results have been obtained in countries where newborn screening is available. While there is general agreement on the prescription of penicillin prophylaxis and vaccinations, few data are available in Europe on patient adherence to prophylaxis and vaccination coverage, as well as resistance to pneumococcus in children undergoing prophylaxis. Research in these areas could be useful to address possible barriers to treatment or to optimise prevention strategies. Annual influenza vaccination is also recommended in many guidelines, but the data on actual vaccination coverage from the various countries is scarce and does not always meet optimal standards.[49-51]

STROKE PREVENTION: TRANSCRANIAL DOPPLER AND CHRONIC TRANSFUSION

Extensive research has been conducted in Europe on the management of cerebrovascular complications in children with SCD.[17,21,22] TCD for evaluation of intracranial circulation is widely recommended and recently, French and UK groups have included the screening of the extracranial circulation[52-53] as part of the protocol for stroke prevention and reduction of silent cerebral infarcts. Despite the strong recommendation to perform TCD screening in every child with SCD starting at 2 years of age, <45% of children with SCD receive adequate screening with TCD in the UK.[54] Data from other European countries are lacking. Several barriers to TCD screening have been identified, including: technical issues due to instrument availability and protocol standardisation (imaging versus non-imaging TCD), lack of trained personnel, and unfavourable schedules with TCD appointments on different days from haematology visits.[31,55] Efforts should be made to investigate actual coverage of TCD screening and efficacy of stroke prevention in the remaining European countries in order to optimise treatment. A multicentre study focussing on the provision of TCD skills was delivered through an educational programme conducted in the UK, Ireland, and Italy, and demonstrated the success of a modular TCD training programme in achieving consistent, standardised, and comparable evaluation in three European countries.[56]

Patients at risk of stroke according to TCD results are offered chronic transfusion through simple top-up or exchange transfusions. The latter reduce iron overload, but are not available in every centre. While TCD allows identification of patients at risk of stroke and the ability to initiate appropriate treatment, it is not useful in screening for the other cerebrovascular complications of SCD, such as silent cerebral infarcts.

DISEASE-MODIFYING TREATMENTS: HYDROXYUREA, RED BLOOD CELL TRANSFUSION, AND BONE MARROW TRANSPLANTATION

HU is currently the only approved and routinely used drug for SCD. It reduces the frequency of acute vaso-occlusive painful crisis and acute chest syndrome, it improves organ function,[5,28-29] and it has recently been demonstrated to have a role in stroke prevention.[57] European centres have participated in proving the safety and benefits of HU in children with SCD.[23,58-60]

Table 3: Practical suggestions for future perspectives.

Expand universal newborn screening to all European countries with sufficient prevalence of disease, whether in the indigenous or the immigrant population
Expand access to minimum standards of care, including vaccinations, antibiotic prophylaxis, and transcranial Doppler for stroke prevention, by increasing the number of skilled personnel and service availability
Increase the use of disease modifying treatments for the paediatric age group, such as hydroxyurea, in formulations that are suitable for children (low-dose tablets or syrups) in all countries
Standardise diagnostic, treatment, and care strategies across European countries
Improve transitional services: more children are surviving into adulthood and the haematology community needs to organise a pathway of care across all ages
Neurological complications go beyond stroke, involving loss of cerebral matter and impairment of cognition; diagnostic tools, severity markers, and targeted treatments need to be developed
Develop wide clinical trials to investigate personalised medicine and new drugs, especially for the management of painful vaso-occlusive crisis, which is still suboptimal

Currently, a study is underway to determine the long-term effects of HU in a European cohort.[61] However, data regarding prescription of HU and adherence to HU treatment in Europe are lacking, and many publications coming from the USA report that there is an under-prescription and underuse of HU;[62] this highlights caregiver and patient barriers, and is a field that warrants investigation. Some countries do not even have a paediatric formulation available, and have to acquire it from abroad or use inappropriate schedules, which is also an urgent issue to resolve.[63]

Red blood cell transfusions are a pivotal treatment of SCD, both for acute emergencies and chronic complications.[5,27-29] Lack of ethnically matched donors is a concerning issue and extensive cell phenotype matching is recommended to avoid alloimmunisation.

The only curative option for SCD is bone marrow transplantation (BMT). The description of the indications for BMT, and of the different types of donor and conditioning regimens that can be used for BMT, goes beyond the scope of this review. It is worth noting, however, that several European centres have the resources to increase the possibility of performing BMT by using one of the parents as a donor (haploidentical transplantation).

OPEN ISSUES AND FUTURE PERSPECTIVES

The past few decades have seen service organisation for patients with SCD in several European countries. While high-level research is conducted in many centres and excellent care is routinely delivered, very few data report outcomes of healthcare delivery and utilisation across Europe. No up-to-date data on the burden of SCD across the EU is available, due to the absence of an organised system of data collection and to the lack of widespread newborn screening in many countries. Information on the rate of hospitalisation, length of stay, readmission rate, outpatient service utilisation, and outcome data, including mortality, are limited to some centres. A great deal of literature on healthcare utilisation, service delivery, and costs of care for SCD is available for the USA, but similar data have not yet been produced from Europe and this is a gap that should be filled to allow better service implementation.

Further aspects of care require co-ordinated action. Cerebrovascular complications are not limited to stroke prevention; silent infarcts and cognitive impairment pose a great burden on the health and quality of life of children with SCD, and will greatly impact them in later life.[64] Investigations and interventions in this field have been performed in some countries but need to be expanded. Transition to adulthood is challenging[65] and the mortality peak is shifting from early childhood to late adolescence and young adulthood. Adequate transition services and adult care are inconsistent across Europe.

In conclusion, as suggested by the EHA roadmap,[16] optimising patient care and clinical trials to address basic aspects of clinical care are key issues in the field of SCD in Europe, together with new profiling of disease severity allowing personalised medicine and investigation of new therapeutic molecules for clinical management. These aspects will affect patient health outcomes and quality of life, as well as national and European healthcare systems by reducing hospital admissions, hospitalisation lengths, and care costs. A few practical suggestions, which are certainly not exhaustive, regarding issues to address and improve in the field of paediatric SCD are shown in Table 3.

REFERENCES

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12.

2. Aguilar Martinez P et al. Haemoglobinopathies in Europe: health & migration policy perspectives. Orphanet J Rare Dis. 2014;9:97.

3. Roberts I, de Montalambert M. Sickle Cell disease as a paradigm of immigration haematology: new challenges for hematologists in Europe. Haematologica. 2007;92(7):865-71.

4. de Montalembert M et al. Sickle cell disease in France in 2006: results and challenges. Arch Pediatr. 2006;13(9): 1191-4.

5. Public Health England. NHS population screening: programme standards. 2010. Available at: http://www.sct.screening.nhs.uk/standardsandguidelines. Last accessed: 1 May 2016.

6. Colombatti R et al. Hospitalization of children with sickle cell disease in a region with increasing immigration rates. Haematologica. 2008;93(3):463-4.

7. Mañú Pereira M, Corrons JL. Neonatal haemoglobinopathy screening in Spain. J Clin Pathol. 2009;62(1):22-5.

8. Gibbons C et al. Sickle cell disease: time for a targeted neonatal screening programme. Ir Med J. 2015;108(2):43-5.

9. Suijker MH et al. [Haemoglobinopathy

in the 21st century: incidence, diagnosis and heel prick screening]. Ned Tijdschr Geneeskd. 2014;158:A7365.

10. Kohne E, Kleihauer E. Hemoglobinopathies: a longitudinal study over four decades. Dtsch Arztebl Int. 2010; 107(5):65-71.

11. World Health Organization. Report A59/9 on sickle cell anemia. 2006. Available at: http://apps.who.int/gb/archive/pdf_files/WHA59/A59_9-en.pdf. Last accessed: 1 May 2016.

12. United Nations. 'Sickle-cell Anaemia as a Public Health Problem' Among Four Adopted Texts. 2008. Available at: http://www.un.org/News/Press/docs/2008/ga10803.doc.htm. Last accessed: 1 May 2016.

13. Rees DC et al. Sickle-cell disease. Lancet. 2010;376(9757):2018-31.

14. Section on Hematology/Oncology and Committee on Genetics; American Academy of Pediatrics. Health supervision for children with sickle cell disease. Pediatrics. 2002;109(3):526-35.

15. Kauf TL et al. The cost of health care for children and adults with sickle cell disease. Am J Hematol. 2009;84(6):323-7.

16. Engert A et al. The European Hematology Association Roadmap for European Hematology Research: a consensus document. Haematologica. 2016;101(2): 115-208.

17. Bernaudin F et al. G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. Blood. 2008;112(10):4314-7.

18. Urio F et al. High Prevalence of Individuals with Low Concentration of Fetal Hemoglobin in F-cells in Sickle Cell Anemia in Tanzania. Am J Hematol. 2016;doi: 10.1002/ajh.24390. [Epub ahead of print].

19. Chaouch L et al. rs11886868 and rs4671393 of BCL11A associated with HbF level variation and modulate clinical events among sickle cell anemia patients. Hematology. 2016;22:1-5. [Epub ahead of print]

20. Telfer P et al. Clinical outcomes in children with sickle cell disease living in England: A neonatal cohort in East London. Haematologica. 2007;92(7): 905-12.

21. Bernaudin F et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. Blood. 2011;117(4):1130-40; quiz 1436.

22. Bernaudin F et al. Long term follow-up of children with sickle cell anemia treated for abnormal transcranial Doppler velocities. Blood. 2016. [Epub ahead of print].

23. Lê PQ et al. Survival among children and adults with sickle cell disease in Belgium: Benefit from hydroxyurea treatment. Pediatr Blood Cancer. 2015;62(11):1956-61.

24. van der Plas EM et al. Mortality and causes of death in children with sickle cell disease in the Netherlands, before the introduction of neonatal screening. Br J Haematol. 2011;155(1):106-10.

25. Díaz-Díaz J et al. [Stroke in paediatric patients with sickle-cell anaemia]. Rev Neurol. 2014;59(4):153-7.

26. Haute Autorité de Santé. Recommandations pour la pratique Clinique. Prise en charge de la drépanocytose chez l'enfant et l'adolescent. 2005. Available at: http://www.has-sante.fr/portail/upload/docs/application/pdf/Drepanocytose_reco.pdf. Last accessed: 1 March 2016.

27. Ministère de la Santé et des Solidarités. Plan National Maladies Rares 2005-2008. Available at: http://social-sante.gouv.fr/IMG/pdf/Maladies_rares_plan_sante_publique_2005_2008.pdf. Last accessed: 13 October 2013.

28. Colombatti R et al. Organizing national responses for rare blood disorders: the Italian experience with sickle cell disease in childhood. Orphanet J Rare Dis. 2013; 8:169.

29. GPOH Konsortium Sichelzellkrankheit. AWMF-S2k-Leitlinie Sichelzellkrankheit (Stand: 12/2014). 2014. Available at: http://www.sichelzellkrankheit.info/behandlungsleitlinie/. Last accessed: 20 February 2016.

30. de Montalembert M et al. ENERCA clinical recommendations for disease management and prevention of complications of sickle cell disease in children. Am J Hematol. 2011;86(1):72-5.

31. Colombatti R et al. Comprehensive care for sickle cell disease immigrant patients: a reproducible model achieving high adherence to minimum standards of care. Pediatr Blood Cancer. 2012;59(7):1275-9.

32. Okpala I et al. The comprehensiveness care of sickle cell disease. Eur J Haematol. 2002; 68(3):157-62.

33. Streetly A et al. Positive screening and carrier results for the England-wide universal newborn sickle cell screening programme by ethnicity and area for 2005-07. J Clin Pathol. 2010;63(7):626-9.

34. Gulbis B et al. Neonatal haemoglobinopathy screening in Belgium. J Clin Pathol. 2009;62(1):49-52.

35. Bouva MJ et al. Implementing neonatal screening for haemoglobinopathies in the Netherlands. J Med Screen. 2010;17(2): 58-65.

36. Cela de Julián E et al. Evaluation of systematic neonatal screening for sickle cell diseases in Madrid three years after its introduction. An Pediatr (Barc). 2007; 66(4):382-6.

37. Bardakdjian-Michau J et al. Neonatal screening for sickle cell disease in France. J Clin Pathol. 2009;62(1):31-3.

38. Rolla R et al. Neonatal screening for sickle cell disease and other hemoglobinopathies in "the changing Europe." Clin Lab. 2014;60(12):2089-93.

39. Venturelli D et al. Sickle cell disease in areas of immigration of high-risk populations: a low cost and reproducible method of screening in northern Italy. Blood Transfus. 2014;12(3):346-51.

40. Ballardini E et al. Universal neonatal screening for sickle cell disease and other haemoglobinopathies in Ferrara, Italy. Blood Transfus. 2013;11(2):245-9.

41. Frömmel C et al. Newborn screening for sickle cell disease: technical and legal aspects of a German pilot study with 38,220 participants. Biomed Res Int. 2014; 2014:695828.

42. Lobitz S et al. Incidence of sickle cell disease in an unselected cohort of neonates born in Berlin, Germany. Eur J Hum Genet. 2014;22(8):1051-3.

43. Kunz JB et al. Significant prevalence of sickle cell disease in Southwest Germany: results from a birth cohort study indicate the necessity for newborn screening. Ann Hematol. 2016;95(3):397-402.

44. Jans SM et al. A case study of haemoglobinopathy screening in the Netherlands: witnessing the past, lessons for the future. Ethn Health. 2012;17(3): 217-39.

45. Kaufmann JO et al. After the introduction into the national newborn screening program: who is receiving genetic counseling for hemoglobinopathies in the Netherlands? Public Health Genomics. 2014;17(1):16-22.

46. Peters M et al. One-third of the new paediatric patients with sickle cell disease in The Netherlands are immigrants and do not benefit from neonatal screening. Arch Dis Child. 2010;95(10):822-5.

47. Thuret I et al. Neonatal screening for sickle cell disease in France: evaluation of the selective process. J Clin Pathol. 2010;63(6):548-51.

48. de Montalembert M et al. Ethical aspects of neonatal screening for sickle cell disease in Western European countries. Acta Paediatr. 2005;94(5): 528-30.

49. Howard-Jones M et al. An audit of immunisation status of sickle cell patients in Coventry, UK. J Clin Pathol. 2009;62(1):42-5.

50. de Montalembert M, Lenoir G. Antibiotic prevention of pneumococcal infections in asplenic hosts: admission of

insufficiency. Ann Hematol. 2004;83(1): 18-21.

51. Colombatti R et al. Lessons learned from the H1N1 pandemic: the need to improve systematic vaccination in Sickle Cell Disease children. A multi center survey in Italy. Vaccine. 2011;29(6):1126-8.

52. Deane CR et al. Extracranial internal carotid arterial disease in children with sickle cell anemia. Haematologica. 2010;95(8):1287-92.

53. Bernaudin F et al. Chronic and acute anemia and extracranial internal carotid stenosis are risk factors for silent cerebral infarcts in sickle cell anemia. Blood. 2015;125(10):1653-61.

54. Deane CR et al. Transcranial Doppler scanning and the assessment of stroke risk in children with HbSC [corrected] disease. Arch Dis Child. 2008;93(2): 138-41.

55. Padayachee ST et al. Problems with implementing a standardised transcranial Doppler screening programme: impact of instrumentation variation on STOP classification. Pediatr Radiol. 2012;42(4):470-4.

56. Inusa B et al. The Impact Of a Standardised Transcranial Doppler Training Programme In Screening Children With Sickle Cell Disease: A European Multicenter Perspective. Blood. 2013;122(21):983.

57. Ware RE et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, open-label, phase 3, non-inferiority trial. Lancet. 2016;387(10019):661-70.

58. de Montalembert M et al. Long-term hydroxyurea treatment in children with sickle cell disease: tolerance and clinical outcomes. Haematologica. 2006; 91(1):125-8.

59. de Montalembert M et al. Three-year follow-up of hydroxyurea treatment in severely ill children with sickle cell disease. The French study group on sickle cell disease. J Pediatr Hematol Oncol. 1997;19(4):313-8.

60. Gulbis B et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: the Belgian experience. Blood. 2005;105(7):2685-90.

61. de Montalembert M et al. Implementation of a European Cohort to Follow Sickle Cell Children and Adults Treated with Hydroxycarbamide. Blood. 2014;124(21):564.

62. Stettler N et al. Proportion of adults with sickle cell anemia and pain crises receiving hydroxyurea. JAMA. 2015;313(16):1671-2.

63. Colombatti R et al. Hydroxiurea prescription, availability and use in children with sickle cell disease in Italy: results of the Italian Association of Pediatric Hematology Oncology (AIEOP) Multicenter survey. Abstract P378. Congress of European Hematology Association, Vienna, Austria, 11–14 June 2015.

64. Brousse V et al. How I manage cerebral vasculopathy in children with sickle cell disease. Br J Haematol. 2015;170(5): 615-25.

65. de Montalembert M et al. Transition from paediatric to adult care for patients with sickle cell disease. Br J Haematol. 2014;164(5):630-5.

UPCOMING EVENTS

18th Meeting of the European Association for Haematopathology (EAHP 2016)

3rd–8th September 2016

Basel, Switzerland

Internationally renowned speakers will be discussing the issues and controversies that have recently emerged in the field of haematopathology. The event will focus on the topics of bone marrow and lymphoma, both of which will be explored extensively in a series of symposia and workshops. Alongside in-depth coverage of the scientific and practical issues within these topics, the congress will also feature an array of 'Meet the Professor' sessions.

49th Annual Meeting of the German Society of Transfusion Medicine and Immunohaematology and the 24th Annual Meeting of the German Society for Immunogenetics (Joint Congress DGTI & DGI 2016)

7th–10th September 2016

Nuremberg, Germany

The scientific organising committee can proudly announce a range of distinguished experts from around the world in addition to the young scientists from Germany's own national institutions, featuring at the congress in Nuremberg. It is the first joint congress held between these two long-established organisations, offering an interdisciplinary discussion of the current research results, developments, and practical applications in each of their areas of expertise.

2nd International Conference on New Concepts in B Cell Malignancies

9th–11th September 2016

Estoril, Portugal

Covering all aspects of B cell malignancies, from molecular pathogenesis to state-of-the-art treatment, this conference will encourage scientific exchange between scientists and clinicians working on lymphoid malignancies across the globe. Through plenary presentations, round table discussions, 'meet the expert' sessions, and poster presentations, the event aims to improve the understanding of B cell disorders, including its molecular pathogenesis and range of prognostic markers.

15th International Congress on Antiphospholipid Antibodies

21st–24th September 2016

Istanbul, Turkey

This triennial event will discuss the recent advances and future directions in antiphospholipid antibodies and antiphospholipid syndrome. The general topics addressed at the event will include antiphospholipid antibody-mediated thrombosis, association between antiphospholipid syndrome and other systemic autoimmune diseases, and new antithrombotic agents, to entice all researchers and clinicians interested in the field. Its broad programme will also provide task force presentations, covering the controversial aspects of diagnosis and management.

10th Anniversary Conference Academy for Sickle Cell and Thalassaemia Conference (ASCAT)

5th–7th October 2016

London, UK

This conference will provide an important space for discussion and interaction between leading experts and healthcare professionals on the topic of sickle cell disease and thalassaemia. Each day of the event will provide an invaluable opportunity to explore a range of prominent topics covering these diseases, including the role of molecular diagnosis in sickle cell disease and psychosocial and adherence issues in haemoglobinopathies.

7th International Eurasian Hematology Congress (EHC)

13th–16th October 2016

Istanbul, Turkey

Last year's congress saw participants from 42 different countries, pushing it ever-closer towards becoming one of the world's leading international conventions. This year is a continuation of the organisers' efforts to serve both Europe and Asia with high-quality scientific discussion and exchange in the field of haematology. Offered in the programme is a range of sessions exploring the varying treatment strategies for a wide selection of blood diseases and disorders.

7th International Conference on Myeloproliferative Neoplasms

27th–29th October 2016

Estoril, Portugal

Scientists and clinicians will present and discuss the latest research on the cellular and molecular biology of myeloproliferative neoplasm (MPN) disorders in order to contribute to its improved diagnosis and treatment. This specialist event will feature a range of topics in alignment with the learning objectives of the event, with a focus on novel therapeutic approaches, targeting the MPN stem cell, and understanding the genetic and cellular pathogenesis of MPNs.

22nd Congress of the European Hematology Association (EHA)

22nd–25th June 2017

Madrid, Spain

The European Hematology Association (EHA) has always striven to promote excellence in patient care, research, and education in haematology. The EHA 2017 Congress will provide a welcome opportunity for the organisation to continue these efforts. This annual event has continued to build in popularity over recent years, with EHA 2016 boasting over 10,000 attendees and a record number of over 2,000 abstracts: a success that will prove to be no exception. Over the past two decades, the EHA Congress has established itself as an unmissable event for haematologists across the board. EHA 2017 will be a confident progression from these previous successes when it returns in June next year.

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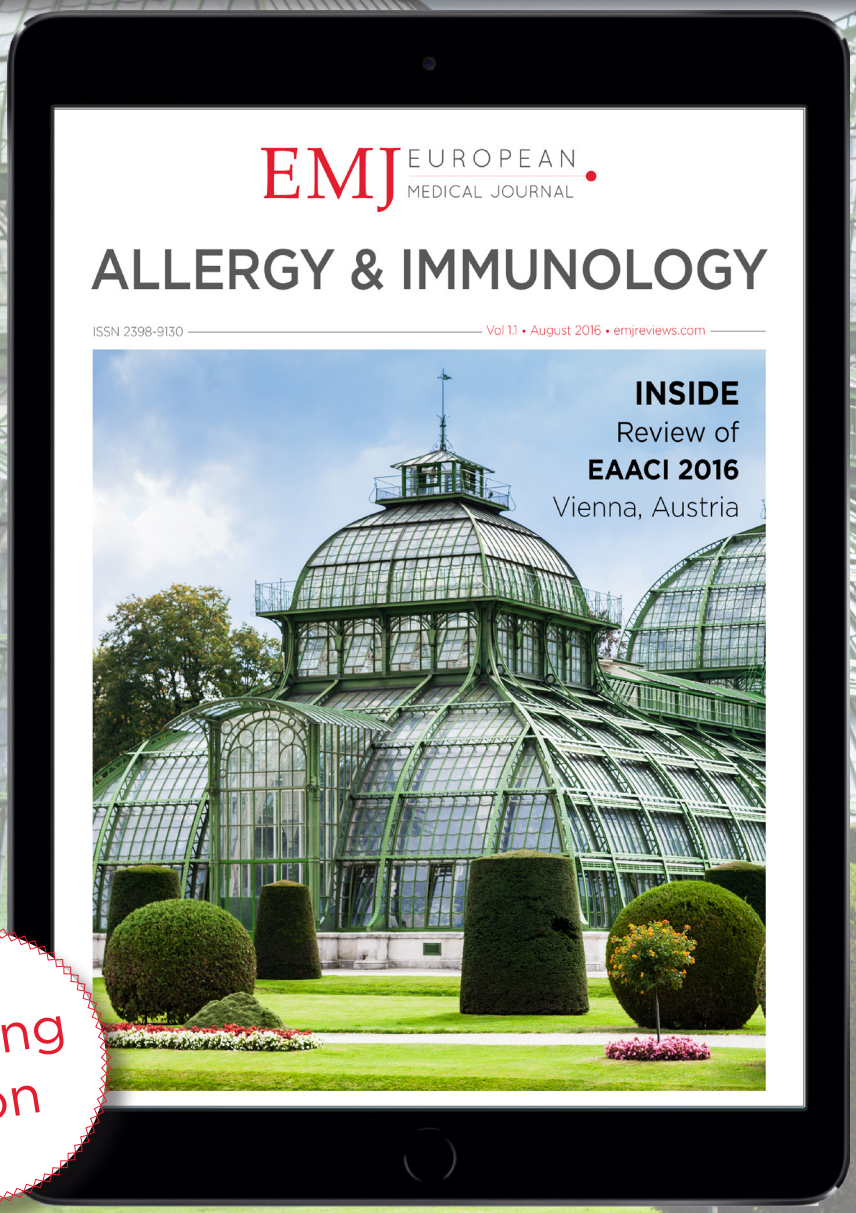

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