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HEMATOLOGY

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INSIDE

Review of

EHA 2015

Vienna, Austria

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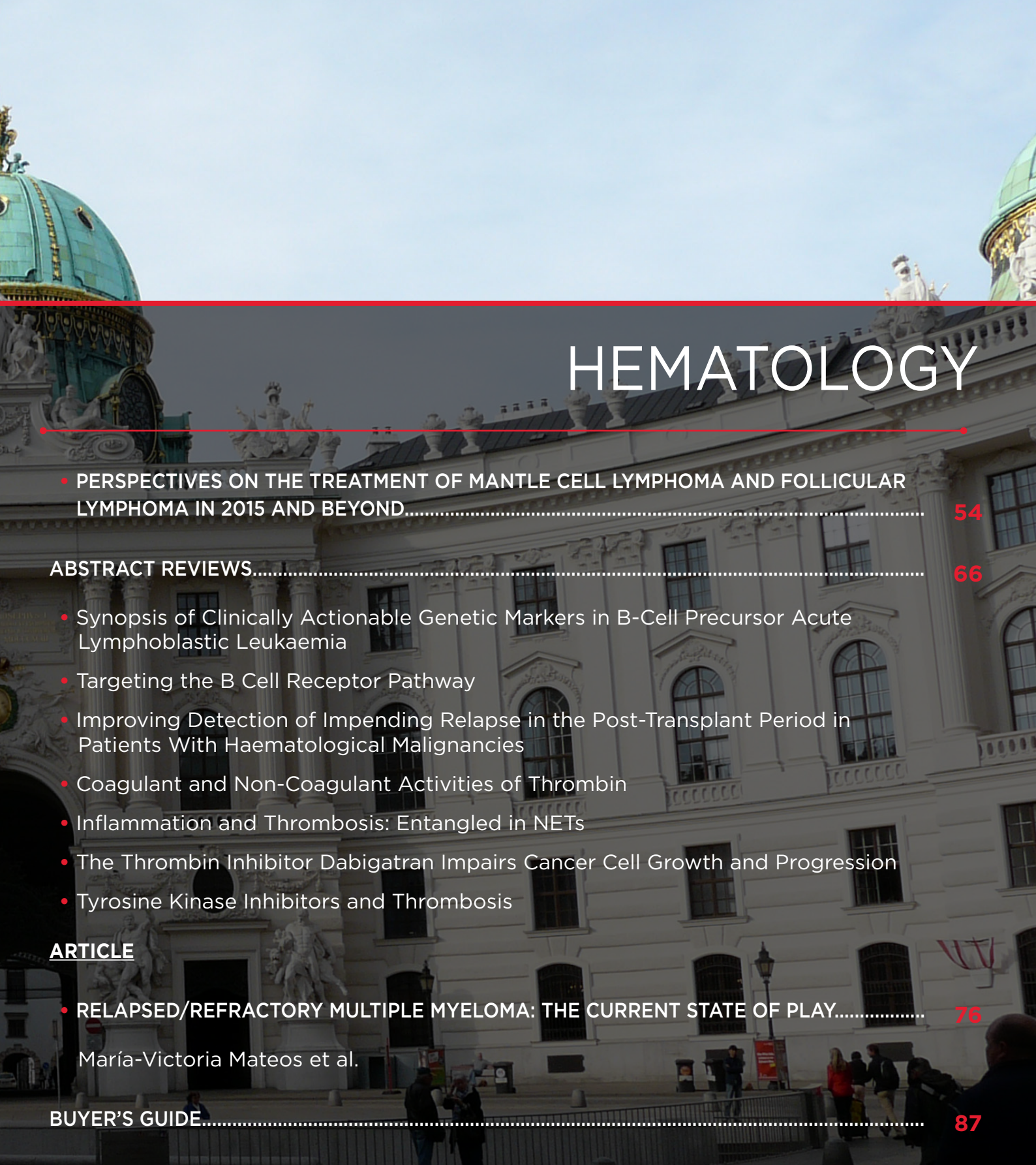

HEMATOLOGY

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Hematology

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Patients with diarrhoea and vomiting should be managed using standard-of-care treatment, including an antidiarrhoeal or antiemetic medicinal product and/or fluid replacement. In addition, these events can also be managed by withholding bosutinib temporarily, dose reduction, and/or discontinuation of bosutinib (see SmPC sections 4.2 and 4.8). The antiemetic agent, domperidone, has the potential to increase QT interval prolongation and to induce "torsade de pointes"- arrhythmias; therefore, co-administration with domperidone should be avoided. It should only be used if other medicinal products are not efficacious. In these situations an individual benefit-risk assessment is mandatory and patients should be monitored for occurrence of QT prolongation. Treatment with bosutinib is associated with myelosuppression, defined as anaemia, neutropenia, and thrombocytopenia. Complete blood counts should be performed weekly for the first month and then monthly thereafter, or as clinically indicated. Treatment with bosutinib may be associated with fluid retention including pericardial effusion, pleural effusion and pulmonary oedema. Patients should be monitored and managed using standard-of-care treatment. Elevation in serum lipase has been observed. Caution is recommended in patients with previous history of pancreatitis. Bosutinib may predispose patients to bacterial, fungal, viral or protozoan infections.Automated machine-read QTc prolongation without accompanying arrhythmia has been observed. Bosutinib should be administered with caution to patients who have a history of or predisposition for QTc prolongation, who have uncontrolled or significant cardiac disease including recent myocardial infarction, congestive heart failure, unstable angina or clinically significant bradycardia, or who are taking medicinal products that are known to prolong the QT interval. Monitoring for an effect on the QTc interval is advisable and a baseline ECG is recommended prior to initiating therapy with Bosutinib and as clinically indicated. Hypokalaemia or hypomagnesaemia must be corrected prior to bosutinib administration and should be monitored periodically during therapy. Treatment with bosutinib may result in a clinically significant decline in renal function in CML patients. A decline over time in estimated glomerular filtration rate (eGFR) has been observed in patients treated with bosutinib in clinical studies. It is important that renal function is assessed prior to treatment initiation and closely monitored during therapy with bosutinib, with particular attention in those patients who have preexisting renal compromise or in those patients exhibiting risk factors for renal dysfunction, including concomitant use of medicinal products with potential for nephrotoxicity, such as diuretics, ACE inhibitors, angiotensin receptor blockers and nonsteroidal anti-inflammatory drugs (NSAIDs). The concomitant use of bosutinib with potent or moderate CYP3A inhibitors/inducers should be avoided as an increase/decrease in bosutinib plasma concentration will occur. Grapefruit products, including grapefruit juice and other foods that are known to inhibit CYP3A should be avoided. **Drug interactions:** The concomitant use of bosutinib with potent (e.g. ketoconazole, grapefruit products including grapefruit juice) or moderate CYP3A inhibitors should be avoided, as an increase in bosutinib plasma concentration will occur. Refer to section 4.5 of the SmPC for further details. If a potent or moderate CYP3A inhibitor must be administered during bosutinib treatment, an interruption of bosutinib therapy or a dose reduction in bosutinib should be considered. The concomitant use of bosutinib with potent (e.g. rifampicin, phenytoin, carbamazepine, St. John's Wort, rifabutin, phenobarbital) or moderate (e.g. bosentan, nafcillin, efavirenz, modafinil, etravirine) CYP3A inducers should be avoided, as a decrease in bosutinib plasma concentration will occur. Caution should be exercised when administering bosutinib concomitantly with proton pump inhibitors (PPIs). Short-acting antacids should be considered as an alternative to PPIs and administration times of bosutinib and antacids should be separated (i.e. take bosutinib in the morning and antacids in the evening) whenever possible. Caution should be used if bosutinib is administered with medicinal products that are substrates of P-glycoprotein (P-gp). An *in vitro* study suggests that bosutinib may have the potential to increase the plasma concentrations of medicinal products that are P-gp substrates. Refer to section 4.5 of SmPC for examples of P-gp substrates. Bosutinib should be used with caution in patients who have or may develop prolongation of QT, including those patients taking anti-arrhythmic medicinal products or other medicinal products that may lead to QT prolongation. Refer to sections 4.4 and 4.5 of the SmPC for further details. **Fertility, pregnancy and lactation:** Not recommended in pregnancy or whilst breast feeding. Bosutinib has the potential to impair reproductive function and fertility. **Driving and operating machinery:** Bosutinib has no or negligible influence on the ability to drive and use machines. **Undesirable effects:** Very common adverse events are: respiratory tract infection, thrombocytopenia, neutropenia, anaemia, leukopenia, decreased appetite, headache, cough, diarrhoea, vomiting, nausea, abdominal pain, alanine aminotransferase increased, aspartate aminotransferase increased, rash, arthralgia, pyrexia, oedema, fatigue. Commonly reported adverse events are: pneumonia, influenza, bronchitis, nasopharyngitis, febrile neutropenia, drug hypersensitivity, dehydration, hyperkalaemia, hypophosphataemia, dizziness, dysgeusia, pericardial effusion, electrocardiogram QT prolonged, dyspnoea, pleural effusion, gastritis, hepatotoxicity, hepatic function abnormal, blood bilirubin increased, gamma-glutamyltransferase increased, urticaria, acne, pruritus, myalgia, back pain, renal failure, chest pain, pain, asthenia, lipase increased, blood creatinine increased, blood amylase increased, blood creatine phosphokinase increased. Refer to section 4.8 of the SmPC for further information on side effects, including description of selected adverse reactions. **Legal category:** POM. **Basic NHS price:** Bosulif 100mg, 28 tablets [EU/1/13/818/001] £859.17. Bosulif 500 mg, 28 tablets [EU/1/13/818/003] £3436.67. **Marketing authorisation holder:** Pfizer Ltd, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK.

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Gaucher Disease

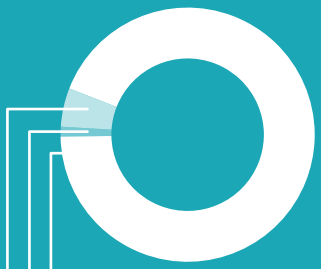
The most common of a family of rare diseases known as lysosomal storage disorders[1]

Gaucher disease is caused by a genetic mutation resulting in the deficiency, absence or incomplete functioning of a lysosomal enzyme called glucocerebrosidase[2]

This leads to a build-up of a fatty waste substance (glucocerebroside) in cells, tissues and organs[2]

Lysosomes are enzyme-rich structures within a cell where waste materials are broken down or recycled[3]

There are three types of Gaucher disease



Type 1 (94%)

Non-neuronopathic – only involves the internal organs and not the central nervous system[4]

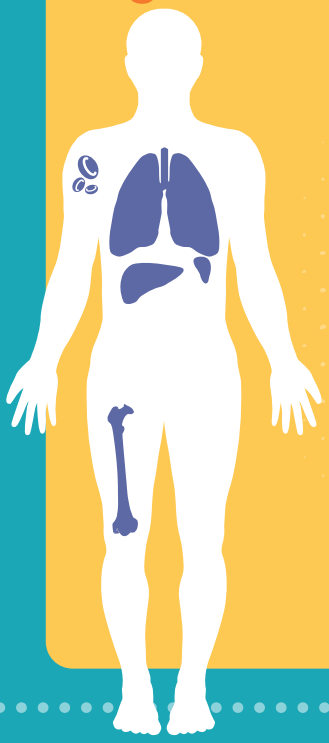
Type 2 (<1%)

Affects the central nervous system; **typically fatal** during infancy[4]

Type 3 (5%)

Affects central nervous system and internal organs; **progresses slowly**[4]

Signs and symptoms of type 1 Gaucher disease



Anemia & fatigue	Reduced hemoglobin[5]
Bleeding & bruising	Reduced platelet count[5]
Abdominal discomfort	Liver & spleen enlargement[6]
Bone pain & fractures	Reduced bone density[7]

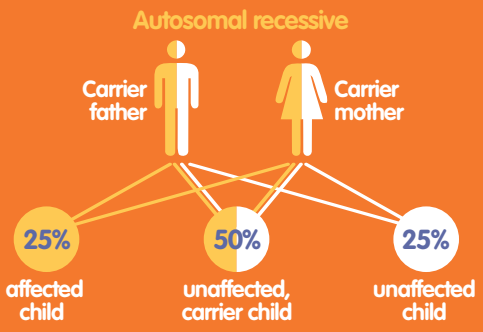
Gaucher disease is usually a **progressive disease**, with symptoms worsening over time

Incidence & inheritance of Gaucher disease

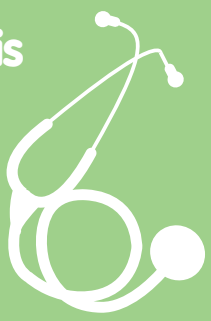
1 in 40,000 people worldwide[8]

But type 1 is more common in people of Ashkenazi Jewish heritage: 1 in 855 people[8]

Both parents must carry the faulty gene to have an affected child, and men and women are affected equally



Diagnosis



Most commonly diagnosed with a blood test to assess glucocerebrosidase enzyme activity[9]

Families with known Gaucher disease may be screened to help identify carriers[9]

Treatment

Currently no known cure for Gaucher disease, but therapies are available to help manage the major symptoms

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Date of preparation: June 2015

Welcome

Hello and a very warm welcome to the new edition of *EMJ Hematology*, the source for the latest developments and innovations relating to the study of the blood, blood-forming organs, and blood diseases. Inside you will find exhaustive coverage from one of the most essential events of the year for haematologists: the 20th Congress of the European Hematology Association (EHA), held in the impressive city of Vienna, Austria, on 11th-14th June 2015. EMJ was there to cover all of the action and our in-depth guide to the event's proceedings is sure to be a vital tool, both for those who attended and those who could not.

Included in our definitive review is a wealth of news stories direct from the congress, including reports on some of the most exciting discoveries and impactful presentations that are changing the face of medicine, and reviews of some of the most innovative symposia. We have also hand picked a selection of summaries of abstracts that were presented over the course of the event, showcasing some of the most interesting topics under debate.

Onset of cancer puts patients at significantly higher risk of venous and arterial thromboembolism. Falanga et al. describe in their abstract '*Tyrosine kinase inhibitors and thrombosis*' how anti-cancer drugs may be associated with vascular complications, and note that identifying patients at high risk is essential prior to treatment initiation. In their abstract '*Coagulant and non-coagulant activities of thrombin*', Spronk et al. describe how a new approach is required to determine the intricacy of the non-coagulant effects of thrombin, as well as the beneficial or damaging long-term effects of inhibiting coagulant and non-coagulant activity. For another perspective on the current state of haematology, we have included interviews with highly esteemed individuals who impart their wisdom and experience of this ever-expanding field, offering a more personal account of a key therapeutic area.

The EHA congress was a big success and its impact will surely be felt throughout the medical community. Hopes are high that the outstanding work displayed in Vienna will be built upon in the coming months, and that these innovations will be transferred through engagement with journals such as *EMJ Hematology*. Finally, we would like to take this opportunity to thank you for reading and wish you the very best of luck for the remainder of 2015 and beyond. Special thanks go to our esteemed editorial board who have once again been a valuable aid over the last year.

Spencer Gore

Team Principal, European Medical Journal

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Foreword

Prof Dr Emili Montserrat

Chronic Lymphocytic Leukaemia (CLL) and Lymphoma Programme, University of Barcelona, Spain

Dear Colleagues,

'Precision medicine' is a relatively newly coined term that refers to an approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle. The term 'precision medicine', incidentally, is much better than the largely employed 'personalised medicine' since the goal of medicine and those who dedicate their lives to their practice has always been to take care of sick and frail persons. Haematology has always been at the forefront of medical research and in translating discoveries from basic investigation to the clinic. Also, progress in genetic studies in the last 40 years has made it possible for a large number of leukaemias and lymphomas to be diagnosed, treated, and their response monitored thanks to specific genetic lesions.

Today, next-generation sequencing techniques and platforms, which allow the deciphering of the genome and identification of genetic and epigenetic lesions associated with a variety of haematological diseases, make it reasonable to foresee a future in which patient management could be largely based on characteristics unique to both the disease and the patient i.e. 'precision medicine'. However, a number of important downsides need to be taken into consideration, and addressed and overcome by future research, including the limited understanding of many of the findings that next-generation sequencing techniques are revealing. Current investigation also needs to be focussed on new forms of cell therapy (e.g. chimeric-antigen-receptor T cells) that might replace, at least in part, allogeneic stem cell transplantation, and the role of small molecules in treatment.

> "Next-generation sequencing techniques [...] make it reasonable to foresee a future in which patient management could be largely based on characteristics unique to both the disease and the patient i.e. 'precision medicine'."

EMJ aims to combine high-quality, peer-reviewed manuscripts with a comprehensive report of the latest breaking news, including first-hand accounts of the biggest medical congresses in Europe. This edition of *EMJ Hematology* provides an informative summary of the findings presented at the 20th Congress of the European Hematology Association (EHA), held in Vienna from 11th-14th June 2015; those unable to attend need look no further for a sufficient and stimulating synopsis.

I hope that you all greatly enjoy this edition and that the content within inspires you in pursuing your professional goals and advancing our knowledge of this ever-changing field.

Yours sincerely,

Emili Montserrat

Chronic Lymphocytic Leukaemia (CLL) and Lymphoma Programme, University of Barcelona, Barcelona Hospital Clinic, Barcelona, Spain; Past-President, European Research Initiative on CLL.

EHA ANNUAL CONGRESS 2015

MESSE CONFERENCE CENTRE
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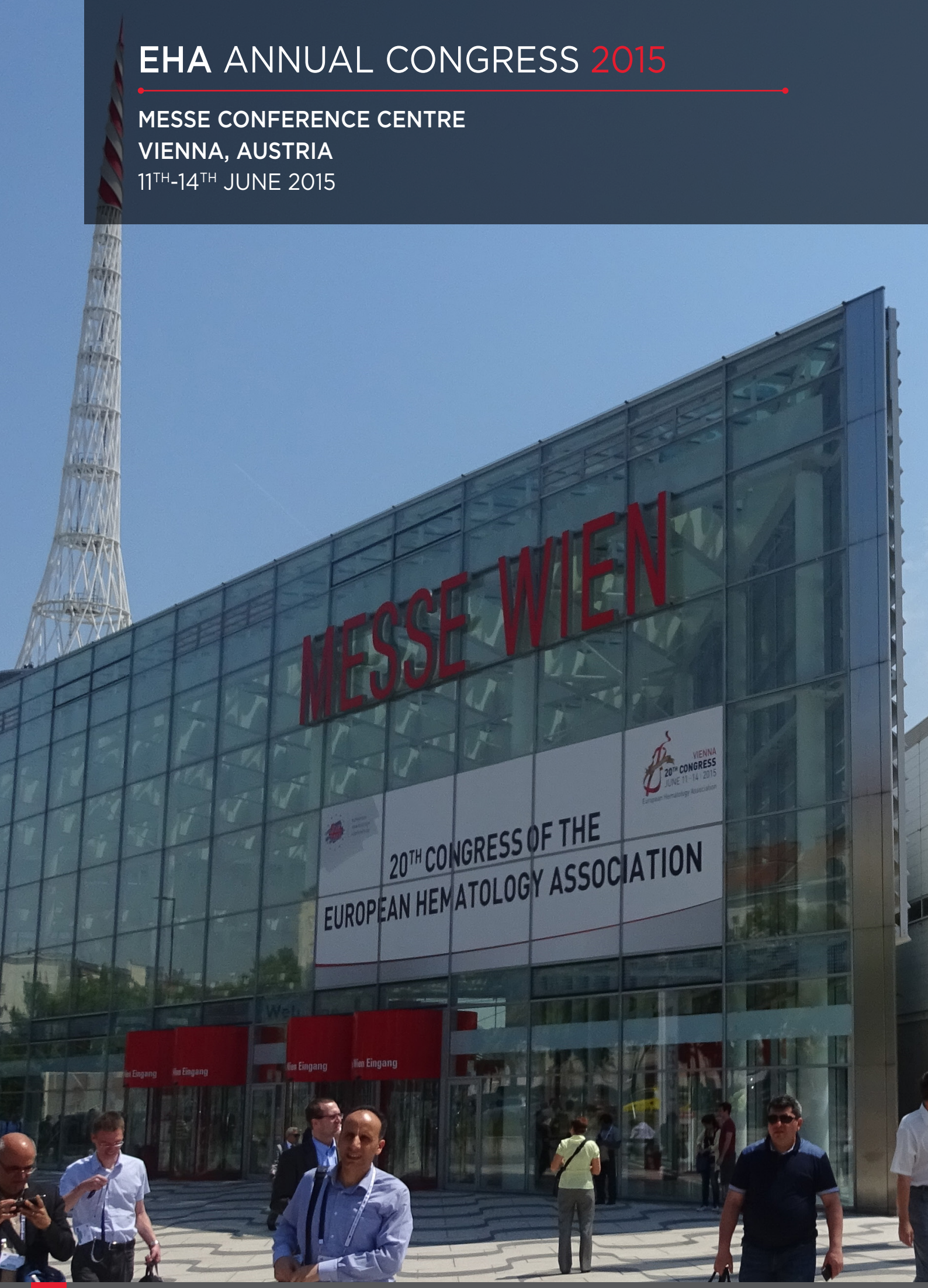


Welcome to the *European Medical Journal* review of the 20th Congress of the European Hematology Association 2015

Austria's grand city of Vienna proved to be a popular venue with delegates attending the 20th anniversary edition of the annual EHA congress this year. Already considered a scientifically historical city as the home of Karl Landsteiner, the pioneering haematologist who discovered human blood groups in 1900 and laid the foundation for the modern medical practice of blood transfusion, the city was definitely worthy of such a momentous occasion. Vienna is also one of the culturally and musically richest cities in the world; the city buzzes with an eclectic, lively mix of cool cafes and bars, galleries, shops, and street markets. Vienna is a city that charms and seduces from the moment you arrive, and entices you to stay.

During the opening ceremony on Friday 12th June, participants were treated to a rousing speech by EHA President Christine Chomienne. She highlighted the theme of the year, 'Innovation in haematology: innovation in research, innovation in education, innovation in policy, and revolutions in technology'. The spotlight also shone on several EHA research fellowships presented, including Clinical: B. Gentner (Italy); Non-clinical junior: I. Triviai (Germany), G. Simonetti (Italy), E. de Pater (Netherlands); Non-clinical advanced: S. Altamura (Germany), D. Kent (United Kingdom); and Short-term visiting award: C. Dufour (Argentina).

EHA's board this year selected Professor Hugues de Thé for the José Carreras honorary lecture, an award that was established in 1999 to honour leading and active investigators in haematological research who have made a significant contribution to haematology. Prof de Thé's lecture was entitled '*Curing APL through therapy - induced PML/RARA degradation*'. Another honorary award presented at the congress was the 8th EHA Jean Bernard Lifetime Achievement Award. This was established in 2008 to honour outstanding physicians and scientists for their lifetime contribution to haematology, and was awarded to the esteemed Dr Volker Diehl.

Approximately 9,000 delegates descended upon Vienna for the event, and 2,200 abstracts were submitted, with 262 selected for presentation in one of the 37 sessions covering all areas of haematology. As such, there was plenty on offer for participants to observe and engage with throughout the congress. Indeed, a rich range of content was displayed at EHA, with developments in topics such as chronic myeloid leukaemia, multiple myeloma, paediatric haematology, stem cell transplantation, acute lymphoblastic leukaemia, myeloproliferative neoplasm, and peripheral T-cell lymphomas, among many others.

In keeping with this year's congress theme of 'Innovation in haematology', novel ways of treating haematological conditions were a prominent part of the proceedings. Prof Andrew Roberts presented the results of a trial reporting that combining the anti-bcl-2 drug venetoclax with the non-chemotherapy drug rituximab stimulates a strong response in the majority of patients with relapsed or refractory leukaemia; there is hope that further investigation will confirm its potential to eliminate this terrible illness. The announcement of a highly sensitive new BCR-ABL1 diagnostic assay also caused a stir, as it has the potential to improve diagnostic accuracy in chronic myeloid leukaemia.

EHA 2015 most certainly lived up to expectations, and provided a fitting celebration for the 20th anniversary of this wonderful congress. The cultural enrichment that Vienna offers was matched by the advances in knowledge made in the field of haematology, which each and every attendee gained from. With this in mind, next year's congress in Copenhagen, Denmark is already highly anticipated!

EHA President Christine Chomienne [...] highlighted the theme of the year, 'Innovation in haematology: innovation in research, innovation in education, innovation in policy, and revolutions in technology'.

HIGHLIGHTS

Kinase Inhibitor Activity Shown in Acute Myeloid Leukaemia Patients

PATIENTS with acute myeloid leukaemia (AML) can be treated with a tyrosine kinase inhibitor (TKI) called sorafenib (SRF), following the results of a trial which has shown the first evidence of kinase inhibitor activity in AML.

Until this study, a kinase inhibitor had not demonstrated activity in AML, despite the success of TKIs in other forms of leukaemia, such as chronic myeloid leukaemia and acute lymphoblastic leukaemia. However, findings on the variety of mutations that fuel AML have led researchers to study SRF, an investigational TKI that blocks the activity of several mutated enzymes that may drive growth of the disease.

"The positive, lasting responses we observed in AML patients receiving SRF represent the first randomised evidence for a clinical benefit of a TKI in this type of leukaemia."

To determine the safety and efficacy of SRF, the researchers enrolled 267 AML patients aged 18-60 years in a Phase II study. They were then randomised to receive either SRF (134 patients) or placebo (133 patients) in addition to a standard protocol. After 3 years of follow-up, SRF-treated patients had a median event-free survival of 20.5 months, a 3-year relapse-free survival rate of 56%, and an overall survival rate of 63%. In comparison, those receiving placebo had a median event-free survival of 9.2 months, a 3-year relapse-free survival rate of 38%, and an overall survival rate of 56%. Although SRF was generally well tolerated among the patients, the treatment did increase the likelihood of side-effects such as fever, rashes, and bleeding.

"The positive, lasting responses we observed in AML patients receiving SRF represent the first randomised evidence for a clinical benefit of a TKI in this type of leukaemia," said lead study author Prof Gerhard Ehninger, Medical Director of the Medical Clinic I, University Hospital Dresden, Dresden, Germany in an EHA press release from 12th June 2015.

Validation in a larger trial is now required to build on these promising results, as well as further evaluation of genetic markers that may predispose some patients to respond better than others to this treatment, to fully maximise SRF's potential.

Successful Results from a Phase I/II Trial of ASP2215 in AML Patients

POSITIVE results from a Phase I/II trial investigating the safety, tolerability, and efficacy of ASP2215, a selective inhibitor of FLT3/Axl, in patients with relapsed or refractory acute myeloid

leukaemia (AML) were reported in a press release from Astellas dated 15th June 2015, that coincided with EHA 2015.

The Phase I/II trial design followed a 3+3 escalation and evaluated doses from 20-450 mg once daily, with a parallel multi-dose expansion cohort initiated based on the efficacy seen in dose escalation. A total of 198 patients were enrolled in the study: 24 in the dose escalation and 174 in the dose expansion cohorts. Preliminary data demonstrated a 57.5% overall response rate (ORR) and a 47.2% composite complete remission (CR) rate (CR + CR with incomplete platelet recovery + CR with incomplete haematological recovery) in 106 patients with *FLT3* mutations who received ≥80 mg doses. A plasma inhibitory activity assay also confirmed sustained *FLT3* inhibition consistently in patients who received doses of ≥80 mg. Further data from the trial showed that the median duration of response was 18 weeks across all doses, and median overall survival was approximately 27 weeks at ≥80 mg in *FLT3* mutation-positive patients.

"ASP2215 is an exciting therapeutic development for relapsed and refractory AML patients with *FLT3* mutations, where there is a significant unmet need."

"ASP2215 is an exciting therapeutic development for relapsed and refractory AML patients with *FLT3* mutations, where there is a significant unmet need," said Dr Alexander Perl, Assistant Professor in the Division of Hematology/Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. "Treatment with ASP2215 has demonstrated a high ORR and promising survival in this group of patients who have highly aggressive leukaemia and historically fared poorly with standard chemotherapy. ASP2215 is quite well tolerated in this setting and provides patients a low toxicity, effective option either to bridge to transplant with curative intent or to maintain quality of life for extended periods."

Following the success of this study, a randomised Phase III trial of ASP2215 at 120 mg per day in relapsed or refractory AML patients is planned.

A Novel Role for *DNMT3A* R882 Mutations for Chemoresistance in AML

RESISTANCE to chemotherapy occurs in most patients with acute myeloid leukaemia (AML) and ultimately leads to refractory, terminal disease. However, researchers from the Memorial Sloan Kettering Cancer Center, New York City, New York, USA, have identified a novel mechanism through which acquired genetic mutations are able to promote chemoresistance and the persistence of leukaemic cells, which may serve as a therapeutic target for reducing the risk of relapse in AML patients. The data were presented by Dr Olga Guryanova at the EHA Annual Congress.

The team performed a multivariate analysis on a large cohort of AML patients and found that *DNMT3A* R882 mutations were, in contrast to other mutations, associated with an increased risk of minimal residual disease following chemotherapy and adverse outcome. In order to try and understand the molecular basis of this increased risk, the researchers

created a mouse model by mutation of the *DNMT3A* gene. Although the introduction of this mutation alone did not cause leukaemia in the mice, haematopoietic stem cells isolated from the mutant animals displayed enhanced survival compared with those from non-mutant animals, especially under conditions of stress, such as following DNA-damaging chemotherapy.

These effects were shown to be mediated by the mutant cells' impaired ability to remodel chromatin, which is necessary for both detection and repair of DNA damage.

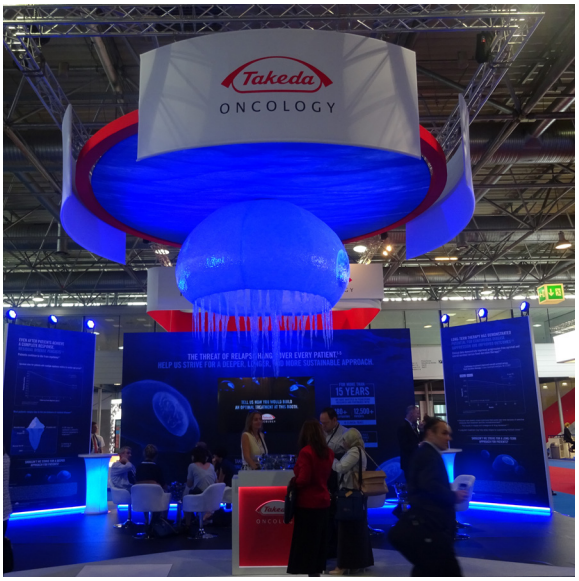
Mutation of *DNMT3A* also accelerated the development of leukaemia in animals with concurrent *Flt3*^{ITD} and *Npm1*^{c} mutations. These effects were shown to be mediated by the mutant cells' impaired ability to remodel chromatin, which is necessary for both detection and repair of DNA damage, and which caused enhanced persistence of the damaged cells and an accumulation of additional genetic mutations. The description of this novel mechanism also helps to provide a mechanistic explanation of how dose-dense anthracyclines are able to provide a therapeutic benefit in AML patients with *DNMT3A* mutations.

Novel Assay Has Potential to Improve Diagnostic Accuracy in Chronic Myeloid Leukaemia

MONITORING of BCR-ABL1 transcripts in patients with chronic myeloid leukaemia (CML) could be greatly improved with the use of a highly sensitive new assay which was launched at the recent annual meeting of EHA in Vienna, Austria. The Quantidex™ BCR-ABL IS CMR Kit was developed by Asuragen, Inc. and reportedly offers a marked improvement over existing tests, attaining a sensitivity of MR4.7 (0.002% International Scale [IS]) while also allowing direct reporting on the IS.

BCR-ABL1 transcripts are derived from a specific chromosomal translocation known as the Philadelphia chromosome, an indicator of CML. With the advent of tyrosine kinase inhibitors (TKIs), CML represents the first cancer type for which a personalised treatment was developed. Quantitative BCR-ABL1 assays are an integral part of this treatment, monitoring the effectiveness of TKI therapy and detecting patient relapse early. However, the lack of clinical reporting on the IS worldwide has complicated patient monitoring and treatment.

20th anniversary of the Annual Congress



With this in mind, the increased sensitivity of this new apparatus allows for the detection of complete molecular response (CMR). In addition, the inclusion of IS reference materials for the creation of a standard curve allows laboratories to report patient results directly on the IS without having to establish conversion factors.

"This product is a major step forward in providing labs with a BCR-ABL kit with class-leading sensitivity that keeps pace with the advances in TKI therapies and the need for IS standardisation," said Dr Matthew McManus, President and CEO of Asuragen, Inc., Austin, Texas, USA in a press release from EHA 2015 published 11th June 2015.

The product was launched at EHA on Friday 12th June at a corporate workshop to demonstrate its effectiveness. With the potential for more accurate diagnostics, as well as integration with the IS, this new BCR-ABL1 assay will undoubtedly be making waves within the haematology community shortly.

Promising Treatment Results for Chronic Myeloid Leukaemia Patients

PATIENTS with chronic myeloid leukaemia (CML) throughout Europe display excellent survival results following treatment, which is in agreement with the remarkable achievements seen in clinical trials, according to new data reported in an EHA press release on 12th June 2015.

The new study, led by Dr Verena Hoffmann, Ludwig-Maximilians-Universität, Munich, Germany, sought to investigate whether all European CML patients are treated in accordance with current guidelines and achieve equally good outcomes compared with those observed in clinical trials, which have demonstrated greatly improved survival times for CML following the introduction of tyrosine kinase inhibitors. However, clinical trials almost always specify many exclusion criteria, and so the researchers involved in the new study wanted to use a population-based registry in order to include all patients, independent of sex, age, or risk profile, and to see whether the outcomes remained similar.

Therefore, the European LeukemiaNet (ELN) and Novartis established a web-based registry to record all

newly diagnosed CML patients in 20 European countries or pre-specified regions covering 92.5 million inhabitants, which represented the first data of this kind to be collected in Europe. In total, data from 2,904 patients with a median age of 55 years were utilised.

The results showed that 94% of patients were diagnosed in the early stages of disease, and 80% received treatment with imatinib according to the ELN recommendations. Newer treatment options were used by patients in clinical trials or with chromosomal abnormalities, while sub-standard treatments were used rarely but mainly in older patients. Although the unadjusted rate of survival was lower in this data set compared with clinical trials, the survival rate of the real-world cohort became similar to clinical trial results after adjustment for the patients' risk profiles: 98% and 95% at 12 and 24 months after diagnosis, respectively.

Overall, this information is highly encouraging, and the researchers expect even fewer CML patients to receive sub-standard treatment following the loss of patent protection for imatinib.

Targeted Combination Therapy May Eliminate Relapsed Chronic Lymphocytic Leukaemia

ELIMINATION of chronic lymphocytic leukaemia (CLL) through a combination of the anti-Bcl-2 drug venetoclax (VEN) and rituximab (RTX) has been demonstrated in an early phase trial, the results of which were reported in an EHA press release on 12th June 2015. The study is the first to combine VEN with another non-chemotherapy drug.

Not only is the administration of VEN + RTX combination safe, but it also triggers strong responses in most patients with relapsed/refractory CLL.

Patients with CLL that has recurred or is not responding to standard treatment require new therapies. However, a new combination of two targeted therapies may be the answer to eliminating CLL in these circumstances.

Led by Prof Andrew Roberts, BMT Physician & Clinical Haematologist, Royal Melbourne Hospital, Melbourne, Australia, the trial combined VEN (formerly ABT-199/GDC-0199) with RTX, forming a non-chemotherapy treatment that is highly active in patients with relapsed/refractory CLL. VEN is a novel once-a-day tablet treatment that kills leukaemia cells by inhibiting Bcl-2, a protein key to CLL cell survival.

The trial enrolled 49 CLL patients receiving VEN at various dosages, and six standard doses of RTX. Prof Roberts and colleagues reported that 41 (84%) patients responded to treatment, while a staggering 20

(41%) achieved a complete response. Furthermore, 27% achieved a complete response with no leukaemia detected using highly sensitive methods, and 6 patients were able to stop VEN treatment completely after achieving complete response (5 of whom remained free of recurrence).

The team were able to conclude that not only is the administration of VEN + RTX combination safe, but it also triggers strong responses in most patients with relapsed refractory CLL. These significant findings are currently being tested in an international randomised Phase III trial, comparing this combination with standard chemotherapy (bendamustine) + RTX.

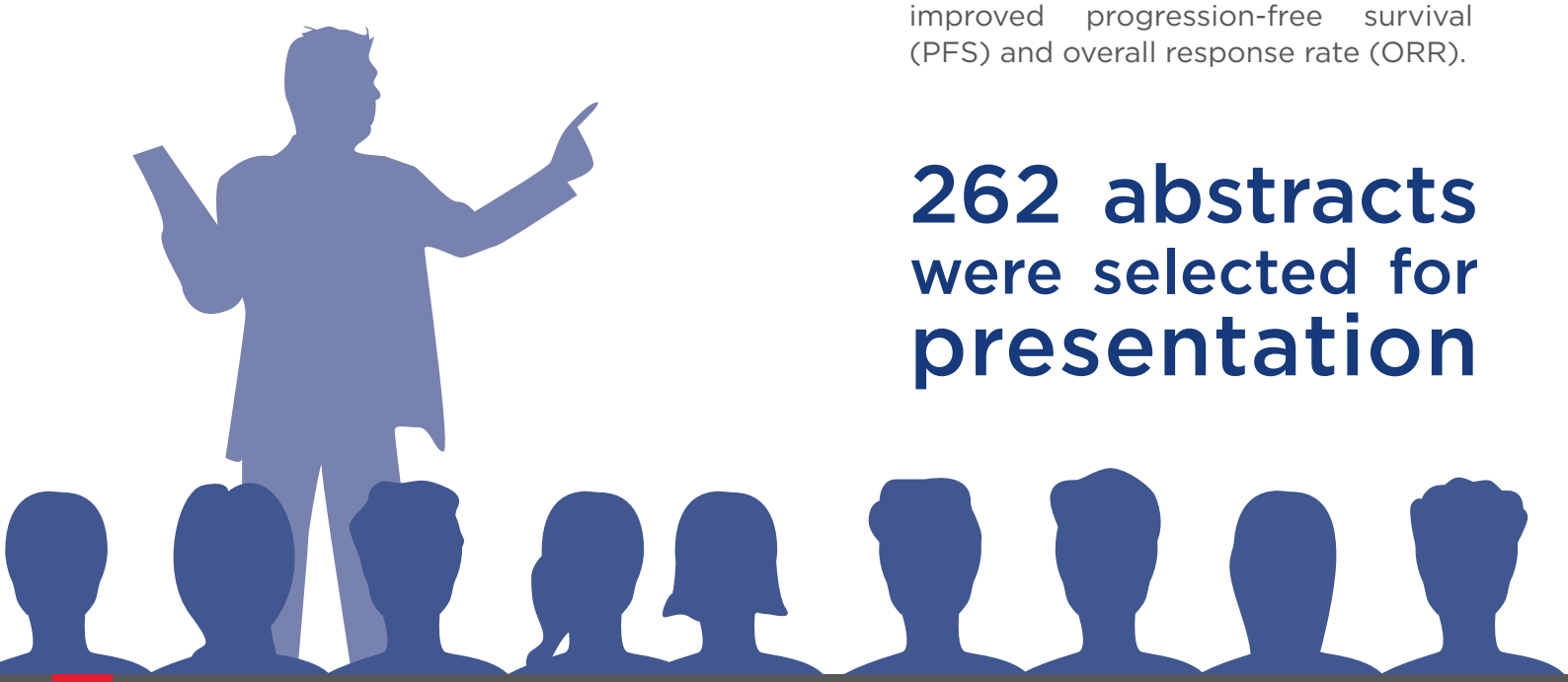
Supplementing Multiple Myeloma Treatment with Elotuzumab Reduces Risk of Disease Progression

ELOTUZUMAB (Elo) plus lenalidomide (LDM) has proven a successful combination in patients with multiple myeloma (MM), according to the results of a study reported in an EHA press release on 12th June 2015.

ELOQUENT-2, which analysed Elo in combination with LDM/dexamethasone (DXM), has become the first Phase III study to prove the advantage of directly activating the immune system in treatment of patients with relapsed or refractory MM.

Elo appears to work in two ways: binding to and directly activating signalling lymphocyte activation molecule (SLAMF7) on natural killer (NK) cells, as well as binding to SLAMF7 on myeloma cells, and flagging them for NK cell recognition and destruction. Led by Dr Meletios Dimopoulos, Professor and Chairman, Department of Clinical Therapeutics, National and Kapodistrian University of Athens School of Medicine, Athens, Greece, ELOQUENT-2 involved 224 sites across 21 countries and enrolled patients with relapsed or refractory MM who had received 1-3 previous treatments.

Patients were randomised to receive either LDM/DXM or Elo + LDM/DXM. Both groups had their treatment repeated every 28 days, and assessments were performed on tumour response and survival every 4 and 12 weeks, respectively. The co-primary endpoints of the study were improved progression-free survival (PFS) and overall response rate (ORR).



262 abstracts were selected for presentation

The results indicate that MM is less likely to worsen or cause death in patients taking Elo + LDM/DXM, triggering superior responses compared with LDM/DXM alone.

The team found that the Elo group displayed a 30% reduction in the risk of disease progression or death, with benefits maintained at 2 years the PFS rate after 24 months was 41%, compared to 27% for the control group. An absolute increase in ORR of 13% at 24 months also occurred in the Elo arm compared with the LDM/DXM-only arm.

The results indicate that MM is less likely to worsen or cause death in patients taking Elo + LDM/DXM, triggering superior responses compared with LDM/DXM alone. Earlier trials prompted the FDA to give Elo breakthrough status for MM patients who have received one or more previous treatments; these results confirm the importance of the unique mechanism of the Elo target SLAMF7 in managing MM.

Increasing Body of Data Shows Value of Ibrutinib, Daratumumab, and Bortezomib

DATA relating to the products Imbruvica® (ibrutinib), daratumumab (DARA), and Velcade® (bortezomib [BTZ]) from the blood cancer portfolio of Janssen-Cilag International NV were presented at the EHA Annual Congress, confirming the importance of these treatments for various haematological conditions.

Imbruvica works by forming a strong covalent bond with Bruton's tyrosine kinase (BTK) to block the transmission of cell survival signals within the malignant B cells. The findings showcased at EHA included data showing that the addition of ibrutinib to bendamustine/rituximab (BR) significantly reduces the risk of progression or death by 80% compared with placebo + BR in previously treated chronic lymphocytic leukaemia/small lymphocytic lymphoma.

The value of DARA was emphasised in three studies presented, with a particularly notable demonstration that in a heavily pre-treated multiple myeloma (MM) population, DARA at 16 mg/kg shows meaningful, durable single-agent activity, with deep responses and a favourable safety profile. DARA is an investigational human anti-CD38 monoclonal antibody in Phase III development in MM.

BTZ is in the specific class of medicines known as proteasome inhibitors, which reversibly interrupt the normal working of cell proteasomes, inducing the cancerous cells to stop growing and die. Among the findings demonstrated at EHA, data indicated that a fixed period (4 cycles) of BTZ consolidation was beneficial in newly diagnosed MM patients with or without prior BTZ exposure.

"At Janssen, we are proud to be driving research and scientific innovation in the haematological malignancy space and the amount of abstracts selected for presentation at this year's EHA shows our continued commitment to improving the lives of patients with these difficult to treat haematological diseases," said Ms Jane Griffiths, Company Group Chairman of Janssen Europe, the Middle East and Africa (EMEA) in a Janssen press release, 26th May 2015. "In particular, we are excited about the investigational DARA monotherapy data in MM and the new data that supports the growing body of evidence for Imbruvica. We look forward to sharing these findings with the clinical community."

Trial Shows Pacritinib Improves Outcomes for Myelofibrosis Patients

DISEASE symptoms and quality of life (QoL) of myelofibrosis patients are significantly improved through treatment with pacritinib (PAC), an oral next-generation multikinase inhibitor with specificity for JAK2 and FLT3, compared with the best available therapy (BAT), according to data that have emerged from the Phase III PERSIST-1 study.

The positive effects of PAC provide hope to myelofibrosis patients, with PAC set to become a valuable new treatment option for the disease in the future.

Most patients with myelofibrosis present with enlarged spleens and also suffer from symptoms such as abdominal discomfort, bone pain, feeling full after eating little, itching, night sweats, and tiredness.

PERSIST-1 was set up in to compare the efficacy and safety of PAC to BAT, which included a range of off-label treatments, in 327 patients with myelofibrosis, regardless of their platelet counts. The study also measured patient-reported outcomes (PROs), which are important for the approval of new therapies as they determine whether the patient's symptom burdens have been reduced and if they felt better after receiving PAC. The findings were presented at the EHA Annual Congress by Dr Ruben Mesa, Mayo Clinic, Scottsdale, Arizona, USA.

The results, displayed in an EHA press release from the 12th June 2015, showed that patients who were treated with PAC experienced a greater degree of relief (median) from symptoms compared with BAT: abdominal discomfort (46% improvement with PAC versus no improvement with BAT); bone pain (32% improvement with PAC versus 8% improvement with BAT); feeling of early fullness (45% improvement with PAC versus 1% worsening with BAT); itching (48.5% improvement with PAC versus 10% improvement with BAT); night sweats (69.5% of improvement with PAC versus no improvement with BAT); and fatigue (27.5% improvement with PAC versus 4% worsening with BAT). In addition, a higher percentage of patients had a reduction in spleen volume with PAC (≥35%), and several measures of patient QoL were improved as a result of the therapy, such as the ability to accomplish usual daily activities.

The positive effects of PAC provide hope to myelofibrosis patients, with PAC set to become a valuable new treatment option for the disease in the future.

Genetic Variants Present New Avenues for Treatment of Bleeding and Platelet Disorders

EIGHT genetic variants in the gene *GFI1B* have been discovered in patients with bleeding and platelet disorders (BPDs), which have the potential for application in novel therapeutic avenues, according to a study presented at the annual meeting of EHA in Vienna, Austria, and outlined in an EHA press release dated 12th June 2015.

The identification of *GFI1B* target genes relevant to megakaryocyte development, α-granule formation, and platelet shedding is certainly promising, and could open the door to novel therapeutic targets for these debilitating bleeding disorders.

The Congress is attended by approximately 9,000 delegates every year

In the event of vessel damage, platelets restrict blood loss through the formation of a clot, or thrombus. A recent study reported the case of a family with a BPD, the cause of which was a defect in the gene *GFI1B*. The patients presented with thrombocytopaenia, a deficiency in platelets, and a lack of α-granules, vesicles in the platelets that stimulate thrombus formation. Another notable finding of this study was that both the patients' platelets and their platelet-producing megakaryocytes expressed the stem cell marker CD34.

With the dual aims of discovering the genetic defect that explains the patients' BPDs, and understanding how this gene regulates platelet development, Dr Anna Marneth, Radboud University Medical Center, Nijmegen, Netherlands, detailed the findings of her teams latest research, and reported eight new variants in *GFI1B* that were discovered in patients with BPDs.

Half of the patients in the study had thrombocytopaenia, and several patients showed similar platelet abnormalities to the earlier reported patients carrying the *GFI1B* defect, such as decreased α-granule numbers and/or platelet CD34 expression. The *GFI1B*-encoded protein is known to regulate gene expression; almost all *GFI1B* variants were still active, while one had lost this function in transcription repression assays. If the new *GFI1B* variants are disease-causing, this would indicate that a molecular mechanism, distinct from the one that was tested, contributes to defective platelet production and α-granule formation, resulting in bleeding complications.

The identification of *GFI1B* target genes relevant to megakaryocyte development, α-granule formation, and platelet shedding is certainly promising, and could open the door to novel therapeutic targets for these debilitating bleeding disorders.

Erythroferrone: Master Regulator of Iron Metabolism in Erythropoiesis?

DISCOVERY of a new role for erythroferrone may represent an end to the search for the 'erythroid regulator' that mediates the body's demand for extra iron during erythropoiesis, according to new research summarised in the most recent newsletter from the EHA Annual Congress.

The absorption of dietary iron is regulated by hepcidin, a peptide hormone produced in the liver, whose expression reduces the availability of iron via down-regulation of an iron

transporter, ferroportin, significantly expressed in enterocytes and macrophages. Due to its massive requirement for iron, the process of erythropoiesis must be capable of modulating these hepcidin-based pathways.

These important findings not only potentially explain a crucial aspect of physiology but may be clinically relevant with regard to the iron overload observed in patients with ineffective erythropoiesis...

To identify new candidates for the erythroid regulator, a team led by Prof Tomas Ganz, Department of Pathology, David Geffen School of Medicine, University of California, Los Angeles, California, USA, examined the gene expression profile of erythroblasts isolated from mice following induced erythropoiesis (via phlebotomy or erythropoietin treatment), and selected candidate genes from the transcripts which encoded secreted proteins and which were highly expressed before a noticeable reduction in hepcidin expression. Among the candidates, the only protein that was responsive to erythropoietin treatment was erythroferrone, previously identified as Fam123b, a member of a large family of proteins related to tumour necrosis factor. Although expressed in several tissues, the increase in erythroferrone following erythropoietin treatment was only observed in the bone marrow and spleen. Erythroferrone directly inhibits hepcidin expression, although the underlying mechanism remains unclear. Although erythroferrone seems to play the erythroid regulator role during expansion of erythropoiesis, the protein probably has a minor role under resting conditions because mice lacking the *Erfe* gene do not show anaemia or iron deficiency unless challenged by phlebotomy, erythropoietin, or inflammation.

Although the extrapolation of these mechanisms to those that occur in humans needs to be made cautiously, these important findings not only potentially explain a crucial aspect of physiology but may be clinically relevant with regard to the iron overload observed in patients with ineffective erythropoiesis, such as those with beta-thalessaemia.

Knock-In Model Reveals New Insights into Homeostatic HSC Biology

HAEMATOPOIETIC stem cells (HSCs) display cell division and differentiation behaviour that differs from previous observations when they are studied under homeostatic conditions, according to new research summarised in the most recent newsletter from the EHA Annual Congress.

The research team, led by Prof Hans-Reimer Rodewald, Division of Cellular Immunology, German Cancer Research Center, Heidelberg, Germany used a novel knock-in mouse model to genetically label approximately 1% of the long-term HSCs (LT-HSCs). Yellow fluorescent protein (YFP) was used as a reporter for the genetic label so that the behaviour of labelled LT-HSCs and their progeny under unperturbed conditions could be monitored.

The proportion of YFP-positive LT-HSCs stayed constant over time, which suggests that self-renewal and differentiation of these cells are balanced under homeostatic conditions. This observation is in contrast to results from transplantation assays (from which much of our knowledge of HSC biology is derived), whereby the degree of LT-HSC labelling following reconstitution can vary widely, suggesting that the normal regulation of HSC fate decisions is dramatically altered in this scenario.

The expression of YFP was not detectable in any other cells except LT-HSCs, including their immediate progeny, short-term HSCs (ST-HSCs), up to 3 weeks post-labelling. Expression of YFP was detectable in multipotent progenitor cells (MPPs) 4 weeks post-labelling and detectable in mature blood cells after 16 weeks. These data were used to construct a mathematical model from which the rate at which cells enter and exit from different differentiation states could be inferred.

Overall, the rate of flux from the LT-HSC population to the ST-HSC population was very low (1 differentiation event per day per 110 LT-HSCs), whereas the rate of flux of ST-HSCs to the MPP compartment was much higher (1 differentiation event per day per 24 ST-HSCs). These findings are also in contrast to the situation observed during haematopoietic development in the embryo and during stress haematopoesis following chemotherapeutic challenge, in which the rates of differentiation from LT-HSCs and ST-HSCs are much higher.

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Viola Popov

Hematology Department, Colentina Clinical Hospital, Bucharest, Romania.

Q: What drew you to the field of haematology? Please give us a brief overview of your career path and how it has led you to where you are now.

A: I was attracted by the possibility of integrating the clinical aspect with laboratory haematology. I completed my haematology training and then started working in the Arges Emergency County Hospital, which is the main hospital where I live. The early activity here was a challenge because I was the first haematologist to start this specialty in the county. With patience, I managed to develop a local network of haematologists and begin clinical trials. I continued research in parallel, with the city where we work being quite close to Bucharest, and this culminated in the award of my PhD. During this time I was able to present my results at several national and international conferences, and I won the DWSA award at the 2011 ISTH conference. The desire to be more involved in research led me to the decision to move to a hospital in Bucharest where I would have more research opportunities.

Q: What changes have you witnessed in the field of haematology since your career began? How has it evolved during that time, and where is it headed?

A: During my training, patients' treatment options were limited. At that time we had just entered imatinib (a tyrosine kinase inhibitor [TKI]) into use for chronic myeloid leukaemia (CML). There was no rituximab, bortezomib, or any other new molecules. I have seen the benefit of these treatments in achieving remission of serious diseases: at the moment I have patients with CML who have been in remission with TKI therapy for more than 10 years.

Q: What is the most common patient indication that you address on a daily basis and how is it treated?

A: I most frequently have outpatients with chronic myeloproliferative neoplasms (MPN). Along with classic treatment with hydroxycarbamide and anagrelide, I have the chance to deal with ruxolitinib. I have patients who have experienced major benefits after starting this treatment, which results in decreased spleen size and a decrease in transfusion requirements.

Q: Tell us a little about your current research — what do you hope to achieve in the next year?

A: My research has been focussed on the membrane properties of platelets in patients with MPN and myelodysplastic syndromes (MDS). I have combined flow cytometry and haemostasis research with fundamental research in the biophysics laboratory, and have been fascinated by the results concerning reactive species and membrane fluidity. These results now require correlation in larger groups of patients and more complex flow cytometry investigations. I want to continue this research and evaluate the role of reactive species in thrombotic complications of MPN or MDS.

Q: Last year you co-authored a review for EMJ Hematology entitled "*The role of JAK2 mutation in thrombotic complications of chronic myeloproliferative neoplasms*" — has there been much progress in this area since the publication of this article?

A: Yes, and by this I primarily mean there have been discoveries in the molecular biology and genetics sphere, and in particular the mutation of calreticulin; I intend to update the initial review this year.

Q: What do medical congresses such as EHA have to offer the field and what do you feel their focus should be?

"There is always a need to discover new molecules and develop advanced methods of genetic and molecular diagnosis."

A: EHA and ASH congresses give us a lot of new information regarding the diagnosis and treatment of haematological disorders. They offer results from clinical trials and alternative treatment protocols, and we can use congresses to obtain insights from the vast experience of our colleagues in other countries.

Q: How do both the prevalence and treatment of haematological conditions differ between Romania and the rest of Europe? What can Europe learn from the Romanian experience?

A: In Romania we can treat patients at the same level as other European countries. The experience of our western colleagues greatly assists us in finding treatment options. With regard to the Romanian experience, I can characterise it by our perseverance and desire to be at the same level as our international colleagues despite the funds available to us being much lower.

Q: What cultural and lifestyle factors have the greatest impact on the health of the blood? What can be done to raise awareness of and address these factors?

A: I think that the greatest impact is the influence of viral infections, especially the effect of viral hepatitis (B or C) and HIV in lymphomas or leukaemias; we closely monitor these patients.

Q: What is the biggest challenge facing haematology practitioners today and what must be done to overcome it?

A: Resistance to chemotherapy causes a lack of therapeutic response in some patients. There is always a need to discover new molecules and develop advanced methods of genetic and molecular diagnosis.

Q: What has been your proudest achievement in medicine to date?

A: Being awarded the title of Doctor of Medical Sciences, which I received in recognition of a large number of presentations and publications.

Emanuele Angelucci

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

Q: What inspired you to concentrate your efforts in the field of haematology?

A: When I graduated I was fascinated by allogeneic bone marrow transplantation, which was at that time (1984) in the early stages of development. Particularly fascinating to me was the idea of acquiring specific skills in an intensive therapy that was able to cure what were, at that time, incurable diseases.

Q: To what extent have improvements been made in bone marrow transplantation since you first began working in this area of medicine?

A: A lot. Just consider that, in 1984, a 36-year-old patient was considered too old for allogeneic transplant and that only transplantation between siblings with identical human leukocyte antigen types was possible.

Q: How much have treatment options improved for patients who suffer from thalassaemia in recent years?

A: Important developments in recent years include: improvements in our understanding of iron-overload pathophysiology, the magnetic resonance imaging approach to iron-overload diagnosis and quantification, and oral iron chelators. Very recently, gene therapy seems to have finally become a realistic approach.

Q: How far has our knowledge of the causes and treatments of leukaemia developed since you first began research into this disease?

"Gene therapy seems to have finally become a realistic approach."

"Blood diseases must be considered in the same way as other diseases..."

A: Not so much in acute leukaemia, but much more in other forms of leukaemia such as chronic myeloid leukaemia.

Q: How feasible is it to expect people to take part in regular screening for blood disorders? What more can be done to encourage people to have blood tests in this regard?

A: This is highly variable and depends upon the pressure applied to, and the interest within, the general population. It is more feasible for genetic diseases, such as thalassaemia major, and much less feasible for acquired diseases.

Q: In your opinion, what is the single biggest challenge facing haematologists today?

A: I believe there are two challenges: the first is professional and the second is medico-social. The professional challenge is to significantly improve the cure rate for acute leukaemia patients, particularly elderly patients. The medico-social challenge is to make treatments available for all the patients who need it.

Q: What advice would you give to people who are concerned about blood disorders?

A: Blood diseases must be considered in the same way as other diseases; today, a lot of them are curable and almost all are treatable.

Q: How does Italy compare with the rest of Europe in terms of the standard of treatment for haematological conditions?

A: Generally speaking, the standard of treatment is similar to the rest of Europe, although highly variable within the country. The problems in Italy are the extremely high cost of the healthcare system and barriers to career development for physicians.

Q: How important is the annual EHA congress for haematologists looking to increase their knowledge of the field?

A: Very important. The EHA meeting is growing so much and is now one of the major resources for improving haematologists' knowledge.

Q: What advice would you give to young medical professionals just starting out in their career in haematology?

A: Work very hard and stay in clinical research!

Sophia Delicou

Thalassemia and Transfusion Unit, Hippokration General Hospital of Athens, Athens, Greece.

Q: What was it that first drew you to the field of haematology?

A: One of the things that drew me to haematology was the possibility to specialise in lots of different areas. The link between the science, the laboratory diagnostics, and the clinical work can be applied to real-life patients, and can save lives.

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

A: Mapping the human genome has provided a potential option for preventive medicine. By understanding the genetic causes and links to disease we can give more and more attention to preventing disease and curing individuals.

Q: How far have treatments for sickle cell disease and thalassaemia improved in recent years?

A: Even though the underlying molecular causes of these diseases were understood more than half a century ago, progress in translating this knowledge into improved patient care has been slow. In the last decade, further progress has been made in sickle cell and thalassaemia research. Researchers have improved outpatient programmes for pain control, identified pulmonary hypertension as a common life-threatening complication of sickle

cell disease, and have found that the use of hydroxyurea works in part by stimulating the body to resume production of fetal haemoglobin (haemoglobin F). In thalassaemia, systematic transfusions have dramatically improved the quality of life of patients, but iron overload from transfusions was a major cause of death and so iron-chelation treatment has given a breath of life to these patients. There are three different chelating agents: deferiprone and deferasirox, which are taken by mouth, and desferrioxamine, the first chelator, which is given via a drip (an infusion inserted under the skin). Furthermore, specialised imaging tests can now detect iron in the heart and allow patients to be treated before they develop iron-related heart failure.

Q: Have you noticed any changes in the types and prevalence of blood diseases that you see in your clinic during the last decade?

A: We have seen sporadic new cases of thalassaemia and sickle cell disease since the National Premarital Screening Program was initiated in my country in the early 1970s.

Q: To what extent has our understanding and knowledge of non-malignant haematology improved since you first began research in this area?

A: The 'classical' diagnostic tool for the detection of new haemoglobin mutations was zone electrophoresis, which separates proteins differing in electrical charge. However, many mutant haemoglobins display normal electrophoretic mobility and must be studied by other methods, such as high-performance liquid chromatography. A number of more sophisticated techniques have been applied to the detection of mutant haemoglobins, including mass spectrometry and sequencing of DNA fragments generated by the polymerase chain reaction, and a database of known mutations of the human haemoglobin gene is now available and so it is easier to associate these with any clinical manifestations.

Q: You recently co-authored an article for EMJ in which you summarised five thrombocytopaenic syndromes caused by platelet-reactive alloantibodies. You stated that increased awareness of these syndromes, together with the greater availability of highly specialised laboratory methods to detect and characterise platelet-reactive alloantibodies, will lead to their more frequent diagnosis. Which do you believe would be the most effective channels for raising awareness of these syndromes amongst healthcare professionals?

A: The risk charts and risk factors should be considered among healthcare professionals, and management guidelines should also be considered.

Q: In what ways can patients remain vigilant and aid the early diagnosis of haematological conditions?

A: They need information that ensures that they are well-informed about their choices, and which is provided in a way that they can understand and by staff who are aware of, understand, and recognise the diversity of social and cultural values and beliefs. Priority should be given to good clinical practice supported by modern technology and equipment.

Q: Are there any other aspects of haematology that you would like to research in the future?

A: The explosion in molecular biology and genomic technology is very exciting and I am very keen on it.

Q: How important is the annual EHA Congress for haematologists?

A: The purpose of this conference is to promote good health and improve outcomes among people at risk for, or affected by, malignant and non-malignant blood disorders through the exchange of experiences. Therefore, the congress is very important and I hope to continue with these activities.

Q: What do you believe is likely to be the next big breakthrough in the field?

A: Gene therapy has the potential to cure genetic diseases. However, the corrective gene must be introduced into a sufficient number of cells and also be adequately expressed in order for its product to correct the deficiency, and this is the next big step for haemoglobinopathies.

Felipe Prósper

Director, Hematology and Cell Therapy, University Clinic of Navarra, Pamplona, Spain.

Q: What was it about haematology that drew you to the field?

A: As usually happens, it had a lot to do with one of my professors during medical school. Prof Rocha, Professor of Hematology, was really great and was able to show us his enthusiasm for this specialty. The fact that I was already interested in research and there were plenty of research opportunities in haematology 'sealed the deal'.

Q: How far has the field developed as a whole since you first began your medical career?

A: Enormously, I think that the development of cell therapy is one of the main contributions of haematology to biomedicine in general.

Q: To what extent has our understanding of stem cell therapy increased since you first began research into this area?

A: Again, our understanding regarding stem cells has improved very significantly. The development of induced pluripotent stem cells and the recent boost in the use of immunotherapy with chimeric antigen receptor T cells constitute what we now call the 'third pillar of therapeutics'.

Q: Do you see stem cell therapy becoming a more prominent and effective treatment for patients in the near future? What further barriers need to be overcome to achieve this?

A: I am completely sure that cell therapy will reach the clinical arena and constitute a new therapeutic weapon in the near future. The hurdles are important, however, because not only do we still need to establish a true efficacy proof of concept, but we also need to develop business models capable of attracting industry to invest the amounts of money required for these treatments to reach patients.

Q: In your opinion, is more funding required for stem cell research?

A: Funding is always necessary, but, as I said before, we need two things: to demonstrate efficacy in some indications, and to develop new business models. Otherwise, we may continue doing research without it ever achieving the main aim, which is to have a positive impact on the health of patients.

Q: How has our understanding of the epigenetic regulation of malignancies developed since you first began research into this area? How has this been translated into effective treatments for patients?

A: Epigenetics was in its infancy when I started in research. Since then we have come to the realisation that epigenetic regulation of gene expression is at least as important as genomics with regard to the development and prognosis of haematological diseases (as well as in other cancers), and that it is also quite complicated and involves many different, interconnected mechanisms. Our current knowledge is driving the development of novel small molecules targeting epigenetic regulators, which, in my opinion, represent a great opportunity.

Q: How well are we able to tackle myeloma and leukaemia? What more needs to be done to combat these diseases?

A: These diseases remain, for the most part, incurable and so there is still a lot to do. In myeloma, a surge of new therapies, including monoclonal antibodies, checkpoint inhibitors, immunomodulatory drugs, and next-generation proteasome inhibitors, have recently been developed and tested in clinical trials, and to some extent we are starting to believe that we may be able to cure a percentage of our patients. Some of the current challenges include being able to define the best drug combinations and in which patients to use them, and developing personalised therapies based on the genetic and epigenetic make-up of tumours.

Q: What do you think is the biggest challenge currently facing haematologists?

A: To improve the percentage of patients that we are able to cure and to make it more affordable for society.

Q: How important is the annual EHA congress for healthcare professionals working in the field of haematology?

A: I find this meeting really important for us, not only because of the quality of the presentations but also because it provides a great opportunity to interact with colleagues from other institutions and with industrial partners.

Q: Are there any aspects of this year's congress that you are particularly looking forward to?

A: The plenary sessions; the best abstracts are something I particularly look forward to.

Q: What advice would you give to young haematologists just starting out in their medical careers?

A: It is always hard to give good advice, but I have found that every physician should try to ask questions regarding our patients. For example, why do only some patients respond to treatment, and what are the results that I am getting in my practice every day? This means that we all need to conduct research if we really want to make a difference. This does not mean laboratory-based research; it just means that we need to question what we do and why we do it. So my advice would be that we must try to move out of our comfort zone every day, and we must try to learn something, ask a question, or have a new idea.

"Cell therapy will reach the clinical arena and constitute a new therapeutic weapon in the near future."

Eloísa Urrechaga

Consultant for Clinical Chemistry, Hematology Laboratory, Galdakao-Usansolo Hospital, Galdakao, Spain.

Q: What made you decide to pursue a career in haematology? What has led you to your current position at the Galdakao-Usansolo Hospital?

A: I started my career in biochemistry and working in a medical laboratory. When I moved to the Galdakao-Usansolo Hospital, the head of the department assigned me to haematology in order to improve the harmonisation among the different sections of the laboratory.

Q: What are the main advances that you have witnessed within the field since you began your career?

A: Quality control is improving every year, coagulation testing has evolved, and many tests are now available in automatic analysers. Automation in image analysis is useful for training trainees and technicians.

Q: How much of an impact has the development of new haematological parameters had on the research of anaemia, erythropoiesis, and the glycation of haemoglobin since you first became involved in this?

A: New parameters, although not standardised and possibly lacking external and even internal quality controls, can improve the algorithms for differential diagnosis of common diseases, which helps to improve the quality of a laboratory.

Q: To what extent has the automation of biological parameters in iron deficiency, β-thalassaemia, anaemia, and other erythropoietic disorders improved in recent years?

A: As mentioned above, their potential usefulness is proven by the fact that nowadays every commercial company has developed extended parameters.

"The objective is to maintain our standards of quality..."

Q: Last year you co-authored a paper published in *EMJ Hematology*, in which you described the advances made since Dr Maxwell Myer Wintrobe's pioneering works on red cell indices. You also stated that both laboratory scientists and clinicians need to keep up-to-date with new parameters and methods in haematology, and that a stronger collaboration between these groups would bolster clinical decision-making. Have there been any improvements made in this respect since you completed this paper?

A: This is a 'human factor' matter: a haematologist or a laboratory professional can work with fine counters, only those interested in new parameters will try to extract their own data. The same applies for clinicians: most of them lack interest in the laboratory in general, and it is more difficult trying to add more parameters. Collaboration is only possible when both professionals find it useful to add the extended parameters to the laboratory reports.

Q: In your opinion, what are the biggest challenges facing haematologists over the next 5 years?

A: The budget shortages in my country have led to consolidated laboratories, which means that the professionals have lost control of their own work, with a single company providing everything. The haematology section usually has little to do with the decision-making process.

Q: What advice would you give to members of the public who wish to reduce the risk of developing common blood disorders?

A: They should seek the advice of clinicians.

Q: How would you describe the state of haematological testing in Spain? How does this compare with other countries in Europe?

A. Spain is divided into regions and the situation is different even in the same area, but the general trend is to include haematology within the core laboratory.

Q: How important are the EHA Annual Congresses to those working in haematology and how influential have they been in your own research?

A: Congresses are useful, not only for the presentations of renowned colleagues and being able to visit booths to see the most modern machines, but also for sharing opinions and experiences with colleagues.

Q: What are the main objectives that you have set yourself for the next 5 years?

A: Our laboratory is now becoming a core laboratory and so the objective is to maintain our standards of quality and our level of organisation.

"Congresses are useful..."

Anna Rita Migliaccio

Professor of Medicine, Division of Hematology/Oncology, Department of Medicine, Mount Sinai School of Medicine, New York, USA; Professor of Histology and Embryology, Department of Biomedical and Neuromotorial Sciences, Alma Mater University, Bologna, Italy.

Q: What first attracted you to the field of haematology, and how did you arrive at your current position at the Mount Sinai School of Medicine?

A: An exhaustive answer to this question would require a book. I began my training in haematology in 1978 and I became Professor of Medicine at the Mount Sinai School of Medicine almost 30 years later in 2007. The thing that attracted me to the field of haematology is deeply linked to my personal life. I started my training as a developmental biologist and, in the 1970s, the models of choice for developmental biologists were sea urchins, sponges, and drosophila. I started my career studying all these models but then I fell in love with a haematologist fellow and, during our pillow talks, I discovered that blood is an excellent model for developmental biologists and so I decided to join the laboratory where my husband was doing his training.

I became Professor of Medicine at Mount Sinai by chance. I had returned from the USA to Italy for good in 1997 in order to establish my own laboratory. While back in Italy, I discovered an animal model for a myeloproliferative disease, primary myelofibrosis. This model was included in a programme project proposal submitted to the NIH Cancer Center that aimed to study and search for cures for myeloproliferative diseases, including primary myelofibrosis. When the project was funded, the principle investigator of the programme project, Prof Ronald Hoffman, decided that the Department of Medicine at Mount Sinai would represent the 'optimal hub' to make the programme flourish and proposed that I join him in the adventure. Since I had my own funding, Mount Sinai was more than happy to recruit me at a suitable level, that of full Professor. This started a scientific adventure that is continuing to give me numerous scientific surprises.

Q: What research projects are you currently working on at the Tisch Cancer Institute?

A: I am funded by the NIH to pursue two major areas of research: the pathobiology of primary myelofibrosis using animal models, and the regulation of terminal erythroid maturation and how this is disrupted under pathological conditions.

Q: How much do you think the field of haematology has evolved since you first began your research?

A: It is impossible for fellows training in the field of haematology today to even imagine what it meant to be a fellow in the 1970s. There was limited instrumentation, which was mainly optical (a few lucky fellows even had electron microscopes), and animal and human experimental models were even fewer. Most of the knowledge from humans was provided by the medical consequences of atomic explosions in inhabited areas: the bombing of Hiroshima and Nagasaki, Japan, August 1945, and the accidental damage to the nuclear reactor at the Vinča Research Institute for Physical Science, Yugoslavia, October 1958. The field has evolved so much in a short space of time and has become highly sophisticated. Now we have generated multiple animal models harbouring a variety of genetic modifications, we perform single-cell transplants, we have multiple recombinant growth factors, we perform full-genome sequencing at reasonable cost, and we can use sophisticated techniques for cell reprogramming and gene editing, etc.

Q: In a recent paper that you wrote for EMJ, you summarised current knowledge on the biological activity of CALR and MPL/JAK2 in haematopoiesis, delineated a unifying pathway for the pathogenesis of myeloproliferative neoplasms, and discussed how this pathway may be exploited for therapy. Have there been any updates on this topic since the paper was published?

A: There have been updates and these will be submitted in an abstract format to ASH 2015 and to a scientific journal soon. I cannot update you with the information so as to not jeopardise the publication!

Q: How would you describe the current state of stem cell research? What obstacles do you think need to be overcome in order for this research to realise its full potential?

A: The field of stem cell research is flourishing. There is great potential that the sources of stem cells and the conditioned regimenn for transplantation will be revolutionised in a few years from now. We are analysing the possibility of using genetically modified stem cell sources at various levels and to devise treatments that may make the microenvironment of the host more receptive to the donor cells. The major problems to overcome are related to possible immunogenicity.

Q: How far has our knowledge and understanding of erythropoiesis increased in recent years? How has this knowledge been used to improve patient care?

A: Studies on erythropoiesis are so important because its alterations generate either anaemia or polycythaemia. The product of the process, red blood cells, are very important to deliver oxygen to all the tissues of the body. If you do not have enough red blood cells, or if you have too many, then you feel really uncomfortable even if you have an otherwise mild disease. The major breakthrough in erythropoiesis has been

the discovery of erythropoietin. Recombinant erythropoietin is the growth factor that is most widely utilised in the clinic and it has revolutionised patient care. It does have side-effects, however, and this is why several alternatives have been developed and are in the pipeline for clinical evaluation; it will be interesting to see if there will be a single winner or multiple winners targeted for different pathological conditions.

Q: What advice would you give to patients who wish to reduce their risk of developing blood disorders?

A: My advice is to trust the primary doctor. It is wonderful that the communication media has evolved so much that information on almost anything may be retrieved in a lay form by anybody, but this information is not screened and may not be accurate. It is good to be informed because it guides us to make informed decisions, but these informed decisions must always be taken after having listened to the opinion of a professional.

Q: How important is the annual EHA congress to you?

A: EHA and its congress is a great asset for haematologists in Europe. I am so proud of this organisation and that I am among the founding members of the society; I still have the EHA tie (I did not want the scarf) that Bob Lowember gave to the scientists who helped organise the first EHA meeting in Rotterdam. Unfortunately, EHA comes at a very difficult time of the year for me because it competes with the scientific retreat of one of my major funds. I wish I had the opportunity to participate in the annual EHA meeting more often and perhaps to be able to organise one of the next EHA meetings, maybe in Bologna, Italy, where I recently received a major scientific recognition.

Q: As a prominent member of numerous international haematological associations, how unified would you say strategies for research and treatments are across national borders? Could more be done to improve this?

A: Treatments across national borders are becoming more and more unified by the day. It is the job of medical associations, such as EHA and many others, to make sure that this unification comes to life in a timely fashion.

Q: What are your own personal goals and ambitions for the next 12 months?

A: I am at a stage of my career in which goals and ambitions may not be reached in 12 months. It is certain that in the next 12 months I will have published a few more papers, obtained a few more grants, and educated a few more students. But this is what I have been doing all my life and may not be defined as a goal or ambition. I am 7 years from retirement and I recently obtained a chair for 'distinguished scientific merit' (whatever that means) from the Alma Mater University in Bologna, Italy. My real goals and ambitions for these next 7 years are to organise an exchange programme for medical students at all stages of their education, between the Ischan School of Medicine at Mount Sinai and the Alma Mater University. This may be something that, if I accomplish it, will leave a 'signature' on the future of haematology, at least for a few years after my time.

Q: Is there a question that you would have liked us to ask and we have not?

A: Yes there is: What is the advice that you would like to give to the next generation of haematologists to be successful? My advice would be 'focus on the question and not on the answer'. There are many worthy answers out there that may provide insights on new clinical interventions. However, current technology may address only a portion of them. To be successful, it is important to prioritise questions that can be answered with current technology.

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THROMBOSIS IN HAEMATOLOGICAL DISORDERS: TAILORED MANAGEMENT APPROACHES

Summary of presentations from the Alexion-supported satellite symposium held at the 20th Congress of the European Hematology Association (EHA) on 11th-14th June 2015, Vienna, Austria

Chairperson

Anna Falanga[1]

Speakers

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MEETING SUMMARY

The meeting commenced with a talk from Prof Anna Falanga on the management of thrombosis in both onco-haematological and non-oncological diseases. Adjunct Prof Sakari Jokiranta gave an overview of the complement system and the interplay between the complement and coagulation systems. Dysregulation of complement and resulting disease states were also discussed. The session was concluded with a presentation from Dr Anita Hill on the management of thrombosis in paroxysmal nocturnal haemoglobinuria (PNH).

Management Options for Thrombosis in Haematological Disorders

Professor Anna Falanga

Haematological disorders can be divided into onco-haematological diseases and non-oncological diseases, both of which have a high risk of thrombosis (Figure 1).[1-3] Non-oncological diseases include hereditary haemolytic anaemias (HHAs), antiphospholipid syndrome, thrombotic thrombocytopaenic purpura (TTP), decreased endogenous anticoagulants, abnormalities of fibrinolysis, and acquired haemolytic diseases such as PNH. Onco-haematological diseases include acute leukaemia, multiple myeloma, lymphoma, and myeloproliferative neoplasm (MPN).

Thrombosis is more prevalent in patients with cancer.[4-6] In fact, venous thromboembolism (VTE) is a frequent complication of cancer, including haematological cancer, with an estimated risk of 0.6% per year.[4] Treatment with chemotherapy increases the risk of thrombosis by 6.5-fold.[7] Cancer-associated VTE is linked to increased mortality, increased risk of recurrent VTE and bleeding complications, and interruption of chemotherapy, and also has economic implications.[8,9] Clinical risk factors (such as hypercholesterolaemia, hospitalisation immobility,

previous history of thrombosis, etc.), tumour cells, and host cell response all contribute to increased coagulation activation in cancer patients. Hypercoagulation leads to thrombosis and tumour progression, which in turn promotes hypercoagulation.[10]

Tumour cells constitutively produce signals that activate coagulation pathways, which is a unique pathogenic mechanism of thrombosis.[11] The production of tumour procoagulant activities, inflammatory cytokines, angiogenic factors, and the expression of adhesion receptors induces the activation of blood coagulation. This leads to thrombin generation and fibrin formation, resulting in cancer-associated thrombosis.[11] Different levels of thrombin may be produced depending on tumour types; in an *in vitro* study of human tumour cell lines, promyelocytic leukaemia cells induced the highest levels of thrombin in normal plasma.[12] Many of the oncogenes commonly dysregulated in cancer drive increased expression of clotting proteins.[13,14] To treat coagulopathy effectively, it is necessary to understand the underlying mechanisms promoting coagulation. For example, in acute promyelocytic leukaemia (APL), the PML/RARα genetic lesion is associated with overexpression of procoagulant activity, i.e. tissue factor.[15] Differentiation therapy with all-trans retinoic acid (ATRA) targets the molecular lesion, causing maturation of the affected promyelocytes and a reduction in the procoagulant expression, thus resolving the coagulopathy.[15] As well as the immediate administration of ATRA, management of coagulopathy in APL consists of platelet transfusion to maintain platelets at >50 × 10^9/l and red blood cell (RBC) transfusion to maintain haemoglobin levels >8 g/dl.[16] If cerebral bleeding is suspected, a computed tomography or magnetic resonance imaging scan should be performed immediately, lumbar puncture should be avoided, and the patient should be transferred to the intensive care unit.[16] Other treatments have either not shown a conclusive benefit in trials (tranexamic acid, unfractionated heparin), or have not been tested in this setting (low-molecular-weight heparins [LMWHs], pentasaccharide, newer anti-Xa/anti-IIa agents).

No specific guidelines exist for the treatment of VTE in haematological malignancies, making it necessary to adapt guidance for patients with solid tumours. In patients with solid tumours, initial treatment of VTE is LMWH at 200 U/kg/d for 1 month and subsequently 70-80% of the initial dose for at least 5 months.[17] For haematological malignancies, expert opinion has suggested adapting the dose according to the platelet count: 70-80% of initial dose for a platelet count of ≤70 × 10^9/l or reduced to 50% if platelets are ≤50 × 10^9/l. Therapy should be stopped if platelets are ≤20 × 10^9/l.[18] The 2014 American Society of Clinical Oncology Clinical Practice Guidelines[17] recommend LMWH for patients with cancer who have deep vein thrombosis (DVT) and pulmonary embolism (PE), both for the initial 5-10 day treatment and for prolonged secondary prophylaxis of at least 6 months.

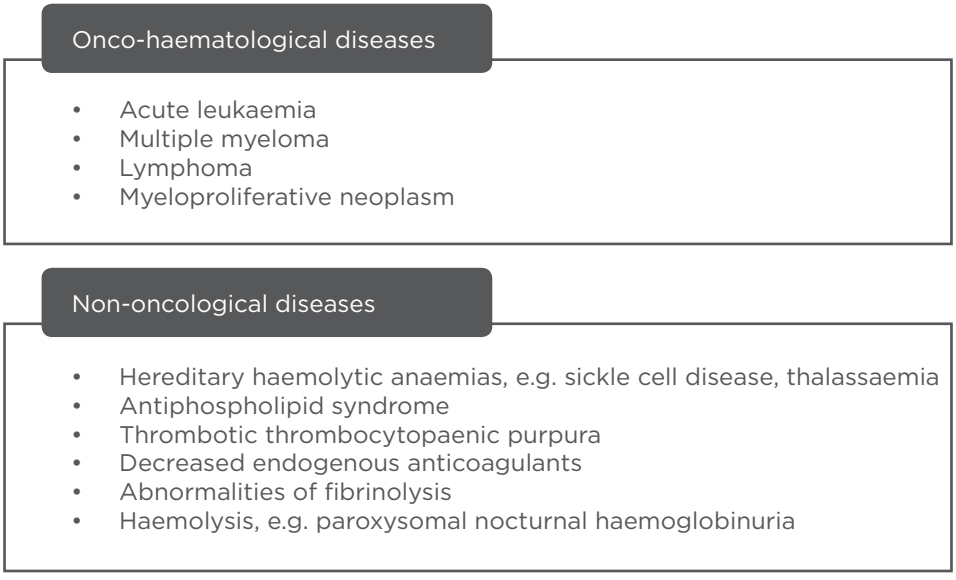


Figure 1: Haematological disorders with high thrombotic risk.

MPNs such as essential thrombocythaemia (ET) and polycythaemia vera (PV) have a high thrombotic risk and management of the disease is dependent on the extent of thrombotic risk.[10] Factors that increase the risk of thrombosis in MPNs include an age of >60 years and previous thrombosis. Other risk factors under active investigation include cardiovascular risk factors, leukocytosis, haematocrit in PV, and the V617F mutation in the *JAK2* gene.[10] Recommendations for a risk-adapted treatment approach in treating thrombosis in ET and PV have been released by Tefferi and Barbui,[19,20] where the treatment regimenn is dependent upon the pathophysiology.

Future management of cancer-related thrombosis is likely to focus more on the pathophysiological approach of targeting the oncogenic molecular lesion, while classic anticoagulant and antiplatelet drugs may be considered in a different light.

Non-oncological diseases such as HHAs also have a high thrombotic risk.[21] The most common forms of HHAs are sickle cell disease (SCD) and thalassaemia. Thalassaemia results from a partial or complete lack of synthesis of one of the major α or β-globin chains of haemoglobin A, whereas SCD is caused by a single amino acid mutation of the β-globin chain. Intravascular haemolysis is a common pathogenic prothrombotic trait in both conditions.[21] Thalassaemia and SCD are caused by the loss of the normal asymmetrical distribution of the RBC membrane phospholipids. Phosphatidylserine is translocated to the external leaflet of the cell membrane resulting in activation of the prothrombinase complex, which facilitates interaction between the RBC and endothelial cells and ultimately leads to a hypercoagulable state.[21] Other factors in thalassaemia contributing to hypercoagulability include reduced levels of nitric oxide (NO) leading to vasoconstriction, increased platelet aggregation, and formation of microparticles from peripheral blood elements.[22]

A high prevalence of thrombotic events (TEs) is observed among thalassaemia patients, particularly those with thalassaemia intermedia.[22] The most notable thrombosis risk factors among thalassaemia patients are advancing age (>35 years old), splenectomy, and serum ferritin ≥1,000 µg/l, as confirmed in the OPTIMAL CARE observational study of over 500 thalassaemia intermedia patients.[22,23] Optimal preventative strategies are not yet established and the roles of antiplatelets, anticoagulants, fetal haemoglobin induction, transfusion, and iron chelation therapy should be further investigated. OPTIMAL CARE identified haemoglobin levels of ≥9 g/dl and transfusion as factors associated with a significantly decreased risk of thrombosis.[24]

Antithrombotics are recommended for treatment of thromboembolism, particularly during acute episodes.[25] The choice of antithrombotic drugs is dependent on the site of thrombosis; aspirin is normally administered for arterial thrombosis and heparin or warfarin for VTE. Regular RBC transfusion has been recommended in thalassaemia patients in order to maintain haemoglobin levels higher than 9 g/dl. For SCD, however, trials of anticoagulants or antiplatelets have been inconclusive. PNH, a further example of non-malignant haematological disease, carries a very high relative risk of VTE compared with other thrombophilic conditions and requires a very specific management approach.[26,27]

In summary, both non-malignant and malignant haematological disorders can carry a risk of thrombosis. In all cases, the risk factors and underlying pathophysiology must drive the decision-making process for the selection of appropriate and potentially life-saving therapy.

Complement-Mediated Thrombosis: A Complex Interplay between Complement and Coagulation

Adjunct Professor T. Sakari Jokiranta

The complement system forms part of the innate immune system. Activation of complement is mediated by >20 proteins circulating in the blood and tissue fluids. In response to a pathogen or to foreign structures, the complement system is activated and results in a sequential activation of proteins and enzymes. This cascade causes the opsonisation of pathogens, which induces a series of inflammatory responses that help fight infection. Complement can be activated via three different pathways: classical pathway, lectin pathway, and alternative pathway. Each pathway is able to cause the activation of C3 and C5 convertases, leading to the activation of a common terminal (lytic) pathway.

The alternative pathway is continuously activated at a low level, with its activity being amplified by various conditions including infection, tissue

damage, surgery, or pregnancy. The alternative pathway is initiated by spontaneous hydrolysis of C3 to form C3(H_2O), allowing generation of fluid-phase C3 convertase which is able to cleave many molecules of C3 to form C3a and C3b. The C3b that is generated is able to attach covalently to the surfaces of host cells or pathogens nearby. C3b that is bound in this way is able to bind complement factor B, which leads to the formation of the alternative pathway C3-convertase (C3bBb) on the target surface, ultimately leading to elimination of the target.

Activation of the complement system results in destruction of the target via three main mechanisms: opsonisation of the pathogen via bound C3b resulting in phagocytosis; generation of C5a, which attracts neutrophils to the site of infection; and creation of pores in the bacterial membrane leading to lysis of the target cell. Damaged cells can activate complement via one or more of the three pathways, and complement-mediated damage can induce further local complement activation.

As the alternative pathway is activated spontaneously, it has the potential to damage host cells if it is not well regulated. Several complement regulatory proteins act to prevent any accidental damage to host cells. Most of these regulators function at the C3 stage within plasma (e.g. factors H and I) or at the cell membrane. A fine balance exists between the activation and regulation of the alternative complement pathway. Impaired regulation, caused by malfunctioning regulators, can cause chronically uncontrolled complement activation leading to organ damage. Similarly, gain-of-function mutations (e.g. mutation in C3 or complement factor B) can result in enhanced activation, which may also lead to organ damage.

Atypical haemolytic uraemic syndrome (aHUS) and PNH are two examples of disease arising from uncontrolled complement activation. aHUS is a rare, life-threatening, systemic disease with a poor prognosis[28] characterised by microangiopathic haemolytic anaemia, thrombocytopaenia, and acute kidney failure.[29,30] Uncontrolled complement activation causes platelet and endothelial cell activation and damage as well as haemolysis.[31] Multiple genes have mutations associated with the disease, six of which are complement proteins.[32-35] Three additional genes: *THBD*, *PLG*, and *DGKE* (encoding thrombomodulin, plasminogen, and diacylglycerol kinase epsilon, respectively) are involved in coagulation or fibrinolysis. Patients with aHUS experience complement activation against all cells in contact with plasma, including platelets, leukocytes, RBCs, and endothelial cells. Complement activation on these cells gives rise to a number of clinical consequences, namely platelet consumption, mechanical haemolysis, blood clotting, vessel occlusion, inflammation, and ischaemia, ultimately leading to systemic multi-organ complications and thrombotic microangiopathy (TMA).[36-40] There is significant clinical overlap with other causes of TMA such as TTP, although TTP is distinguishable through ADAMTS-13 activity: in aHUS it is >5%, whereas in TTP it is always <5%.[41]

Importantly, the complement, coagulation, and fibrinolysis systems are interlinked (Figure 2).[42] Release of C5a, which is a potent anaphylatoxin, acts on endothelial cells and leukocytes, leading to enhanced tissue factor activation and a hypercoagulable state. In turn, the coagulation pathway can lead to complement activation. This can lead to a vicious circle of both pathways activating each other. This continual activation of complement can cause damage to host cells, resulting in organ dysfunction.[42]

PNH is caused by a somatic mutation in the *PIGA* gene (encoding phosphatidylinositol glycan class A) in haematopoietic stem cells, leading to loss of glycophosphoinositol anchor synthesis.[43] This mutation results in the production of abnormal blood cells that lack several cell surface proteins, including complement regulators CD55 and CD59.[44] Absence of CD55 and especially CD59 leads to continual susceptibility of the cells to complement-mediated destruction following spontaneous deposition of C3b on the deficient cells.[45,46] The high incidence of thrombosis in PNH is due to complement activation on CD59-deficient blood cells, activation and aggregation of PNH platelets, and haemolysis of PNH erythrocytes, leading to reduced NO levels.[27,45-49] These effects are responsible for the systemic effects associated with PNH, including renal failure, pulmonary hypertension, abdominal pain, chest pain, dyspnoea, dysphagia, fatigue, haemoglobinuria, and erectile dysfunction.

The importance of the interplay between complement and coagulation is supported by clinical observations in both aHUS and PNH.

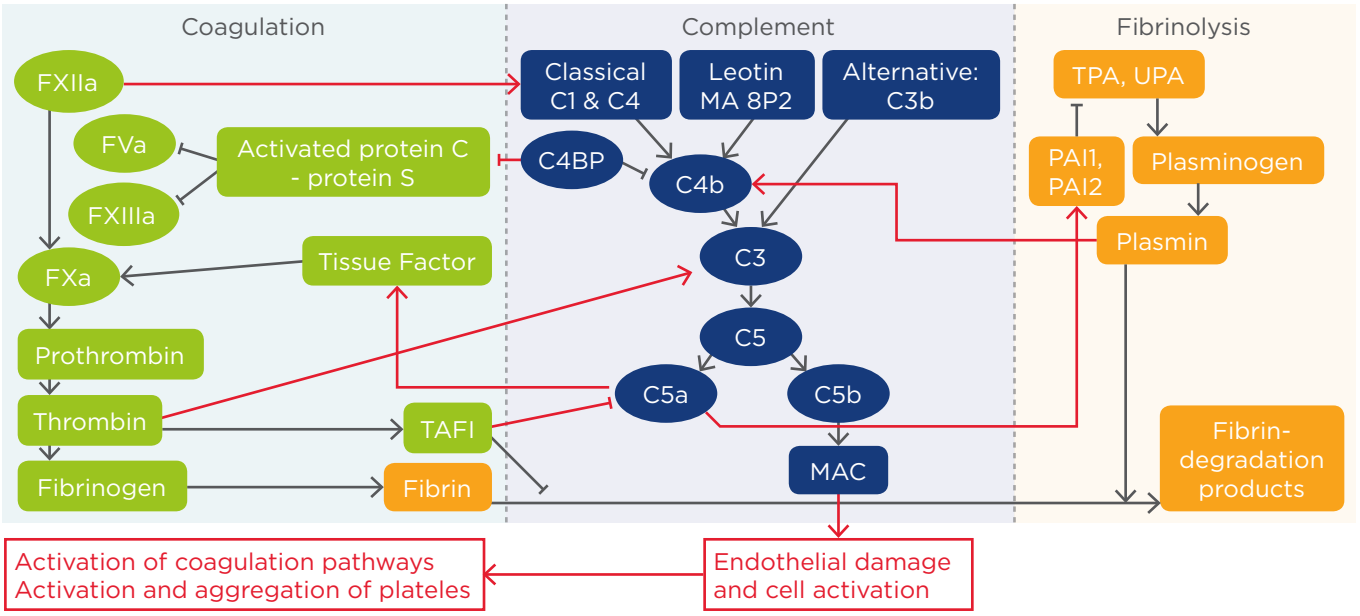


Figure 2: Cross-talk between the complement, coagulation, and fibrinolysis system[a].

[a]Only most relevant links shown; central links shown in red.

Adapted from Rittirsch et al. by permission from Macmillan Publishers Ltd: Nature Reviews Immunology 8, 776-787 (Oct 2008).[42]

Abnormalities in complement regulation without a known abnormality in coagulation or fibrinolysis leads to microangiopathic thrombosis in aHUS.[38] Furthermore, mutations in genes controlling coagulation or fibrinolysis can lead to complement-mediated pathophysiology. For example, *THBD* mutations have been found in 3-5% of aHUS patients[50] and some aHUS patients have exhibited a plasminogen deficiency.[51] PNH is associated with impaired complement regulation on PNH erythrocytes[52] and platelets, and a significantly increased risk of thrombosis.[53] The best evidence of the interplay of complement and coagulation in these two diseases is provided by therapeutic targeting with eculizumab (ECU) of the terminal complement cascade at C5, as this prevents thrombotic complications in PNH[27,54] and has a beneficial effect not only in those aHUS patients with a defect in a complement protein but also in those with a *THBD* mutation.[55] ECU functions in these diseases in two ways: first, by preventing formation of C5a, leading to decreased exhibition of tissue factor activity by endothelium and leukocytes;[56,57] and secondly, the membrane attack complex is not formed on platelets or RBCs, resulting in no hyperactivity of platelets and no intravascular haemolysis or reduction in NO.

Other thrombotic diseases that arise from activation of complement include septicaemia and disseminated intravascular coagulation, ischaemia–reperfusion injury, catastrophic antiphospholipid syndrome, and antibody-mediated rejection.[58-60] This highlights how chronic, uncontrolled complement activation is involved in the pathogenesis of a variety of serious systemic diseases.

Management of Thrombosis in Paroxysmal Nocturnal Haemoglobinuria, a Complement-Mediated Disease

Doctor Anita Hill

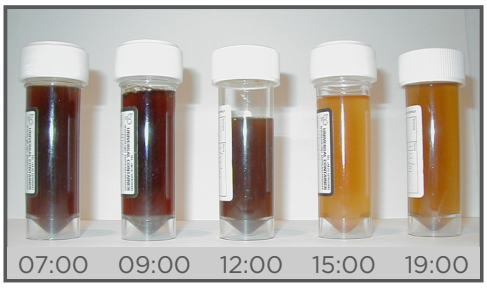
PNH is a rare, acquired, life-threatening disease characterised by chronic, uncontrolled complement-mediated haemolysis and a prothrombotic state (Figure 3).[61] PNH is diagnosed using high-sensitivity flow cytometry performed on peripheral blood.[62] The disease arises from a mutation in the *PIGA* gene against the background of an underlying bone marrow failure, usually aplastic anaemia.[43,63] It is thought that the underlying immune attack of normal stem cells in, for example, aplastic anaemia allows the PNH stem cells to expand. Evidence from clinical practice also shows that these two steps may be reversed: a patient with aplastic anaemia who is initially negative for *PIGA* mutation can later

develop the *PIGA* mutation, leading to the development of PNH.[63] For this reason, the British Society for Haematology Guidelines for the Diagnosis and Management of Aplastic Anaemia[64] recommend testing for PNH upon diagnosis of aplastic anaemia and regularly during follow-up. Other groups of patients who should be considered for PNH testing include those suffering from certain subgroups of myelodysplastic syndromes, patients who develop features of unexplained intravascular haemolysis, and those with unexplained thromboses associated with cytopaenias or evidence of haemolysis.

Although PNH is described as a benign disorder, the survival of patients who remain on supportive therapies has remained unchanged over the decades. As many as 35% of patients with PNH die within 5 years of diagnosis despite best supportive care.[61,65] Unregulated complement activity is the underlying cause of progressive morbidities and mortality in PNH.

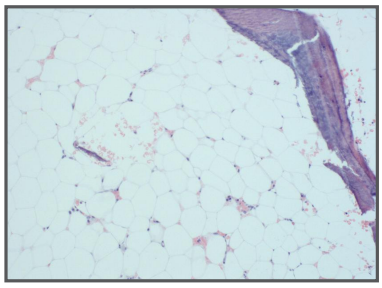
A study of ten patients demonstrated how patients can suffer silent complications of uncontrolled complement activation and thrombosis, such as PE and myocardial infarction.[66] This study highlighted the importance of carefully monitoring patients with high haemolysis (high lactate dehydrogenase [LDH] levels). Importantly, it is recommended that thorough examinations are conducted even in young and fit patients in order to detect silent complications underlying PNH.

Thrombosis is a leading cause of mortality in PNH patients.[54] Common sites include intra-abdominal and cerebral veins, hepatic veins (Budd–Chiari syndrome), DVT of the lower limbs, and cerebral and coronary arterial thromboses.[27] The first TE can be fatal and can also increase the risk of death by 5 to 10-fold.[54] Anticoagulation therapies do not adequately treat thrombosis in PNH.[27,54] Haemolysis and clinical symptoms can help to ascertain the risk of thrombosis in PNH patients. Multivariate analyses confirmed that LDH ≥1.5-times the upper limit of normal (ULN) increases the risk of TEs by 7-fold, when adjusted for age, gender, and bone marrow failure.[67] The combination of elevated LDH with other symptoms such as abdominal pain or chest pain causes a dramatic increase in the risk of a TE.[67] It is therefore necessary to obtain a thorough understanding of patient symptoms alongside close monitoring of LDH levels. Notably, even patients who have had minimal transfusions have an elevated risk of thrombosis,[54] indicating that the risk of thrombosis is independent of transfusion history. Similarly, patients on anticoagulation therapy also have a high risk of TEs.[54] Although PNH is less common than other inherited hypercoagulable states, it has a much higher incidence and relative risk of VTE. The management of the patient changes if PNH is diagnosed in a patient with unexplained thrombosis, hence the recommendation to test.[68] A high LDH can lead to the suspicion of PNH; however, patients with PNH and normal LDH levels can also suffer from thrombosis.



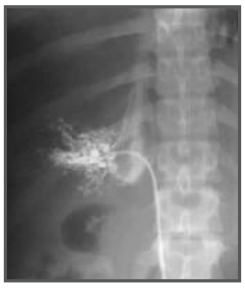
Haemolytic anaemia
Haemoglobinuria
Intravascular haemolysis

Disabling symptoms
- Abdominal pain
- Dysphagia
- Erectile failure



Bone marrow failure
Aplastic anaemia
Myelodysplasia

Often precedes PNH
- Selects for PNH clone



Thrombosis
- DVT/ PE
- Budd-Chiari
- Cerebral
- MI
- ~ 50% patients
- Fatal in 33%

Figure 3: Clinical presentation of paroxysmal nocturnal haemoglobinuria.
Adapted from Hill et al.,[27] Hillmen et al.,[61] and Socié et al.[65]

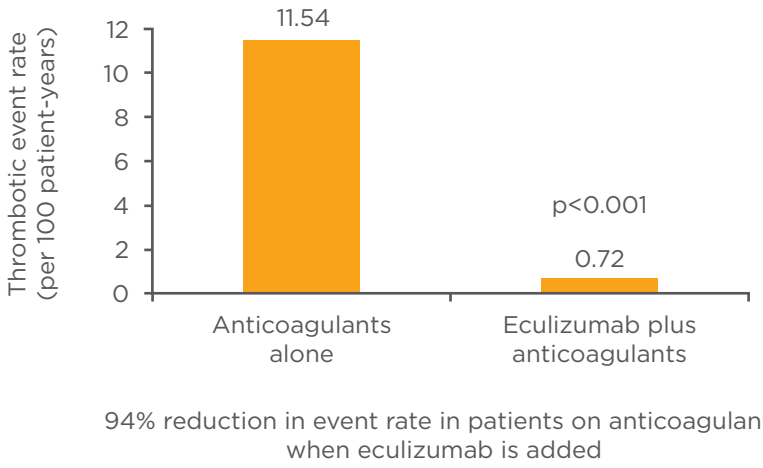


Figure 4: Eculizumab reduced thrombotic events in paroxysmal nocturnal haemoglobinuria patients treated with anticoagulants (n=91).
Reproduced from Hillmen et al.[54]

New oral anticoagulants (e.g. dabigatran, rivaroxaban) are unlikely to benefit patients with PNH as they function at the same points in the coagulation cascade as traditional anticoagulants.[27] In contrast, ECU inhibits the terminal complement pathway by binding to C5 and thus preventing its cleavage into C5a and C5b, thereby impairing prothrombotic mechanisms mediated by the complement system.[56] This mechanism causes a dramatic reduction in the rate of thrombosis: patients treated with a combination of anticoagulants and ECU demonstrated a 94% reduction in TE rate per 100 patient-years versus those treated with anticoagulants alone (p<0.001) (Figure 4).[54]

In the UK, ECU is indicated for transfusion-dependent patients with PNH and transfusion-independent patients who have thrombosis related to PNH, and those who have complications associated with haemolysis, e.g. renal failure, pregnancy, and symptomatic haemolytic PNH.[69] Based on findings from the global PNH registry, the European Medicines Agency has recently updated their approval of ECU to include patients with high disease activity (LDH >1.5 ULN plus one or more specified clinical symptoms), regardless of transfusion history.[56]

In an effort to further our knowledge of PNH, the global PNH registry was initiated several years ago and comprises of anonymised data from more than 3,500 patients from around the world.[70] The main objective of the PNH registry is to collect data to evaluate safety regarding the use of ECU and to characterise the progression of PNH as well as clinical outcomes, mortality, and morbidity in ECU and non-ECU treated patients.

Treatment with ECU appears to impact patient survival, as an analysis of UK data over the last 13 years (up to December 2014) showed that, out of 180 patients treated with ECU, no deaths related to PNH have occurred, which can be compared with the 35% mortality within 5 years seen in patients on supportive therapies.[71]

In summary, the risk of thrombosis in PNH is often underestimated. Patients with unexplained thrombosis with cytopaenia or haemolysis, or recurrent thrombosis despite anticoagulation, should be tested for PNH. Anticoagulation therapy is not sufficient to prevent thrombosis risk in PNH patients; however, ECU therapy has significantly improved survival for patients with PNH.

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WHY BIOSIMILARS MATTER: AN INNOVATIVE SOLUTION TO IMPROVE PATIENT ACCESS

Summary of presentations from the Sandoz-supported satellite symposium held at the 20th Congress of the European Hematology Association (EHA), Vienna, Austria, on 13th June 2015

Chairperson

Felix Keil[1]

Speakers

Mark McCamish,[2] Robin Foà,[3] Karen Van Rassel[4]

1. Hanusch Krankenhaus der Wiener Gebietskrankenkasse, Vienna, Austria
2. Sandoz, Holzkirchen, Germany
3. Hematology, 'Sapienza' University, Rome, Italy
4. Lymphoma Coalition, Toronto, Ontario, Canada

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MEETING SUMMARY

The meeting was introduced by Prof Robin Foà who spoke about the difficulties for patients accessing therapies in the context of rising healthcare costs and reduced budgets. Dr Mark McCamish then explained the biosimilar development process and the analytical techniques involved. Prof Felix Keil discussed the role of biosimilar medicines in haematology using the example of GP2013/rituximab (RTX), and Ms Karen Van Rassel of the Lymphoma Coalition presented the role a patient organisation can play when working with the physician to support a patient's questions and concerns regarding lymphoma.

Introduction

Professor Robin Foà

Prof Foà opened the symposium by highlighting: i) the enormous surge in demand for increasingly expensive treatments, due to an ageing population, and improved biologic age; ii) the rise in long-term chronic conditions, as illnesses become better controlled; and iii) the increased expectations of treatment outcomes by physicians. Providing healthcare is becoming progressively more costly and difficult to manage within the constraints of public resources. The cost of cancer care is increasing at 2-3-times the rate of other healthcare costs; for instance, the average monthly cost of cancer drug therapy has increased from approximately $100 in 1965 to $10,000 in 2013.[1] Patient access to medication is a major problem. An illuminating example is represented by RTX, which has been very successful for the treatment of B-cell chronic lymphoproliferative disorders. In a 2013 global survey of 450 physicians, it was found that patient access was only 39%; cost issues were frequently reported as barriers to RTX access.[2] Biosimilars are approved biologics that are highly similar to their reference product in terms of structure, function, pharmacokinetics (PK), and pharmacodynamics (PD), are comparable with respect to clinical efficacy and safety, and show the same presentation, strength, and mode of action.[3,4]

The first biosimilar was approved in Europe in 2006; currently, there are 19 products approved by the EMA, representing six different biological molecules. In 2013, the first biosimilar monoclonal antibody (mAb) was approved (biosimilar infliximab) and there are many biosimilars currently in development, which may give patients a route to access these important classes of drugs.

Targeted-Directed Development of Biosimilars

Doctor Mark McCamish

Biologics are highly specific and powerful molecules that have revolutionised modern medicine. They are much larger than chemical molecules and smaller peptides, and therefore cannot be chemically synthesised. Synthesis of smaller proteins without sugars can be achieved by inserting the DNA sequence for the protein into a host cell to produce an exact copy, including the protein folding and structure. However, larger glycosylated proteins such as erythropoietin, fusion proteins, and mAbs often experience some post-translational modifications that depend on the cell and the environment, and can vary from one molecule to the next, both during endogenous glycoprotein production in our own bodies and when manufacturing a biologic. Schiestl et al.[5] investigated acceptable levels of change in glycosylation from batch-to-batch and following major manufacturing changes, and found low variability between batches (5% variability) but a large change following a manufacturing change (a specific glycosylation enrichment dropping from 50-30% enrichment). However, this specific glycosylation is not known to impact on biological activity and therefore the change was deemed acceptable by regulatory agencies. Manufacturing changes are monitored closely by the regulating authorities and are only approved when they do not lead to clinically meaningful differences.

There are several reasons for considering the use of biosimilars, including improved patient access, the possibility of using more novel drugs, and reduced healthcare costs. Patient access to biologic medications is suboptimal; for example, only half of patients with severe rheumatoid arthritis (RA) receive biologics in the USA, Japan, and the EU5 countries.[6] In addition, only 30% of patients with moderate severity of RA disease receive biologics;[6] in these patients, the use of biologics could prevent their progression to severe RA, however their cost often limits their use. The use of biosimilar biologics has already been shown to result in large savings in Europe and is estimated to achieve savings of up to $250 billion in the USA by 2024.[7]

The goal of biosimilar development is to engineer a biosimilar to be 'essentially the same' as the reference product.[8] Variability of the reference product is documented over time to establish the variability of various post-translational modifications (such as glycosylations), and this variability helps to establish acceptable variability for the proposed biosimilar. The goal of biosimilar development and production is to reduce the variability to stay within narrow limits as established by the target variability of the reference product, i.e. it is a target-directed development (Figure 1). There are a number of sensitive analytical techniques that can be used to measure variability of the protein structure and glycosylation species; once the comparability of the biosimilar to the reference product has been shown using these techniques, clinical trials can be carried out to confirm biosimilarity, rather than to re-prove efficacy and safety.

To achieve regulatory approval, the goal is to produce a biosimilar that is essentially the same as the reference product. Indeed, the concept of 'sameness' has evolved over time with the development of more advanced techniques and complex molecules. Sameness is demonstrated by combining data from multiple sources evaluating more than 100 individual attributes covering primary structure, post-translational modifications, protein folding, biological activity, and impurities. The process of biosimilar development is very different to that of developing a novel drug or the reference product. During development of the original reference product the analytical tests simply describe the molecule, while clinical testing is substantial and designed to show how the molecule works, ultimately demonstrating clinical safety and efficacy in every indication in the label. However, during biosimilar development it is the opposite: analytical testing forms the basis of development to demonstrate that the molecule is essentially the same as the reference product and, once similarity is established, clinical studies are used to confirm the similarity already demonstrated analytically. The clinical trials are not designed to establish safety or to prove efficacy.

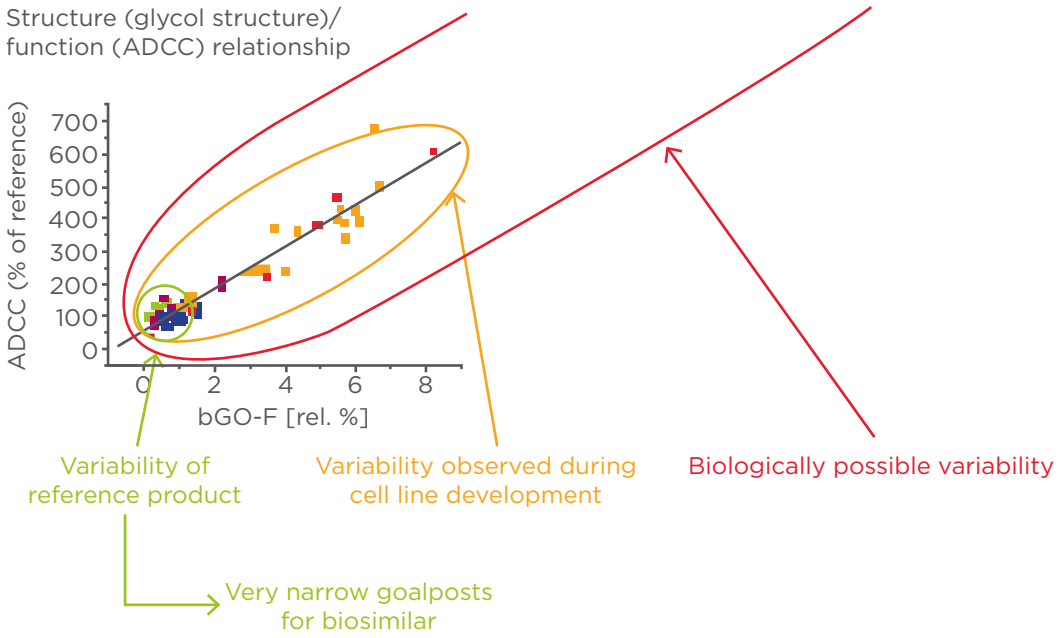

Figure 1: Originator variability is the basis for definition of biosimilarity goal posts.[5]
ADCC: antibody-dependent cellular cytotoxicity.

Biosimilars are recognised around the world as being safe and effective medicines, and have been available for a long time; the first biosimilar medicine was manufactured by Sandoz and approved in the EU in 2006 (biosimilar somatotropin). The EMA and the FDA have developed documents to provide guidance on how to approach biosimilar development; these have evolved over time. The guidelines are similar in both Europe and the USA. There has been more clarity achieved in the USA with the first biosimilar approved by the FDA, biosimilar filgrastim (Zarxio®, Sandoz); this approval demonstrated the development and approval processes in the USA, and has shown that extrapolation for other indications is achievable — a key benefit for biosimilar development. Extrapolation of biosimilar medications for other indications is not based on one small clinical study in one patient population that is then used to extrapolate to other indications on the label; rather, extrapolation is based on the demonstration of similarity between the biosimilar and the reference product in terms of structure, biological function, toxicity, and clinical similarity in a sensitive clinical indication. When it can be proven that the biosimilar molecule is essentially the same as the reference product, extrapolation can be made simply from the biosimilar to all indications of the reference product as they are essentially the same active pharmaceutical ingredients.

During the question and answer session, Dr McCamish commented that production of a biosimilar takes twice as long from target selection to first-in-human studies compared with the reference product, due to the various analytical tests required. In addition, he commented on the benefits of having the experience from the reference product in terms of predicting immunogenicity, as decades of clinical experience define what the potential immunogenicity risks are. Dr McCamish commented that although it is challenging to enrol patients into clinical trials, the motivation for patients to take part include altruism, access to medications that are potentially unavailable or too expensive, and the fact that all patients in the trial would receive the active drug. The concept of interchangeability was also discussed. In the USA, interchangeability is related to the ability of a pharmacist to substitute a biosimilar for a reference drug without the intervention of the prescribing physician. In Europe, the term 'interchangeability' is used to imply the ability of the physician to exchange use of a biosimilar in place of a reference biologic, while the term 'substitution' describes the intervention of the pharmacists as above. Although reference products may have minor changes over time related to manufacturing modifications, they are considered the same if approved by regulators. Although biosimilars that are approved to be interchangeable are designed to be essentially

the same as the reference product and whose function has not shown to alter upon switching, Dr McCamish emphasised that interchangeability is still a regulatory issue. Finally, Dr McCamish discussed the need for the education of physicians on the science of biosimilar production.

Biosimilars in Haematology

Professor Felix Keil

Biologics may be categorised as originators, of which they may be innovators (novel drugs/targets, and a significant step forward in efficacy and/or safety) or biobetters (known target, improved binding etc.), or as non-originators, of which they may be biosimilars (clinically equivalent to the originator, robust regulation) or copy biologics (less stringently regulated, often found in the emerging markets).[9] Biologics account for <1% of all prescriptions, but up to 28% of all medicine costs.[10] While generic medicines can result in price reductions of up to 90% compared with the brand-name version, the same cost savings are not achievable with biosimilars due to the complex nature of their production. In the EU, the median price saving for biosimilar epoetin alfa is 35%.[10] In fact, since the introduction of biosimilar epoetin in 2007, Germany has achieved savings of >€550 million.[11] The global costs of cancer care are high and continue to grow,[12] however, the patents on some of the key oncology therapies have expired or are about to expire,[13] presenting an opportunity for biosimilar medicines to provide the same clinical effects at reduced costs.

RTX has been a successful biologic therapy for the treatment of lymphoma, with approved indications for non-Hodgkin lymphoma (primarily follicular lymphoma, diffuse large B-cell lymphoma [DLBCL], and mantle cell lymphoma), chronic lymphocytic leukaemia, and RA. The incidence of DLBCL and mantle cell lymphoma increases with age,[14] resulting in increasing numbers of patients requiring treatment. The proposed biosimilar RTX GP2013 has been shown to be similar to the originator in terms of non-clinical *in vitro* and *in vivo* studies, as well as PK/PD studies (Table 1).[15]

The ASSIST-FL study is being conducted to compare GP2013 with originator RTX in 618 patients with untreated follicular lymphoma receiving a cyclophosphamide/vincristine/prednisone (CVP) chemotherapy regimenn. The primary outcome of this trial is the overall response rate at 24 weeks. The recruitment of the study is now closed, with results expected next year.

Biosimilars provide opportunities for more affordable and sustainable healthcare, greater access to biologic treatments for patients, and the increased opportunity for clinical studies. Additional clinical studies with RTX are important in order to determine the impact of variations in, for instance, body composition (proportion of fat, muscle, etc.) among patients; although they receive the same dose, the amount retained may not be appropriate for their body composition and thus needs to be personalised. Age and gender have been shown to significantly affect RTX clearance, with older men having greater clearance rates than older women.[16] In addition, increased weight in older male patients with DLBCL results in reduced clearance and increased half-life of RTX.[17] Therefore further studies in this area of personalised dosing are important in order to obtain more information about biologics; the only way to do this is to have more companies conducting more trials.

Table 1: GP2013 and the originator rituximab are pharmacologically similar.[15]

GP2013 and originator rituximab are pharmacologically similar
Pharmacological comparability between GP2013 and originator rituximab were confirmed in preclinical studies using clinical scale drug product: - *In vitro* ADCC potency in lymphoma cell lines - *In vivo* efficacy in mouse xenograft models - Pharmacokinetics and pharmacodynamics (CD20 cell depletion) in cynomolgus monkeys

ADCC: antibody-dependent cellular cytotoxicity; PK: pharmacokinetics; PD: pharmacodynamics.

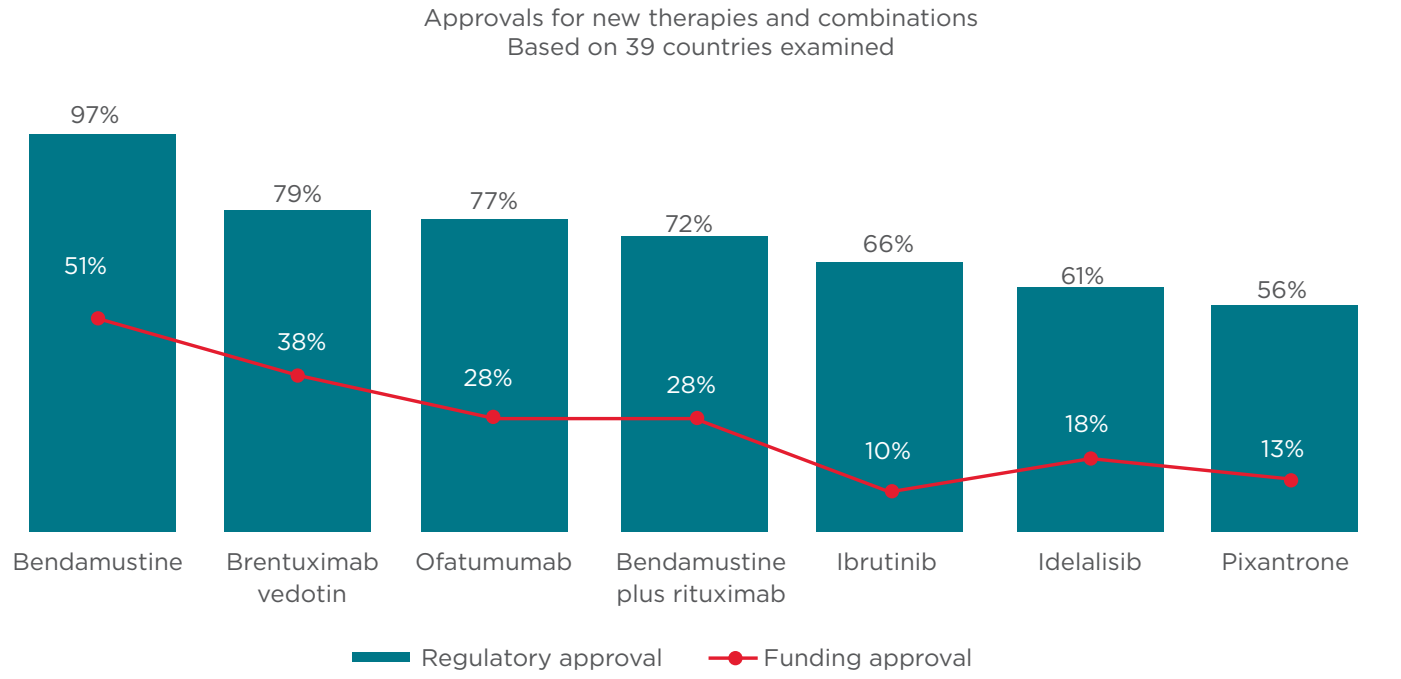

Figure 2: Therapy availability.

The established pathway for biosimilar development ensures quality. Biosimilars may provide increased patient access to medication in a time when global healthcare costs are increasing. RTX biosimilars development may encourage further trials that focus on personalised anti-CD20 treatment in lymphoma. Post-approval surveillance and extrapolation of efficacy to other indications of the reference product remain critical and challenging topics in haematology.

Lymphoma Coalition: Worldwide Network of Lymphoma Patient Groups

Ms Karen Van Rassel

The Lymphoma Coalition was founded in 2002 and has 63 member organisations based across 44 countries. They are managed by a global Board of Directors and have an international Medical Advisory Board. In addition, many of the member patient organisations also have Scientific Advisory Boards that the Lymphoma Coalition are able to access in order to obtain the relevant advice and an understanding of the current clinical situation. This provides an opportunity for patients all around the world to come together to obtain information and share best practice. The goal of the Lymphoma Coalition is to: (1) be a global source of information for lymphoma patients, with considerable statistics and facts; (2) improve awareness and understanding of lymphomas; and (3) build capacity for new and existing lymphoma groups. Information is provided in the context of an algorithm of care, is gathered based around the subtype of lymphoma that patients are diagnosed with, and is broken down into areas such as guidelines for diagnosis, therapies, clinical trials, incidence, mortality, and quality of life (QoL). One of the Lymphoma Coalition's goals is to advise that non-Hodgkin lymphoma is not one disease, rather it is made up of many different subtypes. Information regarding subtypes of non-Hodgkin lymphoma is compiled into case studies that can be used to advise and lobby governments regarding patient needs. This is important as patients need specific information on their illness, which is often difficult to obtain when the umbrella category of non-Hodgkin lymphoma is used.

The Lymphoma Coalition gathers information on each of the categories in the algorithm of care and also on clinical trials for lymphoma and chronic lymphocytic leukaemia, and keeps this information in a global database. The information is also available by country and may be used to build a picture of the disease landscape. For instance, of the 119 approved therapies available for the seven subtypes of lymphoma that the Lymphoma Coalition tracks, 96 are approved in the USA, 72 in the EU, but only 6 in Venezuela. RTX has

regulatory approval in 40 of the Lymphoma Coalition member countries, and is reimbursed or insured in 33, yet it is a component of 40 lymphoma chemotherapy regimenns. Reimbursement is a more accurate measure of patient access to medication compared with regulatory approval (Figure 2). It is still not clear if biosimilars will help funding approvals, however patients do not currently have access to the medications that are available.

There are many clinical trials ongoing, however it is very difficult to find biosimilar clinical trials online. The Lymphoma Coalition identifies trials to give patients the knowledge of where trials (including biosimilar trials) are available. The group also conducts a bi-annual patient survey focussing on specific topics, but designed in general to gain a better understanding of the questions patients with lymphoma from around the world may have, and to answer those questions. It covers topics such as patient and physician understanding of the signs and symptoms of lymphoma, clinical trials, barriers to treatment, QoL issues, and the role of patient support groups. Interestingly, of the >3,500 respondents from the 2014 survey, only 19% reported that they were approached to enter a clinical trial; yet, of those who were approached, 71% agreed to participate. Of those who were not approached, 27% said that they would likely have participated in a clinical trial and only 9% of patients said it was unlikely. The majority of patients who were approached had been approached by a healthcare professional (82%) and, of these, most (63%) fully understood their options. Therefore it is important to discuss clinical trial options with patients. Most patients (65%) said that they had reported QoL issues to their doctor, but only 38% felt that their doctor was able to help. Approximately half of patients were newly diagnosed, and these patients really need support. Outside of their doctor, many patients receive information through the internet, other patients and friends, and patient support groups/organisations. Therefore patient groups play a vital role in supporting physicians in providing information.

When providing guidance to patients, physicians should be mindful that they understand their options, use plain language to explain, direct them to further information or provide it for them, take the time to explain the options fully, and to offer support through patient organisations. The Lymphoma Coalition is learning about developments in biosimilar approvals and is sharing the information with patients. Patients have the right to know about their options and to be assisted in making their choices. The greater understanding of lymphoma biology is leading to the development of new biological molecules and markers. It is important that everyone learns about all new therapies including biosimilars so that they can provide the best information to patients.

During the question and answer session, Ms Van Rassel highlighted that the Lymphoma Coalition provides practical information on entering clinical trials, rather than analysing the trials themselves. She also commented on the utility of internet research but emphasised the importance of physicians helping patients to find the most reliable information.

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PERSPECTIVES ON THE TREATMENT OF MANTLE CELL LYMPHOMA AND FOLLICULAR LYMPHOMA IN 2015 AND BEYOND

Summary of presentations from the Celgene satellite symposium, held at the 20th Congress of the European Hematology Association (EHA), Vienna, Austria, on 11th June 2015

Chairpersons

Martin Dreyling,[1] Gilles Salles[2]

Speakers

Paolo Corradini,[3] Jehan Dupuis,[4] Steven Le Gouill,[5] Marek Trněný[6]

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MEETING SUMMARY

Prof Martin Dreyling opened the symposium by providing an overview of the current status of mantle cell lymphoma (MCL) and the current guidelines for treatment. Prof Steven Le Gouill discussed emerging tools to improve the diagnosis and monitoring of patients such as the assessment of minimal residual disease and the optimal incorporation of new technologies into the treatment pathway. Prof Marek Trněný then spoke about new treatment options for MCL and the improved survival that has been reported from certain combination therapies. Prof Martin Dreyling closed the MCL session.

Prof Gilles Salles introduced the follicular lymphoma (FL) session by explaining how the treatment landscape of FL has recently changed with the advent of anti-CD20 therapies. Prof Paulo Corradini then described the current treatment landscape in FL and Dr Jehan Dupuis spoke about the use of positron emission tomography (PET) at the start, interim, and end of treatment for FL. Prof Gilles Salles described the challenges of incorporating new treatment recommendations and tools for FL within current treatment options, and then summarised and closed the event.

Session 1: Managing Patients with Relapsed and/or Refractory Mantle Cell Lymphoma: Exploring Practical Solutions to Current Challenges

Welcome and Introduction

Professor Martin Dreyling

Prof Dreyling welcomed attendees to the meeting and outlined the challenges in MCL. MCL is a complex, heterogeneous disease that has classical, indolent, and transformed subtypes. Classical MCL constitutes the majority subtype and shows initially high response rates but relapses are also common. Indolent MCL occurs in 10–20% of patients, while 5–10% of patients with MCL have the transformed or blastoid subtype, which can be a difficult disease to treat successfully.

The Current Treatment Landscape in Mantle Cell Lymphoma: Current Guidelines and Remaining Challenges

Professor Martin Dreyling

MCL is a multifaceted disease that has previously been difficult to identify and treat. However, recent advances in the field have shown encouraging results with successes in the diagnosis and treatment of MCL. Only one-third of MCL cases can be accurately diagnosed using histological methods[1] and recent advances have enabled a confirmatory diagnosis of the t(11;14) chromosomal translocation that results in the overexpression of cyclin D1.[2] The indolent subtype of MCL can then be identified by t(11;14) translocation but no additional alterations, while classical MCL will also show impairment of DNA repair through ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHK2), as well as subsequent alterations. Transformed or blastoid subtypes show high levels of the Ki67 antigen, specific alterations in the *p53* tumour suppressor gene, and clinical features that can be evaluated through the Mantle Cell Lymphoma International Prognostic Index (MIPI). A study that stratified patients according to high, medium, or low risk by age (< or >65 years) and by the combined MIPI-c reported a significant difference in overall median survival between the high and low-risk groups ($p<0.0001$).[3]

Treatment decisions are then made according to the age of the patient, with dose intensification used for younger patients and maintenance regimenns for older patients.[4] Patients ≤65 years should be treated with dose-intensified immunochemotherapy (IC) using an alternating regimenn of three rounds of rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) and rituximab plus dexamethasone, cytarabine, cisplatin (R-DHAP) regimenns as first-line treatment. An autologous stem-cell transplantation (ASCT) should be performed after the fourth course if there is no response, at which point total body irradiation, cytarabine (Ara-C), and melphalan should be used and then peripheral blood stem-cell transplantation (PBSCT) as the last action. Continued patient monitoring and follow-up is important to assess the recurrence of MCL. Long-term evaluation of patients treated with the regimenn demonstrated a 20% benefit of progression-free survival (PFS) after 10 years with the alternating R-DHAP regimenn versus the standard R-CHOP course.[5]

Patients >65 years should be initially treated with R-CHOP or rituximab conventional regimenns and then rituximab maintenance,[6] which has been shown to have significant benefits for PFS and overall survival (OS) in patients over a period of 10 years versus a maintenance phase with interferon or no maintenance treatment. The treatment strategy has subsequently become a standard treatment pathway for patients >65 years across most European countries.[6] Although there are set regimenns for the first-line treatment of patients with MCL, relapsed or refractory MCL can be aggressive and difficult to treat successfully due to the multiple pathways that are activated.[7] Newly available therapies include bortezomib, ibrutinib, temsirolimus, and lenalidomide and have been investigated in various studies as shown in Figure 1.[8–15] Ibrutinib plus rituximab treatment has shown overall response rates (ORRs) of 100% (n=34) in a single-centre Phase II study for patients with relapsing remitting MCL who do not show active cell proliferation (Ki67 <50%); however, for patients with active proliferation as indicated by a Ki67 ≥50%, the response rate dropped to 50%.[16] Therefore, treatment combinations may be required for the more aggressive types of MCL that show a Ki67 ≥50%. For example, the TRIANGLE study evaluated the effect of alternating R-CHOP and R-DHAP regimenns followed by ASCT with ibrutinib in patients ≥65 years.[17] In summary, a greater understanding of the MCL cellular

pathways has enabled the development of tools to provide an accurate diagnosis, while novel treatments have been shown to improve the overall and PFS rates of patients with MCL. Future studies are required to assess the efficacy and safety of combination treatments with the new agents.

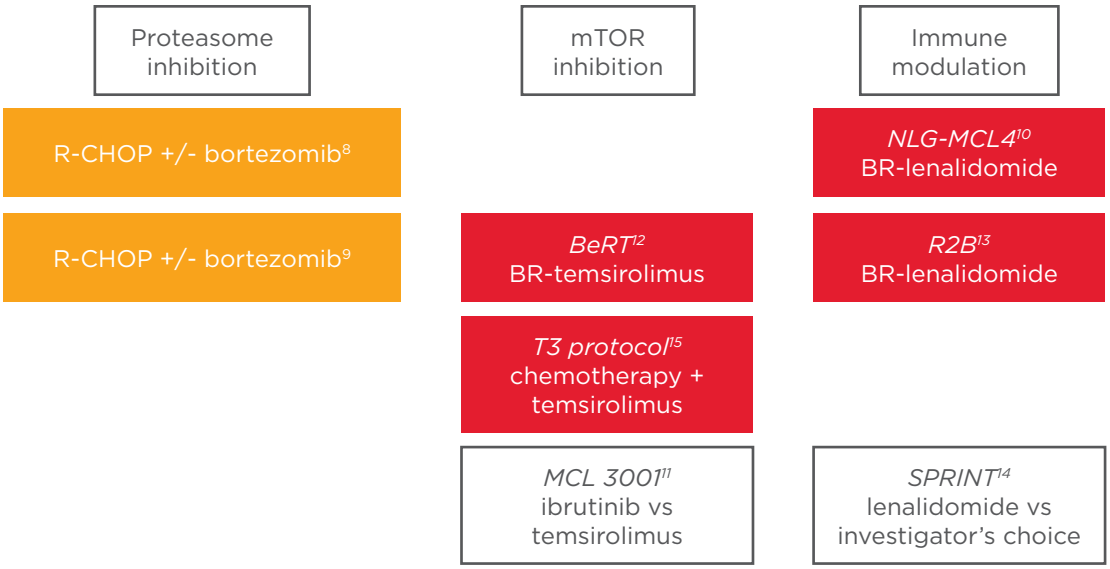

Figure 1: Mantle cell lymphoma studies 2015.[8-15]

From Martin Dreyling, presentation at the Celgene satellite symposium, held at the 20th Congress of the European Hematology Association (EHA), Vienna, Austria, on 11th June 2015.

BR: bendamustine plus rituximab; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisolone; R-HAD: rituximab plus high-dose cytarabine and dexamethasone; mTOR: mammalian target of rapamycin.

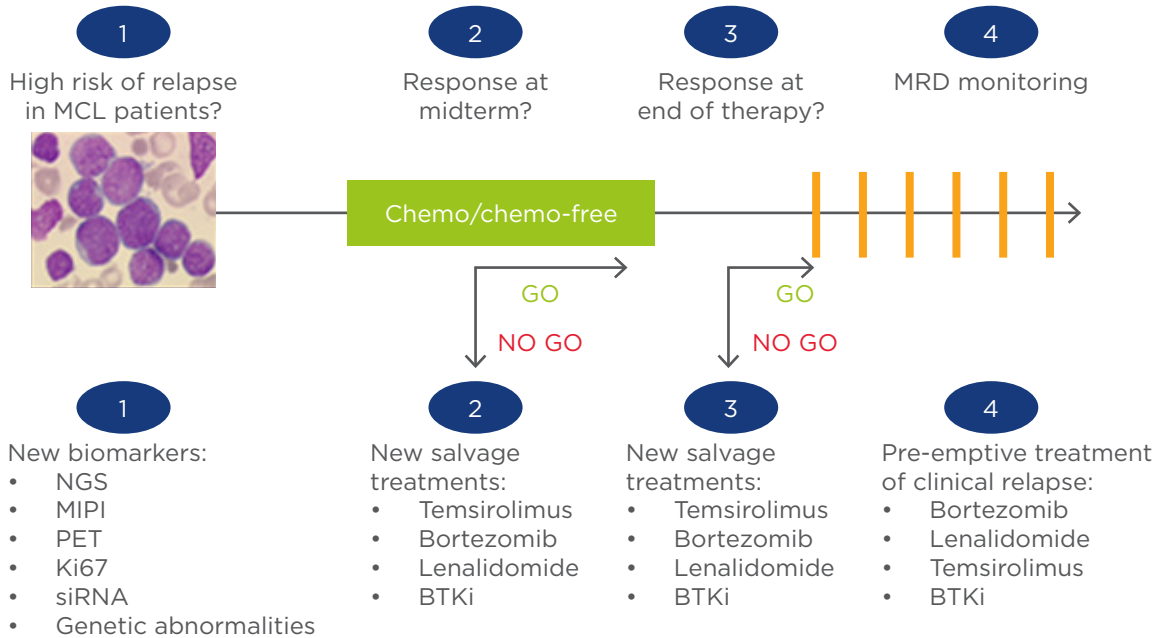

Figure 2: How to use new tools in a risk-adapted targeted strategy over time.

From Steven Le Gouill, presentation at the Celgene satellite symposium, held at the 20th Congress of the European Hematology Association (EHA), Vienna, Austria, on 11th June 2015.

BTKi: Bruton tyrosine kinase inhibitor; MCL: mantle cell lymphoma; MIPI: Mantle Cell Lymphoma International Prognostic Index; MRD: minimal residual disease; NGS: next-generation sequencing; PET: positron emission tomography; siRNA: small interfering RNA.

Emerging Tools for Driving Mantle Cell Lymphoma Treatment

Professor Steven Le Gouill

Along with the availability of new treatments for MCL, tools are currently being evaluated to ensure treatments are administered in the optimal setting to patients who have the highest probability of treatment response. These tools include the assessment of minimal residual disease (MRD) through flow cytometry, polymerase chain reaction (PCR), and real-time quantitative PCR (RQ-PCR), and there are advantages and disadvantages associated with each technique.[18] Although flow cytometry is well known and used, currently there are no standards or optimal settings for this technique in MCL and further validation studies are required prior to its use in routine practice. Comparatively, RQ-PCR has been standardised for MCL, however there is a low availability of this tool in laboratories. PCR is readily available and standardised, analyses of the IGH gene arrangement are detectable in 80–95% of B cell malignancies and the technique provides a short turnover time; however, there is a contamination risk with PCR and the data are qualitative, so interpretation of results is subjective.[18]

Although new treatments have been shown to induce remission in certain patients with MCL, confirmation is required on how, where, and when to evaluated the success of treatment regimenns. The LyMa trial, which recruited 299 patients and evaluate Ara-C (R-DHAP), analysed the MRD of patients prior to and after treatment (n=199). While all patients were high-level positive at the start of treatment, 65% of patients were MRD-negative after induction treatment and 79% were MRD-negative after ASCT treatment.[19] Another study by Pott et al.[20] reported maintained remission at 2 years in the majority of both younger and older patients who showed MRD negativity in peripheral blood and bone marrow samples compared with MRD-positive patients ($p<0.05$, n=259).[20] Remission shown through MRD negativity is a strong predictor of MCL prognosis, with MRD-negative patients demonstrating significantly improved PFS at 92 months ($p<0.001$, n=14) and OS at study end ($p<0.003$) versus MRD-positive patients (n=13).[21]

While the correlation of MRD with remission and improved PFS and OS has been confirmed, the appropriate use of MRD in routine clinical practice still requires verification and the optimal use, timing, and practicalities of MRD are still being studied. MRD can be evaluated upon diagnosis, at treatment interim prior to ASCT, at the end of treatment, and during follow-up. MRD can be assessed through the bone marrow tissue or blood. Although the use of blood to evaluate MRD is less invasive, confirmatory studies are required to compare blood versus bone marrow samples and the effect on patient outcomes. However, MRD is a promising future assessment tool that may minimise further treatment regimenns in patients who show MRD negativity during midterm treatment.

Another promising tool to evaluate the efficacy of treatment for MCL is fluorodeoxyglucose-PET (FDG-PET). Although the use of FDG-PET upon diagnosis is the current gold standard for nodal lymphomas and can be informative for MCL, so far there have been no studies or outcomes from using FDG-PET to optimise the patient treatment plan. FDG-PET can also be used for response assessment to ensure complete remission, but there are limitations of imaging certain areas such as the gastrointestinal tract, while the use of FDG-PET during follow-up is still undergoing experimental studies and requires validation as the false-positive rate with PET scans is over 20%.[22] There are also questions regarding the timing and use of FDG-PET. Although FDG-PET could be a promising technique in MCL, further studies are required to optimise its use for patients to ensure accurate imaging at an appropriate time in the treatment pathway.[22]

In addition to the diagnostic and imaging tools described above, it may be possible to tailor treatment according to the dysregulation of certain pathways in MCL through the use of '-omics'. There are multiple cellular processes that can be dysregulated in MCL that fall under three main areas, namely the NF-κB pathway, PIM1/mammalian target of rapamycin pathway, and epigenetic modifiers.[23] With genomic and proteomic techniques and novel treatments, it will be possible to tailor treatment according to which pathways or genetic processes are dysregulated and therefore target drugs according to the malfunction involved in MCL.

New techniques and modalities will allow the initial staging of patients with MCL to be refined through PCR techniques and FDG-PET so that treatment may be tailored according to the dysregulated pathways as shown by biomarkers. Evaluation of

treatment success via MRD and FDG-PET could show whether a change of treatment is required or not, as illustrated in Figure 2. The follow-up of patients will also be challenging; although new tools will provide the basis for physicians to determine whether treatment should be initiated, i.e. for MRD-positive patients who have not yet relapsed, these promising modalities will be complex and costly to bring into routine practice.

New Treatment Options for Mantle Cell Lymphoma on the Horizon

Professor Marek Trněný

There have been successes with new therapies in improving the outcomes of first-line treatment of MCL using a combination of approaches; however, there are still challenges with the relapsing or refractory forms of MCL. Real-life data report the probability of survival at 6 months as 50% for patients who are relapsing for the second or third time.[24] Therefore, novel treatments that target different points of the dysregulated pathways in MCL are being added on to existing therapies for relapsing patients and include temsirolimus, bortezomib, ibrutinib, and lenalidomide plus other investigational drugs such as ABT-199. In addition to targeting the pathways involved in MCL, the micro-environment and immune-regulation also play an important role in the evolution of MCL, and agents such as lenalidomide and ibrutinib can be used to improve the OS and PFS of patients with MCL.[25,26]

Recent treatments that have shown promising results in relapsed refractory MCL include temsirolimus, bortezomib, ibrutinib, and lenalidomide. Recent Phase III data on temsirolimus showed an ORR of 22% and a median PFS of 4.8 months as an individual agent (n=162).[27] When combined with rituximab and bendamustine, further improvements were seen in ORR (91%, n=11).[12,27,28] Bortezomib reported an ORR of 33% and median PFS of 6.2 months as an individual agent in patients with relapsed/refractory MCL (n=155),[29] and the addition of dexamethasone demonstrated increases in ORR and PFS to ˜80% and 12 months, respectively (n=16).[30,31] Recent studies have shown benefits in combination therapy for induction treatment, with bortezomib and rituximab plus cyclophosphamide, doxorubicin, and prednisone demonstrating superior PFS of 24.7 months (133 events) versus standard R-CHOP treatment that reported a PFS of 14.4 months (p<0.001, 165 events).[8] Ibrutinib inhibits Bruton's tyrosine kinase and has shown an ORR of 67% and median PFS of 13 months as an individual agent (n=111),[32,33] with an ORR of 87% and complete response of 38% when combined with rituximab (n=45).[16] In a Phase II study, duration of response was 17.5 months from a median follow-up of 26.7 months and patients showed an OS of 22.5 months. Ibrutinib demonstrated a good toxicity profile but has certain contraindications.[32,33]

Lenalidomide has demonstrated positive outcomes from studies as both a single agent and when combined with other treatments for patients with relapsed or refractory MCL.[34–39] The ORR is approximately 30% when prescribing lenalidomide alone, with Trněný et al.[36] reporting a PFS of 8.7 months (p=0.004) and ORR of 40% (p<0.001), with a median follow-up of 15.9 months (n=170).[36] The control arm was investigators' treatment choice, which reported a PFS of 5.2 months and ORR of 11% and more than half of these patients were switched to lenalidomide upon relapse. Manageable safety was reported, with mainly haematological toxicities observed. When lenalidomide was used in combination with rituximab, the ORR increased to approximately 55% and PFS to approximately 15%.[34,38,39]

Overall, the ORR of targeted therapies for patients with relapsed or refractory MCL varies from 20-65% and median PFS is between 5 and 13 months, whereas the duration of response is up to 17 months and OS between 13 and 28 months.[27,29,32,36] However, challenges remain in determining the optimal treatment combination for relapsed or refractory patients with MCL and which chemotherapy regimenns should be used, if at all.[40] Promising combinations that are undergoing clinical trials include lenalidomide plus ibrutinib,[41] rituximab plus lenalidomide plus ibrutinib,[42] obinutuzumab plus lenalidomide,[43] rituximab plus lenalidomide plus carfilzomib,[44] ABT-199 plus ibrutinib,[45] and ibrutinib plus palbociclib.[46]

In conclusion, new treatment modalities have already shown significant improvements in patients with relapsed or refractory MCL. Future directions for therapies will include combination treatment with and without chemotherapy, with targeted treatment moving to an earlier phase of disease that includes first-line treatment.

Closing Remarks for the MCL Session

Professor Martin Dreyling

Advances in the diagnosis of MCL have improved the accuracy of recognising and treating the disease. Although molecular markers are required to tailor treatments to the disease characteristics of each patient, future opportunities will be to utilise the available treatments and tools to develop and refine therapeutic algorithms and treatment combinations for patients.

Session 2: Shaping the Landscape in Follicular Lymphoma: How New Approaches will Guide Future Treatment Options

Introduction

Professor Gilles Salles

Treatment options and outcomes of patients with FL have drastically improved over the past 20 years, thereby requiring changes to the treatment pathways. In 1960, the OS of patients with FL was unchanged despite available treatments, with a median survival of around 8–10 years.[47,48] Although these treatments show benefits and can still be used, careful selection of therapies to minimise side-effects and include novel treatments is required. The limitations of classical cytotoxic therapies are cumulative toxicities that can result in the contraindication of these treatments in certain patients. Single-agent rituximab can be used as an alternative, non-cytotoxic method of effectively treating certain patients with FL,[49] as well as other novel agents.

Due to the availability of anti-CD20 antibodies, treatment options have expanded and an improved median survival of around 15–18 years in patients with FL has been reported.[50,51] Anti-CD20 therapies should be evaluated using different treatment combinations in order to maximise their benefit by investigating the therapies in single-arm studies, to be further confirmed in controlled trials. Due to the changing landscape of FL, new endpoints need to be defined that will provide a more informative basis by which treatment decisions are made as well as for the monitoring and follow-up of patients.[52] The availability of new and efficacious therapies requires a rethink of established endpoints and studies should therefore use a range of methods to evaluate clinical outcomes.

High Tumour Burden Follicular Lymphoma: The Current Treatment Landscape

Professor Paolo Corradini

Although there is a range of newly available therapies that have shown improved survival in patient studies, these should be used alongside established therapies in order to maximise clinical outcomes. Current treatments centre on radiotherapy, watch and wait, IC, ASCT, radioimmunotherapy (RIT), and allogeneic transplantation (ATx) for patients who relapse after ASCT.[53] Upon diagnosis of FL, the main treatments are R-CHOP, rituximab plus cyclophosphamide, vincristine, and prednisone (R-CVP) and rituximab plus fludarabine and mitoxantrone (R-FM). The 3-year FOLL05 study[54] evaluated over 500 patients for 3 years and reported significantly improved time to treatment failure with R-CHOP (p=0.003) and R-FM (p=0.006) regimenns compared with the R-CVP treatment. Additionally, bendamustine plus rituximab (B-R) is a novel treatment that has shown non-inferiority to the R-CHOP regimenn across two studies with an acceptable safety profile and fewer toxic effects.[55,56] Although maintenance treatment with bendamustine still needs to be evaluated, three options of R-CHOP, R-FM, and B-R are now available to treat patients upon diagnosis of FL. Other promising therapies include rituximab maintenance treatment, which showed improved PFS versus standard treatment.[57,58] RIT has also reported promising results, with a 100% ORR in patients given a single infusion of irradiated ibritumomab tiuxetan as initial therapy (n=17).[59]

After the initial treatment, ASCT is an option for patients who have relapsed. Guidance states that ASCT is not appropriate to consolidate the first remission in FL responding to IC treatment outside of clinical trials. ASCT is recommended for patients with a short treatment response, duration (<3 months), a high-risk Follicular Lymphoma Prognostic Index (FLIPI) score at relapse and for those previously treated with rituximab.[60] ASCT is also an option at second or subsequent relapse in chemotherapy-sensitive patients, and the decision to use ASCT should be governed by the clinical

course rather than biological and genetic risk factors. Cabanillas et al.[61] reported long-term follow-up of patients with FL who received ASCT and found improved survival of patients who received high-dose cyclophosphamide and total-body irradiation prior to ASCT during the second remission versus a historical control group treated with conventional chemotherapy. Purging with rituximab prior to ASCT does not appear to improve survival;[62] however, the study may have been under-powered. Patients who relapse after the first-line treatments and then also post-ASCT can present a challenge to treat successfully. Allogeneic stem cell transplantation has shown positive results in patients who have failed ASCT when bendamustine was replaced with fludarabine, with improved PFS and OS after 36 months.[63,64]

Along with a greater range of efficacious treatments, improved tools to monitor the progression of patients should be implemented for FL as per other malignancies. Reports have shown that molecular remission as demonstrated by PCR-negative status occurs in a greater proportion of FL patients.[65,66] Therefore, MRD techniques should be integrated into the definition of patient response for FL. In summary, the R-CHOP, R-CVP, and B-R regimenns have shown good outcomes for first-line therapy, while patients who relapse should be considered for ASCT and then allogeneic ATx if subsequent relapses occur. New therapies show promise for FL, however trials need to be carefully designed in order to fully evaluate all treatment options.

The Role of Positron Emission Tomography in Guiding Treatment Options

Doctor Jehan Dupuis

The implementation of novel treatment options requires careful monitoring in order to ensure that the optimal treatment regimenn is given to patients. The imaging modality PET may be a useful tool to ensure correct diagnosis and monitoring, as the technique detected additional lesions in 32% of patients who participated in the FOLL05 study[67] compared with computed tomography (CT) (n=142). Furthermore, of the patients who had initially been diagnosed with radiotherapy for localised disease by CT, 62% of cases were upstaged upon PET examination.[67,68]

The use of PET prior to treatment initiation has been recommended by the International Harmonization Project guidelines in order to interpret the PET results after treatment completion.[69] However, it should be noted that PET imaging cannot replace the use of bone marrow biopsies to assess for transformations but should be used as an additional tool.[67] The use of PET during treatment has not been reported widely, however a study that assessed the use of PET during and after treatment found that end-of-treatment PET is more predictive of outcomes.[70] Therefore, current evidence suggests that the use of PET in the middle of treatment is not recommended (Courtesy of LYSARC). The use of PET after treatment to evaluate treatment success appears to be highly predictive of patient outcomes. Trotman et al.[71] reported a median survival of >6 years in PET-negative patients (n=205) according to the PET scan score versus 1.5 years in 41 PET-positive patients ($p<0.0001$). However, no interventional study based upon PET results has been conducted so far and rituximab maintenance remains the standard of care regardless of the post-treatment PET score.[58]

When transformation is suspected, the relative measure of local radiotracer accumulation in the tissues can be measured with PET using the standardised uptake value (SUV). SUV can vary with biological factors, the method of analysis, and image reconstruction parameters. Transformation should be suspected when a focus of more intense radiotracer uptake in the tissues is identified via PET.[72-74] Higher SUVs have been found to correlate with more aggressive histologies[72,73] and PET can be used to guide the choice of biopsy site, yet the predictions are not certain and therefore biopsies are still required. In conclusion, PET scans should be performed in patients with FL prior to and after treatment, and PET-positive patients should be monitored closely for disease progression.

Challenges for Shaping a New Paradigm of Care in Follicular Lymphoma

Professor Gilles Salles

There has been an evolution in the landscape of FL and recent findings need to be understood in order to optimise treatment pathways. The key events that lead to the development of lymphoma have been described but are not yet fully

understood.[75-77] However, there are standard treatment strategies for the various stages of FL. While the disease cannot be eliminated fully through cytotoxic therapy, the use of ASCT and ATx have shown success with rates of remission and the combination of existing therapies with novel agents may ensure improved PFS and OS in patients with a range of FL staging and severity. Novel agents include immune checkpoint inhibitors and immunomodulatory drugs, aiming to target the cancer stem cell in FL.[78] A recent trial of pidilizumab plus rituximab, which can be directed against PD-1 and/or PD-L1, was suggestive of efficacy in FL. However, as inclusion criteria required patients who were rituximab-sensitive, confirmatory studies are required.[79]

Through analysis of T cells within the FL microenvironment to understand the "immune tolerance" towards tumour cells in FL, reports have shown defects in the ability of T cells to kill the FL tumour cells.[80] Further *in vitro* studies have since indicated that lenalidomide may have a role in restoring the T-cell immune response so that FL cells are targeted by the T cells. Lenalidomide as a single agent or combined with rituximab in relapsing patients with FL has resulted in an ORR of 25–40% and 50–85%, respectively.[81-83] Improvements in CR have also been reported with lenalidomide + rituximab, as 30–50% of patients achieved CR when treated for relapsed or refractory FL.[84,85] Furthermore, Fowler et al.[86] assessed lenalidomide + rituximab in patients with untreated, advanced stage indolent non-Hodgkin lymphoma. The Phase II study from one institution reported an 87% complete response (n=40) of patients with FL. Safety monitoring during the study demonstrated Grade 3/4 neutropaenia incidence in 35–40% of these patients and rashes, myalgia, and thrombosis were also reported, indicating the need for monitoring of treatment side-effects.

From the encouraging PFS data shown with lenalidomide and rituximab, a trial that evaluated various treatment strategies with this combination dropped the single rituximab arm through demonstration of superiority of the combination treatment.[87] Significant improvements were then shown through the CALGB 50401 study (Table 1) across ORR and median event-free survival. Based upon promising results from the combined lenalidomide plus rituximab treatment, the international, multicentre, randomised study RELEVANCE[88] will evaluate standard treatments R-CHOP, R-CVP, and R-B versus lenalidomide + rituximab maintenance.

In closing, the data shown from new agents are changing the landscape of FL and improving outcomes for patients. Development of the immunotherapy approach, combination treatments, and new agents with rituximab could be very promising.

Table 1: Response and event-free survival: CALGB 50401 study.[87]

	Lenalidomide (n=45)	Lenalidomide + rituximab (R2) (n=44)
ORR, %	51.1 95% CI (35.8–66.3)	72.7 95% CI (52.2–85.0)
CR, %	13.3	36.4
PR, %	37.8	36.4
Median EFS (years)	1.2	2.0
2-year EFS, %	27	44

From Gilles Salles, presentation at the Celgene satellite symposium, held at the 20th Congress of the European Hematology Association (EHA), Vienna, Austria, on 11th June 2015.
Median follow-up: 1.5 years (range 0.1–3.6). Unadjusted EFS HR of lenalidomide vs lenalidomide + rituximab (R2) is 2.1 (p=0.010). Adjusted (for FLIPI) EFS HR of lenalidomide vs lenalidomide + rituximab (R2) is 1.9 (p=0.061).
CALGB: Cancer and Leukemia Group B; CI: confidence interval; CR: complete response; EFS: event-free survival; FL: follicular lymphoma; FLIPI: Follicular Lymphoma International Prognostic Index; HR: hazard ratio; NHL: non-Hodgkin lymphoma; ORR: overall response rate; PR: partial response.

Closing Remarks for the Follicular Lymphoma Session

Professor Gilles Salles

While the development of novel agents has been met with intense interest from the FL community, long-term follow-up studies are needed to evaluate the potential benefit of novel agents in prospective clinical trials versus standard treatment. Additionally, the integration of novel tools such as MRD and PET-CT are required to support treatment decisions with novel agents as well as conventional therapies.

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SYNOPSIS OF CLINICALLY ACTIONABLE GENETIC MARKERS IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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The survival of children with acute lymphoblastic leukaemia (ALL) treated with modern protocols now exceeds 90%, but survival is <50% for adults. Improvements in outcome have resulted from optimising the use of a relatively small number of anti-leukaemia drugs, better supportive care, and treatment stratification. Characterisation of somatically acquired genetic abnormalities, one of the key features of ALL, is used in conjunction with information regarding patient age, white cell count, and treatment response in order to guide patient management.[1]

Primary (clonal) abnormalities provide the most robust prognostic biomarkers, and eight chromosomal abnormalities have consistently been associated with superior (n=2) or inferior (n=6) outcomes compared with other patients (Figure 1). The frequency of these abnormalities is strongly related to age, with ~50% of children with B-cell precursor (BCP) ALL harbouring a 'good risk' abnormality compared with ~50% of adults harbouring a 'poor risk' genetic aberration. Patients with high-risk cytogenetics are now treated differently, and patients with *BCR-ABL1* achieve better outcomes when treated with a tyrosine kinase inhibitor in conjunction with standard chemotherapy, whereas patients with intrachromosomal amplification of chromosome 21 (iAMP21) achieve better outcomes when treated intensively. A rare genetic subtype of BCP-ALL characterised by *TCF3-HLF* fusion is associated with a dismal outcome.[1] A comprehensive genomic and drug profiling study reported at EHA 2015 described the genomic landscape of this subtype, and has identified Bcl-2 inhibition as a potential novel therapeutic strategy (Figure 1).[2]

The spectrum of secondary (subclonal) abnormalities is strongly linked to the primary abnormality, and they act as cooperating events to induce leukaemogenesis. In contrast to primary abnormalities, which are often leukaemia-specific, secondary abnormalities are frequently related to generic cancer processes. High hyperdiploidy is associated with a relatively good outcome at all ages, although some patients do relapse. Given the prevalence of this subgroup (30-35% of paediatric BCP-ALL cases), identifying further prognostic or predictive markers is important. A genomic study reported at EHA 2015 identified that high hyperdiploid patients harbouring secondary mutations in both *KRAS* and *CREBBP* genes have a higher risk of relapse and may benefit from targeted therapy.[3] Mutations in Ras pathway genes (e.g. *NRAS*, *KRAS*, *FLT3*) are prevalent in three high-risk genetic subtypes: iAMP21, *KMT2A* (previously *MLL*) translocations, and near-haploidy (Figure 1). The idea of utilising MEK inhibitors to target the resulting activation of Ras signalling was further emphasised by a EHA 2015 presentation on infant ALL, in which *KMT2A* translocations predominate.[4]

The application of genomic technologies to cases without an established abnormality (B-other ALL) has revealed copy number alterations, gene expression profiles, and gene fusions that have shaped our understanding of this subgroup and also started to influence treatment strategies. Deletions affecting genes in key pathways, including lymphoid differentiation, cell-cycle differentiation, and cell proliferation can be used either individually or in combination to subdivide B-other ALL cases into subgroups with distinct outcomes (Figure 1).[1] Gene expression profiling can also be used to dissect B-other ALL into prognostic subgroups, principally through the identification of the poor-risk *BCR-ABL1*-like subgroup. Transcriptomic and genetic profiling studies of the *BCR-ABL1*-like subgroup not only explain the underlying biology, but have also provided novel therapeutic avenues. The *BCR-ABL1*-like gene expression profile is driven by a complex network of chimeric fusion genes centred on *ABL1*, *ABL2*, *PDGFRB*, *CSF1R*, *CRLF2*, *JAK2*, and *EPOR*,

which activate the tyrosine kinase or JAK/STAT pathways.[1] The frequency of individual gene fusions has yet to be established and many have only been reported in single cases. Collectively, however, these and similar genetic lesions are likely to account for approximately 10% of BCP-ALL cases. *In vitro* and *in vivo* studies have demonstrated that *ABL1*, *ABL2*, *PDGFRB*, and *CSF1R* fusions are sensitive to tyrosine kinase inhibitors, while *CRLF2*, *JAK2*, and *EPOR* fusions are sensitive to JAK inhibitors. A small number of patients harbouring *ABL1*, *ABL2*, *PDGFRB*, and *CSF1R* fusions with refractory disease achieved a complete remission following treatment with imatinib or dasatinib. Although these patients were selected and follow-up was limited, these laboratory and clinical observations provide encouraging evidence that targeted therapies could be offered routinely to these patients.

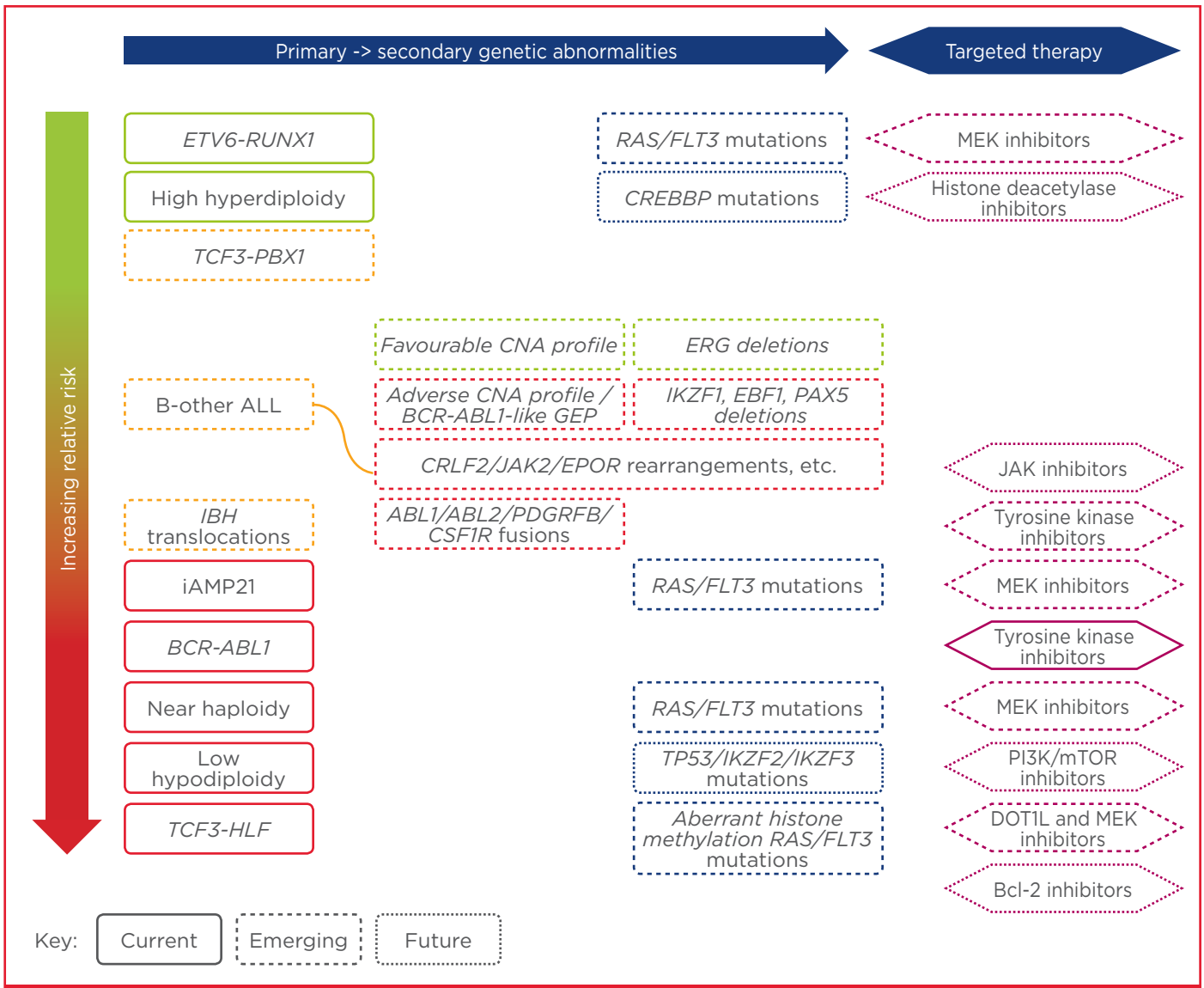

Figure 1: Selected primary and secondary genetic abnormalities in B-cell precursor acute lymphoblastic leukaemia that are current, emerging, or future prognostic or predictive biomarkers. *ETV6-RUNX1*: t(12;21)(p13;q22); high hyperdiploidy: 51-65 chromosomes; *TCF3-PBX1*: t(1;19)(q23;p13); iAMP21: intrachromosomal amplification of chromosome 21; *BCR-ABL1*: t(9;22)(q34;q11); near haploidy: <30 chromosomes; low hypodiploidy: 30-39 chromosomes; MLL: mixed-lineage leukaemia (*KMT2A*) translocations; *TCF3-HLF*: t(17;19)(q23;p13); CNA: copy number alterations; GEP: gene expression profile; JAK: Janus kinase; PI3K: phosphoinositide 3-kinase; mTOR: mammalian target of rapamycin; DOT1L: DOT1-like, histone H3 methyltransferase; MEK: mitogen-activated protein kinase kinase; Bcl-2: B-cell lymphoma 2.

The incorporation of novel targeted agents into treatment protocols will occur in the relapse setting initially. At EHA 2015, we reported that a combination of chromosomal abnormalities, mutations/deletions in *TP53*, *NRAS*, *NR3C1*, and *BTG1*, as well as duration of first remission, can be integrated into a risk index that identifies three prognostic subgroups of childhood relapsed ALL.[5] This type of combined genetic and clinical risk index sets the scene for the coherent inclusion of novel targeted therapies into clinical trials. In conclusion, the continuing investigation into the genomic landscape of ALL is unravelling the underlying biology of the disease and providing a wealth of potential biomarkers that could be exploited to improve patient management.

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TARGETING THE B CELL RECEPTOR PATHWAY

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The B cell receptor (BCR) activates multiple signalling pathways within normal B cells that are essential for a normal immune response. Activation of this pathway, as is seen in mantle cell lymphoma (MCL) as well as other B cell lymphoproliferative disorders, leads to an increase in proliferation, a reduction in apoptosis, and changes in adhesion and migration through an upregulation of genes involved in these processes. There are multiple potential targets within this pathway and many pharmacological agents have been developed to effectively target it. The most active of the approaches to date is inhibition of Bruton's tyrosine kinase (BTK). BTK is an essential component of BCR signalling and B cell activation.[1]

The first-in-class of these agents, ibrutinib (IBR), shows remarkable activity in MCL and has recently been licensed by both the FDA and EMA for use in this disease. When used as a single agent, 70% of MCL patients with relapsed or refractory disease will respond to this oral, once-daily medication. This is double the efficacy of any of the other licensed single-agent drugs for this disease and is achieved with extremely modest toxicity. The long-term data from the original Phase II trial were discussed, and they show that 22% of patients have their remission maintained for at least 2 years, with a median follow-up which is now 26.7 months.[2]

What is encouraging is that there are no new toxicities evident with longer follow-up and the potential concerns about infections have not emerged; in fact, the incidence of infection decreases with time. The addition of rituximab (RTX) to IBR appears synergistic as evidenced by the data from the MD Anderson Hospital in the USA: response rates of 92% were seen in a relapsed/refractory cohort of 50 patients, without additional toxicity. There are multiple ongoing trials evaluating IBR in combination with almost all of the commonly used chemotherapeutic approaches and active agents in MCL. In the UK, a trial randomising patients to either RTX and IBR or RTX and standard chemotherapy (CHOP or bendamustine) will begin soon. These regimenns will be used as front-line therapy in elderly patients, and the study will be the first chemotherapy versus chemotherapy-free regimenn trial in this disease.

There are a number of second-generation BTK inhibitors in early clinical development, which appear to have similar activity to IBR from the limited data available so far. These agents bind to exactly the same cysteine residue (C481) within the phosphorylation site of BTK, which means that

these agents will not work in IBR-failing patients. However, IBR possesses a broad kinome selectivity, which includes irreversible inhibition of several other kinases with important roles in normal and malignant B cell signalling. The newer agents affect fewer other tyrosine kinases and this may lead to a different side-effect profile.

Phosphoinositide 3-kinase (PI3K) is also part of the BCR signalling pathway, and there are multiple drugs under development that target this specifically. In the context of MCL, PI3K inhibition does induce responses, but these are not as complete or durable as seen with IBR. Inhibitors of PI3K are more active in the more indolent lymphoma types and their use in MCL is likely to be explored only in the context of combination therapy with other agents that affect BCR signalling.

We are going to see an explosion of data on agents that affect BCR signalling when used as single agents or in combination with multiple chemotherapeutic and novel agents. The precise role of IBR and how to combine it, if indeed we need to, will become clearer over the next couple of years.

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IMPROVING DETECTION OF IMPENDING RELAPSE IN THE POST-TRANSPLANT PERIOD IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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Over the past two decades, numbers of allogeneic haematopoietic cell transplantations (HCTs) have increased tremendously worldwide, which has been partly associated with the introduction of reduced-intensity conditioning for elderly patients or patients with comorbidities. Transplant-related mortality due to infections, severe graft-versus-host disease, or organ failure has decreased considerably (e.g. due to improved prevention and control of infections and improved donor selection). In contrast, mortality from relapse in the post-transplant period has remained constant over the last few years. Post-transplant relapse became a focus of research in recent years and is now also being discussed at international conferences.[1] Transplant research aims to define markers and methods appropriate to detect imminent relapse in stem cell recipients as early as possible. Detection of an increase or persistence of leukaemia cells on a submicroscopic level would allow practitioners to perform rapid immunotherapeutic intervention (i.e. withdrawal/reduction of immunosuppression or donor lymphocyte infusion) or to use pharmacotherapeutic compounds (e.g. azacitidine in the case of myeloid malignancies) before overt haematological relapse becomes manifest.

Various laboratory methods are able to detect minimal residual disease (MRD) below the microscopic level: most of these rely on molecular genetics, such as quantitative real-time polymerase chain reaction (qRT-PCR) or nested PCR (combining two PCR reactions covering one region of interest). The sensitivity of these molecular techniques may be as high as 10^{-4} to 10^{-6}. High-throughput sequencing (HTS) that allows the parallel investigation of hundreds of thousands of alleles is presently being explored for its potential to detect MRD. Immunophenotyping by multiparameter flow cytometry achieves a lower sensitivity level (10^{-2} to 10^{-4}), but may detect aberrant antigen patterns across the borders of different genetic subgroups, e.g. in acute myeloid leukaemia (AML). In contrast, the molecular genetic methods target distinct mutations that must be suitable for the respective PCR technique.

In the post-chemotherapeutic setting, MRD strategies have been extensively explored. Nevertheless, the thresholds that have been defined for the post-chemotherapy setting cannot

be directly transferred into the post-transplant scenario. This may be illustrated by nucleophosmin (*NPM1*) mutations. In the post-chemotherapy setting, an increase of >1% *NPM1*^{mut}/*ABL1* is able to identify patients at an increased relapse risk, whereas an increase of >10% *NPM1*^{mut}/*ABL1* is most relevant for relapse in allograft recipients.[2] In patients with AML, MRD monitoring in the post-HCT period at present is available for the *NPM1* mutations or reciprocal rearrangements such as *RUNX1 RUNX1T1* or *CBFB-MYH11*. For patients with other mutations, e.g. of *ASXL1* or *DNMTA3*, appropriate monitoring strategies still have to be established. By advanced molecular techniques, mutations are detectable now in the vast majority of patients with myelodysplastic syndromes (MDSs),[3] but these mutations still have to be introduced into the MRD scenario. In patients with myelofibrosis carrying the *JAK2V617F* mutation, the respective mutation has been successfully targeted by qRT-PCR for guidance of post-transplant immunotherapy.[4]

In addition, analysis of chimerism (the ratio of the donor's and the recipient's haematopoiesis) provides an option for identifying patients at an increased post-transplant relapse risk. Different techniques are available such as short tandem repeat PCR or qRT-PCR for donor/patient specific single nucleotide polymorphisms.[5] For patients with AML or MDS, enrichment of the CD34+ (stem cell) compartment increases the safety of the method.[6]

In conclusion, various tasks have to be fulfilled to establish post-transplant MRD monitoring strategies: the MRD thresholds that justify pre-emptive post-transplant intervention have to be defined for individual markers and entities;[1] the molecular MRD marker panel needs to be expanded; HTS should be explored for its contribution to MRD measurement post-transplant; and multicentre studies and central laboratories are needed to optimise molecular surveillance of patients with haematological malignancies in the post-transplant period and to manage the increasing demands on the laboratories in view of more advanced MRD strategies.

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COAGULANT AND NON-COAGULANT ACTIVITIES OF THROMBIN

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Physiological blood coagulation[1,2] is initiated by vascular tissue factor (TF) (expressed on vascular smooth muscle cells [VSMCs], macrophages, and fibroblasts) exposed to circulating coagulation factor (F) VII upon disruption of endothelial cells. Driving the initiation phase of coagulation, binding of FVII to TF leads to the formation of the catalytic complex TF—FVIIa—FXa. A fraction of FXa associates with its cofactor FVa to form the prothrombinase complex, which converts trace amounts of prothrombin into thrombin. Besides its main role in coagulation, the conversion of fibrinogen into fibrin, thrombin executes the

proteolytic cleavage of FXI, FV, FVIII, and FXIII into their active forms FXIa, FVa, FVIIIa, and FXIIIa, respectively. This procoagulant activity of thrombin further enhances thrombin generation in three ways: 1) FXIa activates FIX into FIXa, which in turn generates FXa, 2) the activated cofactors FVa and FVIIIa enhance the proteolytic activities of FXa and FIXa, respectively, and 3) FXIIIa stabilises fibrin by generating covalent bonds between individual fibrin molecules. During this amplification phase of the coagulation cascade, thrombin converts sufficient fibrinogen into fibrin to form the actual clot. Activation of platelets by thrombin stimulates the proper exposure of negatively charged phospholipids in their membranes, thereby providing the physical platform for the assembly of the intrinsic tenase (FIXa—FVIIIa) and prothrombinase (FXa—FVa) complexes. The assembly of the prothrombinase complex on the platelet surface substantially increases FXa proteolytic efficiency and enhances the generation of thrombin, which in turn converts fibrinogen to fibrin. After the amplification phase, the coagulation cascade is terminated through regulated negative-feedback mechanisms as well as by natural inhibitors, the major ones being antithrombin, TF pathway inhibitor, and the protein C pathway, plus heparin cofactor II and protein Z-dependent protease inhibitor, which have a less significant contribution. The protein C pathway[3] is important because thrombin binds to the endothelial cell-exposed thrombomodulin and activates endothelial protein C receptor-bound protein C into activated protein C (APC). Together with the cofactor protein C, APC inhibits the cofactors FVa and FVIIIa. Thrombin generation is strongly attenuated through proteolytic degradation of these cofactors until finally the coagulation cascade ceases.

Activation of platelets through thrombin was first described in 1967,[4] and research by Vu et al.[5] in 1991 identified the cellular thrombin receptor involved in this process as the protease-activated receptor (PAR)-1. PARs are in the family of the G-protein coupled receptors and four subtypes have been identified: PAR-1 through PAR-4, of which mainly PAR-1, 3, and 4 are activated and signal directly in response to thrombin. PARs are found within the cellular membranes of a wide variety of cells, including platelets, endothelial cells, VSMCs, fibroblasts, and monocytes. The basic mechanism of PAR activation by thrombin is through proteolytic cleavage of the N-terminal extracellular domain, thereby generating a new N-terminal tethered ligand domain, which binds and activates the cleaved receptor.[6,7] Different intracellular G alpha subunit ($G_{12/13}$, $G_{i/o}$, or G_q) pathways are activated depending on the ligand (agonist bias) and the location of the receptor. An example of agonist bias is the difference in ligand formation upon activation of PAR-1 by thrombin or APC.[3] Thrombin cleaves PAR-1 at position Arg41 inducing pro-inflammatory signalling, whereas activation at Arg46 by APC results in anti-inflammatory pathways.[8] Other mechanisms that initiate different cellular responses include thrombin-activated PAR-1 donating its tethered ligand to transactivate PAR-2[9] and the formation of homo and heterodimers of PAR-1:PAR-1 and PAR-1:PAR-3 or PAR-1:PAR-4, respectively.[10,11]

Although the precise role of non-coagulant thrombin activities in the involved pathways is not yet known, evidence from experimental studies and clinical observations suggests that the cellular responses upon PAR activation by thrombin play a crucial role in complex pathology, including inflammation, immunity, fibrogenesis, wound healing, cancer, and atherosclerosis.[6,7,12-14] The availability of highly selective inhibitors of coagulation proteases, including mutant molecules that may still signal through PAR pathways but lack anticoagulant activity (such as the recombinant mutant APC3), creates many challenging opportunities to modify complex diseases through coagulation interventions.

In conclusion, despite its well-known coagulant activities, new scientific approaches are required to understand the complexity of thrombin's non-coagulant effects and the possible long-term benefits or drawbacks of inhibition of both the coagulant and non-coagulant activity.

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INFLAMMATION AND THROMBOSIS: ENTANGLED IN NETS

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Neutrophil extracellular traps (NETs) are expelled from activated neutrophils and consist of nuclear DNA with histones and microbicidal proteins. The original discovery of NETs showcased their antimicrobial properties, but the pathological consequences of releasing chromatin into the extracellular space are becoming increasingly evident. Recently, the importance of neutrophils/NETs in thrombus pathogenesis has been appreciated, as the release of NETs within the bloodstream has important procoagulant and prothrombotic implications. NETs can bind platelets and red blood cells and thus participate in the initiation of thrombosis. We have identified prominent NETs in mouse models of deep vein thrombosis and also in venous thrombi harvested from human patients in neutrophil-rich, organising stages of thrombosis.

NET formation (NETosis) is an active and coordinated biological process involving many enzymatic components. One enzyme in particular, peptidylarginine deiminase 4 (PAD4), citrullinates histones and is required for chromatin decondensation during NETosis. We have seen important anti-thrombotic and cardioprotective effects in the absence of NETs using PAD4$^{-/-}$ mice, which do not decondense chromatin or release NETs *in vivo*. PAD4$^{-/-}$ mice were greatly protected from pathological thrombus formation. The mice did not exhibit any defects in haemostasis, and could be induced to produce deep vein thrombi by infusion of wild-type neutrophils that formed NETs as a part of the thrombus scaffold. Therefore, studying PAD4-deficient mice has revealed the impact of NETs in thrombotic/inflammatory disease and identified PAD4 as an attractive therapeutic target.

Intravascular NET formation could be beneficial, for example in septic conditions where containing bacterial spread could be protective for the host. However, the role of NETs in sepsis, particularly the balance between their antimicrobial and cytotoxic actions, remains unclear. The most abundant proteinaceous components of NETs are histones, which are themselves not only procoagulant but also highly cytotoxic to endothelium. The hypercoagulable state and organ dysfunction exacerbated by histones, some of which may originate from NETs, can quickly lead to host mortality. We examined neutrophils from PAD4$^{-/-}$ mice and found that neutrophil functions involved in bacterial killing, other than NETosis, remained intact. We subjected the PAD4$^{-/-}$ mice to mild and severe polymicrobial sepsis produced by caecal ligation and puncture. Surprisingly, under septic conditions, PAD4$^{-/-}$ mice did not fare worse than wild-type mice and had comparable survival as well as similar bacterial burden in the blood and organs. However, PAD4$^{-/-}$ mice did show partial protection from death in lipopolysaccharide-

induced shock, with significantly lower thrombin-antithrombin complexes and less soluble P-selectin in circulation compared with wild-type mice. This suggests that PAD4/NETs may contribute to the toxic inflammatory and procoagulant host response to endotoxin. We propose that preventing NET formation may have beneficial effects in preventing pathological thrombosis and that PAD4 inhibition in inflammatory or thrombotic diseases is not likely to increase host vulnerability to bacterial infections.

Although being investigated, no specific PAD4 inhibitors are yet available and there is reluctance to develop them because of fear of infection. Our results from mouse models indicate that blood infections are not exacerbated in the total absence of NETs and that excessive NET production contributes to nucleosome-induced pathologies in mice. This has important implications for future development of NET-targeted therapeutics, such as PAD4 inhibitors, which could be highly beneficial in prothrombotic patients and unlikely to result in drastic immunosuppressive effects.

THE THROMBIN INHIBITOR DABIGATRAN IMPAIRS CANCER CELL GROWTH AND PROGRESSION

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Advanced malignancy often correlates with activation of the coagulation system, termed cancer coagulopathy, which is associated with increased mortality rates. Several coagulation factors play a pathogenetic role in the induction of such a hypercoagulable state of cancer. Ultimately, the extension of this process to the tumour microenvironment fuels critical cellular events, including cell proliferation, cell adhesion, angiogenesis, and invasion. One of the master regulators of this complex interplay between activation of coagulation and cancer progression is thrombin, a blood-derived serine protease with pleiotropic potential. The pathophysiology behind thrombin and cancer cell interaction is based on the activation of the protease-activated receptor 1 (PAR-1), a receptor highly expressed on the surface of several human cancer types. The activation of this receptor, following binding to thrombin, promotes cancer growth by triggering downstream mitogenic signalling events.

Targeting thrombin with the aim of developing novel anticoagulants has led to the discovery of dabigatran, a selective, direct inhibitor of thrombin currently prescribed to patients with atrial fibrillation or venous thromboembolism (VTE). With this background, it is tempting to speculate that the ability of dabigatran to displace thrombin from PAR-1 binding may be favourably exploited in uncoupling all of the thrombin-driven mechanisms promoting tumour growth.

This was the topic of a presentation at the recent EHA congress. Our group addressed precisely this question: can dabigatran affect thrombin-driven cancer cell progression? Our work started by revisiting the previous observation that thrombin affects cancer cell growth. Using several *in vitro* systems, we confirmed that thrombin significantly increases the proliferation of human cancer tumour cell lines, in particular that of the MCF7 adenocarcinoma and the U87 glioblastoma lines. The mechanism supporting cell proliferation can be attributed to the ability of thrombin to favour cell cycle progression from G1 to S, the phase of DNA synthesis, by modulation of proteins governing the cell cycle, such as cyclin D1, a positive regulator, and p27, a negative regulator. Thrombin also demonstrates neo-angiogenetic properties through its ability to sustain the expression of molecules modulating angiogenetic processes in cancer, such as Twist and GRO-alpha. The expression of angiogenetic factors induced by thrombin is associated with increased branching of endothelial cells, which is a surrogate of neo-angiogenesis. Furthermore, we observed that thrombin is also active in another critical aspect of tumour progression: the ability to metastasise. This property

also relies on the motility of cancer cells, a process that we found augmented when tumour cells were cultured in a gradient of thrombin, suggesting that the activation of coagulation in the vascular system may exploit thrombin generation as a powerful tool to induce cancer cells to migrate from the tumour microenvironment to the intravascular compartment. The most compelling discoveries from our work are the effects of dabigatran on all of these thrombin-driven cancer processes. We could demonstrate that dabigatran dramatically antagonised the proliferative ability of thrombin by restoring a pattern of expression of cyclin D1 and p27 similar to that in unstimulated cells. Dabigatran was also effective in blocking the thrombin-induced expression of angiogenetic factors and this translated into normal branching of human umbilical vein endothelial cells. Furthermore, thrombin was no longer effective in attracting tumour cells when dabigatran was added to cell cultures, thereby restoring baseline cell motility.

Several important questions were raised during the discussion at the EHA meeting. One critical aspect was whether the concentrations of dabigatran we have tested in our *in vitro* system approach the therapeutic plasma levels that are typically reached in patients treated with the thrombin inhibitor according to current indications. The 500 nmol/l dabigatran concentration at which we could observe the antagonistic effect of dabigatran on thrombin is close to the plasmatic level present in patients taking dabigatran for atrial fibrillation or deep vein thrombosis. We cannot draw any conclusion as to whether dabigatran may also reach a substantial concentration in the tumour microenvironment, which is a complex architecture where thrombin can be generated and orchestrate tumour progression.

In an era when new oral anticoagulants are becoming available for the treatment of VTE, it is evident that they may become an additional option to treat VTE in cancer patients. However, although the potential of dabigatran to interfere with mechanisms of tumour progression driven by thrombin is highly appealing, further studies will be needed in order to establish the efficacy and safety of this new agent in this specific population, with particular regard to the efficacy of dabigatran compared with low-molecular-weight heparin, which is the most effective treatment for cancer-associated VTE to date.

TYROSINE KINASE INHIBITORS AND THROMBOSIS

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Patients with cancer have a significant risk of venous thromboembolism (VTE) and arterial thromboembolism (ATE) compared with non-cancer patients. Among multiple risk factors, an important role is played by anti-cancer drugs.[1] Recently, advances in the targeting of unique cancer metabolic pathways have introduced into clinical practice a variety of biological agents that may be associated with vascular complications.[2] These include inhibitors of protein tyrosine kinases (TKs, enzymes that catalyse the transfer of phosphate from adenosine triphosphate [ATP] to tyrosine residues), of which there are two main types: receptor TKs, transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain (e.g. vascular endothelial growth factor [VEGF] receptor [VEGFR]); and non-receptor TKs found in the cytosol, the nucleus, or the inner face of the plasma membrane (e.g. c-ABL). It has been shown that TKs can be highly dysregulated in cancer cells.

Inhibitors of TKs (TKIs) can directly inhibit the catalytic activity of the kinase by interfering with the binding of ATP or substrate, or they can block dimerisation of the TK. Types of TKI include: humanised monoclonal antibodies directed against receptor TKs or their ligands; and small-molecule inhibitors directed against both receptor and non-receptor TKs. Since the approval of the first TKI, imatinib (IMA), in 2001, numerous multi-targeted small-molecule TKIs have been developed. Among them, sunitinib and sorafenib target mainly the VEGFR and are approved for renal cell cancer,

hepatocellular cancer, and gastrointestinal stromal tumour treatments.

TKIs have attracted particular attention because they are perceived to have an improved risk/benefit ratio for patients. Nevertheless, they are associated with adverse effects (AEs) on a number of other System Organ Classes, the cardiovascular (CV) system being the prime determinant of the risk/benefit ratio. A large number of the approved TKIs are associated with a range of serious CV AEs, including ATE and VTE.[3-5] One mechanism leading to thrombosis may result from the anti-VEGF effect of TKIs: blocking VEGF from binding to its receptor may lead to endothelial cell apoptosis. Furthermore, VEGF has specific functions in regulating the balance of procoagulants and anticoagulants, e.g. the induction of endothelial tissue factor (procoagulant) and the induction of fibrinolytic activity (anticoagulant).[4]

TKIs targeting Bcr-Abl have revolutionised the treatment of chronic myeloid leukaemia. After the breakthrough success of IMA, second and third-generation inhibitors (e.g. dasatinib, nilotinib, bosutinib, and ponatinib [PON]) were developed in order to overcome IMA resistance.[6] Among these newer agents, PON stands out as the only approved TKI with activity against the 'gatekeeper' T315I mutation in Bcr-Abl. In the PACE Phase II clinical trial,[7] 9% of patients in the PON arm experienced serious-grade ATEs (treatment-related events: 3%). A further 13 months of PON exposure showed a cumulative incidence of serious ATE of 11.8% (overall: 17.1%). However, ATEs occurred predominantly in patients with previous ischaemic conditions or one or more risk factors. More data are needed to determine the CV risk attributable to PON and the mechanism of action underlying these events.[8]

The possible role of thromboprophylaxis with anti-platelet, anticoagulant, or lipid-lowering drugs is as yet unknown. What is important is the possibility of identifying patients at high risk prior to beginning treatment.

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RELAPSED/REFRACTORY MULTIPLE MYELOMA: THE CURRENT STATE OF PLAY

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ABSTRACT

Multiple myeloma (MM) usually responds to treatment but is incurable. The clinical course is characterised, in most patients, by a series of remissions and relapses. For younger patients, the initial treatment currently usually involves induction with the proteasome inhibitor bortezomib (BOR), alone or in combination, followed by an autologous stem cell transplant (ASCT). Usually only clinical relapses require treatment; the treatment plan should be individualised to take into account factors such as response to previous treatment, duration of the remission, adverse effects experienced, and available treatment options. Evidence suggests that many patients who have responded to BOR will respond to it again. Patients at first relapse should also be considered for a further ASCT or an allotransplant. Clinical studies have led to other drugs being approved for treatment of relapsed MM. These include lenalidomide (an immunomodulatory drug), carfilzomib (another proteasome inhibitor), pomalidomide (an immunomodulatory drug), and most recently panobinostat (a deacetylase inhibitor). The availability of these drugs greatly enhances the therapeutic options available to treat further relapses. Moreover, a bewildering array of other novel agents are at various stages in testing. They include other drugs from the classes already mentioned, as well as monoclonal antibodies, drugs acting on the cell cycle, kinase inhibitors, and signal transduction pathway inhibitors. It seems probable that the introduction of these agents in the coming years will further improve the survival of patients with MM, and may even lead to a cure.

Keywords: Multiple myeloma (MM), relapse, proteasome inhibitor, immunomodulator, monoclonal antibody, deacetylase inhibitor.

INTRODUCTION

Multiple myeloma (MM) is a malignant disease caused by the monoclonal expansion of plasma cells. It affects 6.1 per 100,000 people per year in the USA, where it is the second most common haematological malignancy after non-Hodgkin lymphoma.[1] In the UK, the lifetime risk of developing MM is 1 in 120 for males and 1 in 155 for females.[2] The risk increases sharply in patients >55 years, with the highest rates being in those aged >85 years. There are significant racial differences, with higher rates in black compared with white people. Although MM remains an incurable disease, the survival duration of newly-diagnosed patients has increased markedly in the last decade, mainly due to the efficacy of high-dose melphalan (MLP) followed by autologous stem cell transplantation (ASCT) and novel agents such as thalidomide (THD), bortezomib (BOR), and lenalidomide (LEN). In Europe, either one of the first two agents is usually used as the first-line treatment. In the USA, and increasingly in Europe, LEN is often used as a first-line agent instead of THD. However, all patients eventually relapse and become resistant to these drugs. Almost all patients develop refractory

disease, at which point the median event-free survival time is 5 months, with overall survival (OS) at this stage under a year.[3] This review considers the options available for the treatment of patients with relapsed MM, including those who have become refractory to treatment.

TREATMENT OF RELAPSED AND REFRACTORY MM

Key Clinical Trials

There are a number of drugs and drug combinations approved by both the FDA and the EMA for the treatment of patients with MM who have relapsed or become refractory to treatment. For most, this was based upon the results from Phase III trials,[4-9] although for one drug, carfilzomib (CARF), approval was partly based upon the results of Phase II trials.[10-12] Details of these pivotal trials are summarised in Table 1. Below, we review these and other key clinical trials which currently inform the state-of-the-art treatment of relapsed and refractory MM. The outcome measures for individual studies are the predetermined primary endpoints.

Proteasome Inhibitors

The APEX study compared the use of the intravenous proteasome inhibitor, BOR, with oral dexamethasone (DEX) for the treatment of relapsed MM.[4] The median time-to-progression (TTP) (progression-free survival [PFS] times) were 6.22 versus 3.49 months ($p<0.001$). In a separate trial, BOR alone was compared with the same BOR regimen with the addition of intravenous pegylated liposomal doxorubicin (PLD) for the treatment of relapsed MM.[5] The median PFS durations were 9.3 versus 6.5 months, respectively ($p=0.000004$).

CARF is a second-generation proteasome inhibitor. It was approved by the FDA, but not the EMA, on the basis of results from three Phase II trials for its use as a single agent in patients with relapsed MM.[10-12] These trials had very different designs and study populations. Overall response rates (ORRs) to intravenous CARF of 17.1%, 59.3%-64.2%, and 23.7% were obtained in patients previously exposed to BOR,[11] patients naïve to BOR,[12] and in a mixed group of patients, some of whom had been exposed to BOR,[10] respectively. All three studies concluded that the results demonstrated that CARF was potentially effective for treating relapsed MM. FOCUS was a Phase III trial that compared CARF single agent with low-dose corticosteroids and optional cyclophosphamide (CYC) in patients with relapsed MM. The primary endpoint was OS; this was not reached, although there were significant differences in some secondary endpoints.[13] Subsequently, a Phase III trial (ASPIRE) compared the use of intravenous CARF combined with LEN and DEX with a control group of patients treated with LEN and DEX alone.[14] The median PFS times were 26.3 versus 17.6 months, respectively. Recently, the pre-planned interim analysis of a Phase III trial (ENDEAVOR) that compared intravenous CARF combined with DEX and BOR combined with DEX for relapsed MM showed that PFS was significantly better with the former (18.7 versus 9.4 months). The dose of CARF used in the ENDEAVOR trial[15] (56 mg/m^2) was significantly higher than that used in most previous studies, including ASPIRE (27 mg/m^2). These findings suggested that CARF may be the best in its class for the treatment of relapsed MM.

Numerous new third-generation proteasome inhibitors are currently being investigated for MM. They differ both in terms of the catalytic subunits of the targeted proteasome and in the reversibility of the inhibition. It is hoped that they will have similar or superior efficacy rates to BOR, be better tolerated, and be able to overcome BOR resistance. A Phase III trial of ixazomib (IXZ), which is given orally weekly, has recently been completed. It compared IXZ with placebo, in combination with LEN and DEX. Press releases suggest that patients treated with the active drug had longer PFS times compared with those treated with placebo. Oprozomib, a structural analogue of CARF, is also given orally.[16] Both oprozomib and marizomib, which is given intravenously, appear to confer promising outcomes in early clinical studies.

Immunomodulatory Drugs

Two very similar studies, one from North America and the other from a consortium encompassing Europe, Israel, and Australia compared the combination of oral LEN, an immunomodulatory drug, and DEX with placebo and DEX for the treatment of relapsed MM.[6,7] The median PFS times in the two studies were 11.1 versus 4.7 months ($p<0.001$) and 11.3 versus 4.7 months ($p<0.001$), respectively. The median TTP was not significantly related to the previous exposure to THD in either study of patients receiving LEN. LEN combined with DEX (40 mg weekly) is the

control arm in a number of ongoing Phase III trials investigating the efficacy of novel agents. Depending on the results of these, three-drug combinations may become increasingly utilised.

Table 1: Key clinical studies leading to FDA and EMA approval.

Drug	Study	Patient group	Treatment	Comparator	Principle outcome measure	Other outcome measures (selected)	Adverse events (selected)
BOR	Multicentre, randomised, OL, Phase III[4]	N=669; 1-3 previous treatments	BOR 1.3 mg/m^2 on d. 1, 4, 8, & 11 for 8 3-wk. cycles, then on d. 1, 8, 15, & 22 for 3 5-wk. cycles	DEX 40 mg on d. 1 through 4, 9 through 12, & 17 through 20 for 4 5-wk. cycles, then on d. 1 through 4 for 5 4-wk. cycles	Median time to DP: 6.22 vs. 3.49 mth. (HR for the BOR group, 0.55; p<0.001)	RR: 38% vs. 18% (p<0.001) 1-year SR: 80% vs. 66% (p=0.003) Median DR: 8 vs. 5.6 mth.	Grade 3 or 4 adverse events: 75% vs. 60%
BOR-PLD	Multicentre, randomised, OL, Phase III[5]	N=646; >1 previous treatment	BOR 1.3 mg/m^2 on d. 1, 4, 8, & 11 of an every 21-d. cycle, + PLD 30 mg/m^2 on d. 4	BOR 1.3 mg/m^2 on d. 1, 4, 8, & 11 of an every 21-d. cycle	Median time to DP: 9.3 vs. 6.5 mth. (HR for the PLD-BOR group 1.82)		Grade 3 or 4 adverse reactions: 80% vs. 64%
LEN	Multicentre, randomised, DB, OL, PC, Phase III[7]	N=293; >1 previous treatment and measurable disease not resistant to DEX	LEN 25 mg on d. 1 to 21 of a 28-d. cycle + 40 mg DEX on d. 1 to 4, 9 to 12, & 17 to 20 for the first 4 cycles. Then DEX 40 mg only on d. 1 to 4	Placebo 25 mg on d. 1 to 21 of a 28-day cycle + DEX on d. 1 to 4, 9 to 12 & 17 to 20 for the first 4 cycles. Then DEX 40 mg only on d. 1 to 4	Median time to DP: 11.1 vs. 4.7 mth. (HR for the LEN group 0.35)	RR: 44% vs. 41% (n.s.) 15 mth. SR: 76% vs. 65% (p=0.03) Median DR: 10.2 vs. 7 mth. (p=0.0008)	Grade 3 or 4 adverse reactions: 85% vs. 73%
LEN	As above[6]	As above, except N=351	As above	As above	Median time to DP: 11.1 vs. 4.7 mth. (HR for the LEN group 2.85)	RR: 61% vs. 20% (p<0.001) Median SR: 29.6 vs. 20.2 mth. (p<0.001) OS sig. improved in the LEN group in those on prior THD (p=0.03)	The primary toxic effects of LEN were haematologic, and were manageable
CARF	Multicentre, SA, OL, Phase II[10]	N=266; >2 previous treatments	CARF 20 mg/m^2 x 2 weekly for 3 of 4 weeks in cycle 1, then 27 mg/m^2 for <12 cycles		RR: 24%	RR: 60% vs. 24% (p<0.001) Median time to DP for those on prior THD: 8.4 vs. 4.6 mth. (p<0.001)	Adverse events were 'manageable'

Table 1 continued.

Drug	Study	Patient group	Treatment	Comparator	Principle outcome measure	Other outcome measures (selected)	Adverse events (selected)
CARF	Multicentre, SA, OL, Phase II[12]	N=129; 1-3 previous treatments; naïve to BOR	Cohort 1: CARF 20 mg/m^2 for all treatment cycles	Cohort 2: CARF 20 mg/m^2 for cycle 1 and then 27 mg/m^2	RR cohort 1 vs. cohort 2: 42% vs. 52% (lower bound of the 95% CI was not exceeded)		
CARF	Multicentre, SA, OL, Phase II[11]	N=35; 1-3 previous treatments; BOR non-naïve	CARF 20 mg/m^2 in a twice-weekly, consecutive-day dosing schedule for 12 monthly cycles		Response rate: 17%	Median DR: 7.8 mth. Median OS: 15.6 mth.	
CARF	Multicentre, OL, randomised, Phase III[14]	N=792; 1-3 previous treatments	CARF – d. 1, 2, 8, 9, 15, & 16 (starting dose, 20 mg/m^2 d. 1 & 2 of cycle 1; target dose, 27 mg/m^2 thereafter) during cycles 1 through 12 and on d. 1, 2, 15, & 16 during cycles 13 through 18.+ LEN & DEX as for comparator	LEN 25 mg on d. 1 through 21. DEX 40 mg on d. 1, 8, 15, & 22	Median PFST: 26.3 vs. 17.6 mth. (HR for progression or death 0.69)	RR: 87% vs. 67% 24 mth. SR: 73% vs. 65%	Grade 3 or higher adverse events: 84% vs. 81%
POM	Multicentre, OL, randomised Phase III[8]	N=455 Relapsed on at least 2 consecutive cycles of BOR and/or LEN	28 d. cycles of POM 4 mg/day on d. 1–21, orally + DEX 40 mg/d. on d. 1, 8, 15, & 22 until progression or toxicity	28 d. cycles of POM 4 mg/d. on d. 1–21, orally + DEX 40 mg/d. on d. 1-4, 9-12, and 17-20	Median PFST: 4.0 vs. 1.9 mth. (HR 0.48)		
PAN	Multicentre, randomised, PC, Phase III study[9]	N=768; 1-3 previous treatments	21 d. cycles of PAN 20 mg on d. 1, 3, 5, 8, 10, 12, orally) + BOR 1.3 mg/m^2 on d. 1, 4, 8, 11 + DEX 20 mg on d. 1, 2, 4, 5, 8, 9, 11, 12	As before, but substitute PAN for placebo	Median PFST: 12.0 vs. 8.1 mth. (HR 0.63)	RR: 61% vs. 55% (p=0·09) CRR or NCRR: 13.4 vs. 10.9 mth. (p=0·00006) Median DR: 13.4 vs. 10.9 mth.	Serious adverse events: 60% vs. 40%

BOR: bortezomib; PLD: pegylated liposomal doxorubicin; LEN: lenalidomide: CARF: carfilzomib; POM: pomalidomide; PAN: panobinostat, DEX: dexamethasone; THD: thalidomide; PC: placebo-controlled; OL: open label; DB: double blind, SA: single agent; HR: hazard ratio; RR: response rate; CRR: complete response rate; NCRR: near-complete response rate; SR: survival rate; OS: overall survival; DR: duration of response; DP: disease progression; PFST: progression-free survival time; d: day(s); wk: week(s); mth: month(s); CI: confidence interval; n.s.: not significant; vs: versus.

Pomalidomide (POM) is a second-generation immunomodulatory drug. Early clinical trials have demonstrated that it has limited efficacy for the treatment of relapsed MM patients when used as a single agent, but showed possible synergistic effects when combined with DEX. The NIMBUS trial compared oral POM combined with low-dose DEX with high-dose DEX in patients with relapsed and refractory MM that have exhausted treatment with BOR and LEN.[8] The median PFS time was significantly better in the former group than in the latter (4.0 versus 1.9 months). The findings from STRATUS,[17] a larger Phase IIIb study of POM and low-dose DEX, were comparable. Current clinical trials are investigating POM and low-dose DEX combined with other agents, such as CYC, BOR, and PLD.[18,19]

Deacetylase Inhibitors

Deacetylase inhibitors are not effective treatments for MM when given as single agents, but act synergistically with other agents, including proteasome inhibitors. Panobinostat (PAN) is an oral pan-deacetylase inhibitor. When combined with BOR and DEX (PANORAMA-1 trial), the median PFS time was significantly better than that in controls who were given placebo and combined BOR and DEX (11.99 versus 8.08 months).[9] In contrast, a Phase III trial of vorinostat and BOR recently reported no improvement in OS.[20]

Combinations of PAN and second-generation proteasome inhibitors and immunomodulatory drugs are also being evaluated. A Phase I/II study of PAN and DEX with CARF found an ORR of 82%,[21] and a Phase I study is exploring PAN and DEX with IXZ.[22] Rocilinostat is a deacetylase inhibitor with a narrower spectrum of activity than most other agents. It is hoped this may be associated with fewer adverse effects (AEs), whilst maintaining efficacy.[23]

Monoclonal Antibodies

There is a bewildering array of monoclonal antibodies (mAbs) currently being tested for the treatment of MM patients. They are specifically directed against antigens present in the surface of tumour cells. Thereafter, they have a number of different mechanisms of action which include direct cytotoxicity by inducing apoptosis, direct cytotoxicity as a consequence of conjugation to radioisotopes or toxins, and enhancing the immune response through antigen-dependent cellular cytotoxicity or via inducing complement-dependent cytotoxicity. Other novel mechanisms include targeting and sequestering of interleukins and targeting B-cell activating factor, which promotes the survival of malignant B cells. mAbs are being investigated both as single agent treatments and in combination with other drugs.

The most thoroughly investigated mAb for MM to date is elotuzumab (Elo). Its results are particularly encouraging when used with LEN and DEX; an ORR of 82% was found in a Phase I study.[24] When combined with BOR in another Phase I study, an ORR of 48% was obtained.[25] The ELOQUENT2 trial was a Phase III trial which compared LEN and DEX with and without Elo.[26] The rates of PFS at 1 and 2 years were 68% versus 57% and 41% versus 27%, respectively, and the median PFS times were 19.4 versus 14.9 months, respectively ($p<0001$).

Daratumumab (DARA) is a mAb directed against CD38. It appears to have at least three separate mechanisms of action. In a Phase II study of DARA as a single agent, an ORR of 29.2% was obtained.[27] It has been designated by the FDA as a breakthrough therapy that is considered to have the potential to address an important area of unmet clinical need.[27] Phase III trials are evaluating it in combination with LEN and DEX (MMY3003-POLLUX) and in combination with BOR and DEX (MMY3004-CASTOR).

Treatment of First and Second Relapses

When a patient with MM relapses, it is important to first determine if this is a biochemical or a clinical relapse. CRAB symptoms (elevated calcium, renal failure, anaemia, and bone lesions) should be assessed.[28] Purely biochemical relapses generally do not require treatment, but the patient should be monitored closely for evidence of clinical relapse. Exceptions to this rule include patients with particularly aggressive disease at diagnosis, and those with a rapid increase in paraprotein concentration.

The principle factors to consider when determining the appropriate treatment option for the first relapse in patients with MM are as follows: the treatment regimen already used, the adequacy and duration of the response obtained, any AEs that occurred and that may be ongoing, the nature of the relapse, and the available treatment options. Most patients with MM who are considered to be suitable transplant candidates will have received an ASCT during their initial treatment.

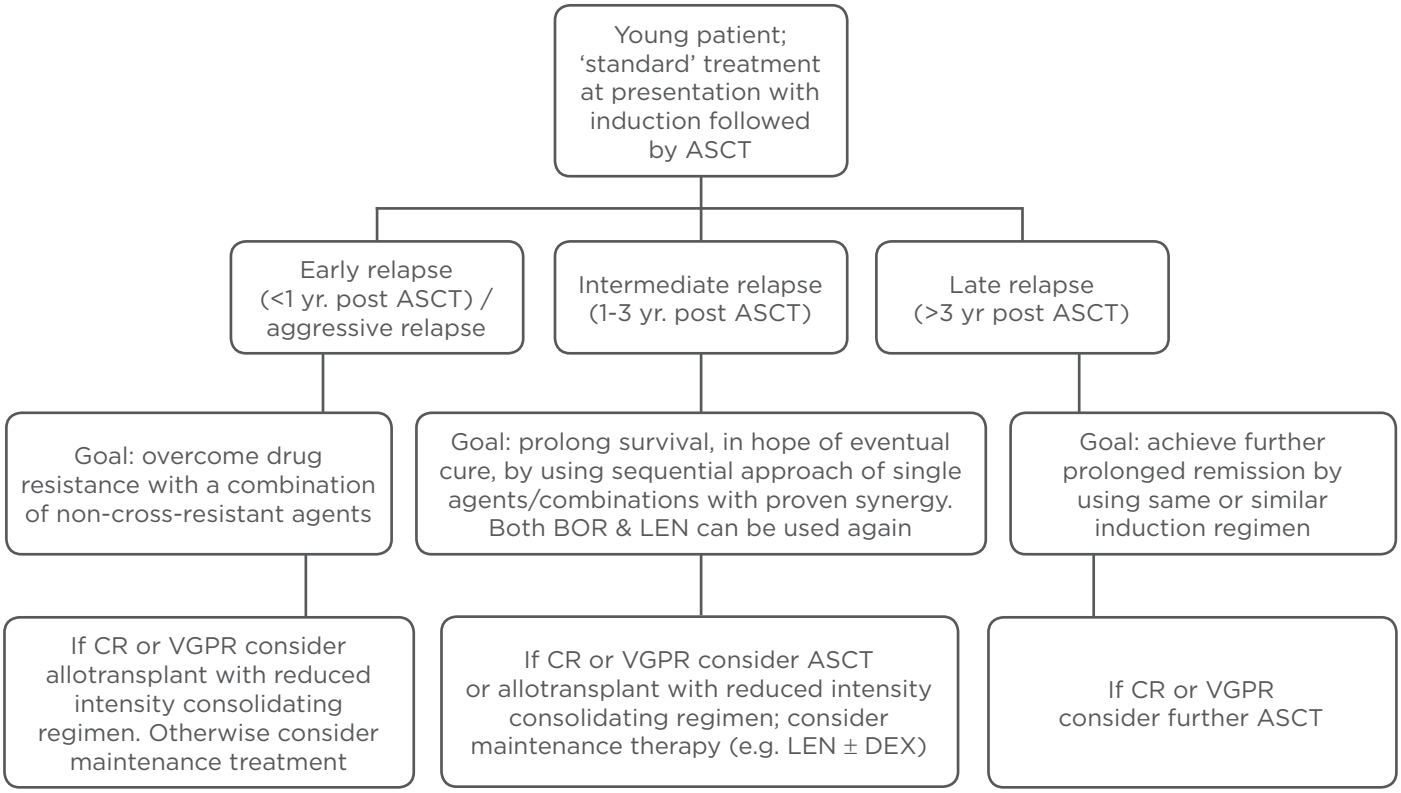

Figure 1: Treatment algorithm for first relapse in multiple myeloma.
ASCT: autologous stem cell transplant; BOR: bortezomib; CR: complete response; LEN: lenalidomide; VGPR: very good partial response; DEX: dexamethasone; yr: year(s).

There has been much debate as to whether, with the availability of modern drug treatments, it is necessary to include ASCT at first presentation of MM; the alternative is that it might only be used after the first relapse. A recently reported Phase III open-label, randomised study compared high-dose MLP + ASCT with MLP-prednisone-LEN (MPR), and also compared LEN maintenance therapy with no maintenance therapy in patients with newly diagnosed MM. Both PFS and OS durations were significantly longer with high-dose MLP + ASCT than with MPR. The median PFS was significantly longer with LEN maintenance than with no maintenance, but 3-year OS times were not significantly prolonged.[29]

Patients treated recently are likely to have received BOR and possibly also LEN and/or MLP. Those treated some time ago may have received THD or agents such as vincristine or doxorubicin (DOXO). It is important to consider the initial regimen in detail, the response to the various agents in it, and AEs. The nature of the response to initial treatment helps to determine the time to disease progression; this is likely to be short in those who showed only a minimal response to initial treatment, intermediate in those who achieved a complete response (no detectable monoclonal protein and <5% of plasma cells in the bone marrow), and longest in those who achieved an immunophenotypic complete response, in which multiparameter flow cytometry fails to detect any myeloma cells.[30-32] However, patients with a rapid and major response but with high-risk genetics can have a rapid and aggressive relapse. An aggressive relapse favours the use of multidrug combinations.

The patient's bone marrow reserve should be considered, as should issues related to previous AEs, such as peripheral neuropathy and deep venous thrombosis (DVT). Other patient factors which may be relevant include comorbidities such as diabetes mellitus and cardiac disease, physical fitness, quality of life (QoL), renal function, and social support. In a young patient (defined as <60 years) who has an early relapse (<1 year post ASCT), the goal should be to overcome drug resistance using a combination of non-cross-resistant agents (Figure 1).[33] Until recently, this situation pertained to around 5-10% of young patients with MM. There are a number of options available, including the following: the VDL-

PACE regimen (BOR with DEX, THD, and a 4-day continuous infusion of cisplatin, DOXO, CYC, and etoposide); the VRD regimen (BOR, LEN, and DEX); the VRD regimen with the addition of CYC; and the RAD regimen (LEN, adriamycin, and DEX).[34-36] In those patients with a complete or very good partial response, this treatment may be followed by an allotransplant by a reduced-intensity consolidating regimen, or else with a maintenance regimen.[37]

In a young patient with a late relapse (>3 years post ASCT), re-induction with the same initial regimen or a novel combination regimen followed by a further ASCT is appropriate (Figure 1).[33] At least until recently, approximately 10% of young patients with MM fell into this category. With the recent early use of new drug treatments, including in combination and of ASCT, the current median first remission time, of around 5 years, is significantly longer than it was just a few years ago. Consequently, more patients are likely to be candidates for a second ASCT.

Recently, the role of re-ASCT at the time of first relapse was investigated in a Phase III study of patients with MM who had suffered a relapse ≥18 months after their first ASCT.[38] Patients received BOR, DOXO, and DEX induction therapy and were then randomised to high-dose MLP 200 mg/m^2 + salvage ASCT or oral CYC (400 mg/m^2 per week for 12 weeks). The median TTP was significantly longer in those who received a further ASCT compared with those who did not (19 versus 11 months). Although the results support the wider use of re-ASCT in selected patients, it should be noted that 41% of potentially eligible patients were not randomised because of a failure to collect the necessary stem cells to allow ASCT, as a consequence of comorbidities, or because consent was withdrawn.

For the remaining ≈80% of young patients who relapse 1-3 years after initial treatment, the aim should be to prolong survival, hopefully until a curative treatment is available.[33] Until recently, this would probably have been achieved by the use of novel agents not used during the initial treatment. However, Phase II studies have demonstrated that retreatment with BOR,[39] and also with LEN,[40] is often successful with acceptable toxicity. Consequently, treatment of the first relapse can reasonably involve a further course of either of these agents for the majority of patients (Figure 1).

A further consideration is whether to treat for a fixed number of cycles, or with continued therapy until disease progression. The former is favoured in patients with indolent disease, and in those in whom further therapeutic possibilities exist. The latter is favoured following aggressive relapses and if treatment options are exhausted.[37] LEN with DEX is therefore a good choice; this two-drug combination remains a standard treatment for relapses. However, combinations of three drugs are being increasingly advocated, informed by trials such as ASPIRE14 and ELOQUENT2.[41] This trend may be changed to a two-drug combination such as CARF + DEX, according to the results of the ENDEAVOR trial.[15] However, in taking this decision, prior therapies and their efficacies must be considered.

In patients who have exhausted BOR and, more especially, LEN, the combination of POM + low-dose DEX would be a good choice, possibly optimised by the addition of a third agent (CYC or BOR) depending on the results of ongoing clinical trials. Relapsed elderly patients, who are not considered suitable for ASCT, should be assessed clinically as to whether they are suitable for active treatment. If so, the approach should be similar to that described for younger patients, but often using smaller drug doses. Both BOR and LEN have been shown to be effective in the elderly, as have the combinations of agents investigated in the ASPIRE, ENDEAVOR, and ELOQUENT studies. For others, treatment with oral CYC and prednisone should be offered.

Subsequent Relapses

In patients with MM who have further relapses, the recent availability of second and third-generation proteasome inhibitors, as well as immunomodulatory drugs and the deacetylase inhibitor PAN, adds significantly to the therapeutic possibilities. Once again, the possibility of using drugs to which the patient has responded to previously should be considered. When deciding whether to use single agents or combinations of drugs, the evidence for true synergy, rather than a purely additive effect, should be considered. Trial evidence suggests synergy between immunomodulatory drugs and DEX and between proteasome inhibitors and deacetylase inhibitors.

Future Possibilities

Despite the marked improvement in the survival of patients with MM over recent years, the condition

remains incurable; relapse is all but inevitable, even in those with the most favourable prognostic indicators. The relapsing nature of MM means that existing licenced treatment options will eventually become exhausted. There is therefore an on-going unmet clinical need for new treatments to be developed and made available. Currently, a plethora of potential novel treatments are emerging for relapsed disease, some still in the early stages of development, and others that may soon be approved. The following is a brief summary of some of the more promising agents in development, emphasising the breadth of different drug classes under investigation. More comprehensive reviews can be found elsewhere.[42]

Not all agents under investigation are new. For example, bendamustine (BDM) is an alkylating drug that has been investigated in MM patients over many years; it is now undergoing clinical trials in relapsed patients.[43] Furthermore, MLP-flufenamide is a dipeptide prodrug of MLP which appears to have greater potency than the parent drug.[44] However, a number of novel drugs have shown early promise, including filanesib,[45] which arrests cells in mitosis, tanespimycin, an Hsp90 inhibitor, combined with proteasome inhibitors,[46] and drugs that block signalling pathways, such as perifosine (an AKT inhibitor), and everolimus and temsirolimus (which target the mammalian target of rapamycin pathway).[47] Less promising have been studies of tyrosine and serine kinase inhibitors, and attempts to synchronise tumour cells with seliciclib, rendering them more susceptible to BOR.

CONCLUSIONS AND TREATMENT STRATEGIES

The treatment of patients with MM has moved from the era of chemotherapy to that of targeted drug therapy. This has been accompanied by improved survival outcomes. However, MM remains an incurable disease and the drugs used to manage it, although often less toxic than past chemotherapeutic regimens, still often cause significant morbidity as a consequence of AEs, including bone marrow suppression, DVT, and peripheral neuropathy, amongst others. As new treatments are developed to address the clear clinical need for these patients, a focus on safety and tolerability should be emphasised, as well as efficacy. It is crucial that attempts to prolong the patient's life do not ignore the importance of their QoL. The principal role of the treating clinician is to choose management strategies that maximise the therapeutic potential of the new agents available, whilst minimising the negative impacts on the patient and their family. This may mean taking different approaches in patients with similar disease profiles.

There is no widely accepted standard treatment pathway for patients with MM. The development of an internationally accepted, evidence-based treatment pathway for patients with MM would not only be welcomed by patients and clinicians alike, but would go some way to highlight the treatment gaps that patients with MM face. In those patients who can tolerate ASCT, initial treatment with an induction regimen with the first-generation proteasome inhibitor, BOR (alone or in combination with, for example, an immunomodulatory drug) followed by an ASCT is probably the most common current approach.

When the first relapse occurs, the most suitable response requires a detailed consideration of a range of factors described above. Usually, only symptomatic relapses are treated. In those patients who respond optimally to the initial treatment, re-induction with the same or modified regimen as used before, followed by a further ASCT, is an appropriate strategy. On the other hand, if the initial response was poor and short-lived, and/or if the relapse is aggressive, overcoming resistance using combinations of three or more drugs, including a proteasome inhibitor and an immunomodulatory drug, is appropriate. Making the choice between available proteasome inhibitors and immunomodulatory drugs should take into account what was used initially and the consequent AEs. For example, for patients previously exposed to BOR, either using the second-generation proteasome inhibitor CARF or the immunomodulatory drug LEN would be a good choice. In a patient treated with THD who had developed peripheral neuropathy, LEN or even POM would be suitable. In responders, a subsequent ASCT or allotransplant with a reduced-intensity conditioning regimen may be considered.

The approach during subsequent relapses is similar. Fortunately, the availability of third-generation proteasome inhibitors, immunomodulatory drugs, and PAN greatly increases the options available. The role of older drugs, such as MLP and BDM, should not be forgotten, and novel agents should be considered as they become available. The most promising of these include the deacetylase

inhibitors, the mAbs Elo and DARA, and signal transduction pathway inhibitors.

To conclude, it seems probable that for the treatment of MM the next decade will prove even more exciting than the last. Treatments that are becoming available offer the prospect of radically changing the survival curve for MM. While this curve may not plateau in the near future, the rate of relapse will become very low. The aims of researchers within this field are to bring to the bedside a range of safe drugs with low toxicity profiles and proven efficacy, which are capable of establishing a prolonged period of remission and even a cure for a subset of patients with MM.

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