EMJ EUROPEAN MEDICAL JOURNAL

HEMATOLOGY

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INSIDE

Review of

EHA 2014

Milan, Italy

Local Prescribing Information and full name and address of disclosing Takeda entity to be inserted according to local regulations

ADCETRIS:
INNOVATIVE CD30-TARGETED THERAPY

DE

ADCETRIS, a novel CD30-targeted antibody-drug conjugate, provides antitumour efficacy for relapsed or refractory Hodgkin lymphoma and sALCL patients[5]

- For the treatment of adult patients with relapsed or refractory CD30+ Hodgkin lymphoma:
  1. following autologous stem cell transplant (ASCT) or
  2. following at least two prior therapies when ASCT or multi-agent chemotherapy is not a treatment option
- For the treatment of adult patients with relapsed or refractory systemic anaplastic large cell lymphoma (sALCL)

- Generally well tolerated in
- Patients who achieve stab of 16 cycles (approximate
- Contraindicated for patien

Abbreviated Prescribing Information: Adcetris ▼ (brentuximab vedotin)

(Refer to Summary of Product Characteristics (SmPC) before prescribing)

Presentation: 50 mg powder for concentrate for solution for infusion. **Indication:** Treatment of adult patients with relapsed or refractory CD30+ Hodgkin lymphoma (HL) following autologous stem cell transplant (ASCT) or following at least two prior therapies when ASCT or multi-agent chemotherapy is not a treatment option; treatment of adult patients with relapsed or refractory systemic anaplastic large cell lymphoma (sALCL). **Dosage & Administration:** Adcetris should be administered under the supervision of a physician experienced in the use of anti-cancer agents. Recommended dose is 1.8 mg/kg administered as an intravenous infusion over 30 minutes every 3 weeks (If the patient's weight is more than 100 kg, the dose calculation should use 100 kg). Treatment should be continued until disease progression or unacceptable toxicity. Patients who achieve stable disease or better should receive a minimum of 8 cycles and up to a maximum of 16 cycles. Complete blood counts should be monitored prior to administration of each dose of this treatment. Patients should be monitored during and after infusion. For reconstitution and administration instructions please refer to SmPC section 6.6 **Dose Adjustments:** If neutropenia develops during treatment it should be managed by dose delays (see SmPC). If peripheral sensory or motor neuropathy emerges or worsens during treatment patients may require delay and dose reduction or discontinuation of Adcetris (see SmPC). Renal or hepatic impairment: No data available regarding use in renal or hepatic failure. Older patients (≥65yrs): No data available. Paediatric patients (<18 yrs): No data available. In nonclinical studies thymus depletion has been observed. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients listed. Combined use of bleomycin and Adcetris causes pulmonary toxicity. **Warnings and Precautions:** Progressive multifocal leukoencephalopathy (PML) has been reported in patients who received Adcetris after receiving multiple prior chemotherapy regimens; patients should be closely monitored for new or worsening neurological, cognitive, or behavioural signs or symptoms, which may be suggestive of PML. Adcetris dosing should be held for any suspected case of PML and permanently discontinued if a diagnosis of PML is confirmed. Acute pancreatitis has been observed in patients treated with brentuximab vedotin. Fatal outcomes have been reported. Close monitoring of new or worsening abdominal pain is advisedBrentuximab vedotin should be held for any suspected case of acute pancreatitis and should be discontinued if a diagnosis of acute pancreatitis is confirmed. Cases of pulmonary toxicity have been reported in patients receiving brentuximab vedotin. Although a causal association with brentuximab vedotin has not been established, the risk of pulmonary toxicity cannot be ruled out. In the event of new or worsening pulmonary symptoms (e.g., cough, dyspnoea), a prompt diagnostic evaluation should be performed and patients should be treated appropriately. Patients should be monitored during treatment for the emergence of possible serious and opportunistic infections. Immediate and delayed infusion-related reactions (IRR), as well as anaphylactic reactions, have been reported. Monitor patients during and after infusion. Adcetris should be immediately and permanently discontinued if anaphylactic reaction occurs. Infusion should be interrupted if infusion reaction occurs (see SmPC). Patients with rapidly proliferating tumour and high tumour burden are at risk of tumour lysis syndrome; these patients should be monitored and managed according to be neuropathy which is reversible in most cases. Patien Patients experiencing new or worsening peripheral or discontinuation of Adcetris (see SmPC). Grade 3 can occur with Adcetris. Refer to SmPC for dose ad be monitored for fever and managed according to b If Stevens-Johnson syndrome occurs, treatment wi medical therapy administered. Any patient who exp their serum glucose monitored and managed app moderate and severe renal impairment, and by low contains a maximum of 2.1 mmol (or 47 mg) of available. Studies in animals have shown reproduct be using two methods of effective contraception d 6 months after treatment.. There are no data as to excreted in human milk. A risk to the newborn/infant Adcetris treatment has resulted in testicular toxicity, administration of Adcetris with strong CYP3A4 and the incidence of neutropenia; with rifampicin, a stron

References: 1. Younes A, et al. *J Clin Oncol* 2012; **3.** Pro B, et al. *J Clin Oncol* 2012; 30: 2190-2196. Product Characteristics 2014.

EMONSTRATED EFFICACY

In relapsed or refractory Hodgkin lymphoma with prior ASCT[1,2]

75% ORR | **33.3%** CR | **40.5** MONTHS MEDIAN OS (95% CI. 28.7 to not estimable)

In relapsed or refractory sALCL[3,4]

86% ORR | **58.6%** CR | **33.4** MONTHS MEDIAN OS NOT REACHED (95% CI. 21.3 to not estimable)

ASCT: autologous stem cell transplant; ORR: overall response rate; CR: complete remission
OS: overall survival

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n the clinical trials

ble disease or better should receive a minimum of 8 cycles and up to a maximum
ely 1 year)

nts who are hypersensitive to the active substance or any of the excipients

est medical practice. Adcetris may cause peripheral
ts should be monitored for symptoms of neuropathy.
neuropathy may require delay and dose reduction
3 or 4 anaemia, thrombocytopenia and neutropenia
djustments if neutropenia develops. Patients should
est medical practice if febrile neutropenia develops.
th Adcetris should be discontinued and appropriate
periences an event of hyperglycaemia should have
propriately. MMAE clearance might be affected by
serum albumin concentrations (see SmPC). Adcetris
sodium per dose. Pregnancy & lactation: No data
ive toxicity. Women of childbearing potential should
uring treatment with brentuximab vedotin and until
whether brentuximab vedotin or its metabolites are
cannot be excluded. Fertility: In non-clinical studies,
and may alter male fertility. **Drug Interactions:** Co-
P-gp inhibitors, such as ketoconazole, may increase
g CYP3A4 inducer, did not alter the plasma exposure to Adcetris however, it reduced exposure to MMAE; and midazolam, a CYP3A4 substrate, did not alter the metabolism of midazolam therefore, Adcetris is not expected to alter the exposure to medicines that are metabolized by CYP3A4 enzymes. **Adverse Effects:** Very common (>10%): Infection, neutropenia, peripheral sensory neuropathy, diarrhoea, nausea, vomiting, alopecia, pruritus, myalgia, fatigue, pyrexia, infusion-related reactions. Common (≥1/100 to <1/10): Upper respiratory tract infection, herpes zoster, pneumonia, anaemia, thrombocytopenia, hyperglycaemia, peripheral motor neuropathy, dizziness, demyelinating polyneuropathy, cough, dyspnoea, constipation, rash, arthralgia, back pain, chills. Uncommon (≥1/1000 to <1/100): Oral candidiasis, Pneumocystis jiroveci pneumonia, staphylococcal bacteraemia, Tumour lysis syndrome, Stevens-Johnson syndrome, acute pancreatitis. Frequency not known (cannot be estimated from the available data): Progressive multifocal leukoencephalopathy, febrile neutropenia, anaphylactic reaction. Serious adverse drug reactions in the phase 2 population were: neutropenia, thrombocytopenia, constipation, diarrhoea, vomiting, pyrexia, peripheral motor neuropathy and peripheral sensory neuropathy, hyperglycaemia, demyelinating polyneuropathy, tumour lysis syndrome and Stevens-Johnson syndrome. **Pharmaceutical Precautions:** Store vial in a refrigerator (2°C-8°C), protected from light. After reconstitution/dilution, chemical and physical in-use stability has been demonstrated for 24 hours at 2°C-8°C. **PI Date of Preparation:** February 2014. **Legal category:** POM. **MA Number:** EU/1/12/794/001. **Name & Address of Marketing Authorisation Holder:** Takeda Pharma A/S, Langebjerg 1, DK-4000 Roskilde, Denmark. **Adcetris®** is a registered trademark of Millennium Pharmaceuticals Inc. *Adcetris has received a conditional marketing authorisation in Europe. A conditional marketing authorisation is granted to a medicinal product that fulfils an unmet medical need when the benefit to public health of immediate availability outweighs the risk inherent in the fact additional data are still required. The European regulatory agency will review new information on Adcetris at least every year and the summary of product characteristics will be updated as necessary.*

Please refer to the summary of product characteristics for details on the full side-effect profile and drug interactions of Adcetris. Adverse events should be reported to your local Takeda office.

EU/ADC-010103(1) Date of preparation March 2014

30: 2183-2189. **2.** Gopal AK, et al. Poster abstract no. 4382 presented at American Society of Hematology, December 2013, New Orleans, LA, USA. **4.** Pro B, et al. Poster abstract no. 1809 presented at American Society of Hematology, December 2013, New Orleans, LA, USA. **5.** Adcetris Summary of

ADCETRIS®
brentuximab vedotin

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HEMATOLOGY

Editorial Board

Hematology

Editor-in-Chief: Prof Emili Montserrat

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

Welcome

Welcome to the *European Medical Journal – Hematology* 2014 edition. Building upon our success from last year we have included more high-quality peer-reviewed papers, more content from the European Hematology Association (EHA) Congress, and more reports on other updates within the field. All content reflects the hottest topics within hematology this year.

The EHA Congress this year presented 200 abstracts in 40 oral sessions covering all topics within hematology. One of the most encouraging updates was the advancement in treatment for multiple myeloma (MM) patients. In our 'Congress Review' section we have reported on the results of Velcade® (bortezomib), a novel first-in-class proteasome inhibitor, which has proved to be a breakthrough in the treatment of MM, for those patients who are ineligible for stem cell transplantation. Velcade is of short duration and results in a longer time until chemotherapy is needed.

Dr Patrizia Tosi also evaluates the efficacy of the new drugs which are being developed to treat MM patients in the paper: '*Autologous stem cell transplantation in multiple myeloma: is it still the right choice*?' Dr Tosi assesses the effects of both short-term consolidation and long-term maintenance therapy results in disease eradication at the molecular level, which will thus increase patient survival. The paper indicates that thalidomide, lenalidomide, or bortezomib increased the percentage of patients achieving a complete response, which, in turn, gives hope for cures to be developed.

Another revolutionary study, reported in our 'What's New' section, describes how a patient's stem cells can be used to grow noses, ears, and blood vessels, combining the use of 3D printing, stem cell research, and nanotechnology. Tear ducts, blood vessels, and windpipes have already been made and transplanted into several UK patients.

Chronic lymphocytic leukaemia (CLL) is another topic which deserves much attention. Dr Michele Dal-Bo addressed the issue of the functional and physical interactions of CD49d with other microenvironmental receptors, in his paper: '*The role of CD49d in chronic lymphocytic leukaemia: microenvironmental interactions and clinical relevance*.' Dr Dal-Bo suggested that microenvironments are responsible for the growth and survival of supporting singles which influence CLL prognosis and therapeutic options.

The *European Medical Journal* aims to advance learning, knowledge, and research worldwide. In particular, this journal strives to provide a wealth of knowledge for all hematologists. It is due to the help of our editorial board that we have created a journal which covers a wide range of some of the hottest topics that are currently bracing modern hematological research. The ultimate goal of this journal is to provide the very latest information to healthcare providers so that patients can receive the treatment that they deserve.

Spencer Gore

Director, European Medical Journal

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EMJ GORELY NEW MEDIA

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Foreword

Prof Emili Montserrat

CLL and Lymphoma Programme,
University of Barcelona, Spain.

Dear Colleagues,

These are exciting times for Hematology. Always at the forefront of biomedical research, it is now one of the clearest examples of an individualised approach in the management of a wide range of medical disorders, ranging from anaemias to blood cancers. Molecular markers and new treatment molecules are rapidly changing the outlook of many hematologic disorders with dramatic improvements in survival and quality of life, as well as a reduction in treatment-related toxicity.

The knowledge published in the *European Medical Journal-Hematology* edition can be accessed worldwide thanks to an open access platform of medical publishing, which allows readers to freely access all articles without charge. To ensure the quality of the published articles, EMJ has gathered an editorial board of international experts and all articles are peer reviewed. The journal calls for basic, translational, and clinical investigators to submit their work for publication, covering current therapeutic and diagnostic developments and novel techniques in all aspects of Hematology.

> “Molecular markers and new treatment molecules are rapidly changing the outlook of many hematologic disorders with dramatic improvements in survival and quality of life, as well as a reduction in treatment-related toxicity.”

The journal also reports on the highlights from the outstanding international European Hematology Association annual meeting that recently assembled more than 10,000 delegates in Milan, Italy. The event was a great success due to its varied lectures and symposia and the introduction of the new EHA Learning Centre, which brings online learning to doctors globally. Additionally, breaking news stories of particular interest are reported in the journal, and this feature is already integrated into social media platforms, providing immediately updated information.

The road ahead for Hematology represents an exciting yet formidable challenge; an array of exciting and innovative developments lie around the corner in basic, translational, and clinical areas in most blood disorders. On behalf of the Editorial Board, I am eagerly anticipating the assembly of *EMJ-Hematology* into a creative forum for the transmission of hematological knowledge to an international audience.

Yours sincerely,

Emili Montserrat

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

EHA ANNUAL CONGRESS 2014

MICO MILANO CONGRESSI, MILAN, ITALY

12TH-15TH JUNE 2014

Welcome to the *European Medical Journal* review of the European Hematology Association Congress 2014

EMJ EUROPEAN MEDICAL JOURNAL

EHA ANNUAL CONGRESS 2014

MICO MILANO CONGRESSI, MILAN, ITALY
12TH-15TH JUNE 2014

Welcome to the *European Medical Journal* review of the European Hematology Association Congress 2014

The 19th Congress of the European Hematology Association (EHA), held from the 12th-15th June 2014, was a spectacular event, held in the beautiful city of Milan, Italy.

Milan is one of the most historic cities in Europe, its history and heritage stretching far beyond 20 centuries. The stunning architecture enclosed in the city can distract one from identifying that it is a city of knowledge, packed with universities and research centres. It is fitting therefore, that ground-breaking discoveries and updates within the discipline of hematology were witnessed here.

The EHA meeting boasts to be the leading meeting place for all hematologists in Europe - a statement that was vindicated by the >10,000 delegates in attendance this year. Speaking of why they joined the EHA, one member from the UK, said: "I joined EHA because I believe that we need a strong European organisation to promote basic and translational research in hematology in the European Union in order to maximise the benefit of our patients."

Age and ageing in blood disorders was the theme of this year's Congress. Due to an ever-ageing population, more people are beginning to develop age-related disorders, which have implications on economics, research, and clinical care. Chronic lymphocytic leukaemia (CLL), the most common type of leukaemia in older people, was one of the topics discussed this year.

For patients with CLL or small lymphocytic lymphoma, ibrutinib has proven to be an effective therapy. The RESONATE study demonstrated that patients who received this treatment displayed good overall survival as

well as impressive response rates. In many ways the trial highlighted that ibrutinib was more effective than ofatumumab. Prof Stephan Stilgenbauer, Department of Internal Medicine III, Ulm University, Alber-Einstein-Allee, Ulm, Germany, said: "When this data evolves and is confirmed, and probably more functional data lays are added, it may inform our treatment choice with regards to new agents that really target pathogenic principles of the disease and really drive the further development of compound development in the disease." For this patient population, ibrutnib can prove to be a breakthrough.

Another topic which was discussed throughout the Congress was personalised and targeted treatment in rare diseases. Results from an international study on paediatric immune thrombocytopaenia revealed the use of eltrombopag in children was safe and effective. The results highlighted that 75% of patients responded to this treatment within 12 weeks, compared to the placebo group who only achieved a 21% response rate.

Hematology is in the top five specialities that suffer from a lack of availability of medicines; for this reason the problem of access to essential medicines was discussed. A Turkish study compared costly tyrosine kinase inhibitors to cheaper generics and found that the generics were non-inferior to the original molecule, but more data are needed to further cement these results.

These data are encouraging as we enter an age where diseases which were once labelled as fatal can now be reclassified as chronic.

EHA ANNUAL CONGRESS 2014

MICO MILANO CONGRESSI,
MILAN, ITALY
12TH-15TH JUNE 2014

Rising to the challenge of acute lymphoblastic leukaemia

The results of the study indicated that the CR rate was 43% whereas in patients who had a stem cell transplantation prior to this study the CR rate was 45%.

BLINATUMOMAB, a bispecific antibody, demonstrated anti-leukaemic activity in adult patients with acute lymphoblastic leukaemia (ALL) who previously had a poor response or relapsed after prior therapies.

ALL is a rare type of blood cancer, and is mainly treated by intensive chemotherapy. However, if the disease is resistant to initial treatment it is likely that the disease will reoccur (relapse); the chances of patient survival after this event are very poor as the leukaemia becomes resistant to chemotherapy. Long-term survival is most commonly achieved after complete remission (CR) and a subsequent stem cell transplantation.

This study, which included 189 patients all with relapsed/refractory B-precursor ALL, evaluated the efficacy and toxicity of blinatumomab as a single-agent treatment. Blinatumomab has been designed to connect to one side of a surface marker on the leukaemia cells (CD19), the other side then attracts the patient's T cells, which in turn kill the cancerous cells.

The primary endpoint of the study was for patients to achieve CR, meaning that no leukaemia cells are detected in the bone marrow, or at other sites in the body. An exploratory endpoint was to measure the minimal residual disease (MRD) response, indicating low levels of leukaemia, within the first two cycles.

Response to ALL therapy is most commonly measured by microscopic analysis of the bone marrow; the detection level of leukaemia cells using this method is 5%. The sensitivity is further increased with the use of molecular biological tests.

All patients were 18 years of age or older, and had either experienced their first relapse/remission within 12 months or relapsed within 12 months of allogeneic hematopoietic stem cell transplantation. Treatment was given as a 4-week continuous infusion, followed by a 2-week break, and then another 4 weeks of treatment.

The results of the study indicated that the CR rate was 43% whereas in patients who had a stem cell transplantation prior to this study the CR rate was 45%, while median relapse-free survival was 5.9 months. The most frequent adverse events were: fever (59%), headache (35%), and low white blood count associated with fever (29%).

The study concluded that blinatumomab, while demonstating anti-leukaemic properties, also resulted in high MRD response rates of 82%. The next stage will be to analyse the correlation of MRD response with different types of cytologic response.

Minimal residual disease will reside no longer

> "Sequenta's ClonoSIGHT™ test has been validated in five lymphoid cancer indications, and we are working with collaborators from around the world to expand this powerful assay to additional clinical settings."
>
> *Dr Tom Willis, CEO of Sequenta, Sequenta Inc., San Francisco, USA*

CANCER detection using a next-generation sequencing-based assay named ClonoSIGHT™ can be used to potentially detect minimal residual disease (MRD) in lymphoid cancers.

A small amount of residual cancer cells that remain in the patient with lymphoid cancer after treatment are described as MRD. These cells are present at levels undetectable by traditional microscopic examination of blood, bone marrow, or a lymph node biopsy.

Early detection of MRD can be extremely useful in assessing whether other cancer treatments have been successful, and can also provide important information about patient prognosis to help guide additional treatment decisions.

The ClonoSIGHT™ technology is simple in premise, it uses a two-step process that involves firstly identifying DNA sequences in a diagnostic sample, then obtaining more samples after treatment to detect MRD. Clinical validation studies have shown that the ClonoSIGHT™ offers significant improvements in sensitivity and performance over traditional MRD detection methods.

"Sequenta's ClonoSIGHT™ test has been validated in five lymphoid cancer indications, and we are working with collaborators from around the world to expand this powerful assay to additional clinical settings," said Dr Tom Willis, CEO of Sequenta, Sequenta Inc., San Francisco, California, USA. "Sequenta is empowering physicians caring for patients, and companies developing new therapies, with the sensitivity and scalability of next-generation sequencing-based MRD detection and quantification."

ClonoSIGHT™ also has another clinical use; research has shown that it could potentially be used to test for follicular lymphoma, possibly allowing the complete replacement of repetitive, time-consuming imaging for assessment of disease status.

It is hoped that the development of this new technology will help reduce the number of deaths from recurrent cancers by implementing a method that results in a more thorough removal of cancer cells in the affected area.

Polycythaemia vera remedy sets pulses racing

EXCITEMENT is building in the face of the imminent availability of Jakavi® (ruxolitinib) - the first ever JAK 1/2 inhibitor for polycythaemia vera (PV) patients - which is outshining its competitors in maximising disease control.

PV is a chronic, incurable blood cancer associated with an overproduction of blood cells in the bone marrow that affects roughly 1-3 people per 100,000 globally. It is typically characterised by an elevated hematocrit, which often leads to severe cardiovascular (CV) complications such as heart attack and stroke.

"Uncontrolled PV in patients who are refractory or resistant to standard therapy can lead to CV complications and other systemic manifestations, and currently patients and their physicians have few options to treat them," said Dr Alessandro M. Vannucchi, study author and Associate Professor of Hematology, Department of Hematology, University of Florence, Florence, Italy.

Fortunately, Jakavi has shown immense promise in the pivotal Phase III RESPONSE trial, inhibiting the Janus kinase 1/2 (JAK1/2) and halting cytokine signalling.

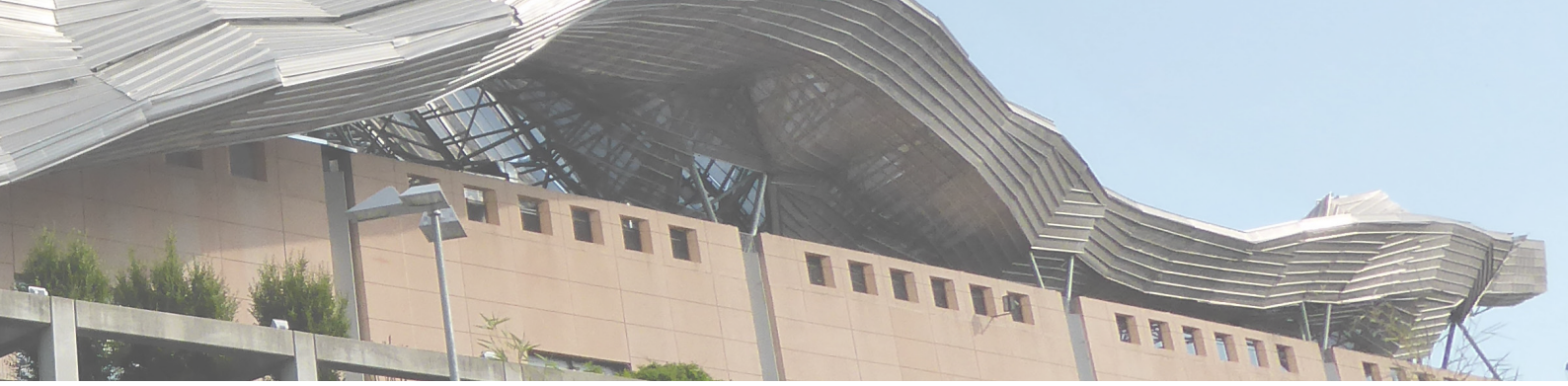
After 32 weeks of treatment with ruxolitinib, 77% of patients achieved one or both of either a reduction in blood cell count or a reduction in spleen size.

The study also showed a 50% or more improvement in PV-related symptoms from when they were receiving absolutely no treatment, compared to only a 5% improvement for those who are currently receiving the best available therapy in the same circumstances.

Ruxolitinib also impressed with its low level of serious adverse effects (AEs). 7.3% of patients developed Grade 3/4 hematologic AEs (such as thrombocytopaenia and anaemia), everybody else exhibited minor symptoms such as diarrhoea and fatigue.

The investigation into this new drug was inspired by the fact that PV is an incurable illness that has not seen much progress in recent years. "New treatments for PV are greatly needed as this is a disease that causes debilitating daily symptoms, which are as severe as symptoms associated with myelofibrosis, and also puts patients at risk for serious CV complications such as stroke and heart attack," said Dr Alessandro Riva, Global Head of Development and Medical Affairs, Novartis Oncology, New York City, New York, USA.

It is hoped that the potential of ruxolitinib will stimulate other pharmaceutical companies to continue research in this area so that rapid progress can be made in an area that has stagnated in recent years.



Defitelio® fighting severe hepatic veno-occlusive disease

THE FIRST and only licenced product for the treatment of severe hepatic veno-occlusive disease (VOD) in hematopoietic stem cell transplantation (HSCT) Defitelio® (defibrotide) has been launched in Europe.

"The commercial availability of Defitelio as the first medicine licensed for the treatment of severe VOD in Europe is an important step forward for patients with this life-threatening condition," said Mr Bruce C. Cozadd, Chairman and CEO, Jazz Pharmaceuticals PLC, Dublin, Ireland.

VOD is classified as a rare disease, affecting fewer than 5 in 10,000 people. Severe VOD is associated with multiple organ failure, thus, it is considered a life-threatening disease resulting in death for over 80% of patients.

Hepatic VOD can be a serious complication in patients who undergo HSCT; data suggest that up to 14% of patients who undergo HSCT develop VOD. Before undertaking this treatment, patients undergo chemotherapy and/or radiation therapy, leaving one susceptible to other diseases.

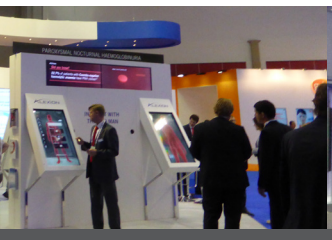
In Europe, the burden of VOD is significant; it places a strain on healthcare services and has a devastating effect on both the patients and their families. HSCT is also associated with large costs, it is resource intensive, and patients often have to spend much of their time hospitalised.

Prof Mohamad Mohty, Professor of Hematology, Saint-Antoine Hospital and University Pierre & Marie Curie, Paris, France, highlighted: "Severe VOD is a complex and unpredictable disease, and its impact on patients, physicians, and resources is substantial. Early and effective intervention is crucial in saving lives and limiting the potentially significant burden of this disease, and physicians have been eagerly awaiting the commercial availability of Defitelio in Europe."

In pivotal clinical trials, Defitelio has been shown to significantly increase survival rates for patients with severe VOD in HSCT. In the group of patients treated with Defitelio there was a 52% increase in survival at 100+ days after transplantation, compared to patients in the historical group.

In the clinical trial, 23.5% of patients treated with Defitelio achieved a complete response at 100 days after transplant, compared to 9.4% of patients in the historical group. Defitelio also performed well in all age groups and can be used as treatment in any patient older than 1 month of age.

International and national guidelines, such as the European Society for Blood and Marrow Transplantation, recommend the use of Defitelio for the treatment of VOD.





Ibrutinib: bringing hope to CLL and SLL patients

Ibrutinib significantly improved PFS, OS, and RR compared to ofatumumab in previously treated CLL/SLL. This study successfully validates ibrutinib as an effective new single agent therapy for CLL/SLL patients.

SIGNIFICANT results in survival and response rates (RRs) were achieved by ibrutinib in the treatment of chronic lymphocytic leukaemia (CLL) and small lymphocytic lymphoma (SLL).

CLL is a type of cancer that forms a primary tumour in the lymphocytes before metastasising through extravasation into the blood and causing secondary tumours elsewhere in the body. CLL is a very gradual cancer that accumulates over time.

Previous research has shown that patients who experience short response duration or adverse cytogenetics have poor outcomes regarding CLL or SLL. To remedy this the development of a new drug called ibrutinib, the first-in covalent inhibitor of Bruton's tyrosine kinase, was produced.

To examine the effectiveness of this new drug, the RESONATE study was founded, which is now in the Phase III clinical trial. This multicentre, international study assessed daily ibrutinib monotherapy versus the anti-CD20 antibody of atumumab.

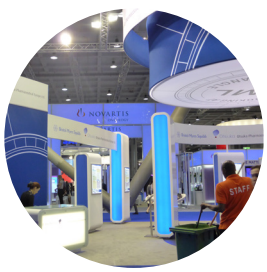
The study showed promising results; at a median follow-up of 9.4 months, the control group showed a median progression free survival (PFS) of 8.1 months, whereas those patients that were being treated with ibrutinib showed absolutely no progression of CLL during that time.

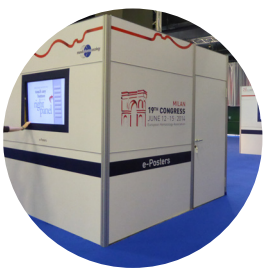
Furthermore, ibrutinib demonstrated an ability to significantly increase overall survival (OS) rates compared to controls that were taking the current standard-of-care.

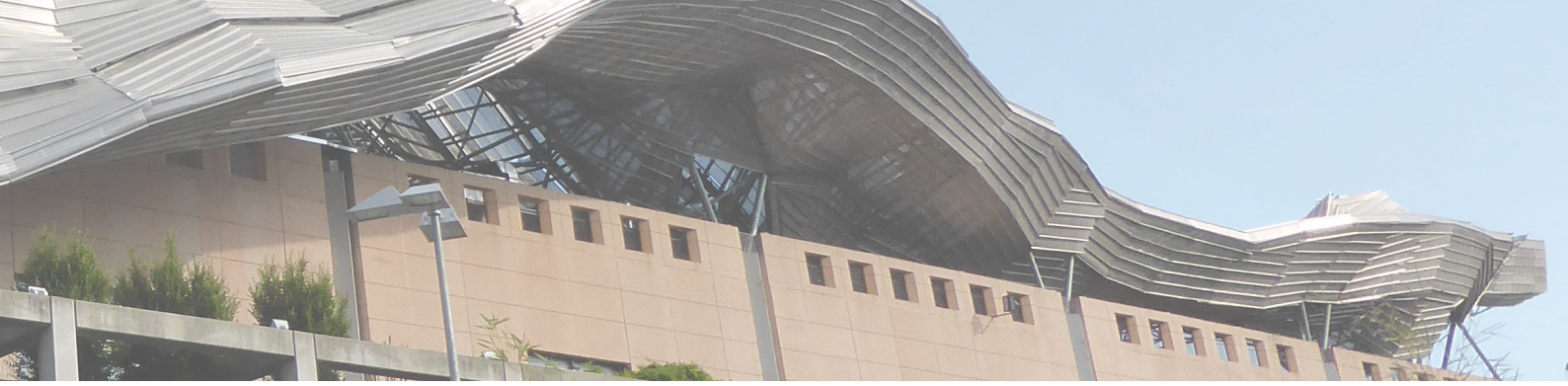
Ibrutinib was also able to improve RRs to treatment; the RR by an independent review committee was 63% including 20% responses with lymphocytosis for ibrutinib, compared to 4% with ofatumumab.

Additionally, toxicities were manageable and did not frequently result in dose reduction or treatment discontinuation, with 86.4% continuing ibrutinib at the time of analysis.

It was shown that ibrutinib significantly improved PFS, OS, and RR compared to ofatumumab in previously treated CLL/SLL. This study successfully validates ibrutinib as an effective new single agent therapy for CLL/SLL patients.





Velcade® reinforces its role as the backbone of multiple myeloma therapy

> "It is encouraging that a relatively short treatment duration with the bortezomib-based regime results in a longer time until the next chemotherapy is needed."
>
> *Prof Tadeusz Robak, Professor of Hematology, Medical University of Lodz, Lodz, Poland*

PROGRESSION-FREE survival in newly-diagnosed mantle cell lymphoma (MCL) patients, ineligible for stem cell transplantation, has been boosted by the introduction of a Velcade®-based combination therapy, which has also established itself as a bedrock for the treatment of multiple myeloma (MM).

MCL is a rare and aggressive blood cancer that usually occurs in older adults, with the median age at diagnosis being 65 years. The disease typically begins in the lymph nodes, but like most cancers it can spread to other tissues such as the bone marrow, liver, and spleen. MCL incidence rates among men and women in Europe are approximately 0.64 and 0.27 per 100,000 persons per year, respectively. Unfortunately, median overall survival is currently as low as 3-4 years, and only 1-2 years after the first relapse has occurred.

Up until now, treatments for MCL have been few and far between. Prof Tadeusz Robak, principal investigator and Professor of Hematology, Medical University of Lodz, Lodz, Poland, said: "MCL is an aggressive blood cancer and treatment options for newly diagnosed patients are limited."

However, Janssen-Cilag International NV announced their Phase III study data for Velcade® (bortezomib) that demonstrated many important facts about how the drug was able to tackle MCL and MM.

The investigation aimed to look at many inherent characteristics of the drug, including overall response rates, speed of response, and the likelihood of patients who had received subcutaneous (SC) Velcade® to reverse their renal impairment (which had been reported by patients suffering from MM).

Interestingly, SC delivery of bortezomib seemed to be the most effective method in terms of response rate (53% for the SC route compared to 31% for intravenous (IV) injection). SC entry also performed better in terms of time-to-response, which was 1 month longer when the IV method was used (2.3 months for SC delivery compared to 3.3 months for IV delivery).

Both delivery methods showed a high level of Grade ≥3 adverse effects, which will need to be monitored closely to ensure the safety of those who will take the drug in the future.

"This study clearly demonstrates a range of potential benefits in using a bortezomib-based frontline therapy in those patients. It is encouraging that a relatively short treatment duration with the bortezomib-based regime results in a longer time until the next chemotherapy is needed," said Prof Robak.

The battle of generics versus tyrosine kinase inhibitors

THE EFFICACY, safety, and quality of generics used to treat patients with chronic myeloid leukaemia (CML) were compared to tyrosine kinase inhibitors in a small retrospective Turkish study.

Tyrosine kinase inhibitors can be very costly and this is a major concern for healthcare payers. 30 tablets of generic imatinib (400 mg/day) are around €100 cheaper than the original molecule; per 10,000 chronic phase CML patients this may save the healthcare payers €12m per year. Moreover, reimbursement policies encourage the use of generics to lower the price, although some cancer specialists question if these savings are worth the associated risk concerning the drugs' quality, efficacy, and safety.

To evaluate the efficacy and tolerability of generics when used in chronic phase CML patients as initial treatment, the study was divided into two groups. Group A included 36 patients (aged 18-83) who received the original imatinib (Gilvec, Novartis), and Group B (aged 17-75) included 26 patients who received generics.

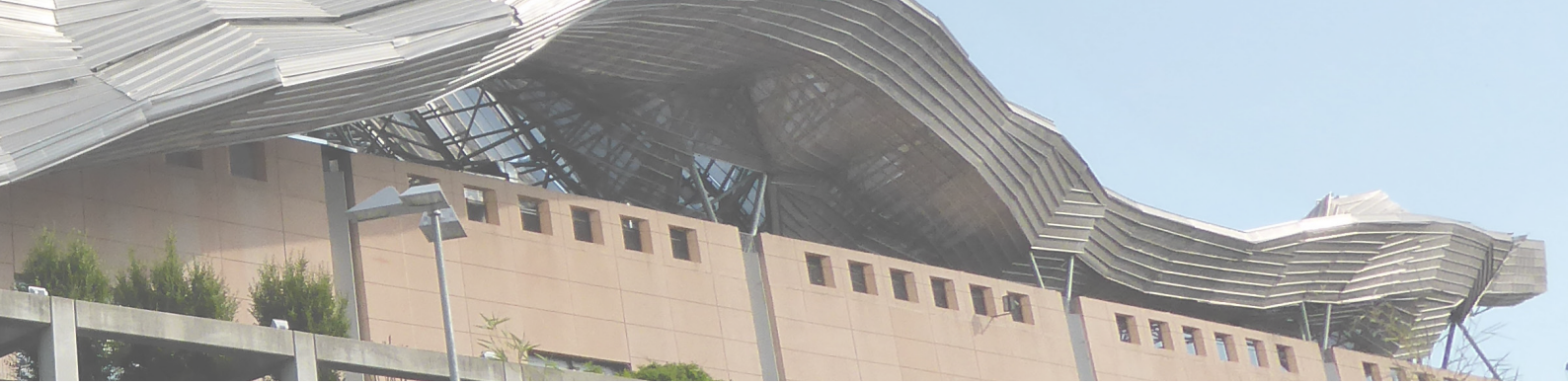
"There is very limited data regarding generics of imatinib, and the results have been conflicting at times," said Dr Zafer Başlar, Division of Hematology, Department of Internal Medicine, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey.

"However, the generics appear to be at least non-inferior to the original molecule regarding efficacy and tolerability when used in the upfront setting, as well as when used subsequently [according to several studies that have yet to published]," Dr Başlar continued.

While only a small subset of patients, the results are reassuring. There was no significant difference between the two groups regarding response and resistance rates, as well as hematological and non-hematological adverse effects.

The generics may have won the battle, but, as of yet, they have not won the war. Dr Başlar has suggested that in order for these data to be proven correct, prospective randomised trials with a larger number of participants are needed.

> "The generics appear to be at least non-inferior to the original molecule regarding efficacy and tolerability when used in the upfront setting, as well as when used subsequently."
>
> *Dr Zafer Başlar, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey*



Eltrombopag safe and successful for all ages

The results of the study showed very good response rates. 75% of patients responded to eltrombopag during the first 12 weeks of treatment, compared to 21% noted in the placebo group.

ELTROMBOPAG, used to treat patients with immune thrombocytopaenia (ITP), has been shown to be effective in adults and children in a new study, the largest drug trial for paediatric ITP ever, with encouraging results.

ITP is a rare disease in children, affecting 5 in 100,000. Most children with the condition will recover quickly without intervention; however, at 12 months 30% will still have the disease. As the condition is characterised by a low platelet count, the risk of life-threatening bleeding in children is real.

While current treatments have been shown to reduce platelet count for a short period, their safety is questionable. Repeated treatments do not always work, and they carry with them significant side-effects and risks. Surgical intervention to remove the spleen is an option but may not always work; the patient can be at risk of long-term infection, blood clots, or other risks deemed unacceptable by physicians and families alike.

Eltrombopag was tested in 92 children (all under the age of 18), at 38 centres, across 12 countries. Those included in the study were children who suffered for ITP for at least 12 months, who had failed previous treatments, and who had a platelet count of <30 Gi/L.

The results of the study showed very good response rates. 75% of patients responded to eltrombopag during the first 12 weeks of treatment, compared to 21% noted in the placebo group. Between weeks 5-12, 40% responded for 6-8 weeks, compared to 3% on placebo. By week 12 there was a 50% reduction in bleeding, which was further reduced to 66% by the end of the study. Moreover, 61% were able to stop or reduce other ITP medications.

Overall, these results are very encouraging. Very high response rates were achieved and maintained and the medication was proven safe for use in children with ITP.

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Sotatercept: potential new therapy for β thalassaemia

REDUCED red blood cell (RBC) production in β thalassaemia patients has shown to increase through the inhibition of GDF-11 (a cytokine known to promote the depletion of RBCs) by a fusion protein, sotatercept.

β thalassaemia is an inherited blood disorder resulting from a mutation in the β chain of hemoglobin (Hb) molecules, which results in the lack of functional RBCs; ranging from severe anaemia (β thalassaemia major) to a less severe form (β thalassaemia intermedia), which is dictated either by both of the β genes being affected, or just one.

Globally, it is estimated that 1 in 100,000 are affected, and approximately 1,000 people in England are suffering with the severe form, with an estimated 214,000 carriers of the disorder.

Sotatercept (ACE-011) is a novel first-in-class ActRIIA fusion protein that acts on late-stage erythropoiesis to increase release of mature erythrocytes into circulation.

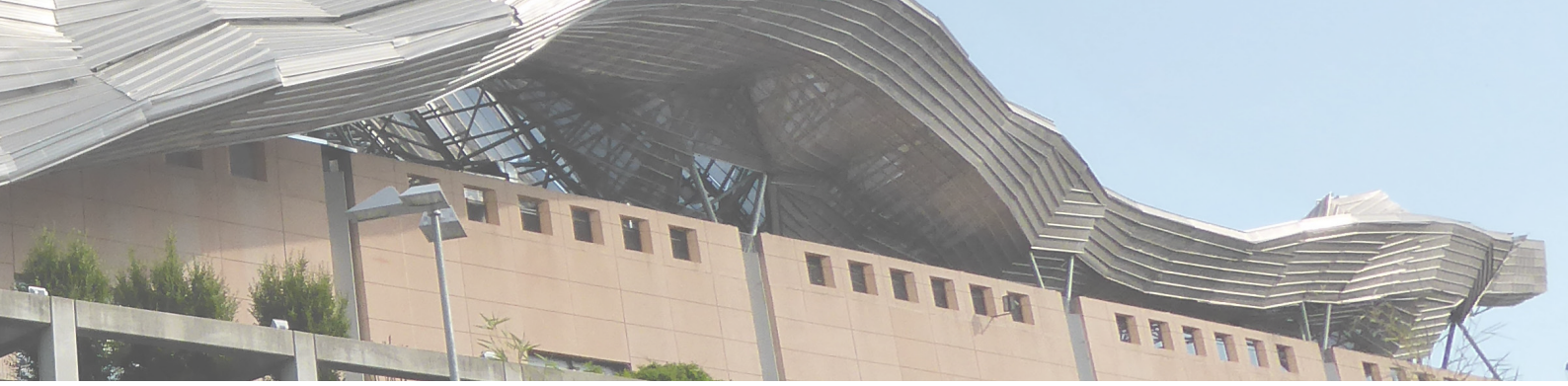
A Phase IIa, open-label, dose-finding study involved 32 β thalassaemia adult patients who received 0.1 (n=8), 0.3 (n=9), 0.5 (n=8), or 0.75 (n=7) mg/kg of the drug subcutaneously once every 3 weeks. Efficiency was assessed based on the increase of Hb from baseline of ≥1 g/dL sustained for 12 weeks in non-transfusion-dependent patients and reduction of RBC transfusion burden (≥20%) in transfusion-dependent patients. The assessment of pharmacokinetic parameters of the drug were included among the secondary endpoints of the investigation.

The results revealed that there were three (9%) patients that succumbed to Grade ≥2 treatment-related adverse events (i.e. bone pain, superficial thrombophlebitis, and ventricular extra systoles).

It was observed that in non-transfusion-dependent patients (n=22), there was maximum Hb increase ≥1 g/dL in the 0.3 (67%), 0.5 (83%), and 0.75 (100%) mg/kg doses in comparison to the 0.1 mg/kg dose group (0%).

In the ten transfusion-dependent patients, there was a significant reduction in transfusion burden ≥20% in 0.3 (33%), 0.5 (50%), and 0.75 (67%) mg/kg doses against the 0.1 mg/kg dose group (0%).

Due to its relatively safe drug profile, sotatercept can potentially benefit β thalassaemia patients but more research is needed to determine the effects of possible long-term drug toxicity. By potentially increasing the RBCs, this drug can be used in other anaemia-related diseases such as: myelodysplastic syndromes, diamond blackfan anaemia, chronic myelomonocytic leukaemia, myelofibrosis, and end-stage renal disease.



New drug multiplies survival chances for multiple myeloma

COMBINATION therapy with LBH589 (panobinostat), bortezomib, and dexamethasone has shown significant improvement in progression-free survival (PFS) in multiple myeloma (MM) patients.

MM is a cancer of the plasma cells, whereby uncontrolled cell proliferation causes an abnormal plasma cell level in the blood, overwhelming the production of healthy cells. MM is known to typically occur in individuals >50 years old, with few cases occurring in those who are <40 years old.

MM is a relatively common cancer that is, like most cancers, most prevalent in the Western world. MM affects approximately 1-5 in 100,000 people every year and has a 5-year survival rate of only 44%.

Panobinostat is a potent oral pan-inhibitor of Class I, II, and IV histone deacetylase enzymes. The drug blocks a key class of cancer cell enzymes, leading to cellular stress and the triggering of apoptosis.

"Almost all patients with MM ultimately relapse and become resistant to treatment, so new therapies are critical for continuing to manage the disease and improve outcomes," said Dr Paul Richardson, Clinical Program Leader and Director of Clinical Research, Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Boston, Massachusetts, USA.

The company's pivotal Phase III PANORAMA-1 trial primarily focused on the efficacy of panobinostat when combined with the current standard-of-care (bortezomib and dexamethasone), and the effect on PFS.

> "PANORAMA-1 data show that adding LBH589 to the standard-of-care treatment for patients with relapsed and refractory MM offers an innovative and effective treatment option to address an unmet need."
>
> *Dr Alessandro Riva, Global Head, Novartis Oncology Development and Medical Affairs, New York City, USA*

Promisingly, the drug has shown excellent potential in increasing PFS as in the LBH589 arm of the study the median PFS was 12 months, compared to only 8 months for the placebo arm of the study. There was significant improvement of 37% in PFS, achieving the primary endpoint of the study.

"PANORAMA-1 data show that adding LBH589 to the standard-of-care treatment for patients with relapsed and refractory MM offers an innovative and effective treatment option to address an unmet need," said Dr Alessandro Riva, Global Head, Novartis Oncology Development and Medical Affairs, New York City, New York, USA.

Revolutionary treatment of chronic idiopathic thrombocytopaenic purpura

> "We continue to be very optimistic about eltrombopag. Our ongoing efforts to assess the benefits of both short and long-term eltrombopag treatment of ITP will be part of GSK's commitment to providing patients with a convenient, effective, and well tolerated ITP treatment."
>
> *Dr Michael Arning, Group Medical Director, Oncology Medicine Development Centre for GSK*

REVOLADE® (eltrombopag), produced by GlaxoSmithKline (GSK), has been designed specifically for the treatment of chronic idiopathic thrombocytopaenic purpura (ITP), and has shown promising potential in the EXTEND study.

Eltrombopag is an oral, non-peptide, thrombopoietin receptor agonist that has been shown to stimulate the proliferation and differentiation of megakaryocytes, the bone marrow cells that give rise to blood platelets.

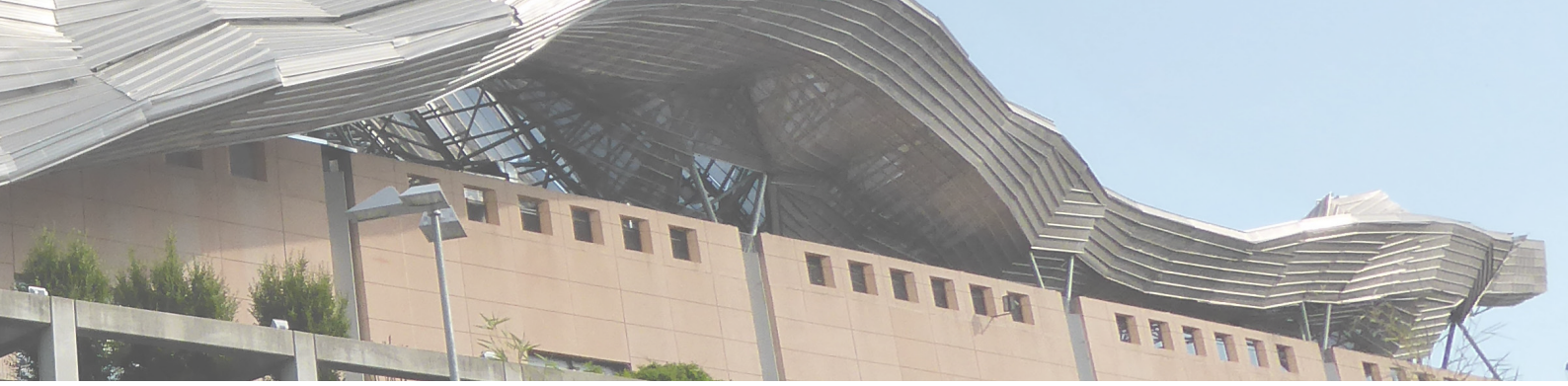
Chronic ITP is a disorder that is characterised by low levels of platelets in the blood, with patients suffering from excessive bruising and bleeding, and in some cases experiencing serious hemorrhages that can be fatal. Chronic ITP is a more serious issue in older patients who have a 5-year mortality of 48%, compared to only a 2% 5-year mortality for younger patients.

Eltrombopag targets the main manifestation of chronic ITP, which is a substantial drop in platelet levels. An impressive 80% of patients achieved a platelet count of >50,000 μl at least once, with 78% maintaining this level for >50% of the study.

Additionally, the trial showed that eltrombopag was well tolerated with no loss of efficacy; however, 17% of patients reported a headache, and 61% of patients reported a different side-effect.

In the REPEAT study, it was found that intermittent use of eltrombopag produced consistent and predictable responses in patients with chronic ITP, generating similar platelet counts with each subsequent cycle. 82% of patients positively responded to treatment in cycle one, with 88% of these patients achieving the primary endpoint (a consistency of response in cycles two and three).

"We are extremely encouraged by the continued progress in our eltrombopag clinical development programme," said Dr Michael Arning, Group Medical Director, Oncology Medicine Development Centre, GSK, London, UK. "We continue to be very optimistic about eltrombopag. Our ongoing efforts to assess the benefits of both short and long-term eltrombopag treatment of ITP will be part of GSK's commitment to providing patients with a convenient, effective, and well tolerated ITP treatment."



Mutation determining evolution of leukaemia

MUTATION of the DNA-methyltransferase enzyme (DNMT3A^{mut}) is generating significant interest in determining tumour evolution in acute leukaemic myeloid (AML) patients.

"These results could pave the way for dramatic changes in how we treat AML," said Dr Edwin Hawkins, postdoctoral researcher, Division of Cell and Molecular Biology, Imperial College London, London, UK.

The study was a comparison between mutated genes commonly present in the AML population and their non-malignant counterparts. T lymphocytes from the same patients were utilised to pinpoint the initiating mutation source. It was revealed that DNMT3A^{mut} was present in AML samples along with a secondary mutation, NPM1c, while the seemingly healthy T cells also contained the DNMT3A^{mut}.

Further investigations using a panel of cell surface markers and flow cytometry also revealed that the DNMT3A^{mut} was present throughout multiple lineages and progenitors. This significant result suggested that the mutation must be present in a common antecedent, hematopoietic stem cell, which gives rise to the myeloid and lineages. The secondary mutation is only confined to myeloid lineage.

The frequency of the mutations in association with the impact of chemotherapy was investigated in a comparative study using samples at diagnosis, remission, and relapse. The remission and relapse samples had a higher frequency of DNMT3A^{mut} than in the diagnosis sample, suggesting a competitive advantage and resistance to chemotherapy.

"Whether this resistance is due to a protective role of the mutation itself, the presence of an additional mutation that was not included in the candidate screen, or the inherent capacity for these progenitor populations to remain quiescent during chemotherapy and [survival] is unclear," said Dr Hawkins.

The mutation of interest can be the main focus in the future treatment of AML, where its frequency can be tracked and the possibility of relapse can be calculated. More emphasis needs to be placed on developing therapies to target the primary mutation, which could possibly lead to eradication of the disease.

> "These results could pave the way for dramatic changes in how we treat AML."
>
> *Dr Edwin Hawkins, Imperial College London, London, UK*

BCL-2 inhibitor could potentially halt leukaemia tumour progression

SELF-DESTRUCTION cancer drug for high-risk chronic lymphocytic leukaemia (CLL) has shown promising results in a Phase I study.

CLL is a rather interesting type of cancer that is diagnosed in approximately 5 in 100,000 people per year. Whereas many cancers are associated with rapid proliferation of tumour cells, CLL develops as a gradual accumulation, where each cell has a profoundly long life-span.

The mechanism that allows a cell to effectively become cancerous, and therefore defy the conventional regulation of the cell cycle, involves escaping the normal process of physiologic programmed cell death, apoptosis. The B cell lymphoma-2 (BCL-2) gene and its corresponding protein bind to the BH3-binding element, and act as a negative regulator of apoptosis. This is due to being a 'pro-survival' protein that is grossly over-expressed in CLL cells, causing deregulation of the cell cycle control mechanism and, therefore, rapid cell proliferation.

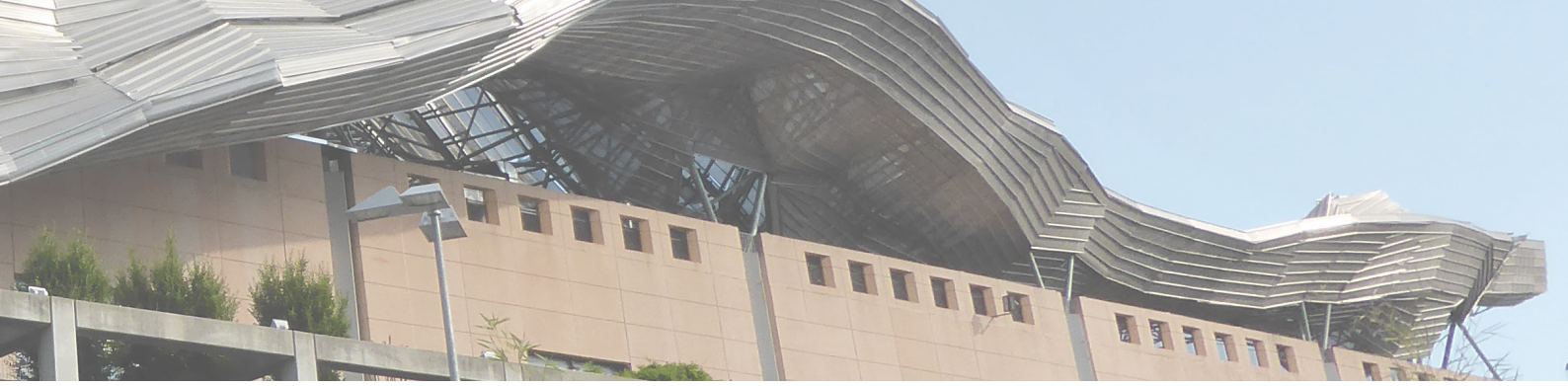
An oral drug known as ABT-199, developed jointly by AbbVie and Genentech, has been designed to exclusively mimic the binding of this BH3 structural element to the BCL-2 protein. This process restores the regulatory process that tells cancer cells to trigger apoptosis.

The results from the Phase I ABT-199 trial have been overwhelmingly positive, showing a remarkable safety profile when delivered using a carefully monitored step-wise dose escalation schedule, as well as outstanding efficacy in patients with CLL (patients with relapsed or refractory CLL showed a response rate of 77%).

Additionally, ABT-199 seems to produce little-to-no side-effects, with few patients refusing to continue the drug due to toxicities beyond the first few weeks of dosing.

This novel, bioavailable BCL-2 inhibitor was also successful in eradicating minimal residual disease (i.e. removal of a tumour which has left extremely low numbers of cancerous cells in the body), which can continue to proliferate and form new tumours. This is one advantage over invasive biopsies which provide no guarantee that some cancerous cells will not remain in the body.

As cancer continues to plague the Western world, it is hoped that this novel BCL-2 inhibitor will provide a method to prevent the deregulation of apoptosis by competitively binding to the BH3 domain.



Hematopoietic stem cell reprogramming: a vice versa case

> "Such efforts require a deeper understanding of the cell biology of HSCs and the niche that fosters these cells, together with better mechanistic insight into intracellular signalling pathways regulating HSC function."
>
> *Dr Ana Cvejic, University of Cambridge, Cambridge, UK*

SELF-RENEWAL and differentiation capacity can be attained by reversing adult hematopoietic stem cells (HSCs) to the foetal form with the upregulation of Lin28b and Hmga2.

As HSCs are able to give rise to other blood cell types they are of vast clinical importance because they can be used in transplants for the treatment of a wide variety of blood and immune system disorders. There are two populations that arise from HSCs: adult and foetal. Adult HSCs differentiate into mature blood cells while foetal HSCs remain undifferentiated.

Adult HSCs are characteristically quiescent in nature while their foetal counterparts have the increased capacity to differentiate and the ability for self-renewal.

Influencing the key developmental changes in HSC properties is the responsibility of the cell-intrinsic pathway called Lin28b-lrt-Hmga2 axis. It was reported that foetal HSCs are associated with increased expression of Lin28b and Hmga2, highlighting that these are the required ingredients for activating self-renewal ability in adult HSCs.

This hypothesis was tested and it was revealed that the enforced elevation of Lin28 increased self-renewal divisions in adult HSCs. Similarly, overexpression of Hmga2 caused the opposite action in mice models where the self-renewal activity of foetal HSCs had differentiated into premature adult-like characteristics.

"It is also worth noting that these effects of increased Lin28 and Hmga2 levels in adult HSCs were obtained in the absence of marked changes in their differentiation, proliferation or survival properties," said Dr Ana Cvejic, Department of Haematology, University of Cambridge, Cambridge, UK.

Understanding these reversal mechanisms can provide more information in treating paediatric and adult human hematopoietic malignancies, and also help in the development of therapies for cord blood transplantations through manipulation properties of the HSCs.

"Such efforts require a deeper understanding of the cell biology of HSCs and the niche that fosters these cells, together with better mechanistic insight into intracellular signalling pathways regulating HSC function," said Dr Cvejic.

EHA ANNUAL CONGRESS 2014

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EHA 2014 AWARDS

Cutting-edge research, integrity, and dedication are the lifeblood of modern hematologists, many of which were in attendance at this year's EHA congress. The opening ceremony sought to reward those who showed exemplary work within the field, work that will undoubtedly improve our understanding of contemporary medicine. Those who received prizes and awards demonstrated outstanding research, and the difficulty in selecting the winners was a testament to the overall quality of the submissions at this year's EHA congress.

NEW EHA HONORARY MEMBERS

Prof Freda Stevenson, UK

Prof Hartmut Döhner, Germany

EHA RESEARCH FELLOWSHIP AWARD WINNERS 2014

Clinical Research Fellowship

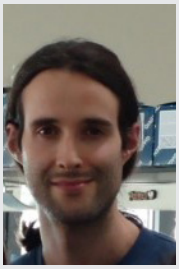
Dr Dimitrios Mougiakakos, Germany

Mitochondria as targets for specific cancer treatment for specific cancer treatment in chronic lymphocytic leukemia.

Junior Non-Clinical Research Fellowship

Mr Marek Mraz, Czech Republic

The role of microRNAs in BCR signalling and microenvironmental interactions of malignant B cells.



Non-Clinical Advanced Research Fellowship

Mr José Ignacio Martín-Subero, Spain

Functional and clinical impact of enhancer DNA methylation in mantle cell lymphoma.

FIND POTENTIAL IN A DIFFERENT ANGLE

You may think you know Levact, but take another look and it could surprise you. Levact has a balance of efficacy and tolerability, so it could be a good fit for your patients.

Levact is indicated for first-line treatment of CLL (Binet stage B or C) in patients for whom fludarabine combination chemotherapy is not appropriate. For additional indications, see Prescribing Information.

Levact® 2.5 mg/ml, powder for concentrate for solution for infusion. Prescribing Information. United Kingdom. Please read the Summary of Product Characteristics before prescribing. Presentation Powder for concentrate for solution for infusion. White, microcrystalline powder. One 26 ml vial of powder contains 25 mg bendamustine hydrochloride. One 60 ml vial of powder contains 100 mg bendamustine hydrochloride. **Indications** First-line treatment of chronic lymphocytic leukaemia (Binet stage B or C) in patients for whom fludarabine combination chemotherapy is not appropriate. Indolent non-Hodgkin's lymphomas as monotherapy in patients who have progressed during or within 6 months following treatment with rituximab or a rituximab containing regimen. Front line treatment of multiple myeloma (Durie-Salmon stage II with progress or stage III) in combination with prednisone for patients older than 65 years who are not eligible for autologous stem cell transplantation and who have clinical neuropathy at time of diagnosis precluding the use of thalidomide or bortezomib containing treatment. **Dosage and administration** For i.v. infusion over 30 – 60 minutes. Monotherapy for CLL, 100 mg/m2 on Days 1 & 2, every 4 weeks. Monotherapy for indolent NHL, 120 mg/m2 on Days 1 & 2, every 3 weeks. MM, 120 – 150mg/m2 on Days 1 & 2, 60 mg/m2 prednisone i.v. or per os on day 1 to 4, every 4 weeks. For further details please refer to the SmPC. **Contra-indications** Hypersensitivity to the active substance or excipients, during breast feeding, severe hepatic impairment, jaundice, severe bone marrow suppression and severe blood count alterations, major surgery (less than 30 days prior to start of treatment), infections, yellow fever vaccinations. **Precautions and warnings** Myelosuppression, infections, skin reactions, patients with cardiac disorders, nausea, vomiting, tumour lysis syndrome, anaphylaxis, contraception, extravasation. **Interactions** No in vivo interaction studies have been performed. Combined use with myelosuppressive agents may potentiate effects on bone marrow. Combination with cyclosporine or tacrolimus may result in excessive immunosuppression. Risk of infection following live virus vaccination which may be fatal. Potential for interaction with CYP1A2 inhibitors exists. **Pregnancy and lactation** Not recommended. **Side-effects** The most common adverse drug reactions are haematological reactions (leukopenia, neutropenia, thrombocytopenia), dermatologic toxicities (allergic reactions), constitutional symptoms (fever), gastrointestinal symptoms (nausea, vomiting), infection, tumour lysis syndrome, haemorrhage, anaemia, hypersensitivity, insomnia, cardiac dysfunction (such as palpitations, angina pectoris, arrhythmia), hypotension, hypertension, pulmonary dysfunction, diarrhoea, constipation, stomatitis, alopecia, skin disorders, amenorrhea, mucosal inflammation, fatigue, pyrexia, pain, chills, dehydration, anorexia, haemoglobin decrease, creatinine increase, urea increase, AST increase, ALT increase, alkaline phosphatase increase, bilirubin increase, and hypokalemia. Other side-effects that could be serious are sepsis, pneumonia primary atypical, haemolysis, anaphylaxis, somnolence, aphonia, paraesthesia, peripheral sensory neuropathy, anticholinergic syndrome, ataxia, encephalitis, pericardial effusion, tachycardia, myocardial infarction, cardiac failure, acute circulatory failure, pulmonary fibrosis, GI haemorrhage, hyperhidrosis, infertility, multi-organ failure, Stevens-Johnson syndrome, toxic epidermal necrolysis and encephalitis. Please refer to the SmPC for further details of other uncommon side-effects. **Legal category** POM. **Package quantities and price** 26 ml vials containing 25 mg bendamustine are supplied in packs of: 5 vials £347.26. 20 vials £1379.04. 60 ml vials containing 100 mg bendamustine are supplied in packs of: 5 vials £1379.04. **Marketing Authorisation numbers** PL 14427/0026. **Marketing Authorisation holder** Astellas Pharma GmbH, Postfach 50 01 66, D-80971 Munchen, Germany. Phone: +49 (0) 89 45 44 01. Distributed by: Napp Pharmaceuticals Ltd, Cambridge Science Park, Milton Road, Cambridge CB4 0GW, UK. Tel: 01223 424444. For medical information enquiries, please contact oncologymedinfo@napp.co.uk **Date of preparation** June 2010. ® Levact and the NAPP device are Registered Trade Marks. © 2010 Napp Pharmaceuticals Limited. PI Code UK/LEV-10023. PI Approved March 2012.

Date of preparation: July 2014. MINT/LEVA-14004c

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard. Adverse events should also be reported to Napp Pharmaceuticals Limited on 01223 424444.

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BIOSIMILARS IN HEMATOLOGY: INCREASING CHOICE, EXPANDING ACCESS

Summary of Presentations from the Sandoz Biopharmaceuticals-Sponsored Educational Session, which was part of the 'Updates-in-Hematology' Programme, held at the 19th EHA Congress, Milan, Italy, on 11th June 2014

Chairperson

Pier Luigi Zinzani[1]

Speakers

Joerg Windisch,[2] Steffen Thirstrup,[3] Wojciech Jurczak,[4] Paul Cornes[5]

1. Institute of Hematology and Medical Oncology 'L. e A. Seràgnoli', University of Bologna, Bologna, Italy
2. Chief Science Officer, Sandoz Biopharmaceuticals, Kundl, Austria
3. Director, NDA Regulatory Advisory Board
4. Department of Hematology, Jagiellonian University, Krakow, Poland
5. Bristol Haematology and Oncology Centre and Bristol Royal Hospital for Children, Bristol, UK

Disclosure: Pier Luigi Zinzani has a financial interest/relationship or affiliation in the form of consultancy for Bayer AG and Sandoz and has participated in speaker bureaus for Celgene International Sàrl, MundiPharma International Ltd, Pfizer Inc., and Takeda Pharmaceutical Company Limited. Pier Luigi Zinzani has received honoraria from and served on scientific advisory boards (SABs) for Celgene International, Sàrl, F. Hoffmann-La Roche Ltd., GlaxoSmithKline, MundiPharma International Ltd, and Takeda Pharmaceutical Company Ltd., and honoraria from Pfizer Inc. and SAB for Bayer AG and Gilead. Joerg Windisch is an employee of Sandoz Biopharmaceuticals. Steffen Thirstrup is a full-time employee at NDA Regulatory Services Ltd., which has a contractual agreement for his participation in this educational session. Wojciech Jurczak has received research funding from Celgene, Eisai, Gilead, Janssen, Pharmacyclics, Pfizer, Roche, Sandoz–Novartis, Spectrum, Takeda, and Teva, and has served on SABs for MundiPharma, Sandoz–Novartis, Spectrum, Takeda, and Teva. Paul Cornes has received salary from the UK NHS and honoraria from Roche, Janssen, Sandoz, Lilly, European Generics Association, Teva, and Hospira.

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MEETING SUMMARY

Professor Pier Luigi Zinzani

Biologicals have revolutionised modern medicine by offering vital therapeutic options to treat or prevent complex, disabling, and life-threatening diseases. Between 2013 and 2018, seven of the top ten pharmaceuticals worldwide will be biologicals; however, growing demand, combined with historically-limited competition, will continue to strain healthcare budgets and limit patient access to these treatments. Since 2006, when the first biosimilar Omnitrope® was approved in Europe, 18 other biosimilars, including the first biosimilar monoclonal antibody (mAb), infliximab (approved in 2013), have received marketing authorisation with many others currently in development. There is now extensive clinical experience with biosimilar epoetin (EPO) and filgrastim in patients with cancer, and many studies have reported comparable efficacy with the originator products, no unexpected safety concerns, and significant economic savings. Nevertheless, misconceptions concerning biosimilars remain. This educational session discussed these issues and gave an overview of biosimilar use in hematology.

Dr Joerg Windisch highlighted the particular challenges and considerations associated with

the development of biosimilars while Prof Steffen Thirstrup covered the approval of biosimilars from the regulatory perspective. Dr Wojciech Jurczak gave a presentation on the development of biosimilars in hematology, with a particular focus on rituximab from a clinical perspective. Dr Paul Cornes concluded with the opportunities that the introduction of biosimilars offer in terms of health economics and improved patient access to care.

Target-Directed Development for Biosimilars

Doctor Joerg Windisch

Biologicals are useful in a wide variety of difficult-to-treat diseases and provide the opportunity to target disease pathways in a highly specific and systematic manner. The average marketed biological is around 20-times more expensive than a small molecule drug, and for this reason almost one-quarter of 46 European countries do not provide access to biologicals for the treatment of rheumatoid arthritis.[1] Cancer patients in the USA are twice as likely as the general population to go bankrupt a year after their diagnosis[2] and costs are considered an important barrier for biological use in psoriasis by 19–24% of European and Canadian dermatologists.[3]

The costliness of biologicals stems partly from their complexity, since biologicals have a 20-800-times higher molecular weight than small molecules they cannot be readily synthesised and are therefore expressed in bacteria or in the case of complex glycoproteins, such as mAbs, in mammalian cell lines. Once the relevant gene is inserted into the cell line, cells are grown in large bioreactors. The biological is isolated, purified to a typical level of at least 99.99%, before being formulated with stabilisers and filled into the final dosage form. Currently there are >40 methods, based around chromatography and mass spectrometry that can provide a quality profile on a mAb with >100 attributes, such as glycosylation, glycation, and higher order structure.[4] Some therapeutic mAbs function by simply blocking their target, but most also act via 'effector functions' to activate the immune system or trigger programmed cell death. These functions can be tested, alone or in combination, using a number of sensitive biological assays.[5-7]

Variability in glycosylation is a normal feature of naturally occurring glycoproteins, and recombinant glycoproteins are no different. Individual batches of proteins contain a mixture of differentially glycosylated sites that have slightly different levels of biological activity,[8] which can occur due to variability in the manufacturing process. These differences in attributes are often greater than batch-to-batch variability; they are stringently controlled by regulators and are approved only if they do not lead to clinically meaningful differences.[9,10] Biosimilars are approved biologicals with comparable safety, quality, and efficacy to a reference product with no clinically meaningful differences. A non-comparable or alternative biological is not a biosimilar and will not be approved for use in highly regulated markets.[11,12]

Once an effective biological enters the market the development of a biosimilar starts by assessing the quality of this 'reference product' and defining the target for technical development, which will stem from variability of the reference product. The structure, function, biological activity, and final dosage form must match that of the originator. The biosimilar must have comparable pharmacokinetics (PK) and pharmacodynamics (PD), and efficacy and safety must be confirmed via tailored Phase III studies. Examples of comparable glycosylation, higher order structure, and biological activity of biosimilar rituximab have been reported.[13]

Ultimately, a biosimilar must match its originator in terms of primary structure (amino acid sequence), post-translational modifications (particularly glycosylation pattern), higher order structure, biological activity, and purity level, although here a biosimilar can surpass the original biological (Figure 1).[4] Following preclinical toxicology studies, biosimilarity is then confirmed in the clinic in studies designed to detect subtle differences between the biosimilar and the original product, which is the essence of biosimilar development. PK and PD studies are crucial and can require recruitment of up to 300 individuals or patients to provide the power and sensitivity to detect these subtle differences. Tailored Phase III studies demonstrate biosimilarity using an equivalence-based design.[14] It is not a requirement to use the same primary efficacy endpoints as the reference product, but rather to choose those endpoints which are most sensitive in detecting potential differences.[15] Immunogenicity should be investigated in a comparable manner to the original product

and, as with product purity, this can potentially be improved upon without invalidating the claim of biosimilarity.

Extrapolation of indications can arise due to the biological having different uses and indications in different patient populations. However, if a biosimilar has been tested for one indication, regulatory authorities do not automatically grant approval for the use of others without separate justification. Justification for extrapolation includes mechanism of action, patient related factors, the relationship between the structure and the target, and PK and PD in different populations.[14,16,17] Crucially, the justification is not based on comparisons between one indication and another; it is always between the reference product, for which safety and efficacy has been established in each indication, and the biosimilar. When extrapolating, one must select a sensitive indication in a patient population that is fully immunocompetent and also exhibits a sufficient effect size to ensure potential differences are detected.[15,18,19] When developing a novel biological, the majority of key data come from clinical studies and the information from analytical studies is relatively unimportant. Conversely, biosimilar development relies heavily on analytical studies, which provide the necessary high sensitivity, with clinical studies providing confirmation.

In conclusion, current analytical methods allow a deep understanding of biologicals that facilitate the development of safe and effective biosimilars of highly complex molecules. Biosimilars must meet the same quality standards as the original products and must be approved by the EMA/FDA with extrapolation of indication building upon the entire similarity paradigm.

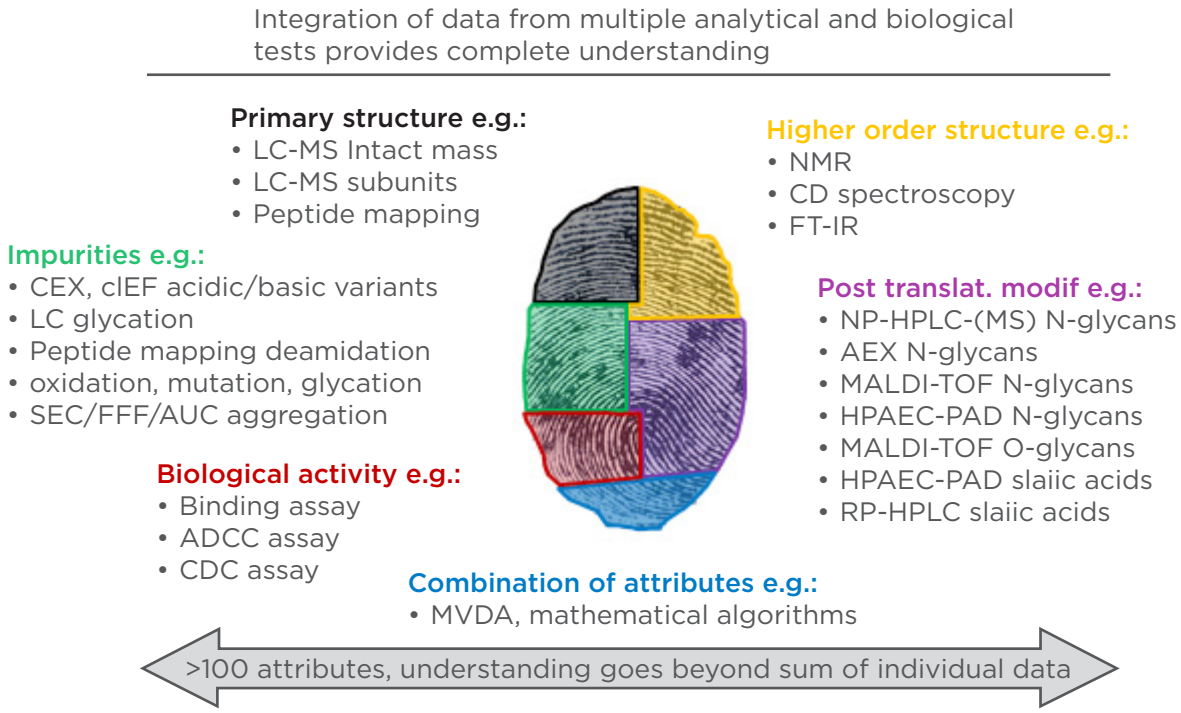

Figure 1: Analytical and biological tools used to characterise biopharmaceuticals and assess biosimilarity. ADCC: antibody-dependent cell-mediated cytotoxicity; AEX: anion exchange; AUC: analytical ultracentrifugation; CD: circular dichroism; CDC: complement dependent cytotoxicity; CEX: cation exchange; cIEF: capillary isoelectric focusing; FFF: field flow fractionation; FT-IR: fourier transform-infrared; HPAEC-PAD: high performance anion exchange chromatography-pulsed amperometric detection; LC: liquid chromatography; LC-MS: liquid chromatography-mass spectrometry; MALDI-TOF: matrix-assisted laser desorption ionisation-time of flight; MVDA: multivariate data analysis; NMR: nuclear magnetic resonance; NP-HPLC-(MS): normal phase-high performance liquid chromatography-(mass spectrometry); RP-HPLC: reverse phase-high performance liquid chromatography; SEC: size-exclusion chromatography.
Modified from Berkowitz et al.[4]

Table 1: Proposal for a more precise terminology.

Terms	Definition	Implications
Biosimilar*	Copy version of an already authorised biological medicinal product with demonstrated similarity in physicochemical characteristics, efficacy, and safety, based on a comprehensive comparability exercise.	Only very small differences between biosimilar and reference with reassurance that these are of no clinical relevance. Extrapolation of clinical indications acceptable if scientifically justified.
Me-too biological/biologic Noninnovator biological/biologic	Biological medicinal product developed on its own and not directly compared and analysed against a licensed reference biological. May or may not have been compared clinically.	Unknown whether and which physiochemical differences exist compared to other biologicals of the same product class. Clinical comparison alone usually not sensitive enough to pick up difference of potential relevance. Therefore, extrapolation of clinical indications problematic.
Second-generation (next-generation) biological/ biologic Biobetter	Biological that has been structurally and/or functionally altered to achieve an improved or different clinical preformance.	Usually stand-alone developments with a full development program. Clear (and intended) differences in the structure of the active substance, and most probably different clinical behaviour due to, for example, different potency or immunogenicity. From a regulatory perspective, a claim for "better" would have to be substantiated by data showing a clinically relevant advantage over a first- or previous-generation product.
*Comparable terms defined by the same/similar scientific principles include the WHO's "similar biotherapeutic products" and Health Canada's (Toronto) "subsequent entry biologicals."		

Modified from Weise M et al.[21]

Regulatory Perspectives on the Approval of Biosimilars

Professor Steffen Thirstrup

An application to market the first biosimilar, a 'generic' version of a growth hormone in Europe, was made in 2001. The application was, however, rejected by the European Commission despite positive opinion from EMA's Committee for Medicinal Products for Human Use (CHMP), since there was no biosimilar legislation at this time. In the aftermath, the European legislators modified the definition of generic drugs to account for biosimilars, leading to the adoption of new directives in 2005. In 2006 the first biosimilar was finally approved. As each new class of biosimilar has emerged the regulators have developed product or therapeutic-specific guidelines, most recently the mAb guidelines in 2012; many of these early guidelines are currently under revision.[20] Similar biosimilar guidelines are now in place globally.[20] The FDA regulations recognise two distinct types:

1) Biosimilars: considered to be a new active ingredient, are not interchangeable, and have no market exclusivity.

2) Interchangeable biosimilars: deemed to have the same active ingredient but are interchangeable and have 1-year market exclusivity.

As discussed above, biosimilar development is not about efficacy and safety per se of the active substance, but to demonstrate biosimilarity.

All biologicals will regularly undergo manufacturing changes that are subject to certain guidelines, which do not differ substantially from those that govern biosimilar development. For instance, Remicade®'s manufacturing process has changed 37 times since the product was first approved in the European Union (EU).[8] A biosimilar product will have to meet all the requirements of the manufacturing process in addition to the regulations governing biosimilarity.

The first wave of biosimilars consisted of EPO-alfa, growth hormone, and filgrastim, small biologicals that can be regarded as having tested the maturity of the emerging regulations. More biosimilars are now on the market but some have since been withdrawn and others have failed to meet the regulatory requirements.

There are currently contentious issues surrounding the terminology of biosimilars and the nature of the regulatory review process in Europe. There are a number of definitions and a number of related terms such as 'me-too biological', 'follow-on biological', and 'biobetter' (Table 1), which all describe products that may not be regarded as biosimilars according to European standards.[21] Inappropriate use of the term 'biosimilar' has contributed to misconceptions about the regulatory requirements and the efficacy and safety of such products. Use of the term 'biosimilar' should only be done in relation to products approved in accordance with EU or equivalent regulatory standards. Studies have shown that the review time for a biosimilar is neither abridged or accelerated,[8] nor is the process more lenient.[22] Another unresolved issue in Europe, unlike in the USA, is that of interchangeability. In Europe, there are currently no requests for 'switching' studies, and practices governing interchangeability are decided on a national basis and, where applicable, left to the discretion of the prescribing doctor. In addition, a biosimilar will not automatically be granted a licence for all indications of the reference product, whereas it may be possible for biosimilars to be developed for new indications beyond those approved for the originator.

For >10 years Europe has led the way in quality, science-driven regulation, and manufacturing of biosimilars. Similar regulatory principles govern manufacturing changes and biosimilars. Biosimilars can drive innovation by competing with the established biological industry.

Developing Biosimilars in Hematology: a Clinical Perspective

Doctor Wojciech Jurczak

The development of biosimilars will reduce the mounting costs of developing new treatments and pass savings on to healthcare providers and ultimately to patients. As discussed above, the 'art' of biosimilar development is to demonstrate, within current technical and scientific limitations and taking into account the inherent variability of biologicals, that all relevant functional and structural aspects are as close to the reference product as possible.[8,18] Biosimilars, unlike 'copy biologicals,' have similar biological activities, enabling physicians to treat patients confidently,[12] and the focus below will be on growth factors,[23,24] rituximab, and EPO.[11]

Phase I studies demonstrated the biosimilarity of Zarzio® and Neupogen® in terms of PD and PK profiles, over doses ranging from 1-10 μg/kg, following both subcutaneous and intravenous administration. A Phase III clinical trial further confirms the efficacy and safety of filgrastim biosimilar Zarzio® in neutropaenic breast cancer patients.[23-25] Furthermore, safety data for Zarzio® were consistent with the well-known safety profile of the granulocyte colony stimulating factor (G-CSF) class and the 21% incidence of musculoskeletal pain (8.8% bone pain) is comparable to incidences reported with Neupogen®.[24] Local tolerability was good and none of the patients developed anti-G-CSF antibodies.[24] As a result, the latest European Organisation for Research and Treatment of Cancer (EORTC) G-CSF guidelines recognise the use of biosimilar G-CSFs in Europe.[26]

Following the introduction of filgrastim biosimilars in 2008, the use of growth factors as primary prophylaxis has increased by 25%, despite the introduction of newer, more sophisticated drugs.[27] As biosimilars are made available and their usage increases, costs fall, thereby increasing access to therapy for more patients.[7] Manufacturers currently produce 13 EPO and G-CSF biosimilars, registered in Europe. They have achieved a market share of >50% in some countries such as the UK, but other countries, for example Italy and Spain, have been slower in adopting biosimilars.[28]

The patents for EPO and filgrastim have already expired in the USA, and those for pegfilgrastim

will expire soon in both the EU and USA.[29] Rituximab's EU patent has expired and its US patent will expire soon,[29] making it a prime target for biosimilar development, especially when one considers its efficacy.[30-32]

Rituximab was first approved for relapsed or refractory, low grade, CD20-positive B cell, non-Hodgkin's lymphoma (NHL) in 1997 before being approved as a frontline treatment for these patients in 2004 in the EU and in 2006 in the USA. In 2010 in the EU and 2011 in the USA, rituximab was approved as a frontline maintenance therapy for follicular lymphoma (FL). Sandoz began clinical trials for its biosimilar rituximab, GP2013, in 2011. GP2013 is intended for treatment in all indications currently approved for rituximab and, as of 2014, three clinical trials are in progress. Other companies are developing rituximab biosimilars, as shown in Table 2.

The aim of biosimilar development is not to establish patient benefit per se but to demonstrate high similarity to the reference product convincingly. This has been achieved in the case of GP2013, which is pharmacologically similar to the originator rituximab,[33] and shows high similarity in physicochemical and functional characteristics.[13] GP2013 is currently in Phase II trials in rheumatoid arthritis (ASSIST-RA)[34] and Phase III trials in FL (ASSIST-FL).[35] ASSIST-FL is running in >120 sites across 22 countries, comparing GP213-CVP against R-CVP in 618 previously untreated adult patients with advanced stage (Grade 1, 2, or 3a) CD20-postitive FL.

In the 6 years since their introduction in the EU, biosimilar use is increasing and establishing confidence with respect to safety and efficacy. Rituximab has changed the treatment of NHL but access remains an issue for many patients. Biosimilar rituximab candidates are now being developed to meet stringent regulatory requirements for quality and safety. GP2013 is currently being tested against originator rituximab in a sensitive population of FL patients receiving CVP (cyclophosphamide, vincristine, prednisone) chemotherapy. Biosimilars can be considered the 'generics' of the 21st century, allowing the reallocation of resources for 'biobetter' drugs. Nevertheless, the introduction of biosimilars is highly dependent on their acceptance by physicians, patients, and especially key opinion leaders.

Introducing Biosimilars to Expand Patient Access to Care

Doctor Paul Cornes

Cancer is now the world's biggest killer and has the most devastating economic impact of any cause of death in the world. It is responsible for 16.7% of all 'healthy' years lost in the EU, and ~83 million years lost worldwide.[36] The total economic impact of premature death and disability from cancer worldwide was $895 billion in 2008.[36]

The good news is that basic cancer science is delivering one medical paper a minute to the PubMed US National Library of Medicine,[37] and from this come innovations and advances in cancer care.

Table 2: Selected rituximab biosimilars in clinical development.

Company	Drug	Immunology	Oncology	Status
Amgen	ABP-		X	Announced
Boehringer Ingelheim	BI1695500	X	X	Ph III active
Celltrion & Hospira	CT-P10	X	X	Ph I/III active
Merck	MK-8808	X	X	ACTIVE; NOT RECRUITING
Pfizer	PF-05280586	X	X	Ph I/II active
Samsung	SAIT101			PROGRAM HALTED
Sandoz	GP2013	X	X	Ph I/II/III active
Teva	TL011			PROGRAM ABANDONED

Modified from 'Biosimilars: 11 Drugs to Watch.'[72]

Moreover, deaths from cancer in the G7 countries are falling.[38] Impressively, in the last three decades the median survival of cancer patients has increased from 1 to 10 years[39] due to innovations in drug development that will see almost 70 new drugs on the market by 2020.[40] Targeted therapies have already delivered on their promise, showing striking advances in otherwise hard-to-treat cancers. They have tripled survival rates in acute promyelocytic and chronic myeloid leukaemia, as well as medullary thyroid cancer.[41]

However, the costs of cancer treatments are increasing[42-44] and there are two main drivers behind this. Firstly, the rates of cancer are predicted to increase as the population ages,[45-47] and secondly, the innovations in cancer treatment come at an increasing cost, with cancer drug prices rising five-times faster than other classes of medicine.[42] Of the 12 cancer drugs approved by the FDA in 2012, 11 were priced above $100,000 per year.[48] This inflation puts enormous strain on health services worldwide,[49] which provides the incentive for the adoption of 'Value-Based Medicine' in oncology. While the budget to treat increasing numbers of patients rises annually, there is no evidence that more spending will consistently improve health;[50] instead, this investment needs to be directed to where it can be most beneficial.

One example of lost resource is from doctors prescribing branded drugs when a generic equivalent is just as good.[51] Generic drug promotion in the USA is estimated to have saved $1 trillion between 2002 and 2011,[52] but the uptake of generic drugs within Europe varies considerably, with some countries missing out on this benefit.[53]

The use of generic drugs may bring treatments into reimbursement that would otherwise be unaffordable, increasing access.[54] Biosimilars offer the same possibilities; for instance, many oncologists in the USA, Brazil, Mexico, and Russia would offer more trastuzumab to breast cancer patients if a lower cost biosimilar was available.[55] Skane University Hospital in Sweden made an annual saving of €650,000 by switching to biosimilar human growth hormone (rhGH) with no loss of efficacy or increase in adverse drug reactions, providing evidence that cost savings from biosimilars are real and increase access to treatment.[56] University College London Hospital's NHS Trust, UK, made annual savings in excess of €240,000 by switching patients from originator rhGH to biosimilar rhGH.[57] G-CSF access has improved in the UK since the introduction of biosimilar G-CSF; use has now surpassed that of the originator[58,59] and standards of care have improved.[49] Net savings of €2 million (representing 4–5% of the total drug budget) followed the switch to biosimilar G-CSF in southern Sweden, despite a 5-fold increase in daily G-CSF usage.[58]

Throughout the EU, G-CSF biosimilar use compared with the originator averages 71%, but the use in many countries remains much lower.[49] Value Based Medicine is not directed simply at cutting costs, but at improving care. Poorly targeted budget cuts may cost more than they save. There is evidence from Germany to suggest that originator filgrastim is frequently under-dosed in an effort to cut costs;[60] however, the drug is extremely dose-sensitive and ineffectual at suboptimal dosage.[61] Clearly a cheaper alternative would be of great benefit both therapeutically and financially. Adoption of biosimilars to a significant extent throughout Europe has the potential to make >€30 billion available for healthcare reinvestment.[62,63] In the USA, replacement of the top 12 biologicals with biosimilars was predicted to yield savings of up to $380 billion over 20 years.[62] These enormous savings make the widespread introduction of biosimilars a high priority in health economic terms.[64]

Safety is paramount when switching to biosimilars and concerns have been raised about the potential to miss rare events due to the small numbers of patients in trials performed to date; however, one meta-analysis encompassing 12,039 patients did not reveal any safety concerns.[65] In patients taking the original EPO-alfa, rates of pure red cell aplasia (PRCA) rose from the natural incidence of 1/100,000 to 50/100,000 due to the failure of the molecule to remain in suspension.[66] A study of biosimilar EPO-alfa is currently underway[67] and initial results suggest a similar association of biosimilar EPO-alfa and PRCA is unlikely.

Despite the financial and therapeutic benefits that biosimilars provide, many physicians remain poorly informed. A survey of 470 European prescribers from France, Germany, Italy, Spain, and the UK revealed that 25% cannot define, or have not heard of biosimilars, and only 22% consider themselves as very familiar with them.[68] Similar findings were reported from a survey in the USA.[69] The methods for promoting the use of generic drugs and biosimilars differ throughout Europe and remain inconsistent even between countries that have a high uptake, such as in Germany and

the UK (Figure 2). This suggests that much of the drive to promote better value comes from individual physicians and guideline groups.[53] Currently in the USA the majority of medical societies explicitly take cost-effectiveness into consideration when making their recommendations, which was not the case in 2002.[70,71]

'Biosimilar' is a specific regulatory term used by the EMA. Biosimilar drugs offer another chance for cost-savings and increased access of Europe's patients to innovative treatments, without compromising safety or efficacy.

	Rules and Incentives														
Market Rules	Mandatory price reduction	✓	✓					✓		✓	✓				✓	
	Patient co-pay		✓	✓			✓			✓	✓				✓	
	Price referencing			✓	✓	✓	✓		✓		✓	✓	✓			
	Pharmacy-level substitution				✓	✓				✓	✓	✓	✓	✓		✓
Market Incentives	At the pharmacy				✓	✓			✓	✓	✓		✓	✓		✓
	With the health insurers						✓			✓			✓	✓		
	With wholesalers										✓					
	With payers	✓	✓			✓										
	Favouring brands							✓				✓				
	Favouring generics	✓		✓			✓		✓		✓			✓	✓	✓

Figure 2: Differing rules and incentives for use of generic medicines across EU markets.
Modified from Sheppard A.[53]

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NEXT STEPS IN FRONT-LINE TREATMENT OF LYMPHOMA: THE ROAD AHEAD

Summary of Presentations from the Celgene-Sponsored Satellite Symposium, held at the 19th EHA Congress, Milan, Italy, on 12th June 2014

Chairperson

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Speakers

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MEETING SUMMARY

Prof Zelenetz opened the symposium on the evolving front-line treatment options for follicular lymphoma (FL) and discussed the potential of novel agents to replace chemotherapy. Prof Zelenetz presented the heterogeneity of diffuse large B cell non-Hodgkin's lymphoma (DLBCL) with regard to the diagnosis and subtypes of DLBCL to describe the specificity of new agents towards certain DLBCL subgroups, whilst Prof Dreyling spoke about the current diagnosis and treatment pathways for mantle cell lymphoma (MCL), and briefly described recent trial results. The final presenter, Prof Coiffier, discussed the lack of efficacy of front-line chemotherapy regimens for peripheral T cell lymphoma (PTCL), and highlighted potential new treatments based upon CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone). He then addressed the use of transplantation for first-line and refractory disease, and called for research to optimise therapy using existing agents.

Front-line Therapy for FL: a Shift towards Chemotherapy-Free Options?

Professor Bruce Cheson's presentation given by Professor Andrew Zelenetz

A large number of alkylating agents are currently used as first-line management of non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukaemia (CLL); however, recent data suggest that although chemotherapy improves time-to-treatment failure and progression-free survival (PFS), it does not impact overall survival (OS).[1] Bendamustine appears to improve PFS when compared to rituximab and CHOP (R-CHOP) in indolent NHL and MCLs; however, OS was not altered.[2] PFS can be improved by the addition of rituximab in the maintenance phase; however, the 6-year data again showed no difference in OS for patients with NHL or MCL.[3] Although therapies based on alkylating agents do improve PFS, there are acute and late toxicities including leukemogenesis, and many patients cannot tolerate the treatment. With the advent of novel biological combinations, the current first-line therapy for indolent lymphoma may be revised.

The Cancer and Leukaemia Group B (CALGB) undertook a series of studies that looked into immunotherapy doublets, the first of which was a Phase II trial of galiximab, an anti-CD80 monoclonal antibody (mAb), with rituximab but without chemotherapy (CALGB 50402).[4] Patients with low-risk disease according to the Follicular Lymphoma International Prognostic Index (FLIPI) showed the highest overall response rate (ORR) of 93%, with a complete response rate (CRR) of 75%, and patients with low-to-intermediate risk had a PFS in excess of 4 years with rituximab and galiximab. Patients with high-risk disease showed an ORR of 55% and a CRR of 27%.[4] The CALGB group found similar results when combining epratuzumab - an anti-CD22 mAb - with rituximab,[5] which resulted in an 86.5% response rate and a CRR of 42% after a median time of 9.2 months. However, further studies are necessary for both galiximab and epratuzumab when combined with rituximab as first-line therapy.

Chemotherapy can be used in a more targeted manner by combination with an antibody to specifically bind to the tumour cell and deliver the drug. Inotuzumab ozogamicin, an anti-CD22 mAb conjugated to calicheamicin,[6] was combined with rituximab and resulted in excellent overall and complete responses in patients with FL of 87% and 62%, respectively (n=39), whilst PFS was 39 months.[7] Other emerging antibody-drug conjugates include anti-CD22 and anti-CD79b conjugated to auristatin.[8-10] A recent study in patients with FL using two different combination therapies, showed a higher overall or objective response with anti-CD79b-rituximab (70%, n=14)[9] compared with anti-CD22-rituximab (59%, n=13)[8] and CRR (40%, n=8 versus 9%, n=2);[10] however, further evaluation is required due to the low number of patients. Grade 3/4 neutropaenia was reported by around 15% of the patients, as was some Grade 1 and 2 peripheral sensory neuropathy.[8-10]

Another approach to treat indolent lymphomas is to target tumours that have chronic active signalling through the B cell receptor and are dependent on downstream signalling pathways, which can be targeted via Bruton's tyrosine kinase (BTK), and therefore inhibited by ibrutinib.[11] Ibrutinib is known to affect other kinases; extrapolated data showed optimal CRR (33.3%, n=9) and improved PFS in patients given doses >5 mg/kg/day in a Phase I study (n=15).[12]

Activation of the B cell receptor initiates the PI3-kinase/AKT pathway, which can be targeted by numerous drugs such as idelalisib - a PI3-delta-specific kinase inhibitor. Promising initial results from Phase I and II studies[13,14] led to a small Phase Ib study to evaluate the safety of combining idelalisib with rituximab (n=32), or bendamustine (n=28), or both (n=10).[15] Improved PFS and CRRs confirmed the safety of combining idelalisib with other treatments. A further promising gamma/delta inhibitor is IPI-145, which demonstrated an ORR of 68% in patients with indolent lymphoma.[16]

Lenalidomide is another encouraging drug that has been shown to improve the immune synapse between B cells and T cells, enhance antibody-dependent cell-mediated cytotoxicity, and influence the microenvironment and angiogenesis. The wide-ranging properties of lenalidomide have a broad activity across various cancer types, as shown in Table 1.[17-20] A Phase II study of lenalidomide alone versus lenalidomide + rituximab in patients with relapsed/refractory FL showed that, although ORR was higher in the combination arm, CRRs were almost three-times higher in the lenalidomide arm, with a corresponding improvement in the event-free survival (EFS) from 1.2-2 years. Another multicentre trial of lenalidomide + rituximab for front-line treatment of FL showed a CRR of 71%.

Table 1: Response rates of lenalidamide.

	SLL (N=27)*	Marginal (N=27)*	Follicular (N=46)*	All Patients Eval (N=103)	All Patients ITT (N=110)
ORR, n (%)	24(80)	24(89)	45(98)	93(90)	93(85)
CR/CRu	8(27)	18(67)	40(87)	66(64)	66(60)
PR	16(53)	6(22)	5(11)	27(26)	27(25)
SD, n (%)	4(13)	3(11)	1(2)	8(8)	8(7)
PD, n (%)	2(7)	0	0	2(2)	2(2)

*7 patients were not evaluable for response – 5 patients due to an adverse event in cycle 1, 1 patient from non-compliance, and 1 patient withdrew consent.
CR: complete response; CRu: complete response unconfirmed; Eval: evaluable patients; ITT: intention-to-treat population; ORR: overall response rate; PD: progressive disease; PR: partial response; SD: stable disease; SLL: small lymphocytic lymphoma.
Modified from Fowler et al.[20]

Adverse events included neutropaenia Grade 3/4 in some patients who had a relatively low risk of febrile neutropaenia and thrombocytopaenia in a few patients, whilst fatigue was a very common side-effect and extensive rash is often seen in patients treated with rituximab and lenalidomide.

In summary, promising novel therapies are emerging that offer specificity as they target cell surface molecules and intracellular pathways, which may result in treatment management strategies that move away from chemotherapy. Combinations selected upon specific tumour types can be developed, thereby tailoring the therapy to each patient.

Optimising Front-line Treatment of DLBCL

Professor Andrew Zelenetz

The 2008 World Health Organization classification of lymphoid neoplasms will be amended next year to reflect the heterogeneity of DLBCL.[21] The heterogeneity of DLBCL was confirmed through gene expression profiling that identified two or three distinct subtypes of DLBCL according to the cells of origin:

1. Activated B cell (ABC) DLBCL

2. Germinal centre (GC) DLBCL

3. Primary mediastinal large B cell lymphoma (PMBCL), which tend to occur in women and show distinct molecular heterogeneity[22-26]

These heterogeneities are demonstrated by differences in patient outcomes, with superior OS and PFS shown in patients with the ABC Type.[21] Further analysis confirmed that EZH_2 overexpression seems to be a critical abnormality within the GC B cell (GCB) DLBCL;[27] whereas, NF-κB activation appears to be the hallmark of the ABC DLBCL, and the PMBCL have a unique molecular pathway. Further to the findings, there can be acquired mutations that are unique to a specific subtype; for example, only the ABC Types show mutations to CD79b.

The cells of origin can also be determined by a variety of models that include the Hans model,[28] the Choi model,[29] or the Tally model,[30-32] but these methods have a significant risk of misclassification compared to gene expression profiling on tissue. Digital multiplexed gene expression profiling uses RNA on a platform that can identify up to 800 genes per run. The Lymphoma/Leukaemia Molecular Profiling Project's Lymph2Cx assay uses formalin-fixed paraffin-embedded tissue and showed accurate assignment of ABC versus GC Types compared to the current gold standard, Affumetrix analysis, and was independently verified by two different laboratories.[31] Additionally, it provided superior identification of PFS and OS in patients compared with the Affymetrix platform,

can be performed in <36 hours, and is economically viable.[31] Fluorescence *in situ* hybridisation (FISH) can be applied to a defined set of genes where mutations have been recorded during baseline studies to further refine the profiling on several hundred DLBCL.[33]

Identification of the DLBCL subtype allows selection of the appropriate therapy, as seen in Table 2.[34] EZH2 mutations are particular to the GC DLBCL subtype, with a prevalence of ~22%. Small molecules that inhibit EZH2 are currently in preclinical testing and early clinical development.[27,35,36] ABC DLBCL are generally associated with the worst outcome; however, it was found recently, after categorising tumours for cell origin by the Hans model, that lenalidomide demonstrated an ORR of >50%.[37]

Lenalidomide not only inhibits NF-κB but also inhibits the complex IRF4 transcription factor pathway, resulting in inhibition of cell proliferation and an enhancement of cell death that has been named as synthetic lethality on ABC lymphomas.[38] A study conducted at the Mayo Clinic in elderly patients evaluated the effect of lenalidomide given for the first 10 days at 25 mg along with R-CHOP on the first day of each cycle.[39] Patient outcomes were compared to well-matched historical controls. The ORR was 98% and the CRR was 83% according to the positron emission tomography (PET) criteria (n=47).[40] Additionally, outcomes from GC and non-GC tumours were indistinguishable. Toxicity was mostly hematological with Grade 3 and 4 neutropaenia rates of 18% and 70%, respectively, 40% Grade 3/4 thrombocytopaenia, and a febrile neutropaenia rate of 10%.[39] The efficacy and safety of lenalidomide with R-CHOP has therefore led to the prospective randomised trial ECOG1412.[38,39,41]

Other targetable pathways include the B cell receptor pathway,[11] which can be treated using ibrutinib through BTK. In relapsed/refractory patients, most of the patients who responded to treatment had the ABC subtype, whilst only one patient with GC BCL responded.[42] The study further reported that a CD79b mutation upstream of BTK led to a higher ORR of 71%, whereas, a CARD11 mutation downstream of BTK predicted no response to treatment, suggesting that ibrutinib sensitivity does not require a BTK mutation. A Phase Ib study of ibrutinib with R-CHOP has since shown an ORR of 100%, with a CRR of 91% (n=22).[43] Bortezomib is another promising treatment that was combined with R-CHOP[44] and has led to three prospective randomised trials (NCT00931918, NCT01040871, and NCT01324596).

Lastly, a recent trial evaluated the effect of a sequential R-CHOP programme. After PET analysis of patient biopsies, participants then received R-CHOP for four doses followed by three doses of ifosfamide, carboplatin, and etoposide (ICE).[45] Among the first 200 patients, only 8 then had a transplant (personal communication). The LNH03 study then compared a cycle of rituximab and CHOP to rituximab combined with doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (R-ACVBP) with sequential consolidation,[46] which demonstrated improved EFS for non-GC tumours only when using the R-ACVBP treatment.[47]

In conclusion, the advent of targeted agents may overcome the adverse impact of non-GC large cell lymphoma. Although these agents require further larger trials to be incorporated in existing guidelines and intensive chemotherapy may achieve similar results at lower cost, it is an exciting time for DLBCL treatment.

Table 2: Targeted therapy in DLBCL - dependence on cells of origin.

Target	Example agent	GCB	ABC
NF-κB	Bortezomib		+
PI_3K	Idelalisib		+
PKCβ	Enzastaurin		+
BTK	Ibrutinib		+
SYK	Fostamatinib		+
Multi-target/ angiogenesis	Lenalidomide	-/+	++
EZH_2	EZH_2 inhibitors	+	
BCL_2	$ABT-_{199}$	+	+

DLBCL: diffuse large B cell lymphoma; ABC: activated B cell lymphoma; GCB: germinal centre of B cell lymphomas.
From Andrew Zelenetz's presentation at this symposium.

Update on Current Trends in MCL Front-line Treatment

Professor Martin Dreyling

Diagnosis of MCL based upon pathology has been difficult due to the similarities with other types of cancer, which had previously led to an accurate identification of MCL cancers in only one-third of cases.[48] However, overexpression of cyclin D1 that is characteristic of MCL can now be used to confirm diagnosis, improving the accuracy to 98%. Once diagnosis is confirmed, risk factors should be assessed to optimise treatment. Hoster et al.[49] showed that patients with Ki-67 proliferative antigen levels >30% have significantly worse outcomes, even when treated with intensified treatment. Identification using a standardised method[50] and treatment of patients with high Ki-67 levels has therefore been included in the current European Society for Medical Oncology (ESMO) recommendations for MCL.[51] The Mantle Cell International Prognostic Index (MIPI) – an automatic web calculator that requires four parameters: performance status, age, elderly age, and leukocyte count – has been confirmed by the major study groups worldwide[51-54] and has been recommended for use in clinical routine as well as trials. Overall, within MCL, indolent cases have a prevalence of 10–15%, whilst 'classical' MCL accounts for 80% of cases, and 'transformed' types account for 5%.[55]

Current treatment recommendations include dose intensification (Figure 1), as a meta-analysis of randomised trials showed PFS and OS benefits.[55,56] Merli et al.[57] used cyclophosphamide, vincristine, doxorubicin, and dexamethasone treatment alternated with high-dose methotrexate and cytarabine treatment (HYPER-CVAD/MA); however, only one-third of patients completed the trial due to toxicity. A European trial with nearly 500 patients substituted the three cycles of R-CHOP with three cycles of rituximab with dexamethasone, cytarabine, cisplatin (R-DHAP) and found that molecular remission improved from 37% to 70%, as well as significant improvement of PFS.[58]

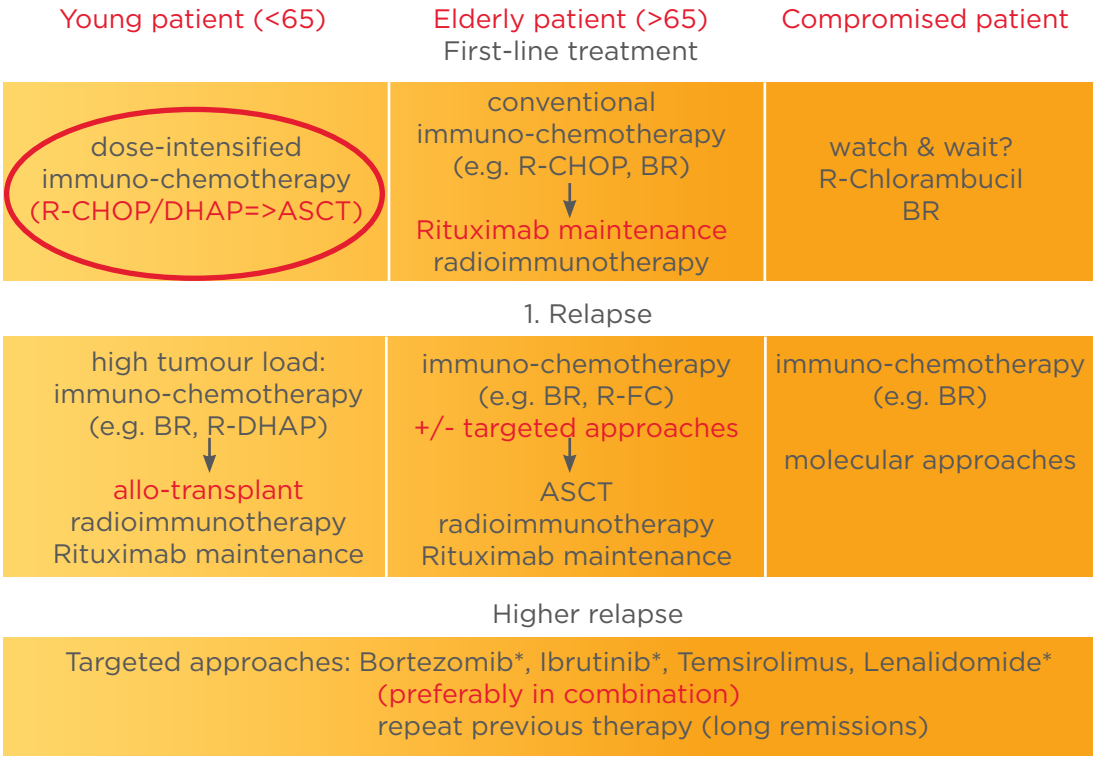

Figure 1: Treatment options for mantle cell lymphoma.
ASCT: autologous stem cell transplantation; BR: bendamustine and rituximab; DHAP: dexamethasone, cytarabine, and cisplatin; R-CHOP: rituximab + cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone; R-DHAP: rituximab + DHAP; R-FC: rituximab + fludarabine and cyclophosphamide.
From Martin Dreyling's presentation at this symposium.

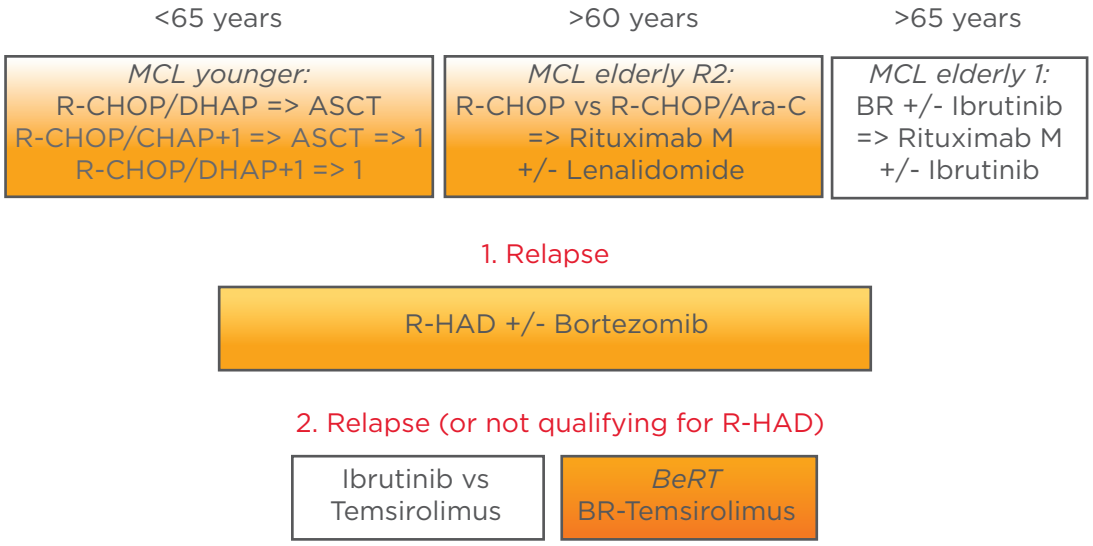

Figure 2: European Mantle Cell Lymphoma Network: Study generation 2014.
Ara-C: cytarabine; ASCT: autologous stem cell transplantation; BR: bendamustine and rituximab; DHAP: dexamethasone, cytarabine, and cisplatin; R-CHOP: rituximab + cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone; R-DHAP: rituximab + dexamethasone, cytarabine, and cisplatin; R-FC: rituximab + fludarabine and cyclophosphamide; R-HAD: rituximab, high-dose Ara-C, and dexamethasone.
Modified from European Mantle Cell Lymphoma Network.[76]

The treatment of elderly patients constitutes the majority of cases since the median age of disease is ~65 years.[55] A study performed in the 1960s that compared R-CHOP against bendamustine demonstrated the same OS across treatments,[59] suggesting that bendamustine should be evaluated with regard to its suitability in elderly patients as the only treatment given.[2] A study published in 2012 evaluated R-CHOP treatment followed by a maintenance period of either rituximab, IFN-α, or PEG-IFN for 2 months. Results showed that prolonged rituximab after R-CHOP significantly increased the remission duration ($p<0.0001$) and OS,[60] and has since been incorporated into national and international guidelines.

Regarding molecular targeted approaches, bortezomib, ibritumomab tiuxetan, temsirolimus, and lenalidomide have been registered for MCL in either the USA or Europe. There are studies combining bortezomib with R-CHOP, whilst a recent Phase I trial that combined temsirolimus with bendamustine and rituximab demonstrated a strong response rate and is currently being evaluated through a Phase II trial.[61]

Ruan et al.[62] explored an R-squared combination of rituximab and lenalidomide without any chemotherapy and reported an overall ongoing remission around 80%, with a CRR of 55%. Although it was a small study, this is undergoing a further large randomised study across eight European countries. Targeting of the B cell receptor pathway[63] with ibrutinib through BTK demonstrated high compliance, a complete response of 40%, and partial response of 37% in bortezomib-naïve patients (n=30) after a median of 14.7 months with very rare side-effects that included pneumonia, cellulitis, and Grade 4/5 sepsis.[64]

Whilst the first standard of care in younger patients is autologous stem cell transplantation, and in older patients it includes high-dose cytarabine during induction, recent promising clinical trials suggest that the first-line and second-line treatment options may soon be revised, as illustrated in Figure 2 from the European MCL Network.

Extending Treatment Possibilities in PTCL

Professor Bertrand Coiffier

As with DLBCL, PTCL shows a wide heterogeneity and there are currently several types of PTCLs, some of which are targetable with specific therapies. The OS of patients with PTCL is very poor and similar to that of angioimmunoblastic lymphoma.[65]

Current problems with PTCL include the rarity of the disease, causing the cancer types to be combined with other types of cancer when evaluated in clinical trials. Whilst the first-line therapy can be inadequate for many patients, the role of transplantation as first-line or for refractory diseases is not well defined either. As it is known that subtypes of PTCL respond differently, a universal first-line therapy would not be appropriate. For example, enteropathy-associated T cell lymphoma has very good results with autologous transplant as first-line therapy. There is only one randomised study that compared R-CHOP to etoposide, ifosfamide, cisplatin as an alternating treatment with doxorubicin, bleomycin, vinblastine (VIP-rABVD) in 88 patients and demonstrated that although VIP-rABVD therapy was not found to be superior to R-CHOP, around 40% of patients on R-CHOP showed EFS after around 30 months of treatment and 60% of patients therefore required alternative treatment.[66]

Novel investigative drugs for PTCL include brentuximab vedotin (SGN-35), mogamulizumab (anti-CCR4), and pralatrexate as first-line therapy, whilst, romidepsin, panobinostat, alisertib, and crizotinib have been evaluated in relapsing patients. Whilst pralatrexate resulted in a good response rate in one-third of patients and a median survival of 3.5 months, the duration of response was 10.1 months and there was some common toxicities such as mucositis, thrombocytopaenia, and neutropaenia that could render the drug unsuitable for combination with other treatments.[67] Romidepsin is a histone deacetylase inhibitor evaluated in three Phase II multicentre studies that included 150 PTCL patients, where it was reported that the majority of patients had reductions in the tumour volume from baseline.[68] Additional histone deacetylase inhibitors such as vorinostat, panobinostat, and belinostat are also under clinical development. Alisertib (MLN8237) is an investigational small oral aurora A kinase (AAK) inhibitor, undergoing a Phase III trial for relapsed or refractory PTCL.[69] Brentuximab vedotin is an antibody-drug conjugate that resulted in tumour reduction in 79% of patients[70] with relapsed T cell lymphomas, but requires further research due to the low patient numbers (n=29).

Most front-line therapies investigated are based upon CHOP. A Phase I study combined CHOP with romidepsin and reported a 1-year estimated PFS of 63.9% at a median follow-up of 10 months (n=27).[71] This front-line therapy study has progressed to Phase III, comparing CHOP with CHOP and romidepsin over 3–5 years, and plans to enrol 420 patients (NCT01796002). Focusing on the NK/T cell lymphoma subtype, L-asparaginase and methotrexate resulted in improved PFS in 19 relapsed or refractory patients.[72] The SMILE study comparing methotrexate and L-asparaginase + ifosfamide and etoposide demonstrated a 45% CRR, whilst toxicities included a high rate of Grade 4 neutropaenia (92%, n=38).[73,74]

There is another point of debate regarding the role and timing of autologous transplants. There are currently five Phase II retrospective trials in first-line consolidation, suggesting that the 5-year OS is 50%, whilst PFS is around 35-40%. It can be an option for some very aggressive subtypes, however, as yet there are no randomised trials so the true impact of autologous transplantation on survival is still unknown. Another retrospective study found that allogeneic compared to autologous transplants were associated with worse outcomes in nearly all cases due to toxicity.[75]

In conclusion, PTCL first-line therapy for many patients is currently inadequate and although there are promising results from new drugs in development, further trials are necessary to determine which treatments are most suitable for each PTCL subtype. Future combinations will probably be based upon CHOP, whereas the role of transplant for first-line therapy is uncertain but may be appropriate for relapsing patients such as those with DLBCL.

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THE MULTIPLE FACETS OF THROMBOTIC MICROANGIOPATHIES

Summary of Presentations from the Alexion-Sponsored Symposium, held at the 19th EHA Congress, Milan, Italy, on 12th June 2014

Chairperson

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MEETING SUMMARY

The Alexion satellite symposium was introduced by Prof Pier Mannuccio Mannucci who provided an introduction to the history of thrombotic microangiopathies (TMAs). Prof Paul Coppo gave an overview of TMAs and the differential diagnosis of atypical hemolytic-uraemic syndrome (aHUS) and thrombotic thrombocytopaenic purpura (TTP). He emphasised the importance of a suitable differential diagnosis in order to initiate an appropriate treatment as soon as possible, eventually allowing better patient outcomes. Prof Javier de la Rubia discussed the role of the complement pathway and how genetic abnormalities can lead to dysregulation in aHUS. Prof Thorsten Feldkamp concluded by giving an overview of the latest clinical trial data on the efficacy and safety of eculizumab in the management of aHUS.

Differential Diagnosis of TMAs

Professor Paul Coppo

TMAs are a group of heterogeneous and rare diseases (<4 cases per million/year). Each disease is characterised by different features and pathophysiological mechanisms. It is possible to distinguish TTP from other TMAs. TTP is mostly an acquired disorder but can also be congenital. Another TMA is HUS, which is caused by Shiga-toxin producing bacteria (STEC-HUS) or complement dysregulation (aHUS). TMAs can also be observed in other contexts such as in the HELLP syndrome in pregnant women, in catastrophic antiphospholipid syndrome, malignant hypertension in cancer patients, and in transplantation. TMAs all have a specific pathophysiology, a specific management, and a specific prognosis.

The pathophysiology of TTP was first described in 1924 by Eli Moschcowitz[1] following the case of

a young girl who suddenly presented with fever, cerebral manifestations, anaemia, hemorrhage, and renal failure. She experienced heart failure and died after 2 weeks. The autopsy showed thrombi in the arterioles and capillaries of most organs. TTP typically involves the brain but its effects are not limited to the brain. Autopsy studies have shown that TTP is a multi-system disorder involving the heart, pancreas, kidney, adrenal glands, digestive tract, and liver.[2]

The pathophysiology of TTP can be explained by the action of von Willebrand factor (VWF). VWF is synthesised and separated by endothelial cells in the plasma as very large multimers (500–20,000 kDa), which are cleaved by ADAMTS13 to lower molecular weight multimers,[3] decreasing their adhesiveness to platelets. TTP patients have a severe deficiency of ADAMTS13 resulting in the accumulation of larger multimers in plasma causing platelet aggregation.[4,5] This eventually results in the formation of microthrombi in the microcirculation leading to multi-organ failure. ADAMTS13 is a metalloproteinase that belongs to the ADAMTS family. Deficiencies of ADAMTS13 can arise from heterozygous bi-allelic mutations of the gene,[6] corresponding to the rare congenital cases of TTP. More commonly, deficiencies result from auto-antibodies directed against the enzyme. These patients can be treated efficiently with plasma therapy, which allows them to receive ADAMTS13 exogenously.

HUS arises from ingestion of the Shiga-toxin via contaminated food or water. The Shiga toxin crosses the digestive barrier and is transported by leukocytes to the endothelial cells of the renal microvasculature. The toxin is internalised in the endothelial cells, leading to the expression of proaggregant and procoagulant molecules such as VWF and tissue factor, which leads to the formation of fibrin-rich microthrombi. Deposits of complement, such as C3 and C9, are also found on the surface of platelets, monocytes, and circulating endothelial microparticles. These complement fragments may therefore be involved in STEC-HUS as well.

The complement system is composed of three pathways: the classical pathway, which is activated by immune complexes; the alternative pathway, which is activated by microbial surfaces; and the lectin pathway, which is activated by sugars on the surface of microbes. In the last 20 years, dysregulation of complement has been found to be linked to the development of aHUS. Mutations in complement regulatory genes are found in approximately 70% of patients with aHUS.[7]

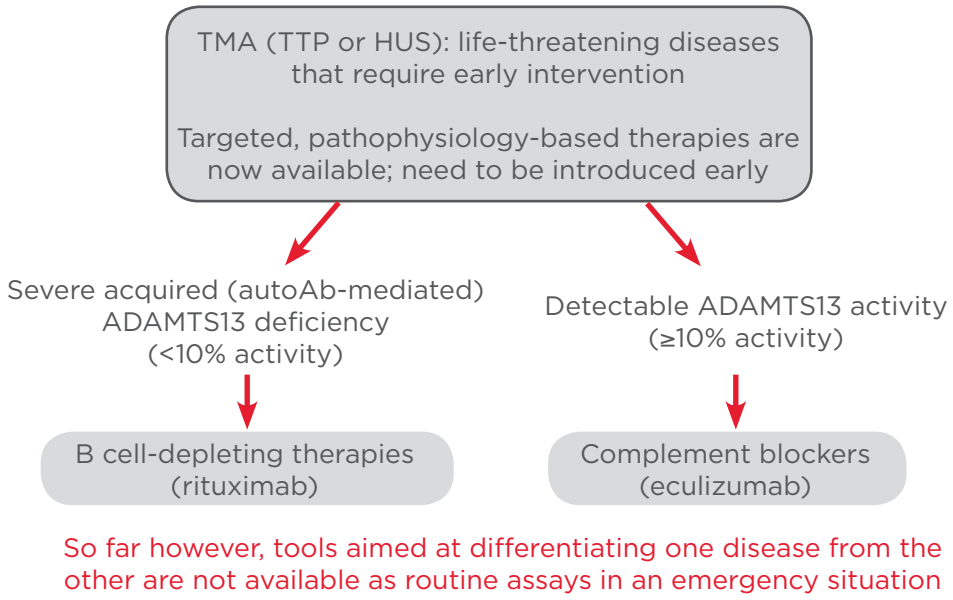

Figure 1: The dilemma of TMA management.
HUS: hemolytic-uraemic syndrome; TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopaenic purpura.
Modified from Sadler[9] and Tsai.[10]

The type of mutation impacts the prognosis of the patient, with mutations in Factor H having the poorest prognosis in terms of renal function, and patients are known to relapse even after renal transplant.[8] The mutation MCP/CD46 is the least severe in paediatric-onset patients. However, all mutations have a strong impact in terms of overall survival and renal function, highlighting the need for new therapies for these patients.

TMAs are life-threatening disorders that require early intervention. In patients with a severe ADAMTS13 deficiency (<10% activity) it is necessary to supply the missing enzyme. If there is an antibody against the enzyme, then immunosuppressive drugs, such as rituximab, are required to be administered particularly in patients who experience a suboptimal response to standard treatment. In patients with detectable ADAMTS13 activity (≥10% activity), complement blockers such as eculizumab are introduced. However, assays to differentiate one disease from another are not yet readily available (Figure 1).[9,10]

One reference method used to measure ADAMTS13 activity is by using the FRETS assay.[11-13] This assay is only available in specialised laboratories and emergency testing is limited by the need to transport the sample. However, the result is available in <1 day.[12] The FRETS assay has been used in a large number of patients from multicentre studies. Commercial kits are also available, which can provide results within a day.[14,15] However, these assays have not yet been validated between laboratories for routine use; therefore, their use for patient care requires further evaluation.

Efforts were made to set up a predictive score to help identify severe ADAMTS13 deficiency. Patients with an idiopathic form of TMA were assessed to see whether it was possible to predict a severe ADAMTS13 deficiency by assessing clinical features and standard biology. The characteristics of patients with a severe ADAMTS13 deficiency were compared with patients with detectable ADAMTS13 activity. The results showed that patients with a severe ADAMTS13 deficiency displayed a more severe thrombocytopaenia compared to patients with detectable ADAMTS13 activity at initial presentation. It was also observed that patients with detectable ADAMTS13 activity had a more severe renal involvement. In fact, 21% of these patients had end-stage renal disease (ESRD).[16,17]

These differences were used to establish a predictive score to identify patients with ADAMTS13 deficiency and ensure an appropriate therapy for treatment, and it was found that creatinine below 200 µmol/L and a platelet count <30,000/mL^3 at presentation were strongly associated with severe acquired ADAMTS13 deficiency.[16,17] Other studies have also confirmed patients with severe ADAMTS13 deficiency are more thrombocytopaenic and have lower creatinine levels at presentation than patients with detectable ADAMTS13 activity.[18-21]

Patients with a diagnosis of TMA often require urgent specific treatment, therefore it is important to identify quickly whether the disease is TTP or aHUS. Briefly, children with a clear diagnosis of aHUS are now usually treated front-line with eculizumab. Adult patients with a newly diagnosed TMA still undergo plasma exchange. An associated context that may impact prognosis and treatment such as pregnancy, transplantation, HIV infection, cancer, or chemotherapy, infection-associated STEC-HUS should be rapidly identified. A patient with an apparently idiopathic TMA responding well to the treatment will show recovery of the platelet count and creatinine level, resulting in a decreased need for plasma exchange sessions. In patients with a suboptimal response by day 5, those with a severe acquired ADAMTS13 deficiency (consistent with the diagnosis of acquired TTP) should be treated with rituximab in association with daily plasma exchange.[17] In contrast, a detectable ADAMTS13 activity suggests the diagnosis of aHUS and administration of eculizumab with plasma exchange interruption should be considered.[17]

In conclusion, individual TMAs can no longer be classified as the same disease; rather the different types of TMA can be differentiated according to their underlying pathological mechanism. Although TMAs such as TTP and HUS are life-threatening, their prognosis may be favourable provided that a rapid diagnosis is made and the appropriate treatment regimen is applied. Measurement of ADAMTS13 activity is the recommended test used to distinguish aHUS from TTP, while taking into account the clinical features of the particular TMA. The rarity of these diseases makes their diagnosis challenging; therefore, it is important for clinicians to be fully aware of the diagnostic features of the different types of TMA.

aHUS and the Role of Complement

Professor Javier de la Rubia

The complement system is part of the innate immune system and is composed of approximately 30 plasma and membrane-bound proteins. The main functions of the complement system are to fight infections, eliminate immune complexes, and destroy and remove autologous damaged cells.[22] The three pathways of the complement system (alternative, classical, and lectin) generate different enzyme complexes, C3 convertases, that cleave C3 to C3a and C3b. C3a is an anaphylatoxin and C3b targets the cells to subsequently be destroyed. The alternative pathway is always active at a low level (tick over). C3b is further amplified via a strong self-amplifying feedback loop. The C3 convertases hold a strategic position, can be exponentially and strongly activated, and control complement activation. C3b then binds to the C3 convertase to form C5 convertase, which cleaves C5 into C5a and C5b. C5b then initiates the assembly of later complement components, including C5b-9, resulting in the formation of the membrane attack complex leading to cell destruction.[23] This sequential activation is potentially dangerous for the host. Fortunately, there is a group of complement components such as complement Factor I and complement Factor H,[23] which perform a regulatory function so that in a normal situation all host tissues are protected against damage by autologous complement (Figure 2). It is precisely this equilibrium between complement activation and regulation that is disrupted in aHUS patients. All complement proteins that have been found mutated in these patients participate in the generation and further inactivation of the C3b molecule through the alternative pathway. Many patients experience prolonged apparently symptom-free periods in spite of having persistent underlying abnormalities in complement activation. These features indicate that multiple, concurrent genetic and environmental triggers are needed to determine complete disease expression. Therefore, external triggers (such as common infection, surgery, pregnancy, or autoimmune diseases) in conjunction with a genetic abnormality in the complement system may cause the onset of the disease.[24,25]

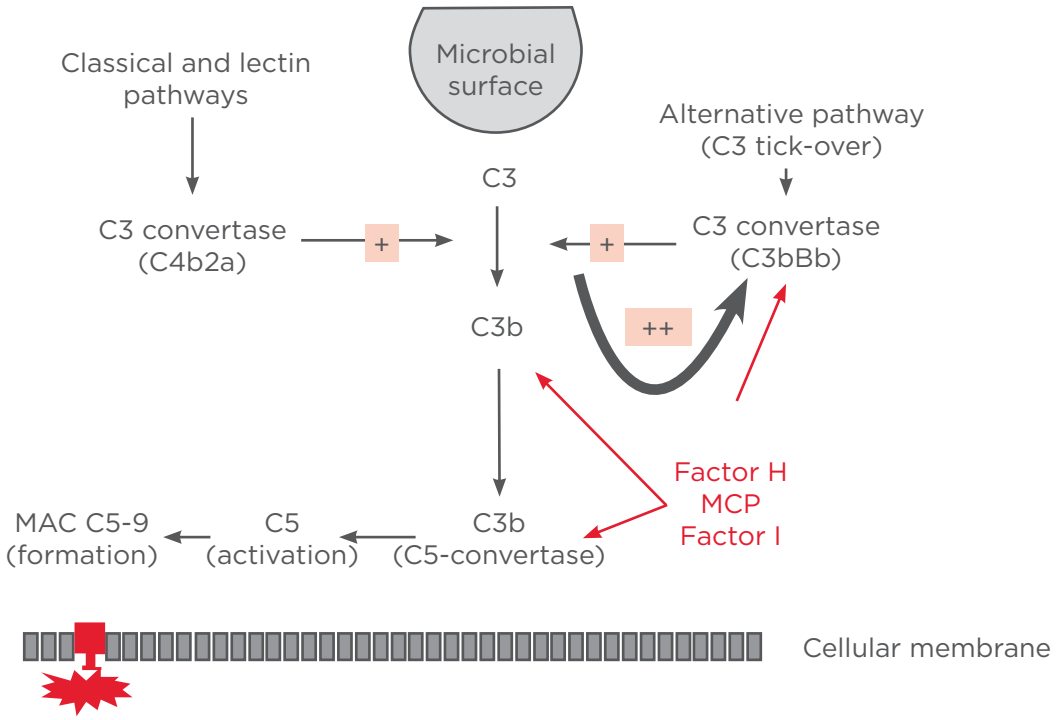

Figure 2: Role of complement in aHUS.
aHUS: atypical hemolytic-uraemic syndrome; MAC: membrane attack complex; MCP: membrane cofactor protein.
Modified from Noris et al.[23]

In aHUS patients, once an environmental trigger initiates the complement cascade beyond a critical threshold, C3b formation and deposition occur on vascular endothelium, which leads to further complement activation through the alternative pathway self-amplifying loop, activation of platelets and leukocytes culminating in microangiopathic injury and thrombosis. There is excessive activation of C3 convertase, causing consumption of C3 and production of C3a and C3b. C3b molecules are then deposited on the endothelial cell surface erroneously (self), targeting them for cell damage and destruction. aHUS is a permanent, ongoing, systemic disease that is defined by the clinical characteristics of TMA; a decrease in platelet count, microangiopathic hemolysis, and organ damage or impairment.

More than 120 different mutations, accounting for 50-60% of cases, have been discovered; the majority in the past 20 years.[26] Proteins such as complement Factor H, complement Factor I, or MCP are complement regulators, and a mutation in these will lead to uncontrolled complement activation. Mutations in C3 and Factor B, can result in the generation of a hyperactive C3 convertase that is resistant to regulation. Not all aHUS patients have an identified underlying genetic abnormality. In fact, complement mutations have been detected in approximately 50% of aHUS patients; therefore, diagnosis of aHUS does not require identification of a genetic mutation.[7,8] Although C3 is consumed in aHUS it is not a reliable marker for diagnosis of the disease as C3 is normal in most patients with aHUS. The reason is that in most cases of aHUS, C3 consumption takes place locally on the surface of the endothelium and thus, it is difficult to detect with classical C3 level measurements.[8,27]

aHUS is a disease of poor prognosis; studies have shown that 33–40% of patients die or progress to ESRD within the first clinical manifestation despite the use of plasmapheresis.[7,28] aHUS is a systemic disease, and so it is important to assess all organ dysfunction when managing patients. Hematologic improvement (platelets and hemolysis) may not necessarily reflect functional improvement of other organs.

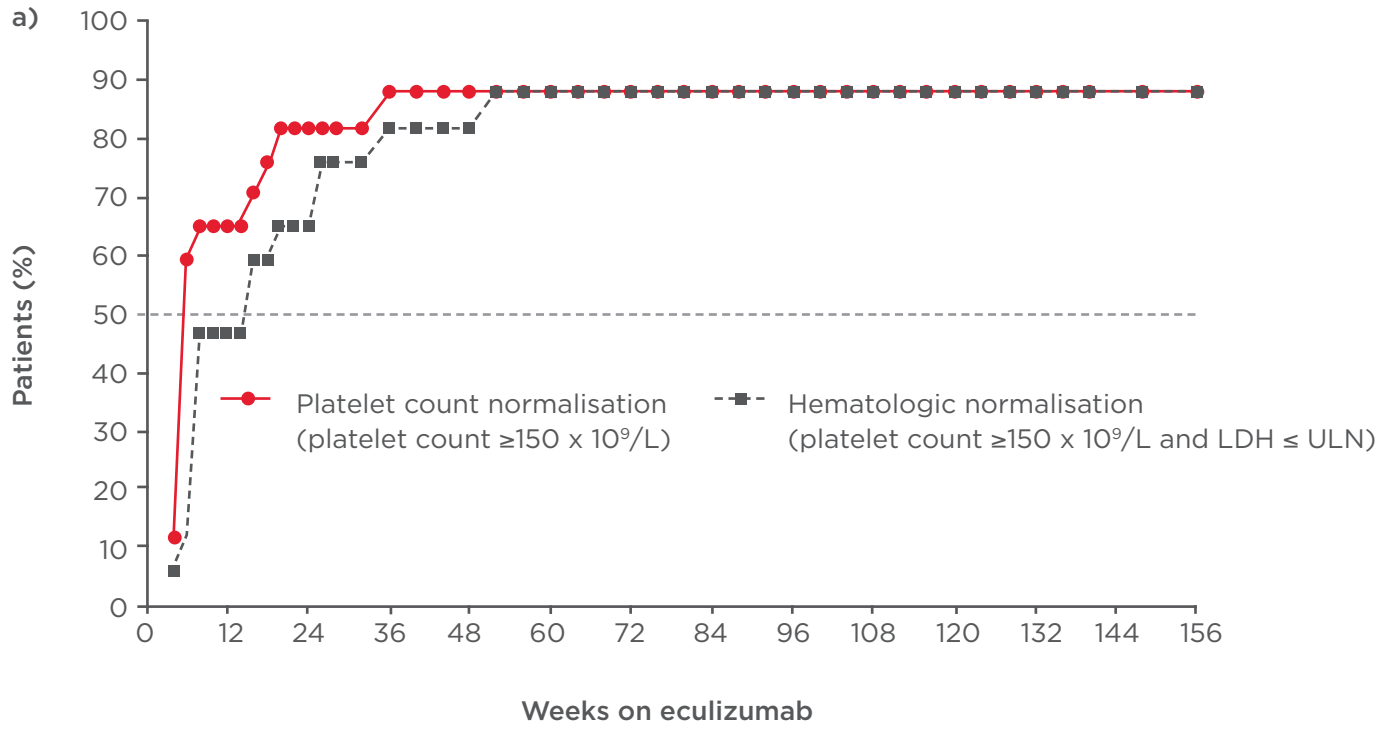

Figure 3: Time to improvement in key hematological parameters in patients with aHUS and progressing TMA (C08-002 trial; n=17).
aHUS: atypical hemolytic-uraemic syndrome; LDH: lactate dehydrogenase; TMA: thrombotic microangiopathy; ULN: upper limit of normal.
Modified from Legendre et al.[29,35]

Management of HUS in 2014

Professor Thorsten Feldkamp

Until recently, plasma exchange/plasma infusion (PE/PI) was the only option available for the management of patients with aHUS. However, many patients were progressing to ESRD despite PE/PI. Studies have shown that 56% of adult patients suffer from ESRD after 1 year, and that after 5 years 64% of patients have ESRD.[8] This is a devastating outcome and although dialysis can be used to compensate for the loss in renal function, it is not an ideal approach due to the associated practical difficulties and its association with extremely high cardiovascular risk and mortality. These factors highlight the need for another treatment option for patients with aHUS.

Eculizumab is a new drug that has been approved for the treatment of aHUS. Eculizumab works by inhibiting cleavage of C5, thus blocking activation of the terminal complement pathway.[23] The clinical development programme of eculizumab consisted of almost 100 patients in total in prospective clinical trials. The outcome of two of these trials supported the approval of eculizumab in 2011,[29] and long-term extension studies are being conducted.[30,31] An aHUS registry is also being developed.[32] This registry will not only include patients from the eculizumab clinical development programme but also all patients that have aHUS, regardless of whether they are being treated with eculizumab. It is anticipated that this registry will, on one hand, broaden our knowledge of eculizumab treatment but also, on the other hand, enhance our understanding of the symptoms and clinical course of aHUS in general.

In terms of the clinical trial patient populations, the first of the three trials (the so-called 'chronic' or C08-003 trial) included patients with a long duration of aHUS and chronic kidney disease (CKD).[29] These patients were undergoing chronic PE/PI and were required to have stable platelets but also had evidence of renal damage and ongoing hemolysis. These patients were switched from PE/PI to eculizumab. The second trial (the so-called 'resistant' or C08-002 trial) enrolled patients with aHUS and progressing TMA.[29] These patients had severe TMA; indicated by evidence of hemolysis, renal damage, and rapid drop in platelets. As a requirement for the study inclusion patients then had to undergo at least four sessions of PE/PI. If these sessions were ineffective, showing that the disease process was plasma resistant, the patients could be included in the trial. In the last study, the C10-004 trial, patients had to meet specific inclusion criteria with regards to platelet count, hemoglobin, LDH, and serum creatinine in order to be enrolled, but there was no specification in terms of need for PE/PI.[33] In all trials, patients were required to have ADAMTS13 activity >5% (to rule out TTP) and no evidence of Shiga-toxin-induced HUS. Identification of genetic mutations in the complement pathway was not a requirement for enrolment.

The key endpoints for the studies were improvement in platelet count from baseline, TMA event-free status (composite endpoint: no decrease in platelet count >25% from baseline, no new dialysis, and no new plasma exchange or plasma infusion for 12 consecutive weeks), hematological normalisation (normal platelet counts and lactate dehydrogenase [LDH] at two consecutive measurements, 4 weeks apart) and TMA intervention rate (number of events [plasma exchange or infusion, dialysis, or both]/patient/day).[29] In patients with aHUS and progressing TMA (C08-002 trial) at 2 years of sustained eculizumab treatment, platelet normalisation was achieved in 87% of patients (Figure 3), TMA event-free status was achieved in 88% of patients, and 88% achieved hematological normalisation. In addition, there was a reduction in TMA intervention rate from 0.88 to 0.00.[30]

In patients with aHUS, CKD, and long disease duration (C08-003 trial) at 2 years of eculizumab treatment, 95% and 90% of patients achieved TMA event-free status and hematological normalisation, respectively. Furthermore, the TMA intervention rate reduced from 0.23 to 0.00 after patients were switched to eculizumab.[31]

Renal function was also assessed over a 3-year period.[34,35] The key endpoints here included a ≥25% decrease in serum creatinine, estimated glomerular filtration rate (eGFR) improvement of >15 mL/minute and a CKD improvement of ≥1 Stage. Data from the C08-002 trial showed that 50% of the patients showed a rapid response within the first few weeks; however, further improvements were also observed after several weeks (Figure 4). Patients who were enrolled in the C08-003 trial and were managed with PE/PI had normal platelet counts at baseline but showed signs of renal damage. Upon switching to eculizumab treatment, these patients showed improvements in serum creatinine,

eGFR rate and CKD Stage. The improvements were not as rapid as seen in the C08-002 trial population. However, over time, and even after 72 weeks, patients showed signs of improvement of renal function indicating that at this time point the kidney is still able to regenerate when excessive complement activation is blocked by eculizumab treatment.

Eculizumab was well-tolerated over a 2-year period. Serious adverse events (SAEs) included peritonitis, influenza, and venous sclerosis at the infusion site. Other adverse events (AEs) included headache, lymphopaenia, leukopaenia, cough, and anaemia. However, these appeared to decrease over time with treatment.[29]

The C10-004 study was a prospective study, which enrolled 44 patients.[33] Study participants had a decrease in platelet count and an increased LDH.[33] Serum creatinine was high (411 μM/L) and 100% of patients had an eGFR of <60 mL/minute/1.73m^2, indicating that they were at least CKD Stage 3. The primary endpoint was complete TMA response (composite endpoint: platelet count and LDH normalisation, and preservation of kidney function [<25% increase in serum creatinine from baseline]). During the 26 weeks of eculizumab treatment 73% of the patients achieved complete TMA response, 88% achieved hematological normalisation (normalisation of LDH and platelet count), and 98% showed normalisation of platelet counts.[33] Assessment of renal function showed a mean change in eGFR of 29.3 mL/minute at 26 weeks.[33] This improvement in renal function is clinically relevant since it will allow most patients to discontinue dialysis. This is supported by the study results where 83% of patients on dialysis at baseline could discontinue dialysis.[33] AEs were consistent with the other eculizumab studies; however, two patients developed meningococcal infection.[33] One patient recovered and withdrew from the study, while the other patient recovered but continued with the trial and eculizumab treatment. There were no deaths in the study.

The eculizumab dosing schedule was 900 mg every week, as an induction phase for 4 consecutive weeks, followed by 1,200 mg every 2 weeks for the maintenance phase.[29,36] As patients on eculizumab treatment are more prone to develop meningitis, it is recommended that a meningococcal vaccination is given before commencing eculizumab treatment.[36]

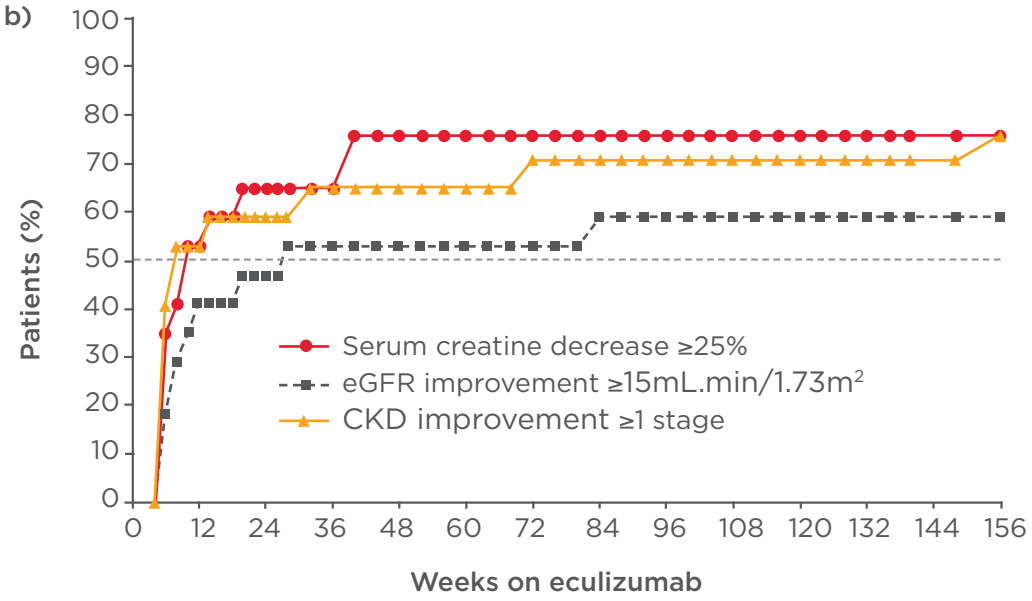

Figure 4: Cumulative percentages of patients achieving renal outcomes in patients with aHUS and progressing TMA (C08-002 trial; n=17).
aHUS: atypical hemolytic-uraemic syndrome; CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; TMA: thrombotic microangiopathy.
Modified from Legendre et al.[29,35]

If eculizumab treatment is to be initiated immediately, then meningococcal vaccination can be administered with antibiotics for 2 weeks to allow activation of the vaccine. Prof Feldkamp's recommendation was that two vaccinations be administered to patients who are about to commence eculizumab treatment to ensure protection against all strains of meningococci (one being the recently approved Bexsero, active against the B strain, which is the most prevalent strain in Europe).

In conclusion, eculizumab has been prospectively evaluated for efficacy and safety in 100 aHUS patients. The three studies presented here included a broad spectrum of patients (patients with long and short disease duration managed with and without plasma exchange; patients who had undergone transplant or were on dialysis). All studies, including the latest (C10-004) and the two pivotal studies that led to the approval of eculizumab, confirm the safety and efficacy of eculizumab for the treatment of aHUS.

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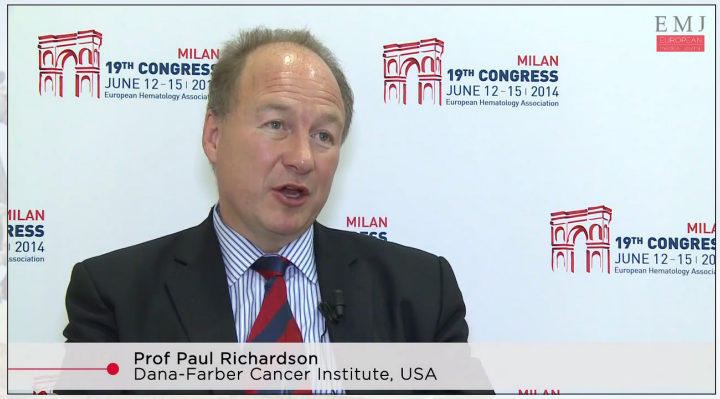

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THE STANDARD OF CARE IN RELAPSED REFRACTORY CD30+ LYMPHOMA

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ABSTRACT

CD30-positive (CD30+) lymphomas are a heterogeneous group of hematological malignancies that share the same antigen. Over recent decades, advances in therapeutic management of these diseases have considerably improved clinical outcomes. Overall, the two main CD30+ lymphomas - Hodgkin's lymphoma and systemic anaplastic large cell lymphoma - are associated with a favourable prognosis after first-line therapy. Nevertheless, optimal therapeutic strategies are needed to manage relapsed or refractory CD30+ lymphomas. The introduction of novel targeted approaches, such as brentuximab vedotin (BV), expands the therapeutic armamentarium and provides new perspectives in terms of clinical efficacy despite heavily pretreated disease, with reasonable toxicity to patients whose quality of life is often impaired by the disease and repeated treatments. The standard of care (SoC) for these malignancies is being refined and will be clarified with results from ongoing and upcoming Phase II/III clinical trials. Clinical studies are currently assessing the use of BV in a broad range of CD30+ lymphomas. Over time, frontline strategies and SoC will be refined in order to improve outcomes for patients with relapsed disease, while allowing clinicians to expand patient selection and provide long-term remission in a wide variety of clinical settings.

Keywords: Lymphoma, CD30, brentuximab, Hodgkin's lymphoma, systemic anaplastic large cell lymphoma.

INTRODUCTION

CD30-positive (CD30+) lymphomas are a heterogeneous group of hematological malignancies that share the CD30 antigen. While advances in the therapeutic management of these diseases have considerably improved clinical outcomes, optimal therapeutic strategies are still needed to manage relapsed or refractory CD30+ lymphomas. This article aims to review the current evidence and rationale on CD30-targeting strategies, including results from brentuximab vedotin (BV) clinical studies, and to provide an overview of the standard of care (SoC) in main clinical settings.

CD30+ LYMPHOMAS

CD30 is a leukocyte activation marker membrane receptor that is physiologically involved in many signalling pathways through its interaction with tumour necrosis factors in activated B and T cells.[1] It is expressed in a range of lymphomas including: Hodgkin's lymphoma (HL), systemic anaplastic large cell lymphoma (sALCL), primary cutaneous ALCL (pcALCL), primary mediastinal B cell lymphoma, adult natural killer (NK)/T cell leukaemia/lymphoma, mycosis fungoides, and some cases of diffuse large B cell lymphoma, primary effusion lymphoma, and anaplastic diffuse large B cell lymphoma.[2-6] This antigen can also be found in other malignancies, such as lymphomatoid papulosis, embryonal carcinoma, and mast cell neoplasm.[6,7] CD30 is consistently expressed in classical HL and sALCL, and is therefore an interesting therapeutic target for those diseases.

HL

HL is a relatively common and highly curable B cell lymphoma characterised by lymphadenopathies spreading in a contiguous manner, causing systemic symptoms and affecting the immune system's ability to fight infection. The large, multinucleated cells and CD30+ Hodgkin's and Reed-Sternberg cells are the hallmark of classical HL.[3] Displaying a bimodal age distribution, HL is the most common malignancy in adolescents and young adults, and also occurs in the elderly.[8]

This disease is highly curable with approximately 80-90% of patients achieving long-term remission following chemotherapy with or without radiotherapy.[9] Young HL patients have better long-term clinical outcomes than elderly patients: 5-year overall survival (OS) rates are of about 96% in the former versus 88% in the latter.[8] Two subgroups of patients carry a particularly poor prognosis: patients aged 60 and older and those with refractory or relapsing disease after second-line therapy.[9,10] These two clinical settings represent an unmet need which is the focus of extensive clinical research.

Despite a generally good prognosis, it is also important to expand the therapeutic armamentarium of HL in children and adolescents, where treatment-related adverse events are of particular concern. A focus on the long-term effects of the treatment and the quality of life (QoL) of survivors is of crucial importance in this subpopulation.[11]

sALCL

sALCL is a rare and aggressive mature T cell non-Hodgkin's lymphoma (NHL) that is characterised by CD30-expressing large pleomorphic cells that can be found in lymph nodes.[4] Another form of ALCL – pcALCL – is distinct from sALCL in terms of both its clinical and biological presentations.[4] A majority of patients, primarily younger, express the protein anaplastic lymphoma kinase (ALK),[12,13] while older adults are more commonly ALK-negative.[14] ALK positivity coupled with low-risk factors is linked to a better prognosis and an overall good long-term response to treatment than ALK negativity with high-risk factors, as evidenced by highly significant 5-year OS differences.[15] Similarly to HL, sALCL patients with refractory or relapsing disease carry a poor prognosis, regardless of ALK status. Novel therapeutic options are required to address these clinical settings.

CD30 AS A THERAPEUTIC TARGET

As previously mentioned, CD30 is expressed in a range of malignancies, but is absent from most normal cells, which allows for specific targeting of cancerous cells with immunotherapy. Under physiological conditions, CD30 is found on the surface of activated T and B cells, but not on their resting forms.[16,17]

CD30-Targeted Immunotherapy

Over the last decades, targeted immunotherapy has become increasingly important in treatment strategies for many malignancies and has enabled a marked improvement of clinical outcomes. The singular expression profile of CD30 led to the development of several monoclonal antibodies in the treatment of refractory or relapsing HL and sALCL.[18]

Despite impressive preclinical results, the first clinical outcomes were disappointing. Two monoclonal antibodies, MDX-060 and SGN-030, were evaluated in Phase I/II clinical trials in patients with either refractory or relapsing HL or sALCL. In both studies, results for MDX-060[19] and SGN-030[20] showed limited activity and objective responses (8% and 9% of patients, respectively), with fewer complete responses ([CR]; 6% and 3% of patients, respectively).

Recent advances in antibody engineering have paved the way for new applications, including the development of novel immunotherapeutic agents called antibody-drug conjugates (ADCs). ADCs are composed of a cytotoxic agent and a monoclonal antibody directed against a specific antigen. BV was the first ADC to be developed for clinical use.

BV (SGN-35)

Chemistry and Mechanism of Action

BV is an ADC composed by an anti-human CD30 antibody and four molecules of monomethyl auristatin E (MMAE), an antimicrotubule agent with high antitumoural potency. BV specifically binds to CD30+ cells and is internalised, triggering the release of MMAE into the cytoplasm, which in turn, inhibits tubulin polymerisation occurring in the G2/M Phase of the mitosis and induces

targeted apoptosis.[21] MMAE has also been found to accumulate in the extracellular compartment, thus pursuing its cytotoxic effects on surrounding cells.[22]

Pivotal Phase II Clinical Studies

Following promising Phase I studies, two single-arm, single-agent, multicentre Phase II studies were conducted. Heavily pretreated patients with either refractory or relapsing HL[23] and sALCL[24] received a single dose of 1.8 mg/kg of BV every 3 weeks for up to 16 cycles. The first Phase II study[23] enrolled 102 patients with relapsed HL after autologous stem cell transplantation (ASCT), while the second study[24] aimed to evaluate the efficacy of BV in 58 patients with refractory or relapsing sALCL. Both studies demonstrated remarkable efficacy for BV with an overall response rate (ORR) of 75% and 86% in HL and sALCL, respectively. CR was achieved in 34% of HL patients and in 57% of sALCL patients for median durations of 20.5 and 13.2 months. Tumour reductions were observed in 94% and 97% of HL and sALCL patients, respectively. In the subgroup of ALK-negative sALCL patients, BV showed similar results with an ORR of 88% and complete remission in 52% of patients.[24]

The most frequent adverse events were peripheral sensory neuropathy, nausea, fatigue, pyrexia, diarrhoea, rash, constipation, and neutropaenia. Most patients who developed peripheral sensory neuropathy showed partial or complete resolution of their symptoms after dose reductions or treatment discontinuation. However, some patients developed persistent neuropathy; this should be taken closely into account when BV is used to treat potentially curable cases of lymphoma, particularly when combined with other neurotoxic drugs. Adverse events led to treatment discontinuation in 20% and 24% of HL and sALCL patients, respectively. No drug-related deaths were reported.[23,24]

Overall, BV showed important antitumoural efficacy with a good risk-to-benefit ratio, which led to accelerated approval by the US FDA in 2011 and conditional approval by the EMA in 2012 for the following indications: HL after failure of ASCT; HL in patients who are not ASCT candidates after failure of at least two multi-agent chemotherapy regimens; and sALCL after failure of at least one multi-agent chemotherapy regimen. Follow-up data on the outcomes for the continuous complete responders from both studies were recently presented at the American Society of Hematology meeting in 2013. After a median observation time of about 3 years, 51 HL patients were alive at last follow-up (including 14 who had a sustained response) were still in remission, and did not start a new treatment cycle.[25] After a median observation time of 33.4 months for the Phase II sALCL, 64% were still alive at last follow-up for an estimated 3-year survival rate of 63%. Patients who had achieved a CR with BV therapy had a higher OS than patients who did not; the median OS for patients with a CR had not yet been reached at the time of analysis.[26]

BV was also evaluated in a range of CD30+ hematological malignancies, such as cutaneous T cell lymphoma,[27,28] other peripheral T cell CD30+ lymphomas,[29] and in diffuse large B cell lymphoma.[30] In January 2012, the FDA issued a revision of the product package insert, with a boxed warning about the risk of progressive multifocal leukoencephalopathy (ML), a potentially fatal brain infection, which has also been associated with the monoclonal anti-CD20 antibody rituximab. To date, three cases of ML with BV have been reported. As BV is contraindicated with concomitant bleomycin due to increased pulmonary toxicity, patients undergoing BV therapy should be closely monitored for symptoms of ML and pulmonary toxicity. Also, recent safety updates have raised caution that BV may be associated with the development of acute pancreatitis.

OPTIMAL STRATEGIES IN RELAPSED/REFRACTORY CD30+ LYMPHOMAS

Standard Management of Relapsed/Refractory HL

Context

In patients with limited-stage HL, first-line chemotherapy (doxorubicin, bleomycin, vinblastine + dacarbazine [ABVD]) plus radiotherapy achieves good clinical results with remission rates ranging from 80-90%.[11] First-line treatment of patients with advanced stage HL with the more intensive BEACOPPesc regimen (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazin, prednisolone) yields superior remission rates and long-term survival, at the expense of more serious toxicity, and also a higher risk of infertility and secondary leukaemia.[31] Nevertheless, a proportion of patients (20-30%

after ABVD and 15-20% after BEACOPPesc) will present refractory or relapsed disease after one or two regimens.[11]

Management of relapsing or refractory HL

The standard management of relapsing or refractory disease (Figure 1) is second-line chemotherapy as induction therapy to ASCT. In about 70% of patients, complete or partial response (PR) is obtained and high-dose chemotherapy (HDC) followed by ASCT can be completed; this therapeutic option is associated to a sustained response in about 50% of patients.[32-34] However, patients who are not eligible for ASCT, or who relapse after two chemotherapy regimens followed by ASCT, seem to benefit from BV.

Outside of clinical studies, real-life data are available from three named patient programmes (NPPs) in Germany, the United Kingdom, and Italy.[35-37] BV showed good ORR (ranging from 60-72%) and CR (ranging from 17%-22%) in heavily pretreated patients.[35,36] Best observed responses were after three-to-four cycles of treatment; consolidation with allogeneic hematopoietic stem cell transplantation (allo-SCT) should be considered early. Clinical outcomes and toxicity profiles were highly similar to those from both pivotal trials,[23,24] suggesting that patients from everyday clinical practices can benefit from the impressive outcomes in the same proportions.

BV as a salvage therapy prior to ASCT in ineligible patients

In the latest National Comprehensive Cancer Network (NCCN) recommendations on HL,[38] the panel advised that BV may be an option in patients who have progressive disease after two chemotherapy regimens, at which point HDC + ASCT is not a recommended option. So far, only a few publications explore the use of BV in transplant-naïve patients with relapsed or refractory HL.

In a retrospective study on a cohort of 24 French patients with relapsing or refractory HL, the ORR was of 66.7% for 45.8% of CRs and 20.8% of PRs. Responding patients underwent a consecutive ASCT or allo-SCT.[39] In another retrospective study of 14 patients, the ORR was 71% with 36% of complete remissions. BV acted as salvage therapy in 36% of patients who subsequently underwent HDC + ASCT. 12-month OS was 69% in the whole cohort.[40] A case series of 20 transplant-naïve patients with relapsed or refractory HL receiving BV reported 6 objective responses and 2 complete remissions, while 3 out of 6 responders underwent ASCT. [41]

Treatment options for patients with multiple relapses

BV retreatment seems to be an option in patients who previously achieved remission with BV. This possibility is most likely due to the persistence of CD30 expression in tumour biopsy immunostaining after BV therapy.[42] Retreatment was evaluated in 21 patients with HL and achieved a response rate of 60% and 30% of CRs (median duration of objective response: 9.2 months) with a similar safety profile[30] as that of the pivotal clinical trials. Retreatment with BV seems to be a good therapeutic option for patients in whom multi-agent chemotherapy side-effects significantly affect QoL.

In patients relapsing after BV treatment, a second ASCT is possible if the previous response to ASCT was sustained for at least 12 months.[43] Prior to ASCT, salvage therapy with chemotherapy or BV may be implemented according to the patient's characteristics. Reduced-intensity allo-SCT is a therapeutic option that can take advantage of the graft versus lymphoma effect and consequently improve clinical outcomes and OS. The role of allo-SCT in relapsed HL remains controversial, but this treatment may represent the only remaining curative option in selected patients. BV can also be used as a bridge therapy to allo-SCT in patients with relapsed or refractory disease following ASCT. This therapeutic option was explored in a retrospective study on 17 patients who had undergone ASCT, and the authors determined that BV provided sufficient disease control prior to allo-SCT.[44] BV was also evaluated after allo-SCT failure in 24 patients; response rates of 50% and 38% of complete remissions were observed.[45]

It should be noted that patients with multiple relapse have a poor prognosis,[46] and the above therapeutic options must be considered and discussed according to the side or late-effects of treatment and the QoL experienced by each patient.[47] If these options are not possible, the patient should be offered enrolment in a clinical trial or palliative chemotherapy.

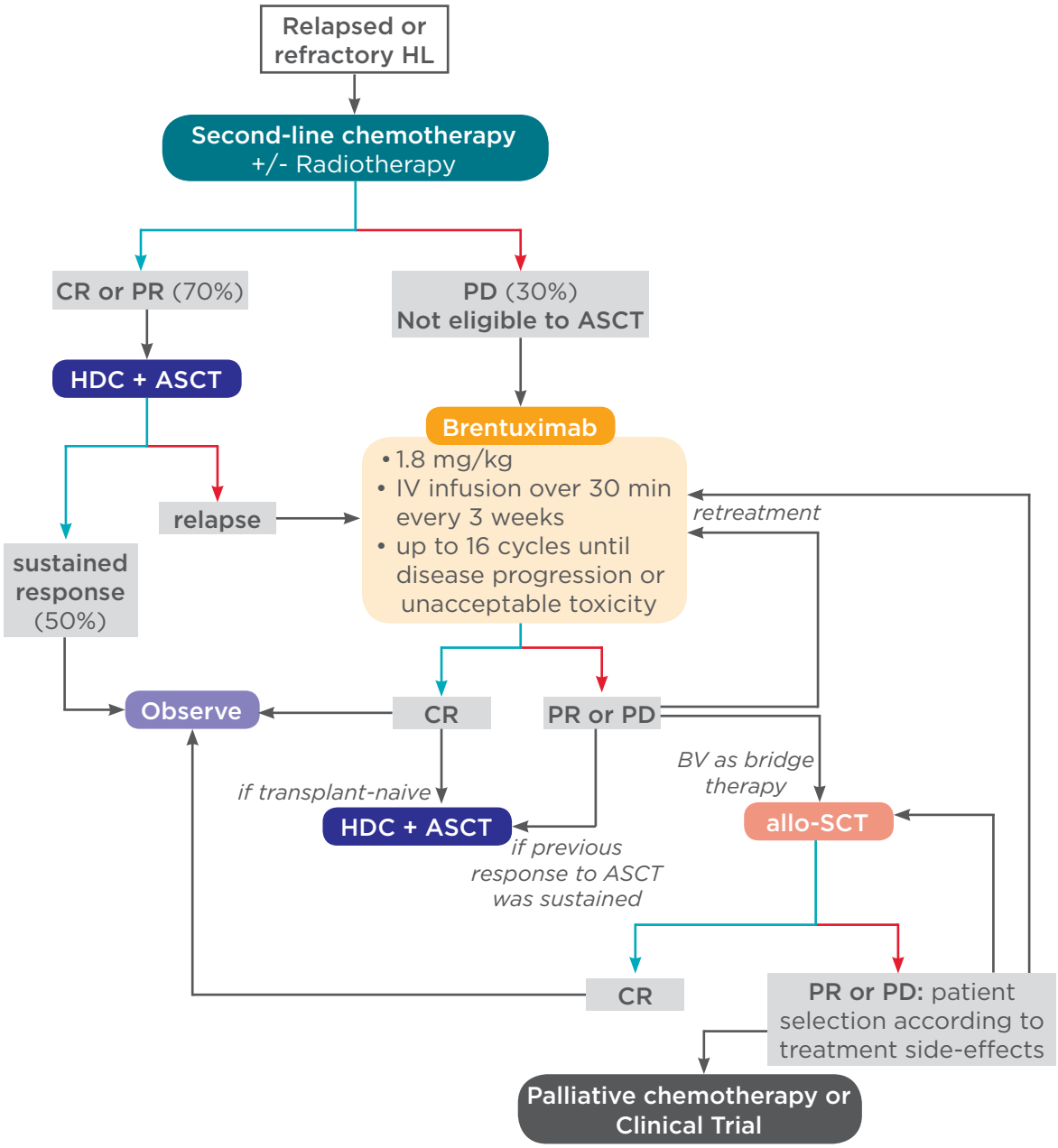

Figure 1: Management of refractory or relapsed HL.

HL: Hodgkin's lymphoma; CR: complete response; PR: partial response; PD: progressive disease; ASCT: autologous stem cell transplantation; HDC: high-dose chemotherapy; IV: intravenous; BV: brentuximab vedotin; allo-SCT: allogeneic stem cell transplantation.

Adapted from Hoppe RT et al.[38]

Standard Management of Relapsed/Refractory sALCL

Context

sALCL has a high remission rate with frontline chemotherapy, which is usually a multi-agent combination (cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]). A long-term study[48] over 8 years revealed an ORR to multi-agent chemotherapy of 86% for ALK-positive patients and 68% for ALK-negative patients, with good survival rates over that same period. The molecular characteristics of ALK-negative sALCL remain obscure.[49] This form is more aggressive[14] with a first-line standard chemotherapy cure rate of only 30%, but the latest studies[14,50,51] on consecutive ASCT have revealed significantly improved outcomes. However, there are few therapeutic options for patients who relapse after first-line therapy, regardless of ALK-status.

Management of relapsing or refractory sALCL

To date, there is no SoC for relapsing or refractory sALCL, and individual patient characteristics must be considered for patient selection towards each of the available therapeutic options. Patients relapsing after first-line therapy could very well benefit from ASCT, but the available data are limited. In a retrospective analysis on 65 patients with peripheral T cell lymphoma (including ALCL),[52] patients undergoing HCT + ASCT or allo-SCT after the first relapse were evaluated in comparison with patients not proceeding to transplant. 4-year OS was 67%, 66%, and 27%, respectively. Crizotinib, an ALK tyrosine kinase inhibitor, is being further investigated in relapsed or refractory ALK-positive sALCL following a Phase I study[53] in which seven out of eight patients achieved complete remission. Several other studies,[54-57] case series or case reports relate the use of crizotinib in relapsed or refractory ALK-positive ALCL.

Over the last few years, several novel agents emerged: pralatrexate (an antifolate), romidepsin (a histone deacetylase inhibitor), and BV; all three products are currently being evaluated in relapsed or refractory sALCL. Pralatrexate and romidepsin are approved by the FDA for the treatment of relapsed or refractory peripheral T cell lymphomas. Pralatrexate showed a 35% response rate in a subset analysis of 17 ALCL patients,[58] while romidepsin therapy achieved an ORR of 25% among 130 refractory T cell lymphoma cases (22 patients had ALK-positive or negative sALCL).[59] BV has shown better response rates and good disease control in patients with CD30+ lymphomas.[24] The drug is being further explored in combination with chemotherapy. In a single centre study, five patients with ALCL who were refractory to at least two chemotherapy regimens received BV therapy. The ORR was of 60% with an identical remission rate.[36]

Treatment options for patients with multiple relapses

As previously discussed in multiple-relapsing HL, a second course of BV is also a possibility in sALCL patients who previously achieved remission with BV. In an open-label, multicentre, Phase II study,[30] retreatment was evaluated in eight patients with sALCL, and achieved a response rate of 88% for 63% of complete remissions (median duration of objective response: 12.3 months).

BV in CD30+ Lymphomas: Ongoing Studies

Clinical studies are currently ongoing to assess the use of BV in a broad range of CD30+ lymphomas.[60,61] A Phase III study that should be completed in 2016 is currently evaluating BV in HL patients at high risk of relapse after ASCT.[62] Another Phase III study is currently ongoing on BV therapy + AVD compared with ABVD therapy in advanced HL.[63] BV is also being assessed in CD30+ mature T and NK cell lymphomas, either in combination with chemotherapy[64] or sequentially.[65] Additionally, further studies are exploring the efficacy and safety of BV in paediatric and adolescent patients with relapsed or refractory HL or sALCL.[66-68]

CONCLUSION AND FUTURE PERSPECTIVES

Overall, the two main CD30+ lymphomas, HL and sALCL, are associated with good first-line remission rates, and patients with relapsed or refractory disease can routinely benefit from multiple or repeated therapeutic options. Nevertheless, significant challenges remain in terms of disease-free survival, long-term remission, and management of chemotherapy-related side-effects in relapsed or refractory disease.

The introduction of novel targeted approaches such as BV expands the therapeutic armamentarium and provides multiple-relapse patients with new perspectives for clinical efficacy without unacceptable loss of QoL. This new drug has impressive anti-tumour activity, but its potential to improve cure rates and long-term OS remains to be seen. Also, as the drug is given in combination with potentially curative first-line or second-line chemotherapy, we need to closely follow the long-term toxicity affecting the patients.

The SoC for these diseases is being refined and will be clarified over time by reliable results from broader-scale Phase II and III clinical trials and by growing clinical experience. Over time, frontline strategies and SoC of CD30+ lymphomas will be targeted in order to improve outcomes for patients with relapsed disease, while allowing clinicians to expand patient selection and provide long-term remission in a wide variety of clinical settings.

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OVERCOMING IMMUNODEFICIENCY IN CHRONIC LYMPHOCYTIC LEUKAEMIA: CURRENT KNOWLEDGE AND PERSPECTIVES

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ABSTRACT

While the standard of care for chronic lymphocytic leukaemia (CLL) leads to high overall response rates and a long progression-free survival, it can be highly toxic for many patients, particularly in the elderly who often present concurrent diseases with associated morbidities. Treatment-related immune system burden and complications are challenging as most CLL patients already show immunodeficiency and are at high risk of infection. The latter are the main cause for increased morbidity and mortality and are correlated with disease severity and type of therapy. In the last few years, many new approaches and innovative agents such as second-generation anti-CD20 monoclonal antibodies, lenalidomide, B cell receptor signalling inhibitors, and novel cellular therapies have advanced the outlook for CLL management. Indeed, novel therapies could soon be addressing the need to promote immune reactivation and re-sensitise the immune system. By doing so, they could reach two main objectives, namely lowering the high proportion of patients at risk of infection, and acting as effective tools for the immune system to overcome its defects and fight malignant cells.

Keywords: Chronic lymphocytic leukaemia (CLL), immunodeficiency, immunotherapy, cellular therapies.

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in adults in the Western world, and is characterised by the progressive accumulation of mature CD5-positive B lymphocytes within the blood, bone marrow, lymph nodes, and spleen.[1-3] Over the past decade, significant advances in the understanding of the pathogenesis of the disease have led to the development of a range of novel treatment options. In young patients without significant comorbidities, immunochemotherapy with fludarabine, cyclophosphamide, and rituximab (FCR) has been established as the first-line standard of care treatment.[4,5] While this regimen leads to high overall response rates (ORR) and a long progression-free survival (PFS), it is unsuitable for certain subgroups of patients; these include 'poor risk patients' with p53 abnormalities[6] and elderly patients with comorbidities unable to tolerate FCR-associated toxicities.[7] In the latter, a recently published pivotal Phase III trial by the German CLL Study Group (GCLLSG) showed that the Type 2 glycoengineered antibody obinutuzumab (also known as GA101) was superior to rituximab when each was combined with chlorambucil.[8] This led to the FDA approval of obinutuzumab in combination with chlorambucil in previously untreated CLL patients. In addition, several recent Phase I and II studies demonstrated that agents interfering with B cell receptor (BCR) signalling are very active for the treatment of relapsed or fludarabine-refractory CLL and

might overcome the issues associated with current treatment approaches.[9] BCR activation is a central stimulus in CLL and increases CLL cell survival by activating different tyrosine kinases such as Bruton's tyrosine kinase (BTK), spleen tyrosine kinase, ZAP70, Src family kinases, and phosphatidylinositol 3-kinase (PI3K), in part via activation of transcription factors such as NF-κB.

Currently, the only curative – yet only suitable for a selected small group of patients - treatment option is allogeneic hematopoietic stem cell transplantation (HSCT).[10] HSCT takes advantage of the graft-versus-leukaemia (GVL) effect mediated by differentiated transplanted effector cells which are capable of mounting an anti-tumour immune response. Targeting the immune system to induce durable disease eradication therefore seems to be an attractive treatment approach, especially if achievable by improving autologous anti-tumour immune responses. This is particularly interesting as CLL is now increasingly understood as a disease that is highly dependent on interactions with its microenvironment and the immune system.[11] Various cellular components such as macrophages, T cells, dendritic cells, and stromal cells provide pro-survival and anti-apoptotic signals and conditions, and influence CLL cell trafficking, survival, and proliferation. These interactions are often bidirectional, and CLL cells have developed several mechanisms to compromise the microenvironment to continuously provide a pro-tumour environment.

Global immune defects, however, are a hallmark of CLL; hypogammaglobulinaemia, increased susceptibility to infections, increased incidence of autoimmune cytopaenias, and impaired responses to vaccinations are observed in the majority of CLL patients and are often aggravated by anti-tumour treatment. In addition, CLL-induced humoral and cellular immune defects often minimise anti-tumour immune responses and enable the malignant cells to escape from immune recognition.[12] Several preclinical studies and early clinical data, however, indicate that some novel agents and immunotherapy approaches have the potential to restore autologous immune responses. This review aims to briefly summarise the current knowledge on intrinsic and therapy-related immune deficiency in CLL and how this can be potentially overcome by novel treatment approaches.

IMMUNE DEFECTS AND DYSFUNCTION IN CLL

The interactions between intrinsic and extrinsic immune defects in CLL and their clinical manifestations are very complex and still not fully understood. In general, immune dysfunction can be both disease and treatment-related, and clinical manifestations include severe and recurrent infections, hypogammaglobulinaemia, autoimmune anaemia, and thrombocytopaenia.[13] Infectious complications are the main cause for increased morbidity and mortality in CLL patients; in retrospective analyses, they account for up to 50% of CLL-related deaths.[14-17] This can be further aggravated by treatment with steroids, cytotoxic drugs, and monoclonal antibodies (mAbs), and several studies indicate that the main risk factor for infections seems to be the number of previously received chemotherapy lines.[18-20] Patients receiving purine analogues (e.g. fludarabine), with and without mAbs, appear to be espcially at risk of prolonged infections and cytopaenia.[21,22]

From a clinical point of view, treatment-related immunosuppression and especially opportunistic infections are a challenge in the day-to-day management of CLL patients. Purine analogues are linked to *Listeria*, *Candida*, *Aspergillus*, and herpes virus infections or reactivations. Alkylating agents frequently cause respiratory tract infections caused by typical and atypical bacteria (e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*).[18-21,23-25] Alemtuzumab has been linked with an increased risk for herpes viruses, *Candida*, and *Aspergillus* infections, while rituximab is commonly associated with reactivation of hepatitis B/C viruses in sero-positive patients.[23,24] Furthermore, cytomegalovirus or cryptococcal infections following combination chemotherapy with mAbs have also been reported.[26-28]

As the underlying mechanisms, several quantitative and qualitative immune defects have been described, and they include both humoral and cellular immune responses. Early data implicated that an impaired complement system might be involved in the pathophysiology of CLL and its infectious complications.[29] Complement plays a crucial role in the control of some bacterial infections, and opsonisation with complement is necessary for subsequent interactions with neutrophils. Although CLL patients appear to

have normal serum concentrations of many complement factors, defects in C3b binding to *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Escherichia coli* were observed.[29] In addition, low activity of the classical complement pathway predicted survival in another study.[30]

Cellular immune defects are observed in nearly all immune cell types. Among B cells, the clinically most apparent defect is hypogammaglobulinaemia.[23] In general, the severity tends to increase with the duration and stage of disease.[23,31,32] More recent data however, indicate that neither hypogammaglobulinaemia nor pure immunoglobulin G (IgG) subclass deficiency are significant risk factors for infectious complications,[33,34] but this needs to be confirmed in larger and more homogenous series.

After early studies showed that clonal CLL cells have a limited ability to present antigen to T cells,[35,36] largely due to an inadequate costimulatory capacity,[37-39] a wide range of defects in the T cell compartment itself has been described. These include altered subset composition, changes in cytokine secretion and surface molecule expression, as well as profound functional defects.[40] T cells from patients with CLL also show severe gene expression profile changes, particularly in genes involved in actin cytoskeleton formation and stabilisation of the immune synapse.[41] To date, it remains unclear as to what extent these global changes represent an immune response to the malignant cells, as opposed to cells that have been compromised to 'help' the tumour cells evade immune recognition. Recently published data highlight the potential role of immune checkpoint pathways such as PD-1:PD-L1, CD160:HVEM, and CD200:CD200R in mediating these defects,[42-44] but the exact underlying mechanisms are still poorly understood.

Several studies have reported functional and numerical alterations in natural killer (NK) cells in CLL patients, which are particularly pronounced in advanced disease[45-47] and an independent predictive factor of disease progression in patients with newly diagnosed CLL.[48] In contrast, a more recent publication indicated that peripheral NK cells from CLL patients maintain partial functionality and are able to degranulate and exert antibody-dependent cellular cytotoxicity (ADCC), although some variability was observed.[49] Cellular immune defects are also observed in neutrophils, monocytes and macrophages, and dendritic cells, compromising their effector functions and phagocytic and bactericidal function as well as migration and chemotaxis.[50-52]

The clinical relevance of peripheral absolute monocyte count (AMC) has recently been demonstrated by the finding that patients with low AMC had a shorter time to treatment (TTT) and immune dysregulation leading to increased infection-related mortality. High AMC patients also had a shorter TTT compared to intermediate AMC patients.[53] NK cells in CLL are downregulated and their action against malignant cells is impaired. The capacity of monocytes/macrophages[53] and neutrophils in terms of phagocytosis, granulocyte function, and chemotaxis is also damaged: this results in an increased risk for bacterial and fungal infections.[50,54-56]

The stromal environment seems to be involved in CLL cell trafficking and homing to lymphoid tissues. The CXCR4/CXCL12[57-59] and CXCL13/CXCR5[60] axes are important therapeutic targets as both are involved in pleotropic effects, survival signals, and enhanced chemotaxis in CLL cells. Several recommendations on the prophylaxis and management of clinical immune defects exist, but these are reviewed elsewhere and will not be further discussed in this article.[13,61]

THERAPEUTIC PERSPECTIVES

As outlined above, a plethora of *in vitro* and preclinical *in vivo* studies have highlighted the importance of interactions between CLL cells and humoral and cellular components of the immune system and microenvironment. While this has led to a paradigm shift in the management of CLL, the mechanisms leading to infections and poor anti-tumour immune responses are still poorly understood. Preclinical data and correlative studies however indicate that novel agents and treatment approaches have the potential to correct immune defects and to promote immune activation, potentially re-sensitising the immune system and restoring its ability to mount anti-tumour and anti-infection immune responses. This review focuses on most clinically relevant drugs, but Figure 1 provides an overview of current and experimental agents.

ANTI-CD20 MONOCLONAL ANTIBODIES

After the first-generation mAb rituximab, several new mAbs targeting the B cell antigen CD20 have

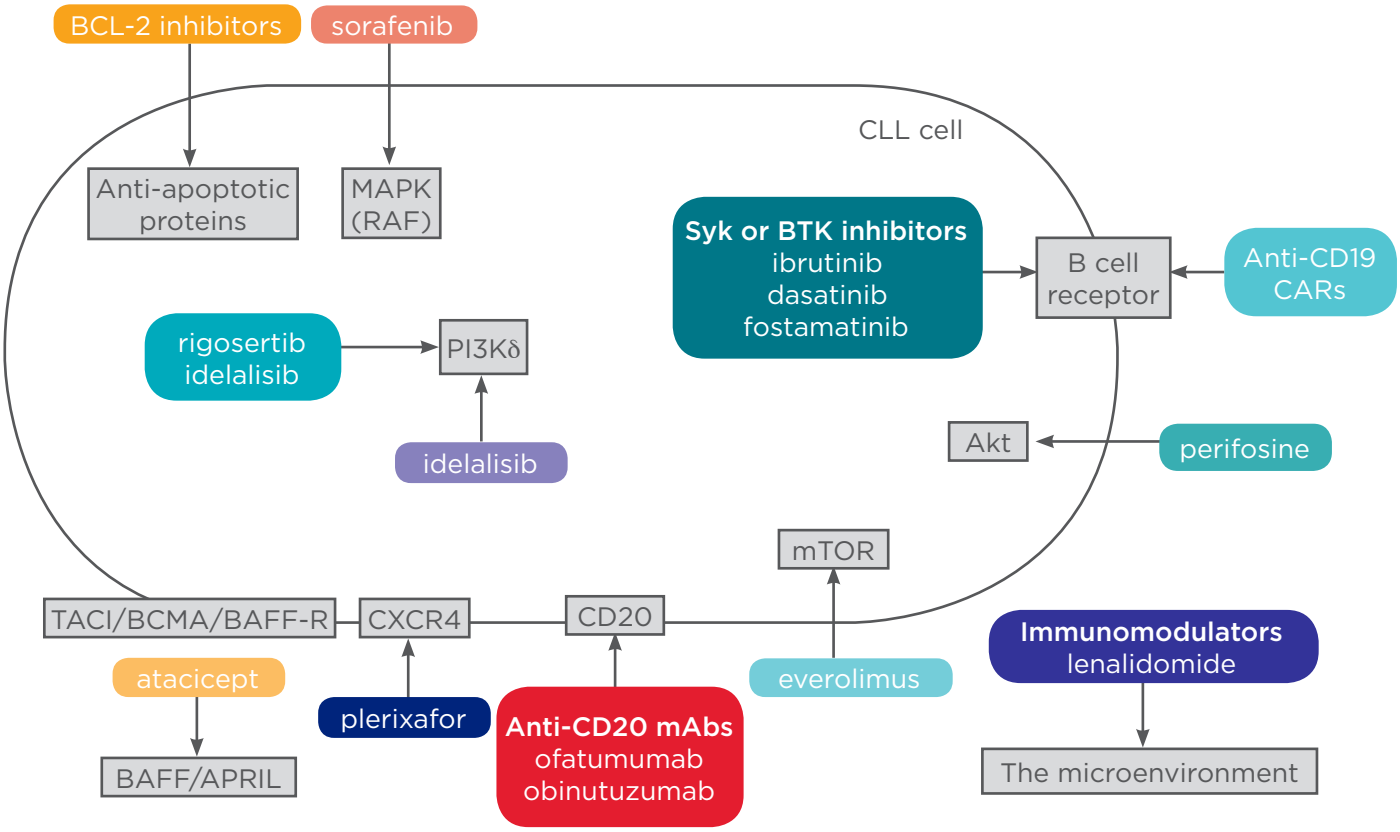

Figure 1: Approved and experimental therapeutic options targeting CLL microenvironment.
CLL: chronic lymphocytic leukaemia; Akt: protein kinase B; APRIL: a proliferation-inducing ligand; BAFF: B cell–activating factor of the tumour necrosis factor family; BAFF-R: B cell–activating factor of tumour necrosis factor family receptor; BCL-2: antiapoptotic protein B cell lymphoma 2; BCMA: B cell maturation antigen; BTK: Bruton tyrosine kinase; CD19: cluster of differentiation 19; CD20: cluster of differentiation 20; CXCR4: chemokine (C-X-C motif) receptor 4; MAPK: mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; PI3Kδ: phosphatidylinositol 3-kinase delta; Syk: spleen tyrosine kinase; TACI: transmembrane activator and calcium modulator and cyclophilin ligand-interactor; CAR: chimeric antigen receptor; mAb: monoclonal antibody.
Adapted from Herishanu Y et al.[53]

been developed. Although representing a 'passive' immunotherapy, they display enhanced anti-tumoural activity by engaging the immune system through increased complement-dependent cytotoxicity and ADCC, and are now an integral component of CLL therapy.[62-64]

Ofatumumab

Ofatumumab is a fully humanised anti-CD20 mAb that has recently been explored in patients with refractory CLL and as first-line treatment.[65-68] In fludarabine or alemtuzumab-refractory patients, ofatumumab yielded an ORR of almost 60%, and was even effective in patients pre-treated with rituximab.[65,68] This led to the accelerated approval by the US FDA in 2010 for the same clinical settings.[69] The combination therapy with chlorambucil is currently assessed in a Phase III study,[70] and other approaches include the combination with targeted agents such as idelalisib or lenalidomide.[71,72]

Obinutuzumab

Obinutuzumab is a Type 2 anti-CD20 mAb presently undergoing clinical investigation, and has demonstrated impressive early results. In the pivotal GCLLSG-CLL11 Phase III trial investigating chlorambucil plus obinutuzumab (Clb-O) or chlorambucil plus rituximab (Clb-R) against chlorambucil alone (Clb) in patients with previously untreated CLL and coexisting conditions, Clb-O or Clb-R as compared with Clb alone increased response rates and prolonged PFS. OS was significantly prolonged with Clb-O compared

with Clb alone. Treatment with Clb-O compared with Clb-R resulted in prolongation of PFS and higher rates of complete response (20.7% versus 7.0%) and molecular response.[8] The FDA approved obinutuzumab in combination with chlorambucil as a first-line therapeutic option in November 2013.[73]

Lenalidomide

Lenalidomide is a thalidomide analogue that has been demonstrated to have pleiotropic effects on immune cells in both preclinical and early clinical studies. Its mechanism of action in CLL appears to function primarily by enhancing anti-tumoural immunity in T cells.[74] It induces T cell cytoskeletal genes and restores the ability to form immunological synapses with B cells both *in vitro* and *in vivo*.[75,76] Lenalidomide also enhances T cell motility and downregulates the expression of T cell-inhibitory molecules in CLL.[42,77] In CLL cells, it induces CD40L (CD154) expression, thus playing a key role in CD80-mediated T cell activation,[75,78] and sensitises cells to TRAIL-mediated apoptosis and costimulatory activation of normal B cells to produce anti-tumoural antibodies.[79] The latter has been consolidated by two clinical studies by the M.D. Anderson Cancer Center, which revealed that lenalidomide therapy can yield a sustained increase in Ig levels.[80,81]

Other clinical trials have demonstrated that this immunomodulatory drug has activity as monotherapy for CLL, but is associated with non-negligible toxicities such as tumour flare reaction and increased risk of opportunistic infections, making the potential combination with drugs such as fludarabine or alemtuzumab difficult.[82-87] The efficacy and safety of the combination with rituximab in relapsed or refractory CLL were explored in a Phase II trial.[84] The ORR was 66% with 12% of CR, for an estimated survival at 36 months of 71%. Severe toxicities included neutropaenia, thrombocytopaenia, and anaemia, while one patient suffered from a Grade 3 tumour lysis.[80,81,87] The combination of lenalidomide with ofatumumab is currently being investigated in relapsed or refractory CLL.[72,88]

BCR SIGNALLING INHIBITORS

Ibrutinib

Ibrutinib is a BTK inhibitor that primarily blocks BCR associated anti-apoptotic pathways. In addition, it affects BCR and chemokine-controlled retention and homing of CLL cells in their growth and survival-supporting lymph node and bone marrow microenvironment.[89-91] Thus, malignant B cells are driven out of their protective niches and are more accessible to cytotoxic therapy and potentially other immune cells.[91] This was supported by early clinical trials where treatment resulted in transient lymphocytosis associated with a decrease in lymphadenopathy, with very good safety profiles.[92]

To further explore these findings, a Phase I/II study was conducted in 85 heavily pre-treated patients with relapsed or refractory CLL.[93] The ORR was 71%, with 18% of PRs with lymphocytosis and 2% of CRs. The progression-free rate at 26 months was 75% with an OS rate of 83%. Overall toxicity was very mild with few serious adverse events. Marked changes were noted in terms of the reduction of lymph nodes and spleen sizes, and platelet and red blood cell counts were also improved. As a consequence, the FDA approved ibrutinib in February 2014 for the treatment of CLL as second-line therapy.[94] Recently published preclinical data indicate that ibrutinib also irreversibly binds the BTK isoform ITK, which is expressed in T cells, and can therefore be potentially used to correct T cell based immune responses.[95]

Idelalisib

Idelalisib is an inhibitor of PI3Kδ, which is also a component of the CLL signalling pathways involved in cell survival, clonal expansion, and malignant cell retention in lymphoid tissues.[96,97] In a Phase I study conducted on 54 heavily-pretreated CLL patients with relapsed or refractory disease, 39% of patients achieved a PR, and 33% of patients achieved a PR with lymphocytosis; the safety profile of idelalisib was acceptable.[98]

A Phase III trial was then initiated in 220 patients with relapsed CLL receiving idelalisib in combination with rituximab versus rituximab plus placebo.[99] Due to overwhelming efficacy, the study was interrupted after the first interim analysis: the ORR was 81% for the combination therapy versus 13% for rituximab monotherapy, while OS at 12 months was of 92% and 80%, respectively. PFS was also greatly improved in the combination arm (93% versus 46%, respectively) and a higher proportion of patients presented a 50% or higher reduction of lymphadenopathy (93% versus 4%, respectively). The safety profiles were similar and acceptable within both groups, with

severe toxicities occurring in 40% and 35% of patients, respectively.

Additional investigation on idelalisib is currently ongoing, especially as a combination therapy with other agents: two Phase III trials on previously treated CLL patients are exploring the efficacy and safety of idelalisib with ofatumumab versus ofatumumab alone,[100] and of idelalisib with rituximab plus bendamustine versus rituximab/bendamustine and a placebo.[101] Preliminary results are expected in late 2015 and late 2016, respectively.

CELLULAR THERAPIES

In contrast with 'passive' immunomodulation as discussed above, active immunotherapy is a reasonable option in some patients, and such strategies include HSCT and chimeric antigen receptor (CAR) T cell therapy.

RIC-HSCT

As previously stated, HSCT is the only curative option for CLL, but is limited to a selected group of patients.[102] Aside from the rarity of available histocompatible donors, myeloablative conditioning is associated with high transplant-related morbidity and non-relapse mortality, as well as the occurrence of graft-versus-host disease (GVHD).[103,104] Thus, non-myeloablative or reduced-intensity conditioning (RIC) approaches have been and continue to be evaluated in CLL patients that would otherwise be ineligible to HSCT, namely elderly patients. Several clinical studies with long-term follow-up have demonstrated that HSCT can provide long-term disease control, even in patients with poor-risk CLL.[104-108] Nevertheless, GVHD remains a complication of RIC HSCT, and translational efforts are now focused on the modulation of GVHD towards the beneficial GVL effect.[109]

Gene Therapy with Chimeric Antigen Receptor T Cells

CAR technology has recently emerged as a novel and promising perspective to specifically target malignant cells with precisely engineered T cells. It uses the single chain variable fragment from an antibody molecule fused with an internal T cell signalling domain to form a CAR, which is then transduced into T cells.[110] A major advantage of this approach is that it eliminates major histocompatibility complex restriction, enabling the same CAR to be used for several different patients.

In a pivotal report, a heavily pre-treated high-risk patient with refractory CLL received autologous T cells that had been modified with CARs directed at CD19, a B cell surface antigen, resulting in remission induction and lasting tumour control.[111] Since then, several clinical trials have reported impressive results with anti-CD19 CARs, both in CLL and acute lymphoblastic leukaemia.[111-115] However, it has also become clear that the success of CAR therapy depends on the inclusion of lympho-reducing conditioning chemotherapy and the choice of CAR design.[111,113,114,116]

In addition, CAR T cell therapy can be associated with severe complications such as cytokine release syndrome, a potentially lethal complication, and lasting normal B cell depletion,[113,117,118] which potentially requires continuous intravenous Ig administration. Taken together, CAR T cells show significant clinical activity but are unlikely to fully restore disease-related immune defects. Further studies are needed to fully investigate the clinical use of CAR T cell therapy and treatment-related toxicities, and its optimal combination with existing treatment approaches.

CONCLUSION

While initial chemoimmunotherapies had offered promising high ORR and OS, real-life settings such as the complex management of comorbidities and complications in vulnerable or elderly patients or in immunosuppressed patients after multiple rounds of therapy is challenging. In the last few years, many new approaches and innovative agents have advanced the outlook for CLL management. Indeed, novel therapies could soon be addressing the need to promote immune reactivation and resensitise the immune system. By doing so, they could fulfil two main objectives, namely lowering the high proportion of patients at risk of infection and acting as effective tools for the immune system to overcome its defects and mount effective, strong, and lasting anti-tumour responses.

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THE ROLE OF CD49d IN CHRONIC LYMPHOCYTIC LEUKAEMIA: MICROENVIRONMENTAL INTERACTIONS AND CLINICAL RELEVANCE

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ABSTRACT

Chronic lymphocytic leukaemia (CLL) is a clinically heterogeneous disease characterised by the accumulation/expansion of a clonal population of neoplastic cells with the morphological appearance of small mature B lymphocytes in blood, bone marrow, and lymphoid organs. Stimulation through the B cell receptor (BCR) plays a prominent role in the selection and expansion of the malignant clone in CLL. On the other hand, other external signals delivered by several cell types including T lymphocytes, macrophages, stromal cells, endothelial cells, and follicular dendritic cells, operating through either direct BCR-independent cell-cell contact or indirect production of paracrine soluble factors, synergistically cooperate in regulating proliferation and survival of CLL cells. In this context, CD49d is known to play a pivotal role in mediating both cell-cell and cell-matrix interactions in CLL-involved tissues, eventually delivering pro-survival signals and protecting CLL cells from drug-induced damages. In the present review, we focused on functional and physical interactions of CD49d with other microenvironmental receptors, including CD38 and BCR, and other specific CD49d-dependent interactions in lymph node and bone marrow microenvironments responsible for growth and survival-supporting signals, eventually influencing CLL prognosis and therapeutic options.

Keywords: CD49d, microenvironment, chronic lymphocytic leukaemia (CLL).

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) represents the most common form of leukaemia in the Western world with an incidence of 3-5 per 100,000 patients. This disease affects mainly elderly people, with a median age at the diagnosis of 70 years, and a little predominance in men.[1,2] CLL diagnosis implies the presence of neoplastic B cells >5000/μL in the peripheral blood; the disease always involves the bone marrow whereas enlargement of lymph nodes, spleen, or liver may be absent. The clinical heterogeneity in this disease is evidenced by the fact that some patients show a refractory disease and die within 2-3 years after the diagnosis, whereas in others the disease has a very indolent course without need of treatment and survival reaching decades. In this context, clinical, genetic, and biological parameters have been introduced to characterise this heterogeneity and to evaluate the prognosis of CLL patients.[2] In particular, specific chromosomal aberrations (i.e. deletion 17p, deletion 11q or trisomy 12), the presence of unmutated immunoglobulin heavy chain (IGHV) genes, as

well as a mutated configuration of the TP53, NOTCH1, SF3B1, and BIRC3 genes, or expression levels for ZAP-70, CD38, and CD49d exceeding the value of an established threshold, have been reported to correlate with a poor clinical outcome in CLL.[2-4]

The biological parameters employed as prognosticators can be molecules involved in the functional interplay of CLL cells with the neighbouring cells which form the tissue microenvironment of CLL cells.[1,2,5] As an example, stimulation through the B cell receptor (BCR) plays a prominent role in the selection and expansion of the malignant clone in CLL, and the prognostic impact of the mutational status of IGHV genes could be considered as a consequence of the relevance of this process in CLL.[1,6] Other external signals delivered by several cell types including T lymphocytes, macrophages, stromal cells, endothelial cells, and follicular dendritic cells (DCs), operating through either direct BCR-independent cell-cell contact or indirect production of paracrine soluble factors, synergistically cooperate in regulating proliferation and survival of CLL cells.[1,7-12]

In this context, in CLL-involved tissues of bone marrow and lymph nodes, CD49d is known to operate as one of the master molecules mediating both cell-cell and cell-matrix interactions by binding respectively to vascular cell adhesion molecule-1 (VCAM-1), non-RGD sequences (Arg–Gly–Asp) of fibronectin (FN), or C1q-like domain of elastin microfibril interfacer-1 (EMILIN-1).[13-17] These features are reflected in the independent prognostic impact of CD49d expression in CLL.[18-20] Moreover, a role of CD49d in proliferative centres of tissue sites can be inferred by studies investigating the expression of CD49d in the bona fide highly proliferative compartment of peripheral blood CLL cells.[21] In particular, CD49d was expressed at a higher level in highly proliferative peripheral blood CLL cells, as defined by the CD5 high/CXCR4 low phenotype, than in cells of the resting compartment.[21]

In the present review, we focused on functional and physical interactions of CD49d with other microenvironmental receptors, including CD38 and BCR, and other specific CD49d-dependent interactions in lymph node and bone marrow microenvironments responsible for growth and survival-supporting signals, eventually influencing CLL prognosis and therapeutic options.

CLINICAL IMPACT OF CD49d EXPRESSION

Recently, in the context of a multicentre worldwide initiative analysing a CLL series of about 3,000 cases,[22] the expression of CD49d - the α chain of the $\alpha_4\beta_1$ integrin heterodimer - emerged as a first level biological prognosticator in CLL, predicting shorter overall survival and progression free survival, along with IGHV mutational status and deletion 17p.[18,23-27] On the contrary, CD38 and ZAP70, as well as the cytogenetic abnormalities deletion 11q and trisomy 12, evidenced a generally lower prognostic impact.

In the same study, when a hierarchical model was restricted to the flow cytometric prognostic markers CD49d, CD38, and ZAP70, CD49d was located at the top of the branching in the entire cohort of patients, as well as in early stages and young patients, thus resulting in the best flow-cytometry-based marker to stratify the prognosis of CLL patients.[22] Given this evidence, testing CD49d expression in routine clinical practice emerged as similarly useful in the baseline prognostic assessment of newly diagnosed CLL, as well as in refining the prognostic evaluation in patients already stratified by CD38 and/or ZAP70 expression. These clinical aspects could be considered as direct and specific consequences of physical and chemical interactions of the CD49d molecule.

CD49d INVOLVEMENT IN THE MICROENVIRONMENTAL CROSS-TALK AND SURVIVAL SIGNALLING

CLL is chraracterised by several functional interactions involving CD49d and specific chemokine-cytokine receptor/ligand pairs. In particular, cell adhesion of CLL cells via the CD49d/VCAM-1 pair, and the subsequent response of adherent CLL cells to the chemokines, CCL21 and CCL19, produced by high endothelial venules (HEV), or by the surrounding lymph node stroma through their receptor CCR7, is involved in transendothelial migration (TEM) of CLL cells across HEV into lymph nodes.[28] In addition, the combined stimulation of CLL cells by vascular endothelial cell growth factor (VEGF), and by CD49d engagement, was shown to be critical for TEM induced by CCL21 and CXCL12 in CLL cells coexpressing CD49d along with the VEGF receptors VEGFR1 and VEGFR2.[29]

Adhesion of CLL cells via CD49d also upregulates matrix metalloproteinase (MMP)-9 production, the MMP-9 proteolytic activity may be enhanced by its localisation at the CLL cell surface.[30] In particular, CLL cells bind soluble and immobilised pro-MMP-9 and active MMP-9 through a cell surface docking complex for MMP-9, composed by CD49d and a splice variant of CD44, conferring a metastatic phenotype that locally causes the growing of tumour cells, and whose expression is associated to tumour progression.[31] MMP-9 is also a functional ligand for the CD44v/CD49d docking receptor, able to provide survival signals independently of its proteolytic activity.[31,32] Interestingly, the pro-survival effect of MMP-9 derives from activation of the Lyn kinase, thus following a distinct and BCR-independent mechanism.[32] Moreover, the LYN/STAT3/MCL-1 pathway, which is elicited by MMP-9 ligation to the CD44v/CD49d docking receptor, is not shared by the CD49d-VCAM-1 axis, suggesting that CD49d may trigger distinct intracellular events depending on the ligand.[32]

CXCR4, the receptor for the CXCL12 chemokine, is also associated with CD49d on CLL cell membrane, suggesting that CD49d and CXCR4 may be functionally linked in CLL,[33] as demonstrated in multiple myeloma or in bone marrow hematopoietic progenitors, where CXCR4, triggering by CXCL12, is able to upregulate CD49d-mediated adhesion to VCAM-1 and FN.[34,35] Of note, as for CD49d, CXCR4 engagement was also shown to upregulate MMP-9 production by CLL cells.[30]

In CLL, ligation of CD49d by FN was demonstrated to prevent *in vitro* onset of apoptosis, likely due to an increase in the BCL-2/BCL-2-associated X protein (BAX) ratio,[36] and to protect CLL cells from fludarabine-induced apoptosis, this effect correlated with an increased expression of BCL_{XL}.[13,37] CD49d triggering is also able to induce spleen tyrosine kinase (SYK) phosphorylation and SYK-dependent protein kinase B (AKT) phosphorylation, through mechanisms distinct from the BCR signalling.[38] The SYK-dependent AKT/myeloid cell leukaemia sequence 1 (MCL-1) pathway is known to contribute to CLL cell survival.[39-42]

Co-culture of CLL cells with endothelial cells determines a significant increase of CD49d expression and enhances CLL cell viability, these effects being mediated by activation of the NF-κB transcription factor RelA.[43] The genes induced by NF-κB to promote survival include the cellular inhibitor of apoptosis FLIP, and the BCL-2 homologous A1 and BCL_{XL}.[44] Alterations in NF-κB signalling cascades have been considered responsible for the differences in the sensitivity to microenvironment stimuli between high and low-risk groups, such as CLL expressing unmutated IGHV and mutated IGHV, or CD49d positive ($CD49d^+$) and CD49d negative ($CD49d^-$) CLL.[45,46]

FUNCTIONAL INTERACTIONS OF CD49d WITH BCR

The binding of CLL cells on stromal cells of microenvironmental niches, mainly occurring through CD49d, reflects the activity of normal B cells where CD49d-driven interactions play a key role in controlling the development of B lymphocytes,[47,48] chemokine-induced transendothelial migration (TEM) of mature B cells during their recirculation and homing,[49,50] and antigen-specific B cell differentiation within germinal centres of secondary lymphoid organs.[51] In particular, during the latter process, B cells that express BCR with high affinity for the antigen are rescued from apoptosis by interacting with follicular DCs through the $\alpha_4\beta_1$/VCAM-1 axis.[52,53] This inside-outside activation of the $\alpha_4\beta_1$ integrin is BCR-controlled through the consecutive activation of LYN, SYK, PI3K, BTK, PLCγ2, IP3R, and PKC. In particular, upon BCR stimulation, $\alpha_4\beta_1$ can be released from a cytoskeletal constraint by Ca^{++}-mediated BCR-dependent calpain activation and mobilised to lipid rafts, this process leading to the formation of $\alpha_4\beta_1$ clusters that, in turn, may become tethered to the actin cytoskeleton, eventually resulting in enhanced $\alpha_4\beta_1$ avidity and adhesion.[54-56] In this model, B cells expressing BCR with high affinity for the presented antigen are preserved in the germinal centre by integrin-mediated signals while, on the contrary, B cells expressing BCR with low affinity for the presented antigen, failing to have sufficient integrin mediated signals, are more prone to apoptosis.[57]

The described BCR-dependent $\alpha_4\beta_1$ functional interaction can be preserved in CLL, where the increased lymph node size is mainly/exclusively dependent from the accumulation of CLL cells due to integrin mediated adhesion to accessory cells and/or extracellular matrix proteins.[57] Inhibitors of kinases, downstream of the BCR such as the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib,[58] the SYK inhibitor fosfamatinib,[59] and the PI3Kδ

inhibitor idelalisib,[60] are promising alternative target therapies for CLL patients that have been very recently employed in clinical trials. These new agents have a clinical activity that appear similar, with a rapid resolution of lymphadenopathy and/or organomegaly, and a redistribution of CLL cells from tissue into the blood, with a subsequent rising of lymphocytosis during the first few weeks of therapy that often slowly resolves. In this context, it has been reported that: 1) the BTK inhibitor ibrutinib strongly inhibits this CD49d-mediated adhesion of CLL cells to VCAM-1 and FN substrates *in vitro*;[61] 2) the PI3Kδ inhibitor idelalisib decreases CLL adhesion to stromal cells by interfering with CD49d/VCAM-1 binding.[62] Thus, these mechanisms of action could be the cause for lymph node shrinkage with the redistribution of CLL cells into the blood observed *in vivo* upon treatment of CLL patients with BTK inhibitors,[61,62] and may also provide the rationale for the use of inhibitors of kinases in combination therapies aimed at targeting CLL cells outside the microenvironmental niches, where they may be more prone to respond to immuno-chemotherapy.

CD49d INTERACTIONS WITH CD38

A functional link between CD49d and CD38 involves CCL3 and CCL4 chemokines that have been found to be overexpressed in CD49d$^+$CD38$^+$ CLL cells, and upregulated upon CD38 triggering.[63] CLL-derived CCL3 and CCL4 have been associated with the recruitment of cells from the monocyte macrophage,[63,64] or T cell lineages,[65] in the context of CLL-involved microenvironmental sites.[63,64,66,67] Moreover, a strong correlation among the CD49d$^+$CD38$^+$ phenotype, infiltration of CD68$^+$ macrophages, and presence of a stromal/endothelial component highly expressing VCAM-1 in the context of lymphoid aggregates in bone marrow biopsies of CD49d$^+$CD38$^+$ CLL, has been demonstrated.[63] In particular, VCAM-1 upregulation has been found due to an overproduction by the infiltrating CD68$^+$ macrophage component of TNF-α, allegedly together with other cytokines.[63] This circuitry may contribute to explain the aggressive clinical course of CLL coexpressing CD49d and CD38, given the pro-survival effects of VCAM-1/CD49d interactions for CD49d-expressing CLL cells.[63]

In CLL, CD49d and CD38 are part of a cell surface macromolecular complex which also includes CD44 and MMP-9, as well as CXCR4,[68] thus characterising a signalling platform in CLL cells of poor prognosis cases. The association between CD38 and the CD49d/CD29 integrin heterodimer, both inside and outside the cell membrane lipid rafts, allows the CD49d/CD29/CD38 complexes to freely shuttle in and out of the specialised cholesterol enriched membrane microdomains, where signalling transduction is organised.[69,70] Moreover, the CD49d/CD29/CD38 complexes are not influenced by the integrity of the membrane structure since the association is unaffected by cholesterol depletion, being joined together by other cellular structures, including cytoskeletal proteins, known to associate with integrins either directly or indirectly.[71]

CD49d-mediated activities are enhanced by the co-expression of CD38; in fact, CD49d$^+$CD38+ cells have higher propensity to adhere and to spread when seeded onto the CD49d-specific substrates VCAM-1 and FN compared to CD49d$^+$CD38$^-$ cells. In this context, CD49d/VCAM-1 interactions exert a more marked anti-apoptotic effect in CD49d$^+$CD38$^+$ as compared to CD49d$^+$CD38$^-$ cells. Moreover, adherent CD49d$^+$CD38$^+$ CLL cells display a distinctive morphology, characterised by a more complex pattern of filopodia-like protusions compared with cells with the CD49d$^+$CD38$^-$ phenotype.[72] CD38 was also demonstrated to be effective in the recruitment of Vav-1, a molecule involved in the integrin pathway, that operates as guanine exchange factor for Rac and Cdc42, two Rho GTPases involved in lamellipodia/filopodia generation in various cell models,[73-75] and that becomes phosphorylated on tyrosine-174 upon integrin engagement. Of note, CD49d$^+$CD38$^+$ CLL cells are characterised by higher levels of phospho-Vav-1 upon adhesion onto CD49d-specific substrates than CD49d$^+$CD38$^-$ CLL cells, resulting in a more robust integrin signalling pathway characterising CD49d$^+$CD38$^+$ CLL.

The physical association between CD49d and CD38 is also responsible for a more marked anti-apoptotic effect exerted upon CD49d/VCAM-1 interactions in CD49d$^+$CD38$^+$ CLL cells than in CD49d$^+$CD38$^+$ CLL cells. This characteristic can depend on a more efficient adhesion of CD49d$^+$CD38$^+$ CLL cells, and consequently a more pronounced activation of the anti-apoptotic machinery,[13,63] also, thanks to the contribution of specific signalling proteins, such as Vav-1,[76] already recruited to the adhesion site.

ASSOCIATION OF CD49d WITH TRISOMY 12

In a recent study by our group,[77] CD49d expression was investigated by flow cytometry in the neoplastic component of 1,200 CLL patients. In this series, using the cut-off of 30% of positive cells, about 40% of cases were classified as CD49d$^+$ cases. Analysis within the major cytogenetic groups showed that a significantly higher percentage of CD49d$^+$ cases (about 90% of cases) is associated with the presence of trisomy 12 cases. Moreover, among CD49d$^+$ cases, trisomy 12 CLL cases are characterised by the higher mean fluorescence intensity levels when compared with cases belonging to the other cytogenetic categories. Additionally, in the context of flow cytometry sorted CD49d$^+$ and CD49d$^-$ subpopulations in CLL cases with bimodal CD49d expression, trisomy 12 abnormality could be detected only in the CD49d$^+$ fraction and it was absent in CD49d$^-$ cells.

In the same study, DNA methylation was analysed within a 5'-UTR CpG island (77 CpGs) of the CD49d gene (ITGA4).[77] In this context, it was found that: 1) CD49d$^+$/trisomy 12 CLL virtually completely lacked methylated CpG, while a significant methylation of CpG was detected in CD49d$^-$ cases; 2) a significant inverse correlation was found between the percentage of methylated CpGs and CD49d expression at both mRNA and protein levels; 3) when highly purified CLL cells from CD49d$^-$ cases were exposed to the hypomethylating agent 5-aza-2'-deoxycytidine (DAC) in the presence of CpG-ODN/interleukin-2 as a proliferative stimulus, the proliferative fraction of DAC treated CLL cells, significantly upregulated CD49d protein levels; 4) consistently, analysis of ITGA4 methylation in these DAC treated proliferating cells revealed lower levels of DNA methylation in ITGA4 5'-UTR CpG-island compared with proliferating CLL cells of untreated cultures.

Overall, these data highlight a direct role of DNA methylation in regulating CD49d expression in CLL. Moreover, the overexpression of CD49d may contribute to explain: 1) the molecular basis of the peculiar biological behavior of trisomy 12 CLL and may predict for the development of additional cytogentic lesions;[78] and 2) the specific tropism toward lymph nodes of trisomy 12 CLL cells and the peculiar clinical features of this CLL subset, in which massive lymph node enlargement is often observed and the final transformation in Richter's syndrome is more frequent than in other cytogenetic categories.[79]

CONCLUSION

In the present review, we have summarised the principal microenvironmental interactions involving CD49d in CLL. In fact, CD49d can be represented as a major factor of a complex interplay with other surface receptors, all expressed by CLL cells, which are able either to potentiate CD49d activities (e.g. CD38, CXCR4, VEGFR1/2, BCR) or are potentiated by interactions with CD49d itself (e.g. CD44, CXCR7). As a consequence of CD49d engagement, pro-survival signals and signals protecting CLL cells from drug-induced damages are delivered (Figure 1).

An interesting observation is the strong correlation between CD49d expression and trisomy 12 since it might anticipate a putative general feature of CLL cells, i.e. the non-random correlation between genetic lesions and microenvironmental receptors. In this context, recent studies by us and others[3,4,80-83] reported the non-random association of specific BCR features, i.e. the expression of the so-called stereotyped BCR, with the novel somatic mutations with prognostic relevance of genes such as NOTCH1 and SF3B1.

The characteristic clinical activity of kinase inhibitors targeting BCR downstream genes consisting in CLL cell redistribution from tissues into the blood emphasise a relevant role for CLL microenvironment not only in CLL pathogenesis but also in the development of new targeted treatment approaches. In particular, the employment of such inhibitors, being non-genotoxic compounds, could also be useful in the context of asymptomatic patients, in which a potential selection of genomic alterations due to DNA-damaging chemotherapy must be avoided, and in which the usual approach is a watch and wait strategy. In this context, the relevance of CD49d expression should be tested in clinical trials similar to the trial planned by the German CLL study group[5] in which ibrutinib is employed as a first-line treatment in patients with early stages of disease (e.g. Binet Stage A). In conclusion, the complex network of CD49d-mastered microenvironmental interactions and/or correlations, as detailed in the present review, may have a relevant role that remains to be established and will be addressed by future studies.

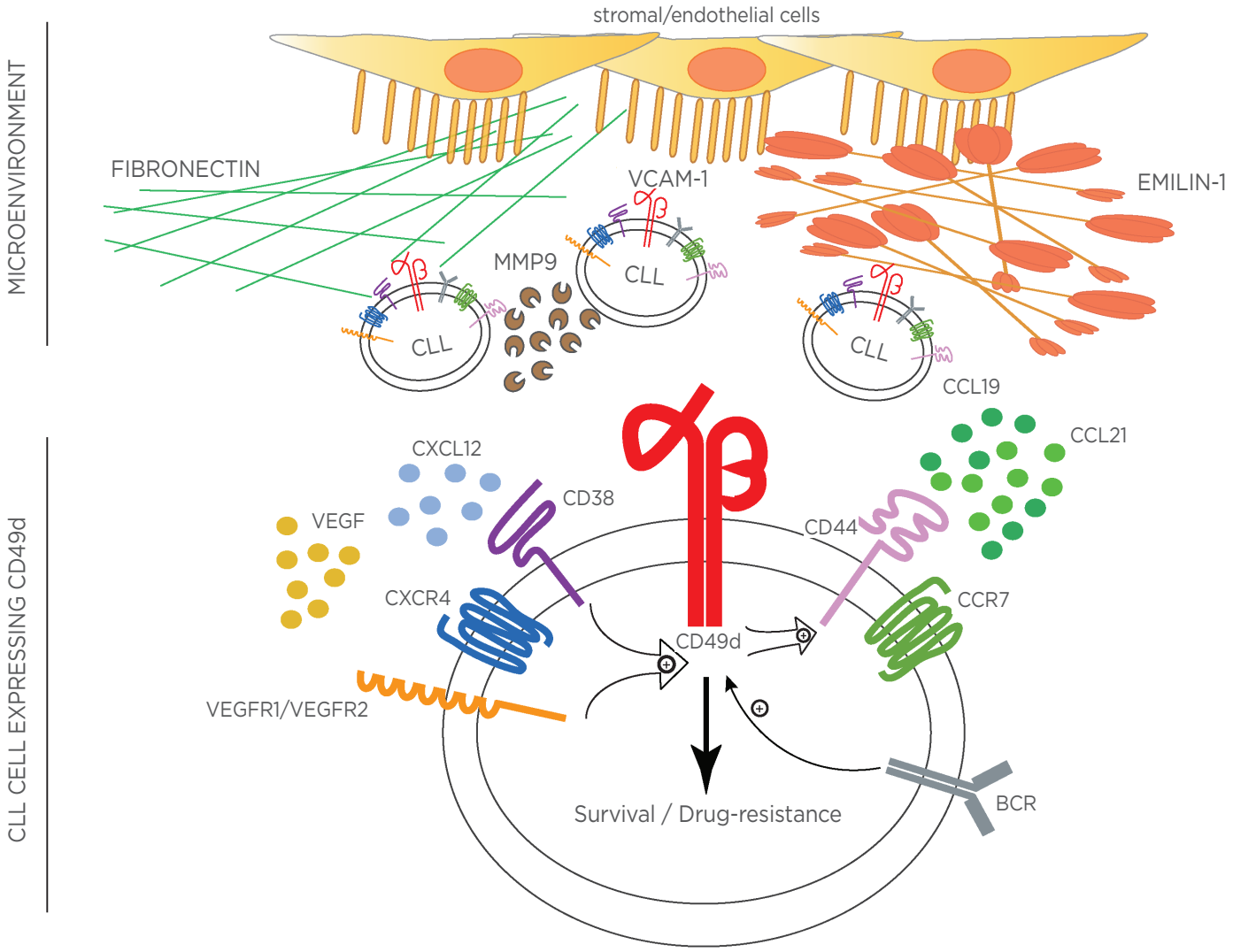

Figure 1: CD49d interactions in CLL microenvironment.
CLL: chronic lymphocytic leukaemia; VCAM-1: vascular cell adhesion molecule-1; EMILIN-1: elastin microfibril interfacer-1; MMP: matrix metalloproteinase; VEGF(R): vascular endothelial cell growth factor (receptor); BCR: B cell receptor.

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CURRENT DEVELOPMENTS AND PERSPECTIVES IN MULTIPLE MYELOMA

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ABSTRACT

In the last decades, advances in the therapeutic management of multiple myeloma (MM) with new drug armamentarium and strategies have significantly improved the outcome and survival of newly diagnosed and relapsed patients. However, the continuing challenges physicians are facing within specific clinical settings and patient subpopulations, whose prognosis with current strategies is extremely poor, call for a paradigm change. New immunomodulators, proteasome inhibitors, histone deacetylase inhibitors, and monoclonal antibodies are being explored to improve first-line outcomes so that a smaller proportion of patients relapse early or fail to respond to induction treatment. Moreover, recent advances and clinical evidence with novel therapies seem to provide patients with relapsed or refractory MM additional survival benefits. Improving clinical outcomes and refining standard of care should help clinicians reduce the burden of multiple and toxic therapy; quality of life (QoL) should be at the core of MM management. Patient selection and stratification needs to be reinforced with the help of comprehensive knowledge on conventional risk factors, and supplemented by molecular pathways in the near future in order to provide tailored options and strategies to patients, including the use of monoclonal antibodies. Numerous drugs are on the horizon and the next few years should witness marked improvements in survival, QoL, and safety of MM management.

Keywords: Multiple myeloma, salvage therapy, targeted therapy, immunomodulators.

INTRODUCTION

Multiple myeloma (MM), also known as Kahler's disease, is an incurable hematological malignancy characterised by the neoplastic proliferation of plasma cells which infiltrate and accumulate in the bone marrow while producing monoclonal immunoglobulins (Igs).[1] The abnormal development of malignant cells in the bone marrow interferes with hematopoiesis and causes considerable bone damage such as osteolytic lesions, osteopaenia, hypercalcaemia, and fractures. In addition to bone pain, the patient can also suffer from anaemia, hypercalcaemia, renal failure, infections, and neurological symptoms.[1]

MM is the second most frequent blood cancer after non-Hodgkin's lymphoma, accounting for 1-2% of all new cancer cases and 10% of hematological malignancies.[2-4] The mean age at diagnosis is 65 years as more than half of newly-diagnosed patients are aged 65 and over, and only a very small proportion of patients are 40 or younger.[5,6] The incidence for MM appears to depend on

ethnicity, with a 2-3-fold higher incidence in Africans and African Americans when compared to Caucasian populations.[7,8] MM accounts for about 2% of all cancer mortality.[9]

The diagnosis is primarily established by the presence of a monoclonal protein in the serum (malignant plasma cells mainly produce IgG, IgA or Ig light chains), the presence of monoclonal light chains (Bence Jones proteinuria) in urine,[10,11] excess clonal bone marrow plasma cells (>10%), and organ impairment.[6,12,13]

The development of novel agents and clinical evidence on combinations have markedly improved the clinical outcomes and overall survival (OS) of MM patients. Over the last decade, median survival has improved from 4-6 years in newly diagnosed young patients,[14-16] while the rates of long-term survival (at 5 and 10 years) in patients aged 50 or younger have increased by 12% and 17%, respectively.[17]

However, the prognosis of MM is still dismal in general and many unmet needs remain unaddressed, as physicians are faced with some challenging clinical settings. This review aims to summarise the current developments and future perspectives of MM management, in which the main objectives are to improve long-term survival with acceptable risk to benefit ratios.

CONTEXT AND CURRENT STANDARDS OF CARE

Management of Newly Diagnosed Multiple Myeloma

The main objective of MM management is to obtain the best possible response, and to maintain it, with acceptable toxicity. Over the last few decades, the OS has been significantly improved by high-dose chemotherapy (HDT) followed by autologous stem-cell transplantation (ASCT), which is the current standard of care (SoC) in medically fit patients aged up to 65-70 years with adequate renal function.[18]

This treatment strategy, when implemented on eligible patients, can yield extended survival and is, therefore, the current SoC for newly diagnosed MM.[19] Indeed, patients achieving a complete response (CR) or a near complete response (nCR) display considerably improved outcomes when compared to those who only achieve a partial response (PR).[20] In a study from the Spanish PETHEMA group,[20] 35% of patients who achieved CR following HDT plus ASCT appeared to benefit from a functional cure. CR in MM was found to be correlated with long-term progression-free survival (PFS) and OS, even in elderly patients.[21] Conversely, Hoering et al.[22] demonstrated that failure to achieve CR and even an early loss of CR is associated with inferior survival, highlighting the importance of achievement of sustained CR.

Overall response rates (ORR) and duration of response were also considerably improved upon the development of novel agents, such as immunomodulatory compounds (thalidomide, lenalidomide) and proteasome inhibitors (bortezomib [BTZ]).[23-29] Combination therapy seems to provide higher ORR and CR than single agent therapy; the most widely used front-line combinations for induction before ASCT are as follows: thalidomide, BTZ, and dexamethasone (VTD); cyclophosphamide, BTZ, and dexamethasone (CyBorD or VCD).[30]

For non-transplant candidates, combination therapies include BTZ, melphalan, and prednisone (VMP) or melphalan, prednisone, and thalidomide (MPT).[31,32] VMP plus thalidomide induction followed by maintenance therapy with BTZ plus thalidomide seems to provide PFS and OS benefits in this patient subpopulation.[33]

Continuous treatment with lenalidomide plus dexamethasone can provide additional survival outcomes in non-transplant candidates. However, longer follow-up is still needed.[34]

The choice of chemotherapy is adapted to the patient's characteristics, patient's choice, and the severity of the disease. Over the course of anti-myeloma therapy, patients should be closely monitored for treatment response, infections, and other treatment-related adverse events, as well as for MM complications.

After ASCT, once an initial response is achieved, consolidation therapy with BTZ or a BTZ-based regimen may be performed in order to consolidate ASCT benefits and result in longer time to progression (TTP) and higher OS (in contrast with maintenance therapy, which is defined by the prolonged administration of low-dose chemotherapy to prevent disease progression).[35,36] Longer progression time and OS have also been

observed with lenalidomide maintenance,[35,37] while short-term consolidation therapy with VTD has been reported to improve PFS after tandem ASCT, but not OS.[25] As of yet, a sequential approach has not been explored (i.e. continuous single agent lenalidomide following single agent BTZ).

Management of Relapsed or Refractory Multiple Myeloma

Nearly all patients with MM will eventually relapse from first-line therapy and experience relapsing or refractory MM (RRMM). Initial or emerging drug resistance is a hallmark of the disease and represents a significant challenge in MM management, as they hinder the efficacy of most agents.

Refractory or end-stage myeloma is associated with a poor prognosis, with an average survival of less than a year, and represents a great challenge to physicians.[38,39] Relapsed MM refers to progressive disease in which at least a PR was previously achieved following first-line treatment or salvage therapy, while refractory MM indicates progressive disease when the patient is either unresponsive initially (primary refractory MM) or following treatment (within the last 60 days).[40]

There is no SoC or optimal choice for RRMM, and therapeutic options must be selected according to initial therapy, TTP, and the patient's condition and quality of life (QoL), while balancing the benefit-to-risk ratio for each case. Retreatment with the initial regimen remains a possibility, as well as switching to other agents. Moreover, a second ASCT as salvage therapy can be an option for patients achieving a good response after their initial ASCT. It should be offered to the patient whenever possible.[41,42] By contrast, a repeat or tandem ASCT, performed within 3-6 months, is a first-line therapeutic option.[41]

Chemotherapy with thalidomide, lenalidomide, or BTZ was demonstrated to be effective in second-line therapy, and prolongs OS in RRMM patients.[18,43,44] Indeed, thalidomide is associated with second-line response rates of 25-35%; these are higher when used in combination with dexamethasone and cyclophosphamide or with conventional chemotherapy, although prolonged exposure to thalidomide is inevitably associated with peripheral neuropathy in the majority of patients.[45-47] One interesting feature of thalidomide is that it does not warrant usual dose adjustments in patients with renal impairment, including patients on dialysis.[48,49]

Lenalidomide, approved in 2006 by the FDA as second-line therapy, yields good ORR (61%) and low toxicity when associated with dexamethasone.[50,51] Celgene has recently submitted an application to the FDA and the EMA for approval of lenalidomide with weekly dexamethasone as therapy for newly diagnosed MM.

Finally, BTZ is a proteasome inhibitor approved as a second-line option since 2008. It is highly effective in RRMM, particularly when combined with other agents.[52-60] Similarly to thalidomide, BTZ does not require dose adjustments in patients with renal impairment.[61,62] Nevertheless, its clinical applicability can be complicated by peripheral neuropathy, although this side-effect can be significantly reduced by subcutaneous administration of bortezomib.

Nonmyeloablative allogeneic stem-cell transplant (allo-SCT) remains debatable in RRMM despite the advantages of the infusion of tumour-free stem cells with a possible graft-versus-lymphoma effect. In a European study on 413 RRMM patients, the OS was 24.7 months for a median PFS of 9.6 months, and a 5-year survival rate of about 30%.[63]

According to the type of transplant, non-relapse mortality varies between 10% and 30%.[38] In relapsed MM, allo-SCT should only be considered for high-risk selected patients with a first treatment-responsive relapse and, at present, it is not recommended outside of clinical trials.[64]

LIMITATIONS OF CURRENT THERAPEUTIC OPTIONS

About 30% of patients will develop renal insufficiency over the course of the disease, and 20% will present renal failure.[65,66] The latter subpopulation is frequently excluded from aggressive strategies and HDT prior to ASCT (as being at higher risk of disease and treatment-related complications), which consequently lowers their prognosis. Novel agents such as BTZ can successfully restore renal function by relieving the MM burden in a proportion of patients, but early detection of renal impairment and prevention are essential to avoid complications.

In addition to being more sensitive to renal impairment, elderly patients over the age of

65 often present concurrent diseases, which exclude them from ASCT eligibility criteria. These patients have a lower physical reserve and are more prone to treatment-related side-effects and toxicities. While standard therapy in this clinical setting used to be melphalan plus prednisone for several decades, the addition of either BTZ[67-69] or thalidomide[30,70] has demonstrated additional benefits in terms of response, PFS, and OS.[71]

Moreover, chemotherapy-related adverse events are challenging and affect health-related QoL; BTZ and thalidomide can induce peripheral neuropathies, while thalidomide and lenalidomide can be involved in the development of deep vein thrombosis and pulmonary embolism.[72] Overall, there still remain patient subpopulations and challenging clinical settings which need to be addressed, and whose prognosis with current strategies is extremely poor. Immunotherapeutic approaches could be one of the emerging and promising frameworks with which to close the gap and provide longer OS and PFS to these patients.

RECENT ADVANCES AND NOVEL THERAPIES

Novel therapies are being explored to improve first-line outcomes so that a smaller proportion of patients relapse or develop refractory disease. They also seem to provide RRMM patients with additional survival benefits (Table 1).

Immunomodulators

Pomalidomide

Pomalidomide is a structural analogue of thalidomide and lenalidomide that was approved by the FDA in 2013 for patients who underwent at least two prior lines of treatment, with disease progression occurring in the first 60 days of the last therapy course. Monotherapy with pomalidomide has demonstrated efficacy in RRMM by overcoming drug resistance encountered with lenalidomide and BTZ.[73-77] When associated with low doses of dexamethasone, the response rates increase and range from 47-63%.[75,78,79]

These results were confirmed by the recent results of a Phase III trial with patients treated with pomalidomide plus low-dose dexamethasone (versus high-dose dexamethasone). The OR was of 32% (versus 11%), with 1% of CR (versus 0%), 6% of very good PR (versus 1%), and 25% of PR (versus 10%), for a median duration of response of 7.5 months (versus 5.1 months).[80] An extension study is currently ongoing to evaluate the pomalidomide monotherapy in subjects who discontinued treatment with high-dose dexamethasone due to disease progression.[81]

This novel immunomodulator has a different and improved safety profile when compared to thalidomide. Indeed, pomalidomide-related peripheral neuropathies are rare, but the most common adverse event is myelosuppression.[44] Pomalidomide can be combined with several other agents including proteasome inhibitors. As an example, updated Phase II results for the combination of pomalidomide plus carfilzomib and dexamethasone were recently presented. In heavily pre-treated patients with RRMM the OR was 70%, with 27% very good PRs, for a median PFS of 9.6 months.[82]

Proteasome Inhibitors

Carfilzomib

Carfilzomib (PR-171) is a novel proteasome inhibitor approved by the FDA in 2012 for patients who have undergone at least two prior lines of treatment with disease progression occurring in the first 60 days of the last therapy course. This approval was a consequence of the very promising results of a Phase II clinical trial.[83] Indeed, carfilzomib has been shown to provide clinically meaningful responses, even in heavily pre-treated and BTZ-refractory patients with RRMM: the ORR was 23.7% with a median OS of 15.6 months for a median duration of response of 7.8 months.

A Phase III clinical trial is currently ongoing (namely the ASPIRE trial)[84] and evaluates carfilzomib plus lenalidomide and dexamethasone versus lenalidomide plus dexamethasone. Another ongoing Phase III study, the FOCUS study,[85] is aimed at comparing carfilzomib with the best supportive care in MM patients who no longer respond to treatment. Enrolment is complete and early results for both studies are expected later in 2014.

Two other clinical trials of carfilzomib are currently recruiting participants: the ENDEAVOR Phase III study[86] will evaluate carfilzomib plus dexamethasone against BTZ plus dexamethasone in patients with MM whose disease has relapsed after at least one, but not more than three prior therapeutic regimens; the CLARION study[87] aims to compare carfilzomib plus melphalan

and prednisone versus BTZ plus melphalan and prednisone in first-line therapy.

Marizomib

In February 2014, the FDA granted the Orphan Drug designation to marizomib (NPI-0052) for the treatment of MM following the early results of four Phase I/II studies.[88-91] A Phase I/II study is currently ongoing in highly refractory MM patients, including those presenting with carfilzomib resistance, in combination with dexamethasone.[92] Another Phase I/II study is evaluating marizomib in combination with pomalidomide and dexamethasone in RRMM, including patients who are resistant to carfilzomib.[80,91,93]

Table 1: Recent findings and future perspectives in MM research.

Class	Compound	Study Type/Name	Clinical setting	Treatment arms	Main findings
Immuno-modulators	**Pomalidomide**	Phase II[71] NCT01464034	Heavily pre-treated pts with RRMM	POM + CFZ + DEX	• OR: 70%, with 27% very good PRs, 36% of PRs • Median PFS: 9.6 months
		Phase III - MM-003; NIMBUS[69] NCT01311687	RRMM	POM + low-dose DEX vs. high-dose DEX alone	• OR: 32% (vs. 11%), with 1% of CRs (vs. 0%), 6% of very good PRs (vs. 1%), and 25% of PRs (vs. 10%) • Median duration of response: 7.5 months (vs. 5.1)
		Phase III (NIMBUS extension study)[70] NCT01324947	Pts who discontinued high-dose DEX (disease progression)	POM monotherapy	• Ongoing
Proteasome inhibitors	**Carfilzomib (PR-171)**	Phase II - PX-171-003-A[172] NCT00511238	Heavily pre-treated and BTZ-refractory pts with RRMM	CFZ monotherapy	• ORR: 23.7% • Median OS: 15.6 months • Median duration of response: 7.8 months
		Phase III - ASPIRE[73] NCT01080391	RRMM	CFZ + LEN + DEX vs. LEN + DEX	• Ongoing
		Phase III - FOCUS[74] NCT01302392	MM pts who no longer respond to treatment	CFZ vs. BSC	• Ongoing
		Phase III – ENDEAVOR[75] NCT01568866	Pts relapsing after 1-3 therapeutic regimens	CFZ + DEX vs. BTZ + DEX	• Ongoing
		CLARION[76] NCT01818752	Newly diagnosed MM	CFZ + MEL + P vs. BTZ + MEL + P	• Ongoing
	Marizomib (NPI-0052)	Phase I/II[81] NCT00461045			• Ongoing
		Phase I/II[82] NCT02103335	Highly refractory MM pts, including CFZ resistance	MAR + POM + DEX	• Ongoing

Table 1 continued.

Class	Compound	Study Type/Name	Clinical setting	Treatment arms	Main findings
Proteasome inhibitors	**Ixazomib (MLN9708-MLN 2238)**	Phase I/II[87] NCT01383928	First-line therapy of newly diagnosed MM	IXA + LEN + DEX	• 95% of responses (21% stringent CRs, 5% CRs, 11% nCRs, 38% very good PRs, and 20% PRs) • Median duration of response of 14 months
	Oprozomib (ONX0912)	Phase Ib/II[88] NCT01832727	RRMM	OPZ + DEX	• Ongoing
Histone deacetylase inhibitors	**Panobinostat**	Phase III - PANORAMA-1[91,92] NCT01023308	RRMM	PAN or placebo + BTZ + DEX	• PAN significantly extended PFS • Full results are still being evaluated
	Panobinostat	Phase II -PANORAMA-2[91] NCT01083602	Relapsed and BTZ-refractory MM	PAN + BTZ + DEX	• Ongoing
	Vorinostat (MK-0683	Phase I/II[93,94] NCT01394354	RRMM	VOR + BTZ + DOX + DEX	• Interim analysis: the ORR was of 65%, for a clinical benefit rate of 89%
	Rocilinostat (ACY-1215)	Phase Ib[95] NCT01583283	RRMM	ROC + LEN + DEX	• 100% of responses, 69% achieved a PR or better (6% CR, 19% very good PRs, 44% PRs)
Monoclonal antibodies	**Elotuzumab**	Phase III - ELOQUENT-1[97] NCT01335399	Newly diagnosed, previously untreated MM	ELO + LEN + DEX	• Ongoing
		Phase III - ELOQUENT-2[98] NCT01239797	RRMM	ELO + LEN + DEX	• Ongoing
	Daratumumab	Phase I/II[99] NCT00574288	RRMM	DAR	• Ongoing
		Phase I/II[100] NCT01615029	RRMM	DAR + LEN + DEX	• Ongoing
		Phase III[101]	RRMM	DAR + BTZ + DEX vs. BTZ + DEX alone	• Ongoing
	SAR650984	Phase I[102] NCT01084252	CD38+ hematological malignancies	Dose-escalation study	• SAR650984 shown encouraging single-agent activity in pts with heavily pretreated RRMM

BSC: best supportive care; BTZ: bortezomib; CFZ: carfilzomib; CR: complete response; DAR: daratumumab; DEX: dexamethasone; DOX: doxorubicin; ELO: elotuzumab; IND: indatuximab; IXA: ixazomib; LEN: lenalidomide; MAR: marizomib; MEL: melphalan; MM: multiple myeloma; nCR: near complete response; OPZ: oprozomib; OR: overall response; ORR: overall response rate; P: prednisone; PAN: panobinostat; PFS: progression-free survival; POM: pomalidomide; PR: partial response; pts: patients; ROC: rocilinostat; RRMM: relapsed or refractory multiple myeloma; VOR: vorinostat.

Preliminary reported adverse events include fatigue, nausea, vomiting, dizziness, weight loss, and shortness of breath, but so far no peripheral neuropathy, anaemia or thrombocytopaenia were observed.[94]

Ixazomib

Ixazomib (MLN9708) is the first oral proteasome inhibitor[95] and has demonstrated a more favourable pharmacokinetic and pharmacodynamic profile when compared with BTZ in pre-clinical studies.[96] In a Phase I/II study of ixazomib in combination with lenalidomide and dexamethasone for first-line therapy of newly diagnosed MM,[97] 95% of the 56 patients achieved a response (21% of stringent CR, 5% of CR, 11% of nCR, 38% of very good PRs, and 20% of PRs) for a median duration of response of 14 months. These results are very encouraging, as observed in similar studies for carfilzomib plus lenalidomide and dexamethasone.

Oprozomib

Oprozomib (ONX0912) is a newly formulated proteasome inhibitor which is an analogue to carfilzomib.[98] It is presently being developed as an oral therapy in a Phase Ib/II study.[99] The optimal administration (2/7 versus 5/14 days) still needs to be determined as the maximum tolerated dose. Gastrointestinal toxicities seem to be the most challenging adverse effects.

Histone Deacetylase Inhibitors

New histone deacetylase inhibitors are under evaluation in MM. Phase I results have shown a very favourable safety profile but their efficacy as single agents is moderate. Phase II clinical trials have established promising results as combination therapies with BTZ and dexamethasone.[100]

Panobinostat

Recent results from a Phase III clinical trial (PANORAMA-1)[101] investigating panobinostat in combination with BTZ and dexamethasone showed that this new combination significantly extended PFS in RRMM when compared with BTZ plus dexamethasone alone. While these results represent a high therapeutic potential, full results from this study are still being evaluated. Additionally, a Phase II study (PANORAMA-2) is currently ongoing to assess the efficacy of panobinostat in patients with relapsed and BTZ-refractory MM.[102] In June 2014, Novartis submitted an application to the FDA for the approval of panobinostat, and the drug was granted priority review.[103]

Vorinostat

Whilst the results of the combination of vorinostat plus BTZ were rather disappointing with a PFS benefit of only 1 month,[104] preliminary results of a Phase I/II study on vorinostat associated with BTZ, doxorubicin, and dexamethasone were recently presented at the 2013 American Society for Hematology (ASH) meeting. A response was observed in 65% of patients. 22% of patients experienced severe adverse events; the most common reported Grade 3/4 adverse events were thrombocytopaenia, neutropaenia, and anaemia.[104,105]

Rocilinostat

This novel agent was assessed in a Phase Ib study in combination with lenalidomide and dexamethasone for RRMM. Early results were reported at the 2013 ASH meeting:[106] 100% of patients experienced a response, with 69% achieving a PR or better (6% CR, 19% very good PRs, 44% PRs). Overall, rocilinostat was well tolerated.

Monoclonal Antibodies (mAbs)

Elotuzumab

mAb therapy in MM is a very promising perspective. Elotuzumab as a single agent shows limited efficacy, but good results were achieved in combination with lenalidomide and low-dose dexamethasone.[107] Two Phase III clinical trials (ELOQUENT-1108 and ELOQUENT-2109) are currently ongoing or recruiting participants to evaluate elotuzumab plus lenalidomide and dexamethasone for newly-diagnosed MM or RRMM, respectively.

Daratumumab

Daratumumab, a very promising anti-CD38 antibody, was granted 'breakthrough therapy designation' from the US FDA for the treatment of patients with MM who have received at least three prior lines of therapy. Daratumumab is currently being evaluated in two Phase I/II studies on RRMM,[110,111] either as a single-agent or in combination with lenalidomide and dexamethasone. Additionally, a Phase III study on daratumumab in combination with BTZ and dexamethasone versus BTZ and dexamethasone alone in RRMM was recently announced.[112] Daratumumab's sponsor has

also announced a high-priority Phase III registration trial of lenalidomide plus dexamethasone versus lenalidomide plus dexamethasone and daratumumab in RRMM.[113]

Other mAbs

Other mAbs such as SAR650984 and indatuximab have displayed impressive single-agent activity in MM and are currently being evaluated in several phases of the disease. SAR650984 was recently evaluated in a Phase I/II study and demonstrated encouraging single-agent activity in heavily pre-treated RRMM patients.[114] Indatuximab is part of a novel approach, an antibody-drug conjugate, where it is combined to the cytotoxic agent DM4. Early results are very encouraging.[115]

Other Emerging Therapies

Other emerging agents for the treatment of MM include filanesib (ARRY-520),[116] a kinesin spindle protein inhibitor, and the Akt inhibitor afuresertib (PKB115125).[117] Bendamustine, an older alkylating agent also continues to be investigated in MM.[118,119]

CONCLUSIONS

Advances in the therapeutic management of MM with new strategies and a developed armamentarium have significantly improved outcomes and extended survival in RRMM patients. However, the continuing challenges within specific clinical settings and patient subpopulations, whose prognosis with current strategies is extremely poor, call for a paradigm change.

The development of new combination strategies and novel therapies is crucial to improve the clinical outcome and to overcome resistance in MM. This should help clinicians to reduce the burden of multiple and toxic therapies, as QoL should be at the core of MM management. Patient selection and stratification need to be reinforced with the help of comprehensive knowledge on molecular pathways, in order to provide tailored options in therapeutic strategies. Numerous drugs are on the horizon and the next few years should witness marked improvements in terms of OS, PFS, QoL, and safety. As of yet, predictive biomarkers as guidance for treatment are largely lacking, making the approach to patients still an empirical one.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: IS IT STILL THE RIGHT CHOICE?

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ABSTRACT

Autologous stem cell transplantation (ASCT) is considered the standard of care for multiple myeloma patients aged <65 years with no relevant comorbidities. The addition of proteasome inhibitors and/or immunomodulatory drugs has significantly increased the percentage of patients achieving a complete remission after induction therapy, and these results are maintained after high-dose melphalan (Alkeran®), leading to a prolonged disease control. Studies are being carried out in order to evaluate whether short-term consolidation or long-term maintenance therapy can result in disease eradication at the molecular level, thus also increasing patient survival. The efficacy of these new drugs has raised the issue of deferring the transplant after achieving a second response upon relapse. Another controversial point is the optimal treatment strategy for high-risk patients, that do not benefit from ASCT, and for whom the efficacy of new drugs is still matter of debate.

Keywords: Autologous transplantation, myeloma.

STATE-OF-THE-ART

For many years the gold standard treatment for multiple myeloma (MM) was the combination of melphalan and prednisone (Deltasone®) (MP),[1] as different polychemotherapy regimens failed to demonstrate a superior efficacy.[2] MP was able to induce a response in >40% of treated patients; complete responses, however, were achieved in <5% of the cases, and overall patient survival did not exceeded 3 years. The first step towards the introduction of autologous stem cell transplantation (ASCT) in MM was the demonstration of a dose-response effect of melphalan in MM cells.[3] The potential to overcome resistance to melphalan by using higher doses of the drug was subsequently explored *in vivo*;[4] 27% of newly diagnosed patients reached a complete response (CR) upon treatment with high-dose melphalan (HDM), and this translated into a prolonged survival, even though treatment-related mortality was unacceptably high. In order to reduce the duration of profound cytopaenia related to the use of HDM, autologous stem cell rescue was then introduced in the clinical practice, initially for relapsed/refractory disease, and then for newly diagnosed MM.[5,6]

The formal demonstration that ASCT is superior to conventional chemotherapy in terms of response, duration of response, and overall survival (OS), came from two randomised trials, from the Intergroup Francophone du Myeloma (IFM)[7] and the Medical Research Council (MRC).[8] In order to ameliorate these results, the application of two subsequent ASCTs was then explored by IFM[9] and by the Bologna Follow-up Group;[10] both studies demonstrated an improvement in response rate (RR) and event-free survival (EFS); however, only the French study was able to show a survival advantage for patients receiving a double ASCT. Further analysis of the IFM trial[9] showed that a second ASCT could result in an increased OS only in patients failing to achieve at least a very good partial response (VGPR) after the first ASCT; these data were in agreement with a subanalysis of the Bologna trial[10] showing an improved EFS after a second ASCT in patients failing to achieve at least a near-CR after the first one. While the use of a

double ASCT is still matter of debate, from the late 90s onwards, a single ASCT has been referred as the standard of care (SoC) for newly diagnosed MM patients <65 years with no relevant comorbidities.

In addition to the clinical benefit offered by ASCT, in recent years the therapeutic results for MM have significantly improved due to the availability of drugs that are active both on neoplastic plasma cells and on bone marrow microenvironment, such as thalidomide (Thalidomid®), lenalidomide (Revlimid®), and bortezomib (Velcade®). Thalidomide was the first agent included in induction therapy for newly diagnosed MM patients eligible for ASCT; the drug was used in combination with high-dose dexamethasone, i.e. thalidomide-dexamethasone (TD), yielding interesting results as compared to conventional chemotherapy in a case-match retrospective analysis[11] or to high-dose dexamethasone in a prospective randomised trial.[12] In a further randomised trial (Total Therapy 2),[13] thalidomide was continuously applied in the various phases of the whole treatment programme until patient relapse; again, an advantage in terms of CR rate and EFS was observed in patients treated with thalidomide as compared to those not receiving the drug, but OS was similar in the two groups of patients. Subsequent trials were designed to evaluate the combination of TD with doxorubicin (Adriamycin®);[14] a significant improvement in RR was observed when compared to conventional chemotherapy (vincristine [Oncovin®]-doxorubicin-dexamethasone [Decadron®] [VAD]). Bortezomib was tested in combination to dexamethasone (VD) in a Phase II study;[15] a VGPR rate of >30% was achieved after induction and upgraded to >50% after ASCT. A further Phase II study was designed with the aim to compare VD to conventional VAD;[16] again the arm treated with the novel regimen showed a significantly higher RR (38% VGPR or better versus 15%) that was confirmed after ASCT. The combination of VD with cyclophosphamide (Cytoxan®) (VCD) was able to induce a VGPR or better in >60% of the patients,[17] similar results were reported using VD+ doxorubicin (PAD).[18] Lenalidomide was studied in a randomised trial in combination to high (RD) versus low (Rd) doses with dexamethasone.[19] After four courses patients were allowed to undergo ASCT or to proceed with the same therapy; even though RR was significantly higher in the RD group, survival was the same due to the higher toxicity experienced by the patients treated with high-dose dexamethasone.

A further improvement in the results obtained with novel drugs ± steroids ± chemotherapy was achieved by combining two novel drugs with dexamethasone. The combination bortezomib-thalidomide and dexamethasone (VTD) was randomly compared to TD as induction therapy prior to ASCT (Table 1), yielding a significant advantage in terms of response, both CR and VGPR.[20] These data were confirmed by a recent study of the Pethema group.[21] A bortezomib + thalidomide-containing regimen was also used in the Total Therapy 3 trial,[22] in the context of a polychemotherapy programme involving induction, ASCT, consolidation, and maintenance; as compared to Total Therapy 2, in which only TD was used,[13] a significant prolongation of EFS was observed. A randomised study conducted by the IFM in newly diagnosed MM patients[23] demonstrated that the triple combination VTD, with reduced dose bortezomib and thalidomide, was superior to VD in terms of response, both after induction and after ASCT. So far, these results indicate that induction therapy in preparation to ASCT should include bortezomib + dexamethasone + an immunomodulating agent, either thalidomide or lenalidomide, that is presently being explored in Phase II trials.[24]

DEBATED ISSUES

Is Complete Remission a Goal to be Pursued?

When MP was the only available therapeutic strategy for MM, the attainment of CR was no matter of concern as only a minority of patients could achieve a minimal residual disease status. The introduction of more aggressive therapeutic programmes including ASCT prompted a better evaluation of minimal residual disease, also including cytofluorimetric analysis[25] and molecular techniques.[26] At present, the International Myeloma Working Group (IMWG)[27] has provided the definition of 'stringent CR' including negative serum/urine immunofixation together with a normal serum free-light chain ratio and absence of clonal plasma cells in the bone marrow. Several groups have analysed the relationship between CR and patient outcome, and have pointed out that CR is a strong predictor of survival,[28] especially when extended over several years;[29] for this reason it is now generally recognised that every effort should be made in order to achieve maximal disease eradication through the various phases of the treatment programme.[30]

Table 1: Results obtained with novel drug combinations used as induction therapy prior to ASCT.

		Induction	Post ASCT		
Author (reference)	Regimen	≥VGPR (%)	≥VGPR (%)	PFS	OS
Harousseau[16]	**VD**	38	54	36 months	81% at 3 years
Reeder[17]	**VCD**	61	74	NR	NR
Sonneveld[18]	**PAD**	42	61	35 months	NR
Cavo[20]	**VTD**	62	82	68% at 3 years	86% at 3 years
Rosinol[21]	**VTD**	60	46 (CR)	56.2 months	74% at 4 years
Richardson[24]	**RVD**	61	NR	75% at 18 months	97% at 18 months
Rajkumar[48]	**Rd**	40	NR	63% at 2 years	92% at 3 years

ASCT: autologous stem cell transplantation; VGPR: very good partial response; PFS: progression free survival; OS: overall survival; VD: bortezomib + dexamethasone; VCD: bortezomib + cyclophosphamide + dexamethasone; PAD: bortezomib + dexamethasone + doxorubicin; VTD: bortezomib + thalidomide + dexamethasone; RVD: lenalidomide + bortezomib + dexamethasone; Rd: lenalidomide + dexamethasone (low dose); CR: complete response; NR: no response.

Can Consolidation or Maintenance Therapy Improve Patient Outcome?

The administration of some kinds of treatment upon completion of major therapy in order to improve/maintain its efficacy represents the SoC in several lymphoproliferative neoplasms, such as acute lymphoblastic leukaemia, low-grade lymphoma, or mantle cell lymphoma, and for this reason it has been considered an attractive option also for MM.

Consolidation therapy is defined as a short course of treatment administered after ASCT, which is aimed at further reducing tumour load. A study from the Nordic group[31] has evaluated the efficacy of a short course of Bortezomib, and an increased percentage of CRs was observed. Two different studies analysed the effects of a short course of VTD administered as consolidation after ASCT, and both trials showed that a molecular response can be achieved in up to 60% of the patients.[32-34] Maintenance therapy is defined as long-term treatment aiming at preventing disease recurrence or progression. Alpha interferon has been widely tested after ASCT, and despite two reports showing an improved survival, side-effects greatly overcome the possible advantage, so that this approach has been definitely abandoned.[35] A limited efficacy was also reported with long-term use of steroids.[36] Thalidomide has been studied in six trials,[13,14,37-40] and in three, the drug was also used in induction phase. Although all the trials showed an advantage in terms of EFS or progression free survival (PFS), an OS advantage for patients treated with thalidomide was observed only in two trials. A major concern regarding the use of this drug as maintenance therapy is the high percentage of patients dropping out due to long-term side-effects, specifically peripheral neuropathy.[36-39] Furthermore, the likelihood of selecting MM clones resistant to thalidomide and responsible for short post-relapse survival should probably be taken into consideration[13,14,40] as well as the limited efficacy of the drugs in case of poor-risk cytogenetics.[39] Due to its favourable toxicity profile, and specifically to the lack of long-term neurological toxicity, lenalidomide has been tested as maintenance therapy in two randomised studies,[41-42] both of which showed a significant advance in time to progression, while OS was significantly improved only in one study.[42] Side-effects were mainly hematological, and a higher percentage of second primary malignancies were observed in lenalidomide-treated patients;[41,42] however, these data need further observation as it is clear that survival benefit outgrows the risk of death from second malignancies.[43] A recent report analysed the role of bortezomib maintenance after ASCT;[18] patients showed a significant advantage in terms of PFS and OS, even though the potential of neurological toxicity should be taken into consideration. Despite these interesting results, however, data are not mature enough to recommend a specific strategy, and the issue of consolidation and/or maintenance treatment remains still debated.

Should ASCT be Performed Upfront or After First Relapse?

Early studies on ASCT in MM were performed in patients with relapsed/refractory disease but, due to the poor results that were obtained,[44] the procedure is now preferentially employed in newly diagnosed patients.[45] Furthermore, a time-dependent application of ASCT seems to be crucial in determining an optimal response.[46] A randomised study from the French group,[47] however, demonstrated a comparable outcome in terms of survival in patients undergoing early versus deferred ASCT (64.4 versus 64 months OS). These data were obtained when only chemotherapeutic agents were available; it is now evident that new drugs, when applied during induction, are able to determine a deeper response than that obtained with conventional chemotherapy combinations. Several groups have thus designed studies aimed at evaluating efficacy of long-term treatment with new drugs as compared to ASCT,[48,49] resorting to transplant only upon relapse. Results that have been published so far failed to show a difference in patient survival even though early ASCT is related to a shorter duration of treatment and drug exposure. A recent retrospective study has shown that, in patients treated with thalidomide or lenalidomide followed by early stem cell mobilisation,[50] comparable results were achieved after early versus late ASCT. Data from further studies are awaited.

Is ASCT Feasible in Elderly Patients?

Patients aged >65 years are not considered good candidates to ASCT as their survival is significantly shorter than that observed in younger patients (50% versus 68%, respectively, at 5 years[51]). Several reports, however, have identified a 'grey zone' represented by patients aged 65-70, who are in good clinical condition, and who could potentially take advantage from this procedure. In particular, a randomised study conducted in these patients has demonstrated that intermediate dosage of melphalan (100 mg/m^2) with PBSC support results in a significantly prolonged EFS and OS as compared to MP.[52] On the other hand, a later study conducted in older patients (65-75 years) failed to show the advantage of intermediate melphalan dose as compared to MP, and both regimens were inferior to the combination MP + thalidomide.[53] At present, however, MP does not represent the SoC for elderly MM patients, and no data can unequivocally establish whether an ASCT program including new drugs can be useful in older patients as it happens in younger ones. Only one Phase II study has been reported which aimed to evaluate the toxicity and the efficacy of bortezomib and lenalidomide included in pre-transplant induction and post-transplant consolidation and maintenance in patients aged 65-75 years.[54] The percentage of patients obtaining a CR increased progressively through the various phases of the treatment programme (13% after induction, 43% after transplant, and 73% during consolidation/maintenance) and hematological and non-hematological toxicities were acceptable. These data indicate that an ASCT programme including new drugs can be safely performed in selected elderly patients, thus representing a possible therapeutic option.[55]

Is ASCT the Best Treatment for High-Risk Patients?

In recent years, many attempts have been made in order to identify patients at high-risk of relapse and poor survival, and several parameters have been taken into consideration. The simplest and cheapest one is the International Staging System prognostic model,[56] designed by the IMWG, based on beta-2 microglobulin and albumin level; a significantly different survival (62 months, 44 months, and 29 months) was shown in Stage 1, 2, or 3 patients, respectively. The major pitfall of this risk stratification is that it does not take into account cytogenetic alterations that are now considered the main parameter affecting patient prognosis. No agreement exists on which - among fluorescence *in situ* hybridisation, comparative genomic hybridisation, and gene expression profile - is the best method to use in order to detect chromosomal abnormalities. However, patients showing t(4;14), t(14;16) deletion 17p[57] or 1q abnormalities[57,58] carry a worse prognosis and should be treated differently from patients with no chromosomal abnormality.[59] Very few data, however, are presently available concerning the efficacy of different therapeutic regimens in poor-risk patients. A bortezomib-containing induction therapy seems to improve the outcome of patients carrying t(4;14).[20,21] This is not the case for thalidomide,[60] especially in maintenance trials,[36] while conflicting results were reported regarding lenalidomide-dexamethasone induction.[61] On the other hand, patients with 17q deletion seem not to benefit from bortezomib followed by ASCT.[62] Dose-dense regimens, upfront myeloablative ASCT,

or novel agents are presently proposed for high-risk patients in the context of clinical trials, which are aiming at finding a proper therapeutic approach.

CONCLUSION

In the last few years the outcome of MM patients has significantly improved with the introduction of novel drugs in the clinical practice. The inclusion of thalidomide, lenalidomide, or bortezomib in various combinations in the different phases of an ASCT programme increases the percentage of patients achieving a CR, thus, potentially leading to patient cure. Data are not mature enough, so far, to establish whether a combination of new drugs, administered for a prolonged period of time, could render ASCT unnecessary. At present, in many US Institutions, both physicians and patients are in favour of a delayed ASCT policy in order to avoid complications related to the period of myelosuppression related to the procedure. It cannot be taken for granted, however, that patient quality of life may be worse in the case of a short time myelosuppression as in ASCT, rather than in the case of a prolonged therapy with any of the new drugs that are presently available and whose side-effects are well known. At present, at least in Europe, ASCT is still considered the SoC for young patients with newly diagnosed MM, and the issue is how the results can be further improved. A number of new drugs are presently being tested in MM, at various disease phases. Among them is carfilzomib (Kyprolis®), an irreversible proteasome inhibitor that, after having proven effective in relapsed/refractory disease, has been tested in combination with lenalidomide in newly diagnosed MM patients[63] inducing up to 40% stringently defined CR. Pomalidomide (Pomalyst®), a thalidomide derivative, has demonstrated to be effective even in lenalidomide or bortazomib-refractory patients.[64] These drugs will probably be included into induction therapy prior to ASCT in order to further improve disease eradication.

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THE ROLE OF JAK2 MUTATION IN THROMBOTIC COMPLICATIONS OF CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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ABSTRACT

Patients diagnosed with myeloproliferative neoplasms (MPNs) often develop thrombotic events as an onset of symptoms or in evolution. The pathogenesis of thrombosis in patients with MPN is multifactorial. There are multiple prognostic score systems, but the presence of JAK2V617F (JAK2) mutation is an independent and strong thrombosis risk factor. Patients with MPN and JAK mutational status usually associate thrombocytosis, increased immature circulating platelets, and leukocytosis, with increased expression of CD62P and CD14, increased levels of circulating microparticles and leuko-platelet microaggregates, and altered endothelial function. This review aims to discuss different factors contributing to the increased thrombotic risk in association with JAK2 mutational status. Also, recent reports incriminate this mutation to have a possible role in spontaneous loss of pregnancy.

Keywords: Chronic myeloproliferative neoplasm, JAK2V617F mutation, thrombosis, leuko-platelet microaggregates.

INTRODUCTION

Patients diagnosed with chronic myeloproliferative neoplasms (MPNs) frequently develop thrombotic complications; patients with polycythaemia vera (PV) and essential thrombocythaemia (ET) in particular are exposed to an increased thrombotic risk.[1] These complications may often be the onset manifestation of MPNs. The annual incidence of thrombotic complications in MPNs is around 1-10%.[2] Age, history of thrombosis, leukocytosis, and JAK2V617F (JAK2) mutation are all considered risk factors for thrombotic complications.[2,3] JAK2 mutation represents a G>T transversion at nucleotide 2,343, resulting in a substitution of phenylalanine for valine (V617F) in the JAK2 protein with tyrosine kinase activity (loss of autoinhibitory control).[4] Clinical studies report a correlation between disease phenotype and JAK2 (V617F) mutant alleles introducing the concept of allele burden, considered to be the ratio between mutant and wild type JAK2 in hematopoietic cells.[5]

A predictive risk score for thrombosis in ET patients was identified. This model provides a better prognostic risk stratification of patients compared with the classic one; the thrombotic risk was estimated to be from 0.95% patients/year (low risk), to 2.86% patients/year (high risk). Risk factors included in this model were: age over 60, history of thrombosis, cardiovascular risk factors, and the presence of JAK2 mutation.[6] If for the first 5 years after diagnosis, leukocytosis, age >60 years, and history of thrombosis were considered to be the most important predictive risk factors for thrombosis, after 5 years of evolution the single most powerful risk factor was the presence of JAK2 mutation. These patients are considered to have a 2-fold increased thrombotic

risk compared with JAK2 negative patients.[7-9] JAK2 allele burden is not a predictive risk factor for thrombosis or acute transformation, but JAK2 allele burden >50% is associated with increased risk of progression to primary myelofibrosis (PMF).[8,9]

Approximately 90% of patients with PV present JAKV617F mutation, whereas in ET and PMF patients, the prevalence of the mutation is around 60%. >50% of refractory anaemia with ringed sideroblasts and thrombocytosis patients are also JAK2 positive, as well as 20% of MPN unclassifiable cases.[10] Other somatic mutations that may appear in MPN are exon 12 JAK mutation (<5% of PV cases) and MPL W515F. The latter has been identified in 10% PMF and 1% ET patients, but not in PV.

Recently the presence of insertions or deletions in exon 9 of the CALRE (endoplasmic reticulum chaperone) has been described. CALRE and JAK2 mutations were not observed concomitantly in the analysed patients, and, among those JAK2 negative, 67% and 88% of ET and PMF patients, respectively, presented CALRE mutation. CALRE positive patients associated an increased number of platelets but with a lower risk of thrombosis and decreased overall survival compared to JAK2 positive patients.[11]

CLINICAL FEATURES

Thrombotic complications in MPN patients are represented by microcirculatory events and venous or arterial thrombosis. Microcirculatory symptoms are erythromelalgia, transient ischaemic attacks, transient hearing or visual impairment, recurrent headache, and peripheral paresthaesia. Less common occurrences are: dysarthria, temporary loss of monocular vision, and mono/hemiparesis. Venous commonly affected sites are abdominal veins (portal, hepatic, and mesenteric) and cerebral venous sinuses; less frequent are deep vein thrombosis (DVT) of lower limbs - associated with high risk of pulmonary embolism - and superficial vein thrombosis of the lower limbs.

50% of Budd-Chiari syndromes and 25% of patients with portal vein thrombosis (PVT) are diagnosed with MPNs.[1] For many of these, intra-abdominal thrombosis may be the early sign of an undiagnosed MPN. At this point, the presence of the JAK2 mutation may be the only indication of undiagnosed MPN.[12] Screening for JAK2 in patients diagnosed with splanchnic vein thrombosis (SVT) and no other hematological changes revealed the presence of this mutation in 17.1% of Budd-Chiari syndrome patients and in 15.4% of PVT patients.[13] Thrombotic events in MPN patients may precede the onset of any hematologic abnormalities within 1-2 years.[14]

JAK2 positive MPN patients may develop not only thrombotic complications of abdominal veins, but also thrombosis of cerebral or retinal veins, DVT of lower limbs, and pulmonary thromboembolism. The incidence of such complications in MPN is similar to the percentage of thrombotic events in the general population.[15] MPN cases diagnosed with Budd-Chiari syndrome present a higher incidence of this mutation compared to MPN patients with DVT.[13] Arterial thrombosis is more common than venous thrombosis.[3] Arterial thrombosis accounts for approximately 60-70% of thrombotic events occurring in MPN, with clinical features of stroke, myocardial infarction, or peripheral arterial occlusions. Strokes are by far the most frequently encountered thrombotic events (30-40%).[1] MPL515 mutation was identified in a small percentage of MPN patients with SVT (<1%). The clinical relevance of the presence of this mutation has not been fully understood.[13]

PATHOGENESIS OF THROMBOSIS

The pathogenesis of thrombosis in MPN patients is multifactorial (Figure 1). The hypercoagulant status is the result of blood cell abnormalities (erythrocytes, platelets, and leukocytes) arising from the clonal hematopoietic progenitor cells, which express a prothrombotic phenotype.[1]

Role of Red Blood Cells (RBCs)

Patients with MPN may present increased blood viscosity due to elevated numbers of RBCs and/or platelets. Elevated hematocrit represents a thrombotic risk factor especially for cerebral circulation.[1] This factor is well documented in PV patients. Along with elevated hematocrit, blood flow speed is also important. In veins with turbulent circulation, such as portal vein, the risk of thrombosis is lower. Hepatic veins present a greater risk of thrombotic complications, explaining the increased incidence of Budd-Chiari syndrome in PV patients.[13] Hepatic vein thrombosis is more frequent in PMF, because of enlarged spleen with compressive effect on portal vein system.[13] JAK mutations may affect cell prothrombotic phenotype, resulting in an increased RBC adhesion by modifying surface adhesion molecules.[1]

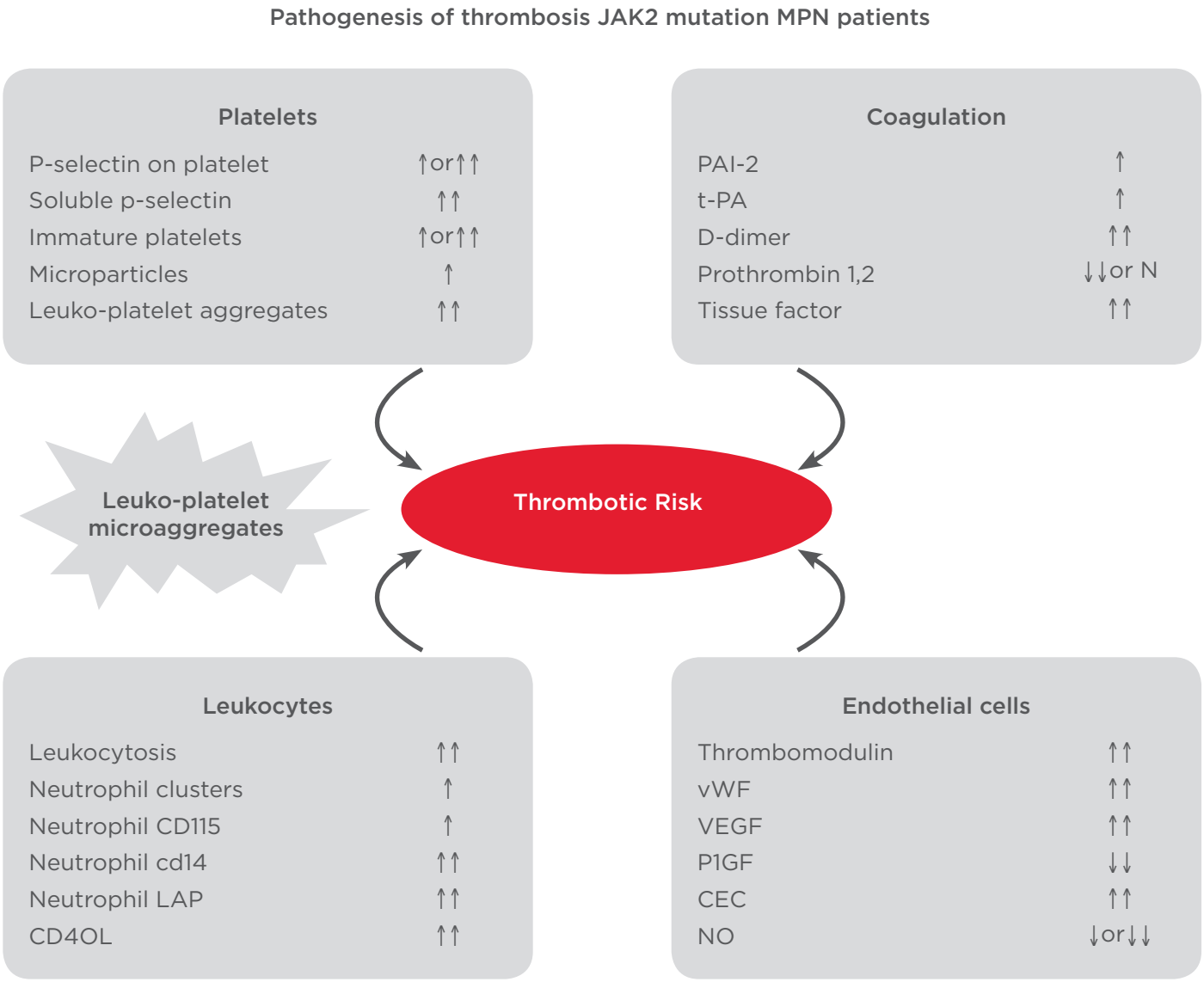

Figure 1: Multifactorial pathogenesis of thrombosis of MPN patients.
JAK: Janus kinase; MPN: myeloproliferative neoplasms; PAI-2: plasminogen activator inhibitor-2; t-PA: tissue plasminogen activator; LAP: leukocyte alkaline phosphatase; vWF: von Willebrand factor; VEGF: vascular endothelial growth factor; P1GF: placental growth factor; CEC: circulating endothelial cells; NO: nitric oxide.

Role of Platelets

Thrombocytosis is a paradoxal contributing factor in thrombosis and the platelet count *per se* has not been significantly correlated with thrombosis. The control of thrombocytosis is correlated with a decrease in the frequency of thrombotic complications. High platelet count (over one million/mmc) is generally protective against thrombosis because of acquired von Willebrand disease. In the ECLAP analysis, antiplatelet therapy, but not cytoreductive treatment, was significantly associated with a lower risk of thrombotic events.[1,3]

In a healthy population, 2% of circulating platelets are immature. Their number reflects the rate of thrombopoiesis. The percentage of immature platelets is increased in MPN patients, associated with an increased response to thrombin and P selectin expression on platelet surface. These platelets are more active in hemostasis. In fact, increased number of such immature platelets is considered a risk factor for thrombotic events. Studies report statistically significant correlations between immature platelets and JAK2 mutational status.[1] Platelets of MPN patients present high levels of CD63 and CD62P associated with acquired dense storage pool disease and ATP release deficiency.[16]

Acquired storage pool disease of dense granules involving ATP, ADP, calcium, serotonin, and pyrophosphate has been reported not only in

chronic MPNs, but also in acute MPNs as well. Defects are the result of granule formation, or the existence of empty vesicles developed after platelet activation. All results suggested that this association is due to chromosomal abnormalities on the megakaryocytic line, leading to a decrease in dense granule formation. The gene coding granule formation is closely located near one gene that in abnormal conditions might be involved in acute leukaemia or myelodysplastic syndrome (MDS) evolution.[17] Acquired storage pool disease of dense granules is associated with an increased secretion from alpha granules in MPN.[18] Increased expression of CD63 on inactive platelet surface and after platelet stimulation in PV patients, the level of P selectin expression on inactive platelet surface and after stimulation with arachidonic acid in ET patients, increased soluble P selectin, soluble CD40 ligand, and tissue factor expression on inactive platelet surface and after stimulation, all represent predictive factors for thrombosis. The level of platelet P selectin (CD62P) is higher in JAK2 positive MPN patients.[19]

JAK2 mutation modulates leukocyte and platelet activation with a great impact on platelet dysfunction. JAK2 mutation leads to an increase in JAK-STAT signalling pathway, activating signalling pathways such as PI3K, RAS, and SAT5 with hypersensitivity to cytokines, increased cell proliferation, and resistance to apoptosis.[20] Exon 12 mutation involves one amino acid located in the pseudokinase domain, suggesting a similar pathogenic mechanism with JAK2 mutation. Platelets of MPN patients present a preactivated conformation as a result of abnormalities of phospho-Tyr527 dephosphorylation with inhibitory effect. Src protein has an important role in this process. Activation of thrombin Src protein-dependent appears before JAK2 activation in normal and pathological platelets.[19,21,22] A strong correlation between the presence of JAK2 mutation and increased activity of platelet-neutrophil with platelet aggregates formation was established. Platelet activated status allows exposure of phosphatidylserine on surface membrane, normally present on the inside of the membrane, thereby providing a catalytic surface for thrombin generation, thus amplifying platelet activation.[23] In MPN patients a high level of plasma microparticles seems to be associated with an increased level of neutrophil - platelet aggregates and CD 62P, especially in those patients with a history of arterial or venous thrombosis.[24]

Platelet and megakaryocyte c-MPL expression is decreased in patients with PV and PMF, but not in patients with ET. Patients with JAK2 V617F mutation have lower levels. The mutation influences both the location and also the stability of c-MPL, contributing to platelet activation.[25-27]

Role of Leukocytes

Leukocytosis was also identified as a potential risk factor for arterial and venous thrombosis.[1] The pathogenic mechanisms are: the active status, the interactions between leukocytes and platelets, endothelial cells, and coagulation factors. The leukocytes contribute to the inflammatory process in atherosclerosis and therefore increase the probability of vascular events. The status of activated leukocytes is proved by CD11b overexpression and increased plasmatic levels of catepsin G, elastase, and myeloperoxidase. The increased expression of integrins and selectins promotes leukocyte adhesion to endothelium and to platelet membrane, thus contributing to the appearance of the aggregates of reactive oxygen species (ROS), and of inflammatory cytokines.[28]

Leuko-Platelet Microaggregates

Leuko-platelet microaggregates are often present in patients with MPN who associate thromboembolic accidents.[29] In JAK2 positive patients, leuko-platelet microaggregates are seen more frequently, as these patients present a higher level of tissue factor, soluble P-selectin, sCD40L, von Willebrand factor (vWF):Ag, and a lower level of free S protein, CD41, and CD42b receptors. Tissue factor expression is highly increased in JAK2 positive patients versus wild type.

Granulocyte-thrombocyte CD11b-CD42b aggregates and monocyte-thrombocyte CD11b-CD14-CD61 aggregates are more common in patients with MPN and MDS.[24,30] The presence of these aggregates does not affect the platelet surface antigen expression.[31] The level of aggregates is correlated with platelet count, P-selectin percentage, thrombospondin (TSP), and GP IV. The incidence is higher in patients with thrombosis and microcirculation abnormalities.[29,32] Alvarez Larran[33] considers that CD11b expression may be used as a marker for PV in patients presenting with Budd-Chiari syndrome. Patients with PMF have an increased risk of spontaneous aggregates. Treatment with aspirin plus hydroxycarbamidum is more effective versus aspirin for the prevention

of leuko-platelet aggregates CD11bCD42b and CD11bCD62P. The important role of hydroxycarbamidum is explained also by the inhibition of endothelin-1 and ICAM-1 expression, and the increased levels of NO, and therefore hydroxycarbamidum, has a strong antithrombotic effect.[25] Trelinski et al.[30] underlined the positive effect of aspirin in preventing leuko-platelet aggregates and the ineffectiveness of hydroxycarbamidum. Microparticles are formed due to alterations of the platelet membrane cytoskeleton and changes in phospholipid asymmetry; the latter ones express phosphatidyl-serine and in some cases active tissue factor, the main coagulation activation.[23]

Aspirin decreases CD11b and neutrophil-platelet aggregates (induced by *in vitro* stimulation), suggesting a lower leukocyte-platelet interaction. G-CSF receptor is connected to JAK2 pathway, and therefore, a constitutive activation of intracellular signalling in the presence of JAK2 mutation might be possible, contributing to the activated leukocyte status. Some markers such as CD14 expression and leukocyte alkaline phosphatase are significantly altered in JAK2 positive patients, while expressions of CD11b and plasma elastase level are not significantly different.[1]

The possible correlation between high levels of C reactive protein and low levels of pentraxin^3 as inflammation markers and thrombotic risk in MPN was studied by Barbui et al.,[34] proving a possible association with high statistical relevance. Both proteins were significantly correlated with increased prevalence of JAK2 mutation in patients with MPN. Still, when pentraxin^3 was >4.5 ng/ml, the thrombotic risk was lower.[28,34] Pentraxin^3 is produced at the inflammation site by endothelial cells, neutrophils, monocytes, and macrophages, and its level increases in sepsis, vasculitis, and autoimmune disorders. Its expression is induced by interleukin-1, tumour necrosis factor-alpha, and low density lipoproteins, but it is not influenced by the level of C reactive proteins.[34]

In patients with MPN, thrombopoietin may induce an increased platelet aggregation and release of dense granule content after exposure to standard stimuli (collagen, epinephrine, ADP). Platelet P-selectin expression and neutrophil CD14 expression are increased in patients with JAK2 positive ET, while CD11b is highly expressed of neutrophils and monocytes of PMF JAK2 positive patients. A higher level of platelet tissue factor and leuko-platelet aggregates was also observed in JAK2 positive ET patients. Also, JAK2 positive patients have increased levels of thrombomodulin and P-selectin.[1] Aside from higher P-selectin, patients with MPN also express a higher TSP level, especially the ones with antecedent thrombotic accidents. These are associated with lower glycoprotein (GP) IIb/IIIa and GPIIb expression.[29] GP IIb/IIIa receptor, an early megakaryocytic marker is higher in MPN, with up to a 33% increase (especially for GP IIIa).[35] While dormant platelets have low GP IIb/IIIa expression, patients with MPN present high levels when the platelets are stimulated with platelet-activating factor (PAF) or PFMA.[36] Fibrinogen induces conformational changes of GP IIb/IIIa receptor, and the altered expression corresponds to the status of activated platelet. Kaplan et al.[37] indicated the presence of a GP IIb/IIIa with normal fibrinogen receptor, but with altered (low) expression in MPN patients. During megakaryocytic maturation, GP IIIa expression decreases.[38] Platelet expression in MPN is correlated with higher thrombotic risk. In addition, patients with MPN present platelet aggregation anomalies due to lower adhesion molecule levels (GP-IIb, GP IIb/IIIa, GPIV, and GPVI) and deficient platelet metabolism (abnormal arachidonic acid metabolism).[1] In ET patients, higher levels of thromboxane A2, B2, PAC-1, PGF1, and PGI2 were observed. These levels are decreased under treatment with Ozagrel, a thromboxane A2 (TxA2) inhibitor, thus, decreasing the thrombotic risk.[39] Recently, an anomaly in the P2Y12 signalling pathway was observed in patients with MPN, which might be one of the contributing pathogenic mechanisms for the bleeding tendency in patients with very high platelet counts. The function of platelet P2Y12 is inversely correlated with white blood cell (WBC) count, platelet count, and JAK2 allele burden.[40]

Role of Endothelial Cells

Besides from the pathogenic mechanisms described above, recent studies have identified the endothelial cell dysfunction as a risk factor for thrombosis.[41] The normal endothelium acts as an antithrombotic surface which inhibits platelet adhesion and coagulation cascade activation. In MPN, it becomes a pro-adhesive and pro-coagulant surface, by exposure to higher levels of ROS, to intracellular proteases released by activated neutrophils, and to cytokines from inflammation site.[1,26] These may induce endothelial cell destruction and release of thrombomodulin, selectin, and vWF.

Selectins are also present on endothelial cells (P-selectin – CD62P, E-selectin – CD62E), platelets (P-selectin), and leukocytes (L-selectin).[1,42] Also, Type 1 intercellular adhesion molecules (ICAM-1, CD54) and vascular cell adhesion molecules (VCAM-1) are expressed, which contribute to leukocyte adhesion to endothelial cells and promote vascular occlusion.[42] The number of different circulating endothelial cells (CECs), the levels of vascular endothelial growth factor (VEGF), of soluble receptors for VEGF (sVEGFR-1,2), and of placental growth factor (P1GF) are all modified in patients with MPN. The number of CECs was significantly higher in patients with ET and PV versus controls, regardless of JAK2 status. Their number correlated with WBC count, probably as a consequence of the existence of a pathological hematopoietic clone.[42] The medium levels of activated CECs were significantly higher in patients with PV and increased WBC (cut off $8.7x10^9$/l). VEGF and soluble receptor sVEGFR-1 plasmatic levels were significantly increased in patients with ET and PV versus controls, while P1GF was decreased. Also, a higher level of D-dimers was observed in patients with MPN. Angiogenesis, reflected by CEC levels, is increased in patients with ET and PV, again regardless of JAK2 status. The presence of an increased level of CECs contributes to the pathogenesis of MPN, reflecting vascular injury. Angiogenic cytokines interact with other known prothrombotic factors.[1,43]

The endothelial cell also releases nitric oxide (NO), generated by oxidation of L-Arginine to L-Citrulline by NO-synthesis. NO mediates vascular relaxation to vasoactive substances, inhibits platelet adhesion, activates platelet secretion, induces platelet de-aggregation, inhibits platelet P-selectin expression, and increases leukocyte adhesion to the endothelium. As a consequence of inflammation, platelets and endothelial cells release small quantities of NO.[28] Deficiency in release of NO – seen in patients with MPN – favours thrombosis.[1] Hydroxycarbamidum increases NO in patients with ET, promoting thrombotic complications, while in PV patients it did modify the prevalence of thrombosis.[1] Endothelial splanchnic cells were found to express JAK2 mutation, possibly as a part of the malignant process.[13] The increase of vWF antigen and serum thrombomodulin in ET may also act as a predictive factor of thrombosis.[19]

Role of Coagulation Factors in Thrombosis

In the assessment of the thrombotic risk, in addition to the quantitative and qualitative changes of the blood and endothelial cells in patients with MPN, abnormalities of the coagulation factors were also observed. MPNs (PV and ET) are associated with acquired resistance to activated C protein.[1,19] Increased levels of prothrombin fragment 1.2 in PMF and tissue factor in ET represent risk factors for thrombosis.[19] Patients with MPN present higher levels of tissue plasminogen activator (t-PA:Ag), plasminogen activator inhibitor-1 (PAI-1:Ag), and D-dimers, indicating the secondary activation of fibrinolysis (t-PA:Ag and D-dimers) and the inhibition of fibrinolysis (PAI-1:Ag). The increase of PAI-1:Ag in MPN is associated with a higher percentage of activated platelets and with altered vascular endothelium.[44]

JAK2 MUTATION IN PREGNANCY

Besides the correlation to an increased risk of thrombotic complications, JAK2 mutation is interestingly correlated with a spontaneous loss of pregnancy in association with ET45 but also independently from other factors.[46,47] Presence of JAK2 mutation was significantly correlated with the risk of pregnancy loss, with odds ratios of 4.63 and 7.20 for embryonic loss and foetal loss, respectively.[46] Nevertheless, there are studies that do not report a negative implication of JAK2 mutation in pregnancy.[48]

CONCLUSIONS

Patients with JAK2 positive MPN have a high incidence of thrombosis and the presence of JAK2 mutation is an important risk factor. The pathogenesis of thrombosis in MPN patients is complex. Platelet and/or endothelial cell dysfunction, leuko-platelet microaggregates, and abnormalities of the coagulation factors are important factors which contribute to the increased risk of thrombosis in MPN. Occasionally, the presence of JAK2 mutation may be the only indication of undiagnosed MPN. The mutation is also interestingly correlated with spontaneous loss of pregnancy, in association with ET or even independently.

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CALRETICULIN IN MYELOPROLIFERATIVE NEOPLASMS: THE OTHER SIDE OF THE ALICE MIRROR

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ABSTRACT

Calreticulin (CALR), a Ca^{2+} binding protein mostly localised in the endoplasmic reticulum, regulates Ca^{2+} homeostasis, chaperones, and other proteins to the nucleus and other cellular compartments. CALR has been implicated in several cellular processes including: signalling, regulation of gene expression, cell adhesion, apoptosis, autoimmunity, and, when expressed on the cell surface, induction of phagocytosis by macrophages. Reports indicating over-expression of CALR in cancer cells suggest that modulation of CALR expression may be exploited to increase their clearance by the immune system. In the hematopoietic system, CALR has been implicated in the activation of the stress pathway as an obligatory partner of the glucocorticoid receptor. More recently, somatic loss-of-function mutations in the CALR gene were discovered in a significant proportion of patients with Philadelphia-negative myeloproliferative neoplasms (MPN) who did not harbour gain-of-function mutations in Janus kinase 2 (JAK2), the first signalling element of cytokine receptors, and myeloproliferative leukaemia virus oncogene (MPL), the thrombopoietin receptor, usually associated with these diseases. This review will summarise current knowledge on the biological activity of CALR and MPL/JAK2 in hematopoiesis, delineate a unifying pathway for the pathogenesis of MPN, and discuss how this pathway may be exploited for therapy.

Keywords: Myeloproliferative neoplasms, calreticulin, JAK2, glucocorticoid receptor.

INTRODUCTION

The Philadelphia-negative myeloproliferative neoplasms (Ph-negative MPN) are a group of clonal stem cell disorders characterised by abnormal proliferation of myeloid cells. These disorders include polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF).[1] These diseases are characterised by hyperproliferation of either erythroid cells, leading to erythrocytosis (PV), or of megakaryocytes (ET and PMF). Hyperproliferation of megakaryocytes may be associated with platelet overproduction (ET) or, when due to delayed maturation, with thrombocytopaenia (PMF). Since their discovery in 1951 by William Dameshek,[2] much progress has been made in our understanding of the pathobiology of myeloproliferative neoplasms (MPN), leading to the establishment of international consensus criteria for their diagnosis by the World Health Organization in 2008;[3] these were refined in 2011.[4]

The discovery in 2005[5-8] that the primary genetic lesion in these disorders was a gain-of-function mutation in the gene encoding a tyrosine kinase, Janus kinase 2 (JAK2), paved the way for development of targeted pharmaceutical inhibition. The positive clinical experience with imatinib, a tyrosine kinase inhibitor that targets the breakpoint cluster region protein/Abelson murine leukaemia viral oncogene mutation associated with Philadelphia-positive MPN (Ph-positive MPN), suggested that a similar approach could be successfully applied for the cure of PV, ET, and PMF as well. In as little as 9 years after the discovery of JAK2 mutations, JAK2 inhibitors have been designed, clinical trials with selected drugs conducted, and the drugs have been approved for clinical use by the FDA.[9] However, in contrast with

Ph-positive MPN that harbour one primary lesion, the genetic lesions found in Ph-negative MPN are heterogeneous, and so concerns were raised whether JAK2 mutations represent the primary transformation event in these diseases.[10]

This review will summarise the biology of the major mutations observed in MPN and discuss the possibility that different mutations may target a common pathway, suggesting that, in spite of their genetic heterogeneity, MPN patients may be treated with the same drugs.

MUTATIONS ASSOCIATED WITH PH-NEGATIVE MPN

The majority of MPN patients harbour acquired gain-of-function mutations in JAK2, the first signalling element common to several hematopoietic growth factor receptors. The most frequent of these mutations are in the region of the gene encoding for the tyrosine kinase domain of the protein (JAK2V617F[5-8] and JAK2 exon 12 mutations[11]). The JAK2V617F mutation is present in almost all PV, ~50% of ET, and ~65% of PMF.

Less common mutations involving myeloproliferative leukaemia virus oncogene (MPL), LNK, TET2, ASXL1, EZH2, IDH1/2, CBL, IKZF1, have been observed in 3-20% of MPN patients.[12-14] These mutations may be found either alone or associated with JAK2 mutations. MPL and LNK mutations also affect the JAK2 signalling pathway.[12] MPL encodes the thrombopoietin receptor, and LNK encodes a chaperone protein that restrains MPL expression on the cell surface.

Other genes affected in MPN encode proteins involved in epigenomic DNA modification (TET2, ASXL1, and EZH2), cell metabolism (IDH1/2), protein stability (CBL), and gene transcription (IKZF1). TET2 encodes a methylcytosine dioxygenase that catalyses the conversion of methyl-cytosine to 5-hydroxy-methylcytosine; ASXL1 encodes a nuclear protein with a N-terminal helix-turn-helix domain associated with an unusual C-terminal domain that binds methylated lysines; EZH2 encodes the catalytic subunit of the polycomb repressive complex 2 (PRC2) responsible for methylation of histone H3 (H3K27) (methylation of H3K27 regulates stem cell renewal); IDH1/2 encodes isocitrate dehydrogenase 1 and 2, $NADP^+$ enzymes that catalyse the conversion of isocitrate to α-ketoglutarate; CBL encodes a protein of the Cbl family of E3-ubiquitin ligases that acts as a negative regulator of cell signalling by promoting ubiquitination, and decreasing stability of proteins involved in this process; IKZF1 encodes Ikaros, a transcription factor that regulates B and T cell development.

More recently, Klampfl et al.[15] and Nangalia et al.[16] used exome sequencing to identify novel mutations in exon 9 of calreticulin (CALR) in the majority of ET and PMF patients who did not harbour JAK2 or MPL mutations. CALR mutations were not found in healthy controls, lymphoid neoplasia, acute leukaemias, or solid tumours, indicating their specificity for ET and PMF. The mutations were either insertions or deletions, the commonest (80-90%) of which were a 52 bp deletion (CALRdel52) and a 5 bp insertion (CALRins5). All of them encode for a mutant protein with a novel C-terminus lacking the KDEL signal localisation domain (see the section of CALR functions). The mutations were detected in hematopoietic stem/progenitor cells and persisted with disease progression.[15] Re-sequencing samples from 1,107 MPN patients confirmed that CALR, JAK2, and MPL mutations were mutually exclusive.[15] Although the mutation rate in MPN is rather low, JAK2 and CALR mutations were found to be associated with other mutations such as loss of heterozygosity in p53 in those MPN patients at high risk of progression toward acute myeloid leukaemia.[17]

JAK2 AND PATHOBIOLOGICAL EFFECTS OF JAK2-MPL MUTATIONS

The mutation most frequently detected in MPN is JAK2V617F and several excellent reviews have been published on this subject.[18,19] Here we will summarise only information necessary to understand the relationship between the biological consequences of JAK2 and CALR mutations.

JAK2V617F constitutively activates the cytokine receptor signalling, including signal transducer and activator of transcription (STAT) proteins, and is largely responsible for the reported cytokine hypersensitivity and cytokine-independent growth *in vitro* of hematopoietic progenitor cells from MPN patients.[19] The addition in culture of JAK2 inhibitors reduces the erythropoietin-independent growth of erythroid colonies from PV patients, a property used for years as diagnostic criteria for this disease.[20,21] This observation provided the rationale for the development of JAK2 inhibitors,

such as ruxolitinib, currently used to treat patients with MPN.[22]

MPL - a gene first identified in a naturally occurring animal retrovirus - is located on chromosome 1p34 and, in addition to MPN, is present in Down's syndrome patients with acute lymphoblastic leukaemia (ALL).[12] The most frequent mutation is a point mutation in exon 10 that induces a tryptophan to leucine substitution in amino acid 515 (MPL^{W515L}). Additional MPL mutations found in a small number of JAK2 negative ET and PMF patients are MPL^{S505N}, MPL^{W515Ki}, and $MPL^{W515Kii}$.[23-27] All these mutations lead to ligand-independent activation of the receptor and of JAK2-STAT signalling, and to cytokine-independent cell growth, providing the rationale to use drugs that target JAK2 also for the treatment of patients carrying mutation in MPL.

JAK2V617F constitutively activates the STAT5 pathways.[8,28] Intermediate and high levels of STAT5 activation favour, respectively, proliferation and maturation of human hematopoietic progenitor cells.[29,30] It may be argued therefore, that the JAK2V617F mutation (by inducing constitutive STAT5 activation) should decrease, and not increase, the intrinsic proliferative potential of erythroid cells. This apparent paradox was resolved by studies indicating that the glucocorticoid receptor (GR) plays a major role in fine-tuning the levels of STAT5 activation expressed by erythroid cells expanded from PV patients.[31]

GR is the stress nuclear receptor that mediates the increased erythroid output occurring under conditions of acute and chronic blood loss,[32,33] and may suppress megakaryocytic maturation.[34] Activation of GRα by its ligand induces its homodimerisation and heterodimerisation with signalling partners, such as phospho-STAT5. GRα/phospho-STAT5 complexes migrate to the nucleus to bind through the DNA binding domain (DBD) of GR specific consensus sequences activating/inhibiting the expression of target genes. However, STAT5 is also downstream to the erythropoietin receptor and, upon receptor stimulation, elicits the signal that allows erythroid cells to mature. Simultaneous activation of the glucocorticoid and erythropoietin receptor impairs the ability of both receptors to phosphorylate STAT5 and ultimately quenches the signal delivered by the erythropoietin receptor leaving the cells immature and capable of proliferation.[31]

In humans, alternative splicing of GR mRNA leads to synthesis of GRβ, a dominant-negative form of the receptor that retains GRα in the nucleus, preventing its interaction with STAT5.[31] A A3669G single nucleotide polymorphism (SNP) stabilises GRβ mRNA, increasing the cellular content of the GRβ protein.[35] The A3669G SNP is present at a frequency greater than normal in patients with PV31 and PMF, and in PMF patients it predicts poor survival.[36] We had determined that erythroid cells, expanded *in vitro* from PV patients, express high levels of GRβ. In addition, erythroid cells expanded from these patients, in spite of constitutive STAT5 phosphorylation, contain low levels of STAT5 in the nucleus (Figure 1) and do not express GR target genes, such as GILZ.[31] Transcriptosome profiling identified similarities in gene expression between erythroblasts expanded from normal donors in the presence of dexamethasone, a synthetic GR agonist, and those expanded from PV patients without this hormone, confirming that in both cases the transcriptional activity of STAT5 was greatly reduced.[37] Surprisingly, reduced nuclear localisation of STAT5 in association with nuclear retention of GRα was observed also in erythroblasts expanded *ex vivo* from JAK2-non mutated MPN patients who did not harbour the polymorphism (unpublished results). The reduced levels of nuclear STAT5 localisation in PV address the paradox described above suggesting that PV expression of GRβ, by sequestering GRα in the nucleus and preventing its interaction with phosphorylated STAT5 present in the cytoplasm, fine-tunes the nuclear levels of constitutively activated STAT5, assuring that these levels remain within the range that elicit a proliferation rather than a maturation response. They also reinforce the general impression that Ph-negative MPN results from genetic mutations that impair the stress response.

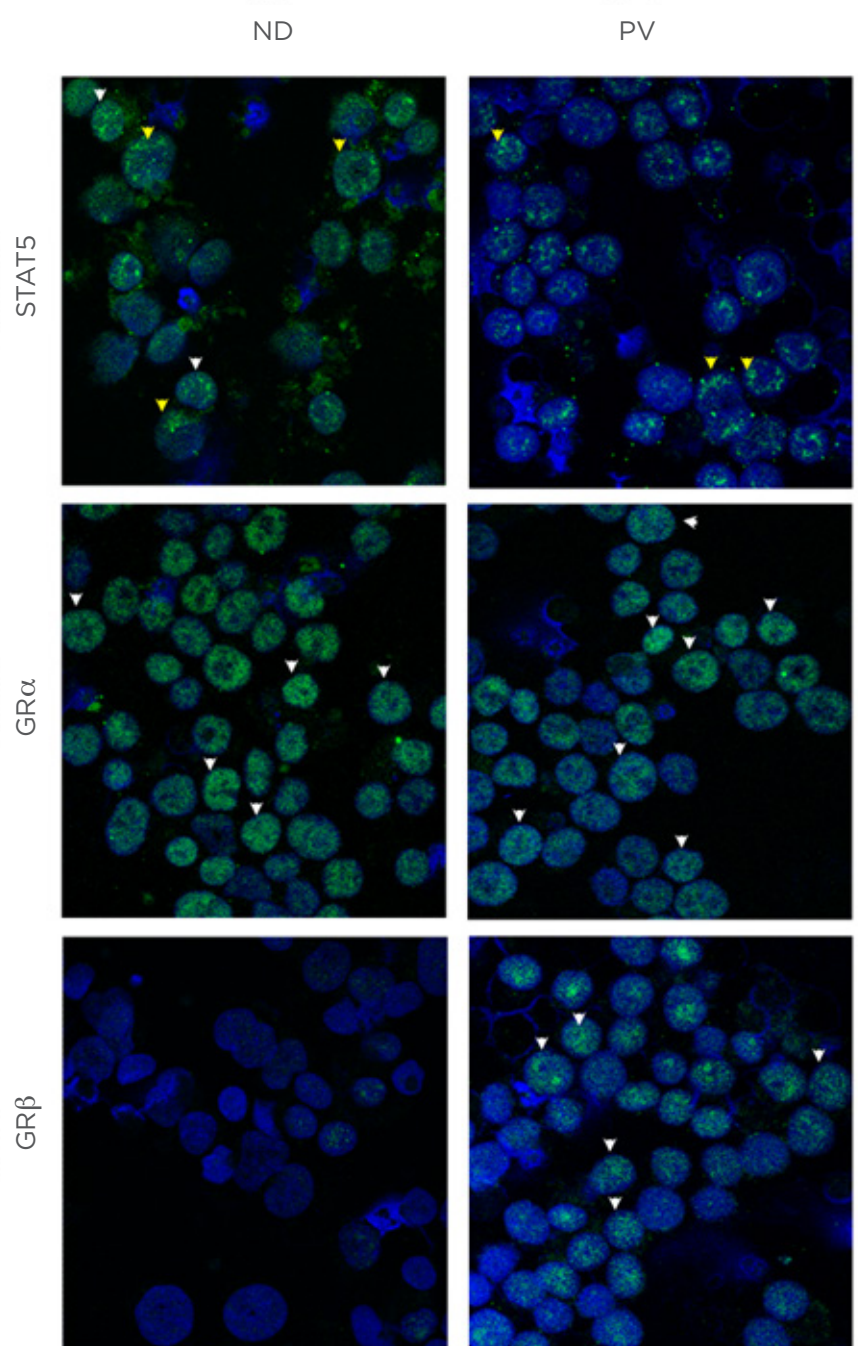

Figure 1: Confocal microscopy showing the cellular localisation of signal transducer and activator of transcription 5 (STAT5), glucocorticoid receptor alpha (GRα), and GRβ (in green) in erythroblasts expanded ex-vivo from Normal Donors (ND) with dexamethasone and from JAK2-mutated polycythaemia vera (PV) patients without dexamethasone.

Nuclei are evidentiated by DAPI staining. Erythroblasts from ND expanded with dexamethasone contain STAT5 both in the cytoplasm (yellow arrow) and in the nucleus (white arrows), GRα mainly in the nucleus, and do not express GRβ. Erythroblasts from PV patients contain STAT5 mainly in the cytoplasm, and GRα and GRβ mainly in the nucleus where the two subunits likely form a transcriptionally inactive complex. For reasons unknown at the time of this publication, a similar cytoplasmic-restricted STAT5 localisation and nuclear-restricted GRα localisation were also observed in erythroblasts expanded from JAK2-negative primary myelofibrosis patients that did not express GRβ (unpublished results).

Modified from Varricchio L et al.[31]

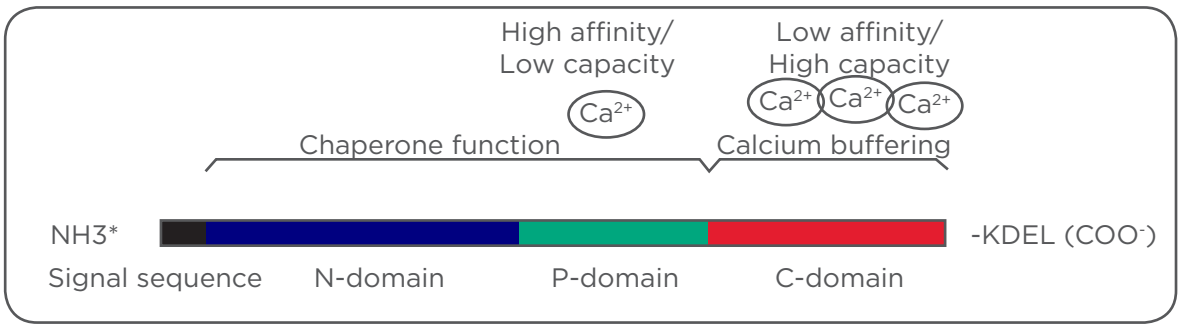

Figure 2: Structure of calreticulin (CALR) protein. Schematic representation of the structural domains of CALR.

The protein has at least three domains: N-domain in blue; the P-domain in green; and the C-domain in red. The protein contains an N-terminal amino acid signal sequence (black box) and a C-terminal KDEL endoplasmic reticulum retrieval signal.

Modified from Michalak et al.[41]

STRUCTURE AND BIOLOGICAL FUNCTION OF CALR

The CALR protein was first isolated by Ostwald and MacLennan in 1974[38] and its gene cloned by Smith and Koch[39] and Fliegel et al.[40] in 1989. The CALR gene is located on chromosome 19p13.2 and contains nine exons.

The CALR protein has three main functional domains (Figure 2). The first amino (N)-terminal domain (residues 1–180) contains a highly conserved amino acid sequence responsible for interactions with other proteins. This domain is homologous to that found in proteins with chaperone functions. The second domain is the central or P domain (residues 181–290) and is rich in the amino acid proline. It contains the region that binds calcium (Ca^{2+}) with high affinity and a lectin-like chaperone domain. The third carboxyl (C)-terminal domain (residues 291–400) is rich in acidic amino acids that confer to CALR its capacity to bind Ca^{2+} with low affinity. This domain regulates the Ca^{2+} levels in the endoplasmic reticulum (ER). This domain also contains the four amino acids KDEL (lysine, aspartate, glutamate, and leucine) motif that serves as a retention protein signal that, in the Golgi, switches the fate of CALR and its associated partners from extracellular secretion to ER localisation.[41]

Within the cell, CALR has multiple localisation sites and shuttles among different compartments (cytoplasm, ER, Golgi, cell membrane, nucleus, etc.) in response to its Ca^{2+} binding levels. The basic function of CALR is to bind Ca^{2+} in the ER (it binds >50% of the Ca^{2+} present in the ER). On the basis of these levels, it binds to newly synthesised proteins, assuring that they are properly folded, become proteasome-resistant, and reach their functional intracellular location. Therefore, CALR may be considered a sensor that, on the basis of the intracellular Ca^{2+} levels, fine-tunes the concentrations of other proteins in the various cellular compartments, allowing them to elicit the programmed cellular response. As such, CALR does not have a 'unique' biological function but cooperates with its multiple partners in the regulation of all cellular functions (proliferation, apoptosis, phagocytosis, gene transcription, etc.).

The role of CALR in the control of cell proliferation has been best described in the endothelial system. CALR expression is specifically upregulated in the heart during the middle stages of embryogenesis[42] and mice lacking CALR have an embryonically lethal phenotype with impaired heart development.[43] The survival functions of CALR are exerted through its partner p53. CALR has been shown to regulate p53 expression, localisation, and function. The observation that mouse CALR deficient embryonic fibroblasts (*calr*$^{-/-}$ cells) express significantly lower levels of p53 protein indicates possible p53 degradation in absence of CALR. These cells are also more resistant to apoptosis induced by ultraviolet light exposure.[44]

For their clinical relevance, the functions of CALR in phagocytosis have been extensively

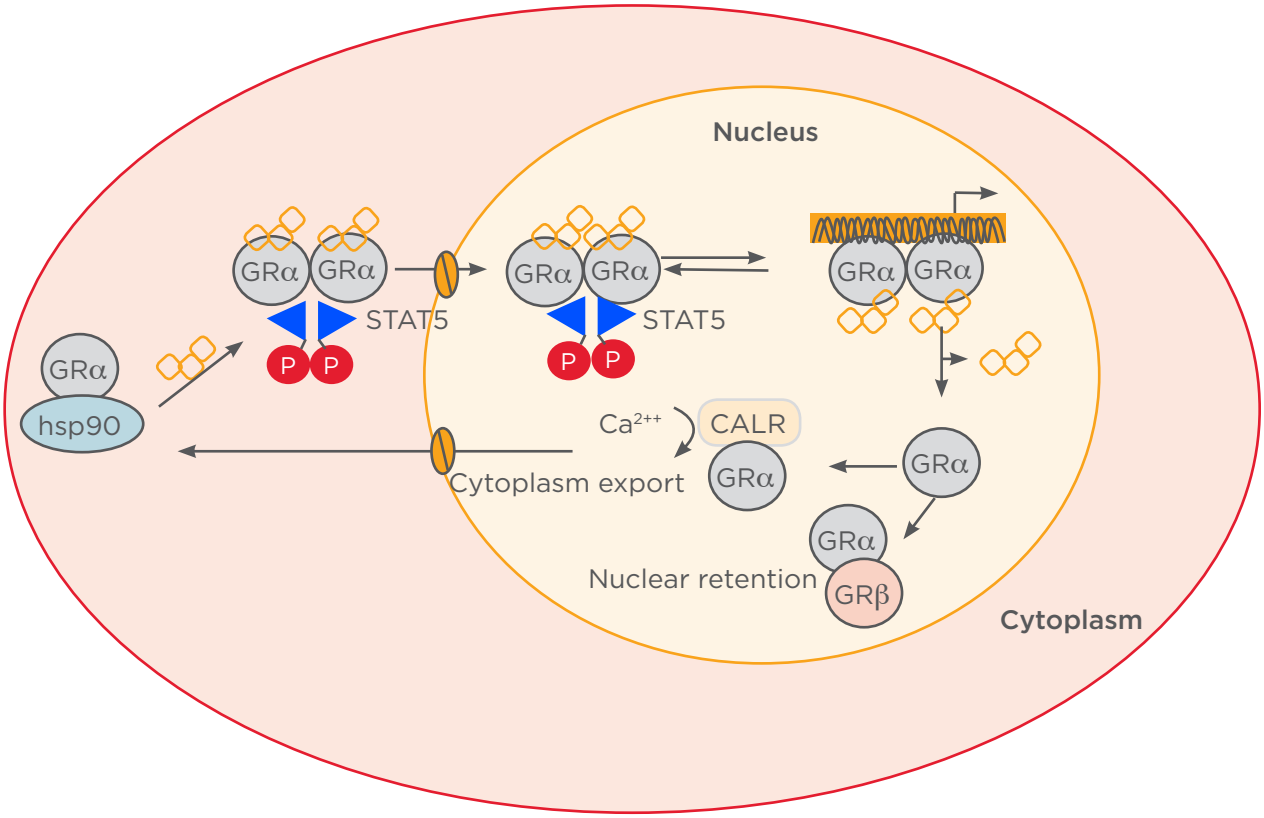

Figure 3: Calreticulin (CALR)-dependent trafficking of glucocorticoid receptor (GR) between the nucleus and the cytoplasm.

This function of CALR is Ca^{2+} dependent. Ligand binding to GRα leads to dissociation of a cytoplasmic complex interacting with GRα. Ligand-bound GRα rapidly translocates into the nucleus where it activates gene expression by directly binding to specific consensus sequences, either as a homodimer or a heterodimer with other transcription factors, such as STAT5. CALR inhibit the transcriptional activity of GR by masking its DNA binding domain and by facilitating its translocation from the nucleus to the cytoplasm. On the other hand, expression of GRβ leads to formation of a GRα/GRβ complex that is constitutively retained in the nucleus.

studied. In eukaryotes, CALR is the second signal - the membrane phospholipid phosphatidylserine being the first - present on apoptotic cells to be recognised by phagocytes. This function is highly conserved and is exerted in organisms as distant as *Drosophila*[45] and man.[46,47] A great variety of human cancer cells, including leukaemia blasts and progenitor cells from myelodysplastic syndrome, express increased levels of CALR; this increased expression exerts a pro-phagocytic signal that counter-balances the 'eat-me-not' signal provided by CD47.[46] In addition, Obeid et al.[47] have shown that when expressed on the cell surface, CALR may serve as an 'eat me' signal that allows dying cells to be recognised, ingested, and processed by dendritic cells. Based on these discoveries, molecules promoting apoptotic clearance of cancer cells, including leukaemic cells, through modulation of CALR/CD47 interaction have been devised.[48]

In contrast with JAK2, much less is known on the function of CALR in hematopoiesis. These functions may be inferred from studies with *calr*$^{-/-}$ cells indicating that CALR exerts an important function in the regulation of nuclear localisation and transcriptional activity of GR,[49,50] the nuclear receptor controlling the response of the hematopoietic system to stress. Both immunocytochemistry and sub-cellular fractionation studies have indicated that CALR may be localised in the nucleus[51,52] where it exerts the function to reset the cellular response of nuclear receptors, including that of GR (Figure 3). On one hand, CALR, through its N-terminal domain, binds to the DBD of GR, preventing its binding to glucocorticoid response elements (GRE) and activation of GR target genes.[49]

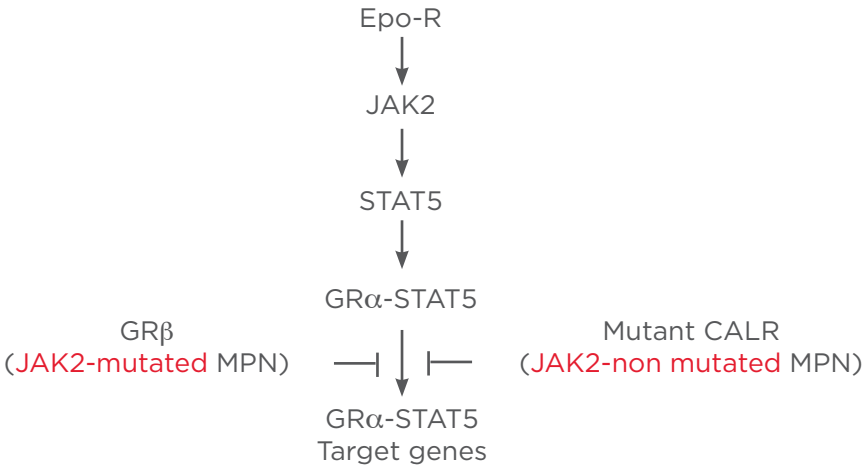

Figure 4: A unifying model for suppression of the expression of glucocorticoid receptor (GR)-target genes in JAK2-mutated (expression of the dominant negative GRβ isoform) and JAK2-non mutated (loss-of-function mutations) in myeloproliferative neoplasms patients.

In both cases, GRα is constitutively retained in the nucleus. In the case of JAK2-mutated patients, GRα is retained in the nucleus by the GRβ isoform. In the case of JAK2-non mutated patients, it is retained in the nucleus because the mutated CALR does not migrate to the nucleus and cannot exert its GR cytoplasm export function.

On the other hand, in response to changes in Ca^{2+} binding, CALR regulates the export of GR from the nucleus to the cytoplasm, enabling GR to respond to novel glucocorticoid stimulation[53] and to shuttle additional STAT5 to the nucleus. The function to export GR to the cytoplasm in response to Ca^{2+} is exerted by the KDEL in the C-terminal domain of the protein.

These data indicate that the role of CALR in the regulation of GRα activity (nuclear export) is antagonistic with that of GRβ (nuclear retention). It may be, therefore, hypothesised that while the function of GRβ in erythropoiesis is to suppress the cellular response to glucocorticoids, that of CALR is to prime erythroid cells to respond to glucocorticoids by increasing the amount of GRα present in the cytoplasm.

BIOLOGICAL CONSEQUENCES OF CALR MUTATIONS IN MPN

The limited number of studies on the function of CALR in hematopoiesis prevents a comprehensive analysis of the pathobiological consequences of CALR mutations in MPN. Some insights on these consequences may be inferred by the biological data included in the recent papers by Klampfl et al.[15] and Nangalia et al.[16] The CALR mutations found in MPN encode a protein lacking the C-terminal regions including the KDEL signal and the low affinity Ca^{2+} binding sites suggesting that this protein is mislocated and responds poorly to Ca^{2+}. In agreement with this hypothesis, myeloid cells from MPN patients carrying CALR mutations, and cells expressing ectopic levels of the mutant genes, express the mutated protein at normal levels in the Golgi and on the cell surface but at levels greater than normal in the ER, where they may acquire novel functions, and lower than normal in the nucleus, where they may instead lose function.[15,16] These reports also showed that CALR mutations increase STAT5 phosphorylation conferring cytokine hypersensitivity, suggesting that in the cytoplasm the mutated CALR acquires the novel function to constitutively activate the hemopoietic cytokine signalling pathway. Further studies are necessary to decipher the mechanisms that result in cytokine-independent signalling activation in hematopoietic cells harbouring CALR mutations.

The extensive data indicating that CALR resets GR signalling by favouring its export from the nucleus suggest that the nuclear function lost by the mutated protein may be the regulation of GR activity. This interpretation is consistent with our unpublished observations that STAT5 and GRα were similarly mislocated in erythroid cells from JAK2-positive PV patients that expressed GRβ and in those from JAK2-negative MPN patients

that did not express GRβ but probably expressed the mutated form of CALR (Figure 1). This interpretation provides a unifying mechanism for the hyperproliferation observed in JAK2-mutated and in CALR-mutated MPN patients, according to which in both cases the hyperproliferative response is elicited by constitutive STAT5 activation quenched within levels consistent with a proliferative response by a mechanism, expression of GRβ or CALR mutation, that restrains the localisation of GRα in the cytoplasm (Figure 4). This hypothesis must be tested by direct experiments.

FUTURE THERAPEUTIC STRATEGIES FOR MPN

The different forms of Ph-negative MPN have a heterogeneous prognosis; this ranges from the benign course of PV, usually well controlled by minimal interventions (phlebotomy), to the more challenging PMF, presently cured mainly by bone marrow transplantation. This variability likely reflects the interaction between the variegation of molecular lesions underscoring the insurgency of the disease and genetic factors, such as GR polymorphism and possibly other gene modifiers.[10,13,36] These considerations suggest that MPN patients may represent suitable candidates for the development of personalised therapies. This concept is exemplified by recent retrospective analyses of European and USA patients that have identified the presence of mutations in EZH2, ASXL1, SRSF2, and IDH1/2 as possible biomarkers to predict overall lower survival and increased risk of leukaemic transformation in PMF.[13]

Unexpectedly, the results of a large clinical trial have recently identified that the JAK2 inhibitor ruxolitinib is effective in reducing spleen size and disease manifestation both in JAK2-mutated and JAK2-non-mutated patients,[54] and the JAK2 inhibitor fedratinib reduced splenomegaly in two CALR-mutated patients.[55] As discussed in Cazzola and Kralovics' paper,[56] these results confirm the concept discussed here that mutations leading to development of MPN occur along a unifying pathogenetic pathway including JAK2 and CALR. Since the clinical response to JAK2 inhibitors is far from optimal, the discovery of CALR, as the other side of the Alice mirror suggests, highlights that drugs that will improve recognition and clearance of the MPN clone by the immune system (and/or target additional mutations [IDH1/2] or the genetic background of these patients) should be considered as candidates in combination with JAK2 inhibitors for personalised therapy in MPN.

CONCLUSION

In conclusion, loss-of-function CALR mutations, and gain-of-function JAK2/MPL mutations identified in MPN both target STAT5, suggesting that CALR and MPL/JAK2 represent the negative and positive effectors of a unique mechanism that controls proliferation of hematopoietic cells in response to stress, the alteration of which results in MPN.

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IRON SUPPLEMENTATION FOR PERIOPERATIVE ANAEMIA IN PATIENT BLOOD MANAGEMENT

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ABSTRACT

In patients undergoing major surgical procedures, preoperative anaemia and perioperative allogeneic blood transfusion (ABT) have been linked to increased postoperative morbidity and mortality, as well as longer hospital stays. A multidisciplinary, multimodal, individualised strategy - collectively termed patient blood management - used to minimise or eliminate ABT is indicated to improve outcomes. This new standard of care relies on detection and treatment of perioperative anaemia (Pillar 1) and reduction of surgical blood loss and perioperative coagulopathy (Pillar 2) to harness and optimise physiological tolerance of anaemia (Pillar 3), thus allowing the use of restrictive transfusion criteria. Normalisation of preoperative hemoglobin levels is a World Heath Organization recommendation. Iron repletion should be routinely ordered when indicated. Preoperative oral iron is time-consuming and poorly tolerated with low adherence in published trials. Postoperative oral iron has been proven to be inefficacious and is no longer recommended. Preoperative and perioperative intravenous iron, with or without erythropoiesis stimulating agents, is safe and effective at reducing ABT rate and hastening the recovery from postoperative anaemia. Intravenous iron does not seem to increase the risk for postoperative thromboembolism, infection, or mortality. Newer intravenous iron formulations demonstrate potentially much lower immunogenic activity, allow complete replacement dosing in 15 to 60 minutes, markedly facilitating care, and may be cost-effective in many clinical settings.

Keywords: Anaemia, surgery, transfusion, intravenous iron, erythropoiesis stimulating agents, patient blood management.

INTRODUCTION

Major surgical procedures (e.g. orthopaedic, cardiac, gynaecological, cancer resection, etc.) may result in a significant postoperative decline in hemoglobin (Hb) levels. As a result, a significant proportion of patients received at least one unit of allogeneic blood for treating acute postoperative anaemia. Evidence of the clinical and economic disadvantages of allogeneic blood transfusion (ABT) has prompted recommendations for its restrictive use,[1,2] and a growing interest in multidisciplinary, multimodal, individualised strategies, collectively termed patient blood management (PBM), aimed to minimise ABT with the ultimate goal of improving patient outcomes,[3] which has been endorsed by the 63rd World Health Assembly.[4]

This new standard of care, which relies on the detection and treatment of perioperative anaemia (Pillar 1) and the reduction of surgical blood loss and perioperative coagulopathy (Pillar 2) to harness and optimise physiological tolerance of anaemia (Pillar 3); thus, allowing restrictive use of ABT, is now being established for elective surgery in several European countries.[3]

In this paper, we will review the diagnosis and treatment options for perioperative anaemia, with a special emphasis on the role of intravenous (IV) iron. The recommendations on the use of a particular therapeutic option will be given in accordance with the updated Seville Document (SD update) on alternatives to ABT and NATA (Network for Advancement of Transfusion Alternatives) consensus statements.[1,5,6] All of the recommendations were formulated according to GRADE methodology, taking into account efficacy, safety, and target patient populations.[7-9]

DIAGNOSIS OF PREOPERATIVE ANAEMIA

Preoperative anaemia is a major, independent, predictive factor for the need of perioperative ABT. Moreover, preoperative anaemia in itself has been linked to increased postoperative morbidity and mortality.[10-12] Therefore, patients scheduled for major surgery should have a full blood cell count (including reticulocyte counts) and iron status (serum iron, ferritin, and transferrin saturation) test, preferably 30 days before the scheduled surgical procedure to allow the implementation of appropriate treatment, if available.[5,6,13] Preoperative hematinic deficiencies without anaemia should also be treated. The diagnosis of an unexpected anaemia should be considered an indication for rescheduling surgery until the evaluation is completed.[6]

Transferrin saturation (TSAT) is a measure of iron in transport, and values of <20% indicate decreased iron availability for the bone marrow (and also the need for parenteral iron in the setting of anaemia treated with erythropoiesis stimulating agents [ESAs]). When used with either the ferritin concentration or red blood cell (RBC)/reticulocyte variables, %TSAT may be useful in the diagnosis of functional iron deficiency (FID).[13,14] Serum ferritin assay is essential for evaluating iron stores (1 μg/L serum ferritin corresponds to 8 mg stored iron). However, it might be accurate when low levels are found but not with high levels since ferritin is also an acute phase reactant.

As for surgical patients, values <30 μg/L indicate absent iron stores (iron deficiency anaemia [IDA]).[13] Importantly, the cause of iron deficiency (ID) should be investigated, and this may include upper and lower gastro-intestinal investigations, screening for coeliac disease and *Helicobacter pylori* colonisation, and/or genito-urinary blood loss evaluation, depending on the patient's age and gender.[13,14] In the presence of inflammation, TSAT <20% and serum ferritin >100 μg/L are suggestive of iron sequestration (anaemia of chronic disease [ACD]), whereas ferritin values <100 μg/L are associated with a high likelihood of iron deficiency (ACD+ID) and a potentially good response to IV iron (Figure 1).

The percentage of hypochromic red cells (%HRC >5) and reticulocyte Hb content (CHr <27 pg) are the best-established variables for the identification of iron sequestration.[14] Mean cell volume (MCV) and mean cell Hb (MCH) values are useful at diagnosis and in assessing trends over periods of weeks or months (treatment follow-up), whereas red cell distribution width (RDW) differentiates IDA from other microcytic anaemias. Zinc protoporphyrin (ZPP) in circulating red cells is increased in conditions that limit iron supply to the erythroid marrow or stimulate porphyrin synthesis. However, due to lack of specificity and low sensitivity to acute iron changes, ZPP may be used to monitor response to therapy, but not as a sole diagnostic test.[14] The soluble transferrin receptor (sTfR) assay is relatively expensive and not widely available, but it may have a role, either alone or in combination with the ferritin assay, if automated measures such as %HRC or CHr are unavailable. The utility of serum hepcidin measurement as a diagnostic tool is currently uncertain, but it could be helpful in identifying IDA or ACD with ID when reduced hepcidin levels are detected, whereas high hepcidin levels would predict unresponsiveness to oral iron.

When anaemia in surgical patients cannot be explained by acute blood loss, IDA, ACD, or ACD+ID, it is important to consider other causes that would demand specific treatment. In these cases, further testing should include B_{12} (especially for those aged >60 years), lactate dehydrogenase, and serum creatinine in order to exclude other nutritional deficiencies, hemolysis, or renal disease. If malabsorption or severe malnutrition, a red cell folate may also be useful.[13,15,16] An easy-to-follow algorithm, which allows for detection and classification of most cases of anaemia and implementation of appropriate therapy in surgical patients, is depicted in Figure 1.

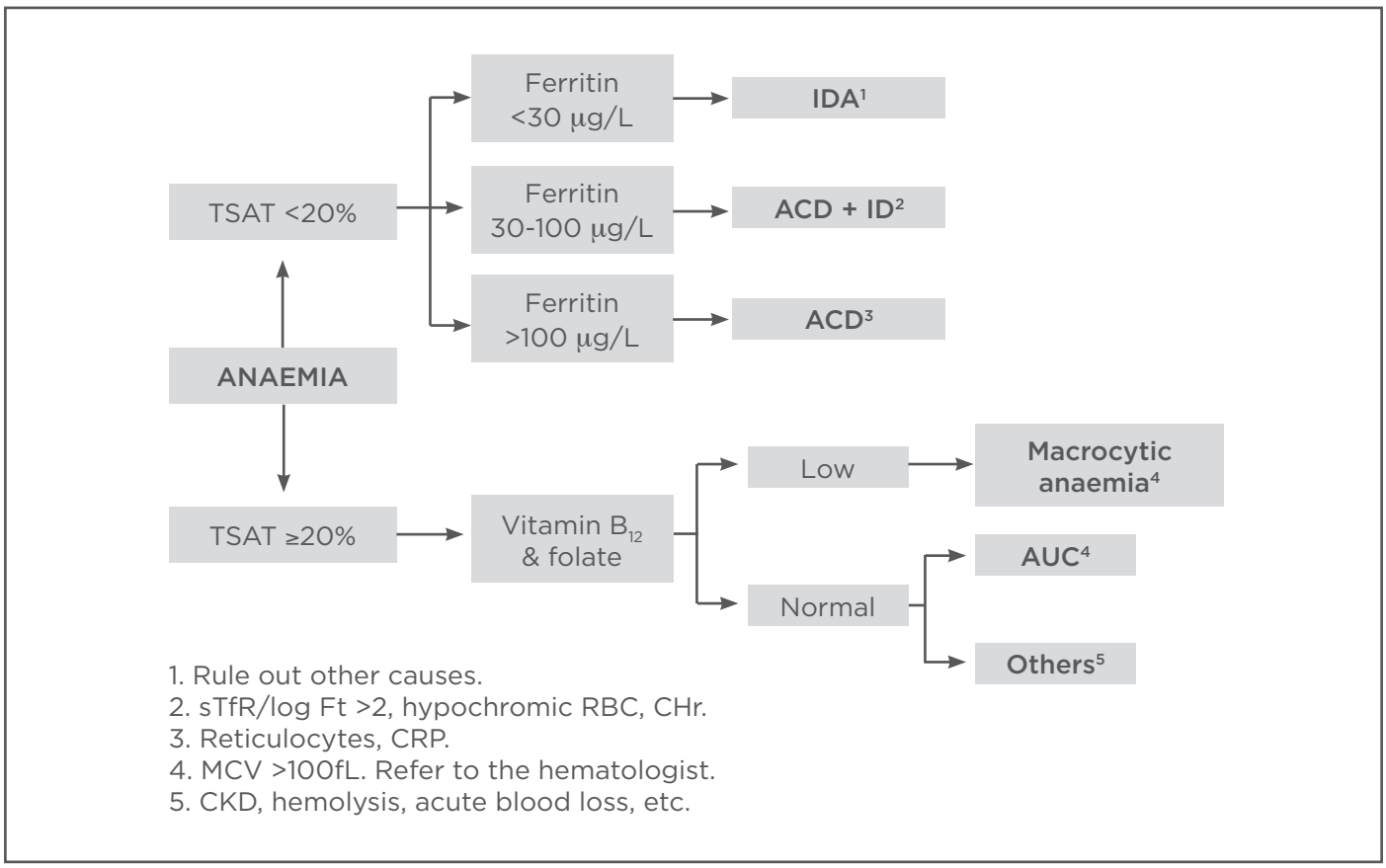

Figure 1: An algorithm for anaemia diagnosis in surgical patients.
IDA: iron deficiency anaemia; TSAT: transferrin saturation; ACD: anaemia of chronic disease; ID: iron deficiency; AUC: anaemia of unknown cause; sTfR: serum transferrin receptor; Ft: ferritin; RBC: red blood cell; CHr: reticulocyte hemoglobin; CRP: C-reactive protein; MCV: mean corpuscular volume; CKD: chronic kidney disease.

MANAGEMENT OF PERIOPERATIVE ANAEMIA

Iron Therapy

Efficacy

Whenever there is enough time and no contraindications, oral iron supplementation should be attempted for IDA treatment (Grade 2B).[1] In patients scheduled for orthopaedic procedures or colon cancer resection, ferrous salts (100-200 mg/d; 2-4 weeks) improved Hb levels, reduced transfusion rates, and, in some cases, the length of hospital stay,[17-21] while others did not.[22] Postoperative oral iron did not hasten the correction of anaemia or reduce the transfusion rate, but was associated with a high rate of adverse effects,[23-29] and is therefore not recommended (Grade -1B).[1] Newer oral iron formulations, such as heme iron polypeptide or liposomal iron, seem to offer advantages over the traditional iron salts even in the context of inflammation, although more studies are needed.[30-33]

If there is poor absorption or poor tolerance of oral iron, or an accelerated response to treatment is required, preoperative IV iron supplementation could be considered. Several IV iron formulations are currently available (Table 1). An IV iron course, starting 3-4 weeks prior to the scheduled procedure, is suggested (Grade 2B)[1] as it increases reticulocyte counts and Hb levels (or corrected anaemia), and may result in reduced ABT requirements.[34-40] IV iron formulations are clearly superior to oral iron in replenishing iron stores. Should this timeframe not be available, short-term perioperative IV iron - with or without ESAs - may be administered, as they have been shown to be efficacious at reducing ABT rate (Grade 2B).[1,5,41-43]

Table 1: Some characteristics of the different intravenous iron formulations.

	Iron gluconate	Iron sucrose	HMWID	LMWID	Ferric carboxymaltose	Iron isomaltoside 1000	Ferumoxytol
Brand name	Ferrlecit®	Venofer®	Dexferrum®	Cosmofer® INFeD®	Ferinject® Injectafer®	Monofer®	Rienso® FeraHeme®
Carbohydrate shell	Gluconate (monosaccharide)	Sucrose (disaccharide)	Dextran (branched polysaccharide)	Dextran (branched polysaccharide)	Carboxymaltose (branched polysaccharide)	Isomaltoside (linear oligosaccharide)	Polyglucose sorbitol carboxymethylether (branched polysaccharide)
Molecular weight (kD)	289-440	30-60	265	165	150	150	750
Plasma half-life (hours)	1	6	60	20	16	20	15
Direct iron donation to transferrin (% injected dose)	5-6	4-5	1-2	1-2	1-2	<1	<1
Iron content (mg/mL)	12.5	20	50	50	50	100	30
Maximal single dose (mg)	125	200-300	20 mg/kg	20 mg/kg	20 mg/kg (max 1000 mg in one infusion)	20 mg/kg	510
Premedication	No	No	TDI only	No	No	No	No
Life-threatening ADE (x106 doses)	0.9	0.6	11.3	3.3	??	??	??

HMWID: high molecular weight iron dextran; LMWID: low molecular weight iron dextran; TDI: total dose infusion; ADE: adverse drug event.

Similarly, IV iron therapy is recommended for treating moderate-to-severe anaemia in postpartum (Grade 1B)[44-50] and inflammatory bowel disease (Grade 1B),[16,51,52] as well as an adjuvant to ESAs at correcting chemotherapy-induced anaemia (Grade 1A).[53-57] In addition, the use of IV iron has emerged as a viable alternative to allogeneic blood transfusions and a valuable tool to face restrictions on ESAs in cancer patients (Grade 2B).[58-61] In contrast, intramuscular (IM) iron administration is no longer recommended.

Safety

Although no serious IV iron-related adverse effects have been described, the number of surgical patients enrolled in the studies analysed is insufficient to draw definitive conclusions, especially in regards to the infrequent severe anaphylactic-type reactions. Future low risk of bias, adequately powered, prospective efficacy and safety trials in various surgical settings would be required to make evidence-based conclusions.[62]

However, data from the chronic kidney disease (CKD) study indicate that the frequency of severe adverse effects and deaths is extremely low except for high molecular weight iron dextran,[63] and significantly lower than the frequency with ABT.[64] More recently, using clinical data from 117,050 patients of a large US dialysis provider merged with data from Medicare's End-Stage Renal Disease programme, Brookhart et al.[65] estimated the effects of iron dosing patterns during repeated 1-month exposure periods on risks of mortality and infection-related hospitalisations during the subsequent 3 months. In 776,203 exposure/follow-up pairs, they observed that maintenance dosing did not associate with increased risks for adverse outcomes, compared with no iron.

Iron dextran complexes may cause well-known dextran-induced antibody-mediated anaphylactic reactions, which are significantly more frequent with those of higher molecular weight (not available in Europe) (Table 1). However, it must be remembered that all IV preparations have been reported to cause anaphylactoid reactions, which are characterised by nausea, hypotension, tachycardia, chest pain, dyspnoea (lung oedema), and bilateral oedema of the hands and feet, and they should not be misread as anaphylaxis.[66] These anaphylactoid reactions are mostly due to transferrin oversaturation and are, therefore, less frequent with the newer, more stable IV iron formulations (Table 1). As with other nanomedicines, complement activation-related pseudoallergy (CARPA), a mechanistic term for infusion or anaphylactoid reactions, could also be observed.

As of 28th June 2013, the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) concluded that the benefits of IV exceed their risks (favourable benefit-risk profile), when appropriately prescribed and dosed, and adequate measures are taken to minimise the risk of allergic reactions.[67] To improve patient safety, CHMP has issued clear recommendations for healthcare professionals, including:

- IV iron medicines should only be administered when staff trained to evaluate and manage anaphylactic and anaphylactoid reactions are immediately available as well as resuscitation facilities.

- A test dose is no longer recommended, as there are data indicating that allergic reactions may still occur in patients who have not reacted to a test dose.

- In the case of hypersensitivity reactions, healthcare professionals should immediately stop the iron administration and consider appropriate treatment for the hypersensitivity reaction.

- Patients should be closely observed for signs and symptoms of hypersensitivity reactions during and for at least 30 minutes following each injection of an IV iron medicine.

- IV iron-containing products are contraindicated in patients with hypersensitivity to a specific active substance or excipients, or other parenteral iron products.

- The risk of hypersensitivity is increased in patients with known allergies or immune or inflammatory conditions and in patients with a history of severe asthma, eczema, or other atopic allergy.

- IV iron products should not be used during pregnancy unless clearly necessary. Treatment should be confined to the second or third trimester, provided the benefits of treatment clearly outweigh the potential serious risks to the foetus such as anoxia and foetal distress.

• All prescribers should inform patients of the risk and seriousness of a hypersensitivity reaction and the importance of seeking medical attention if a reaction occurs.

With regards to the risk of infection, a recent meta-analysis concluded that: "Compared with oral iron and no iron, IV iron effectively increased Hb concentration and reduced RBC transfusions in various settings, but it was also associated with increased risk of infection."[68] However, infection was not a predefined endpoint in many pooled studies, a dose-response association between iron and risk of infection was not detected, further undermining the causal relationship, and rates of mortality, and other serious adverse events were not statistically significantly higher with IV iron. In contrast, when diagnosis of infection was clinically made using pre-established criteria and confirmed by laboratory, microbiologic, or radiologic evidence, no impact of IV iron on the infection rate was detected in large series of orthopaedic or cardiac surgeries.[41-43]

Practicalities and costs

Iron needs should always be calculated on an individual basis to avoid both infra and supra-dosage. The use of newer IV iron formulations (e.g. iron isomaltoside 1,000 or ferric carboxymaltose), which allow the administration of larger single doses (≥1,000 mg) will facilitate a more accurate iron replacement therapy in surgical patients. In addition, these newer IV iron formulations are safer and more convenient both for the patient (e.g. less venous punctures, less time out from work, etc.) and the health system (e.g. less visits to day hospital, less ambulance transfers, etc.).[16] These advantages may clearly out-balance their higher acquisition costs and make them cost-effective, suggesting that novel IV iron formulations are a valuable tool for the efficient and cost-effective treatment of iron deficiency in various therapeutic areas, including surgery.[38,43]

ESAs

Efficacy

In Europe, ESA administration is only indicated for reducing ABT rate in patients with moderate anaemia (Hb between 10 and 13 g/dL) scheduled for elective orthopaedic surgery who are expected to have moderate blood losses[69,70] (Grade 1A). ESA administration is also approved for those included in an autologous blood predonation programme and scheduled for procedures usually requiring three or more units of packed red cells (Grade 1C). The minimum effective dose of ESA in these indications is presently unknown.[71] Off-label use of ESA is suggested in patients undergoing cardiac surgery[72-74] or gastrointestinal cancer resection[75] (Grade 2B), and is not recommended in critically ill patients who do not have a previous indication (Grade -1A).[1,76]

Current European guidelines for anaemia management in CKD patients suggest using ESA therapy to generally maintain CKD patients with Hb values ranging between 10 and 12 g/dL, individualising the value in this target range according to the possible comorbidities of the patients. Hb values >13 g/dL should not be intentionally aimed for during ESA therapy in this setting.[77] However, no specific recommendations were issued for CKD patients undergoing major surgery.

Safety

Various government agencies (US FDA, EMA) have issued alerts on the association between the use of recombinant human erythropoietin and an increased risk of thromboembolic events and mortality in patients receiving long-term treatment for anaemia associated with chronic renal failure[77] or cancer chemotherapy,[54-56] as well as in patients undergoing orthopaedic surgery without thromboembolic prophylaxis.[78] It is important to stress that administration of IV iron alone will never result in supra-physiological Hb levels and thrombocytosis, leading to increased risk of thromboembolic complications, as could be the case with high ESA doses. In surgical patients, it would be, therefore, advisable to adjust ESA dose individually, ensure iron supply to the bone marrow (administering adjuvant iron, preferably IV), and provide adequate pharmacological thromboembolic prophylaxis.[1]

Restrictive Use of Allogeneic Blood Transfusion

After major surgery, perioperative blood loss and postoperative blunted erythropoiesis, due to surgery-induced inflammation, may lead to severe postoperative anaemia, especially in those presenting with low preoperative Hb. In this context, ABT continues to be the most frequently used treatment for acute intra and postoperative anaemia, although its quick and effective increase in Hb levels is transitory, there is a lack of evidence regarding its efficacy for increasing tissue oxygen

consumption or reducing tissue oxygen debt in selected patients, and it is associated with poorer outcomes. Subsequently, ABT should be used restrictively and judiciously in patients for whom pharmacological options are not available or cannot be implemented (e.g. acute severe anaemia with hemodynamic instability).

Accordingly, in making a transfusion decision in euvolaemic, non-bleeding patients: 1) the risk of anaemia and the risks and benefits of red cell transfusion should be carefully balanced for each individual patient; 2) the so-called 'liberal' transfusion protocols (pre-transfusion Hb concentration >9-10 g/dL) should be generally avoided; 3) should ABT be deemed necessary, single unit transfusions are desirable; and 4) patients should be reassessed between transfusions to determine the remaining transfusion needs.[79]

Efficacy

The use of patient-based restrictive transfusion criteria reduces both the frequency and volume of ABT (and, consequently, ABT-related risks) and should be the cornerstone of any PBM. In most surgical patients, ABT could be considered for maintaining Hb concentrations between 7-9 g/dL (Grade 1A); for those with cardiac and/or central nervous system dysfunction, ABT could be considered for patients with symptoms or Hb level of 8 g/dL or less, and given for maintaining Hb concentrations between 8-10 g/dL (Grade 1A).[1,2,79] Nevertheless, whenever possible, avoidance of ABT is preferable.[80]

Safety

Following the seminal Transfusion Requirements in the Critical Care trial,[81] a number of studies have demonstrated that restrictive transfusion triggers reduced transfusion rates and did not increase morbidity or mortality rates or the length of hospital stay in a variety of clinical settings, and could even be beneficial in some aspects.[79,80] However, its effects in high-risk groups need to be tested in further large clinical trials.[82,83] Meanwhile, for patients presenting with acute myocardial infarction, unstable angina, or other organ dysfunctions (heart failure, respiratory insufficiency, sepsis, etc.) it seems sensible to adopt a less restrictive transfusion protocol aimed at maintaining higher hemoglobin levels, although more studies are needed.[84-86]

AUTHORS' PERSPECTIVE

From the analysis of the reviewed evidence and the recommendations issued in several consensus documents, it seems fair to conclude that:

1. Preoperative anaemia should be detected, classified, and treated prior to elective procedures. For non-elective procedures, anaemia should be detected, classified, and treated as soon as possible. Whenever possible, pharmacological treatment should be preferred, whereas ABT should be restricted to those with severe anaemia and/or poor physiological reserve.

2. In elective procedures, preoperative IV iron replacement seems to be safe, results in lower transfusion requirements, and hastens recovery from postoperative anaemia. The use of newer IV iron formulations (ferric carboxymaltose or iron isomaltoside-1000) may facilitate iron replacement and offer additional benefits for both the patient and the health system.

3. If there is no contraindication, the preoperative use of ESAs seems to be justified, especially in those whose anaemia has an inflammatory component, although the minimal effective dose is presently unknown. An adequate iron supply should be ensured when using ESAs.

4. As they are inexpensive and non-toxic treatments, preoperative supplementation with folic acid (5 mg/day, oral) and vitamin B_{12} (1 mg, IM) could be considered to prevent functional or absolute deficiency during anaemia correction, especially if their levels are not routinely measured and/or in patients older than 60 years.

5. In non-elective procedures, the current evidence (mostly in hip fracture) broadly supports the use of IV iron or IV iron plus ESAs in reducing transfusion rates and improving outcome. Therefore, the acceptable safety profile and the ability to be administered without delaying surgery further support its clinical use.

6. Finally, the aim of performing major surgical procedures without the use of ABT and without placing the patient at risk of complications may be better accomplished by combining several blood conservation strategies into a defined PBM algorithm.

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LOOKING BACK TO OUR ROOTS: 80 YEARS OF WINTROBE'S INDICES

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ABSTRACT

This year (2014) we are celebrating the 80th anniversary of Dr Maxwell Myer Wintrobe's pioneer works, one of the most important contributions in clinical laboratory and medicine. Red cell indices continue to provide an essential support to the diagnosis and classification of anaemia. The erythrocyte indices, such as mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin, are called the Wintrobe's indices. Automation in hematology has progressed steadily since Wallace Coulter first applied electrical impedance technology to counting red cells and white cells. Technological advances being incorporated into hematology analysers since then are now allowing access to more cellular information than was ever available before through a 'simple routine complete blood count'. Current research is beginning to demonstrate that this information also has great potential to identify cellular changes that typically occur in several important medical conditions-bringing us all one step closer to using hematology analysers as more than just simple cell counters, but instead as powerful tools for the management of any medical condition that impacts the biology of blood cells. There are increasing amounts of data provided, which require specialist knowledge to interpret as well as to understand the limitations in the measurement of the parameter. Both laboratory scientists and clinicians need to keep up-to-date with new parameters and methods in hematology, implying a stronger collaboration between them to improve clinical decision-making.

Keywords: Anaemia, erythrocyte indices, red cells, hematology analysers.

MAXWELL MYER WINTROBE - THE PIONEER

In 2014 we are celebrating the 80th anniversary of Dr Maxwell Myer Wintrobe´s pioneer works, one of the most important contributions in clinical laboratory and medicine. Red cell indices, the Wintrobe's indices, continue to provide an essential support to the diagnosis and classification of anaemia. From the measurements of the average volume and hemoglobin (Hb) concentration of erythrocytes, the underlying aetiology of anaemia was brilliantly unveiled.[1-4] Anaemia is a disease itself, but it is often symptomatic of other illnesses; every clinician must deal with anaemia in daily practice, taking into account that concomitant anaemia makes the prognosis of the underlying disease worst for those patients.

His text book: *Clinical Hematology*, first published in 1942,[5] remains a prototype of excellence and for many years stood alone as the premier text in the field. He had written and edited the first six editions by himself, though always depending on the critical peer review of his talented colleagues at Utah University, with his wife Becky, as he said, his severest and most helpful critic.[6] '*Hematology, the Blossoming of a Science: A Story of Inspiration and Effort*' was published in 1985,[7] soon before his passing. Writing this book as part memoir, part

history, Dr Wintrobe realised that he could never cover the lives of all who had contributed to what he called 'the Golden Age of Hematology'. When he died in 1986, his distinguished career in medicine had spanned 60 years. His scientific achievements are recorded in >400 publications. He was a world leader in hematology, a role attested by a legion of honours, memberships, and activities in national and international scientific societies; elected to the National Academy of Sciences, he became the first chairman of the section of Human Genetics, Hematotogy, and Oncology.[6]

Wintrobe's method was based on manual measurements of Hb, hematocrit, and erythrocyte count. Reading his articles in the 1930s,[1-4] his concern for the accuracy of the methods and the reliability of the measurements as the first step of his work to classify anaemia was clear; then he brilliantly postulated the link between cell morphology to deduce the real aetiology of each type of anaemia. The erythrocyte indices, which include mean cell volume (MCV), mean cell Hb concentration (MCHC), and mean cell Hb (MCH; derived from precisely measured values of Hb and red blood cell [RBC] count), are collectively called the Wintrobe indices.[8] The hemogram is one of the more required tests by clinicians and the indices are still applied for classifying anaemia. The analysis nowadays is totally automated and the correct interpretation of the results is required to reunite the knowledge about the characteristics of the equipment and the clinical meaning of the results.[9]

THE BIRTH OF AUTOMATION

The first attempt in automation of blood cell counts was the introduction of the Coulter Principle. While under contract to the US Navy in the late 1940s, (though a patent was not awarded until the 20th October, 1953), Wallace H. Coulter developed a technology for counting and sizing particles using impedance measurements. The technology was principally developed to count blood cells quickly by measuring the changes in electrical conductance as cells suspended in a conductive fluid passed through a small orifice. Presently, most automated cell counters incorporate this technology, which is referred to as the Coulter Principle.

Using count and pulse height analyser circuits, the number of particles and volume of each particle passing through the aperture can be measured. If the volume of liquid passing through the aperture can be precisely controlled and measured, the concentration of the sample can also be determined. A typical measurement using Coulter counter instruments takes <1 minute, as counting and sizing rates of up to 10,000 particles per second are possible. The accuracy of the size measurements can be better than 1%.[10]

Automation in hematology has progressed steadily since Wallace Coulter first applied electrical impedance technology to counting red and white cells. By the 1980s, most hematology laboratories were reporting a 7-parameter complete blood count (CBC) and three-part differential obtained from a single aspiration on a stand-alone, bench-top instrument. Eventually, this process was upgraded even further when it became possible to obtain these results without uncapping the sample.

Automated blood cell analysis is traditionally performed using impedance technology that allows the measuring of RBCs. Combined with the determination of blood Hb concentration, basic RBC indices - as defined by Wintrobe - can be calculated: MCV, MCH, and MCHC. An important overlooked fact is that an individual's red cell volume determination in various hematology analysers is dependent on the technology used, and that hematocrit value is calculated from the measured MCV. The most reliable red cell index with all hematology analysers is MCH, which is derived from precisely measured values of Hb and RBC.[8]

The Advance of Technology

In the 1990s, the analysers were capable of quantifying the heterogeneity in distribution of MCV (red cell distribution width [RDW]), adding remarkable useful information to MVC. In order to mathematically describe a set, two types of measures are necessary: centralisation measures (more abundant individuals) and measures of dispersion, giving insight into the heterogeneity of the population. MCV is the mean of the volumes of all erythrocytes; RDW refers to the variety of individual cells with different volumes present in the whole population so the contribution of marginal sized subsets to the calculated mean value can be assessed.[11]

Although RDW is generally understood to be a coefficient of variation of the distributions of erythrocyte volume, some hematology analysers calculate RDW from the direct measurement of

the width of the distribution.[12] Other technologies have been adapted to the automated analysers, enabling them to analyse the physiological and chemical characteristics of cells, for example, flow cytometry. Nowadays most of the counters in the hematology laboratory utilise laser, impedance, and/or flow cytometry. The basic purpose of the optics bench in a flow cytometer is to detect light from cells as they pass through a flow cell illuminated by a laser beam. This light can be scattered from the surface and internal structures of the cell. In addition to volume and conductivity, several additional cellular measurements can be obtained for each individual cell, including different angles of light scatter and pulse time. This information can be used in multiple combinations, allowing discrimination of different cellular elements.[13]

The American Legacy

The flow cytometric optical technology for RBC parameters measurement was first made available by the then Technicon Company in their H* series of instruments, later followed by the Advia® hematology analysers (Bayer Diagnostics, presently Siemens Healthcare Diagnostics, Deerfield, IL, USA). This optical technology enables the measuring, simultaneously and independently, of the cellular Hb concentration of individual erythrocytes as well as their volume, derived from light scatter by isovolumetrically sphered RBC according to the Mie theory.[14,15] Mie theory describes the mathematics of light diffraction by spherical objects; in this case the red cells are transformed into isovolumetric spheres. When using monochromatic laser light, diffraction is only a function of the size and the refractive index of the object (related to its internal structure).

In the red cell/platelet channel, red cells are converted to spheres. Using Mie theory, low-angle (2-3°) and high-angle (5-15°) light scatter is measured and mathematical models in the software use these scatter signals for calculating the size and Hb concentration (CHC) of individual red cells. Based in both measurements, a cytogram can be provided. In this graph, the size of individual red cells is indicated on the y-axis, while their Hb concentration is indicated on the x-axis. The whole RBC population can be classified considering the Hb concentration and cell volume, and four new RBC extended parameters have become available: hypochromic RBC with CHC <280 g/L, hyperchromic RBC, CHC >410 g/L, microcytic with volume <60 fL, and macrocytic with volume >120 fL.

Technicon Instruments was the first manufacturer to offer a complete set of extended RBC parameters in their H*3 hematology analyser[16] and this explains why the majority of literature on these parameters was produced using this analyser and its successors of the Advia series (Siemens Healthcare Diagnostics). More recently, Abbott (Abbott Diagnostics, Santa Clara, CA, USA) introduced extended RBC parameters on the CELL-DYN Sapphire analyser. These are calculated from three-dimensional laser light scatter, applying the principles of the Mie theory, which is basically the same as in the Advia analysers.[17,18]

The Asian Wisdom

Over the past years, other manufacturers have started offering extended RBC parameters, although differences in the technology used make it impossible to directly compare the numerical values of RBC parameters derived from different instruments. Sysmex analysers XE 5000 and XN (Sysmex Corporation, Kobe, Japan) report RBC extended parameters, the percentages of erythrocyte subsets. In the reticulocyte channel hypochromic and hyperchromic erythrocytes are quantified by means of flow cytometry, but it is important to highlight that it is Hb content which is reported. Hypochromic red cells by Sysmex are those with an Hb content of <17 pg, while hyperchromic are those with Hb >49 pg.[19] RBCs are continuously produced in the bone marrow; because of the long lifespan of mature erythrocytes the values of the derived parameters are related to iron status in the last 2-3 months.

MCV is the mean of the volumes of all erythrocytes; RDW refers to the heterogeneity of volumes present in the red cell population. This is not the case for MHC. Calculated from RBC count and Hb, MCH represents the average; the percentage of subsets can give complementary information of the contribution of the cell with extreme values (hypochromic and hyperchromic cells) to the mean values. The percentages reflect how Hb is distributed in the individual cells, in a uniform way or not; if polychromasia is present, the fluctuations of iron availability to the erythron in the previous weeks can be highlighted.[11,20] Similar application of those technologies are being introduced by other companies (Mindray Biomedical Electronics, Nanshan, Shenzhen, China) on their analysers.[21]

In summary, technological advances being incorporated into hematology analysers since Dr Wintrobe´s days are now allowing access to more cellular information than was ever available before through a 'simple routine CBC'.[22,23] The added information potentially contained in the 'new extended hemogram' has proven its usefulness in certain clinical conditions: anaemia of chronic disease, functional iron deficiency, and monitoring the availability of iron during treatment with erythropoietin,[11,17,24-31] thalassaemia trait screening,[32,33] spherocytosis,[34,35] and latent iron deficiency.[36]

A LOOK INTO THE FUTURE

Current research is beginning to demonstrate that this information also has great potential to identify cellular changes that typically occur in several important medical conditions, bringing us all one step closer to using hematology analysers as more than just simple cell counters, but instead as powerful tools for the management of any medical condition that impacts the biology of blood cells.

Bone marrow produces $200x10^9$ cells per day; the body loses the same number through senescence, maintaining the overall steady state in physiological conditions, but little is known about the life cycle of 120 days of RBC. Novel and promising research[37] has added new light on the relationship between Hb content, Hb concentration, and cell volume. Using a systems biology approach, the dynamics of circulating red cells can be analysed, linking the changes of volume and Hb concentration to red cell lifespan and removal. The mechanisms that regulate the number, size, and Hb concentration of normal red cells in circulation are not well understood. It has been established, however, that after their release from the bone marrow, red cells undergo a reduction in their volume and total Hb content.

Higgins and Mahadevan[37] used a theory from statistical physics, together with standard red cell indices derived from electronic cell counters and the information generated by flow cytometry, to develop a master equation for the maturation and clearance of red cells. Their mathematical model implies that the total number of red cells added to the circulation equals the number removed, and suggests that there is a threshold for the mean cellular Hb concentration below which most red cells are cleared from the circulation.[38] The model distinguishes the dynamics of red cell population in healthy subjects from those of patients suffering iron deficiency, thalassaemia, or anaemia of chronic disease.[37]

As has been exposed, manufacturers have developed hematology analysers that achieve good levels of precision and accuracy in cell counting through the examination and identification of thousands, not hundreds, of cells in each sample analysed. There are increasing amounts of data provided, which require specialist knowledge to interpret and understand the limitations in the measurement of the parameter. Both laboratory scientists and clinicians need to keep up-to-date with new parameters and methods in hematology, implying a stronger collaboration between them. Good laboratory practice ensures that reliable results of laboratory tests are reported to the clinician.[39] The modern clinical hematology laboratory is facing strong pressures to provide clinically relevant information that can help the clinicians to make a diagnosis in a fast, cheap, and useful manner, but we must not forget the words by Dr Wintrobe: "And yet, we must not stop exploring and measuring, for there is always more to learn."

Happy anniversary!

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Leukaemia and lymphoma patients' lives enhanced by new drug

"I have yet to come across another class of drugs in my career that has been so successful for leukaemia or lymphoma."

Prof Simon Rule,
Plymouth University Peninsula Schools of Medicine and Dentistry,
Plymouth, UK

TERMINAL leukaemia and lymphoma sufferers will experience improvements in their quality and length of life with the introduction of a cancer inhibiting drug, with minimal side-effects, pushing harmful chemotherapy to the side.

Penned as a major breakthrough in cancer therapy, a new class of Bruton's Tyrosine Kinase (BTK) inhibiting drugs operate by inhibiting the BTK protein, which stimulates signalling, causing cancerous cell growth. Blocking the protein causes the death of cancerous cells while normal cells are left unaffected.

An average survival rate of 4-5 years means the drug is a genuinely life-enhancing option for leukaemia and lymphoma sufferers who have run out of other options.

The first worldwide study in this area was led by Prof Simon Rule, Consultant Haematologist, Plymouth Hospitals NHS Trust, and Researcher, Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, UK.

Massive improvements and positive reactions have been noted in the patients enrolled into the study; the number of patients taking the drug has risen from 4 in September 2012 to 30 currently.

Prof Rule said: "The UK is at the forefront of this drug development and all of the studies into these drugs are being run from Plymouth. This will completely change the way we manage these diseases. We have access to the next generation of the drug to be part of the next trial phases.

"This is not a cure for cancer but it will mean we are significantly improving our patients' life expectancy and quality of life; similar to managing a chronic condition. I have yet to come across another class of drugs in my career that has been so successful for leukaemia or lymphoma."

The next stage is to put the latest BTK drug through its paces, hopes are high that this will signal an end to standard chemotherapy for the treatment of leukaemia.

Nose no bounds: pushing the boundaries in regenerative medicine

CUSTOM-MADE body parts such as noses, ears, and blood vessels are being grown in the laboratory using the patient's stem cells. This promising outcome has already caught the attention of Mr Boris Johnson, the Mayor of London, who hopes that this might attract investment to the UK's health and science sectors.

The body parts are the result of a combination of 3D printing technology, stem cell research, and nanotechnology. The revolutionary technique has already supplied tear ducts, blood vessels, and windpipes to UK patients.

"It's like making a cake," said Prof Alexander Seifalian, lead scientist and Professor, Nanotechnology and Regenerative Medicine, University College London, London, UK. "We just use a different kind of oven."

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"I'm convinced engineered organs are going to be on the market soon."

Prof Suchitra Sumitran-Holgersson, University of Gothenburg, Gothenburg, Sweden

Firstly, the researchers start by making a glass mould of the original organ. Salt and sugar solution is then added to the mould and made to set. Once this is done, the mould is removed leaving behind a honeycomb scaffold which serves as the cartilage's foundation.

Stem cells obtained from the patient's abdominal fat were then obtained and grown in the laboratory before being added to the scaffold. The patient's skin on another area of the body is then gradually stretched using a small inflated balloon and once there is enough space, the ready-made organ is implanted sub-dermally. After several months, the organ is then removed and placed in the correct location.

The researchers custom-made a nose for a patient who had lost his original nose to cancer; it is currently implanted under the skin on his forearm and is waiting for approval to transfer it onto his face.

"I'm convinced engineered organs are going to be on the market soon," said Prof Suchitra Sumitran-Holgersson, Professor, Transplantation Biology, University of Gothenburg, Gothenburg, Sweden. Currently, she has transferred lab-made blood vessels to a few patients and hopes to progress to a larger population, once regulatory approval has been received.

Mini heart pumping it up

"We are suggesting, for the first time, to use stem cells to create rather than just repair damaged organs. We can make a new heart outside of one's own heart, and by placing it in the lower extremities, significantly improve venous blood flow."

Dr Narine Sarvazyan, George Washington University, Washington, USA

RHYTHMICALLY contracting cuff acts as an implanted mini heart to restore blood flow in veins which lack functioning valves. The mini heart is typically comprised of heart muscle cells, and the resulting pulsation pumps the blood through the vein.

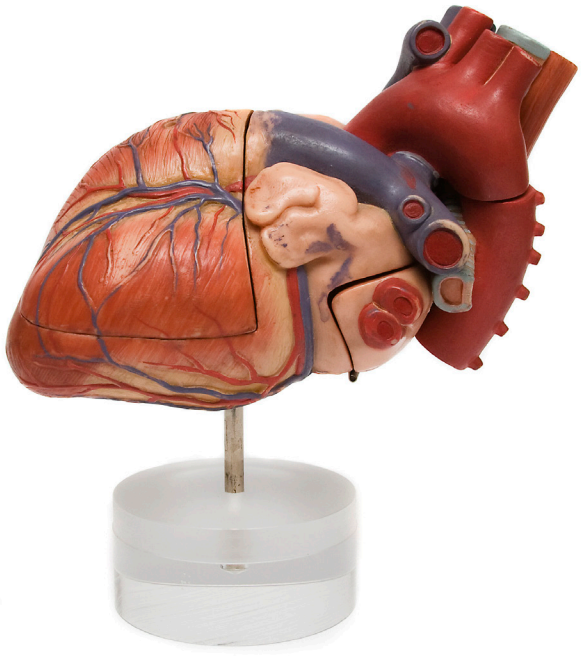
"We are suggesting, for the first time, to use stem cells to create rather than just repair damaged organs. We can make a new heart outside of one's own heart, and by placing it in the lower extremities, significantly improve venous blood flow," said Dr Narine Sarvazyan, Associate Professor, School of Medicine and Health Sciences, George Washington University, Washington, D.C., USA.

This innovative procedure has the potential to treat chronic venous insufficiency (CVI), a long-term condition where the vein struggles to pump blood from the legs back to the heart. The chronic state can be the result of vein blockage or leaky valves, which may be due to deep vein thrombosis (DVT) or blood clots. This condition affects approximately 20-30% of people over the age of 50 and it is more frequently presented in women than men.

Varicose veins, ulcers, and discolouration on the legs and ankles are common symptoms resulting from this condition. If the symptoms worsen to the point of causing skin sores and leg pain, then surgical removal of varicose veins is recommended. In the case of increased swelling, the use of compression stockings can alleviate this issue.

Individuals suffering from 'sluggish' blood flow (such as diabetes), paralysis, and those that are on the road to recovery after undergoing surgery may also benefit from this implantation.

Considered the next diagnostic frontier, this therapeutic option will be pushing the boundaries in the tissue engineering field. Dr Sarvazyan and her research team have demonstrated the success of this technique *in vitro* and are looking to progress onto *in vivo* trials.



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Step aside stubborn blood cancer

"Considering the high number of previous therapies that these patients had received, higher than we sometimes see in comparable studies, the efficacy of idelalisib that we observed was remarkable."

Dr Ian Flinn,
Sarah Cannon Research Institute,
Nashville, USA

A PILL targeting a key regulator of cancer growth may be the answer for relapsed leukaemia and lymphoma sufferers who have exhausted their treatment options.

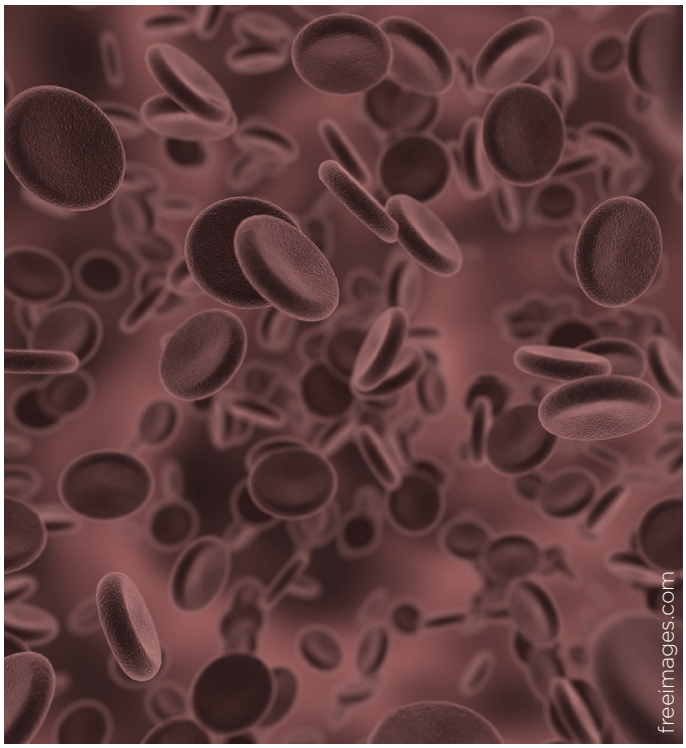
Chemotherapy and immunotherapy are currently offered as a first-line combination therapy to blood cancer patients. However, chemotherapy, although an effective weapon, carries a high risk of side-effects, along with resistance build-up in patients.

Idelalisib - taken as a pill - targets and then blocks the expression of the delta isoform of the PI3 kinase enzyme, crucial for the activation and survival of cancerous B cells. Idelalisib's keyhole targeting of the PI3 kinase delta has differentiated it from the current crowd of treatments, leaving healthy cells alone, and thus, giving a tantalising option to chronic lymphocytic leukaemia (CLL), indolent non-Hodgkin lymphoma (iNHL), and mantle cell lymphoma (MCL) sufferers.

To test the safety and efficacy of idelalisib, 150 subjects with either CLL, iNHL, or MCL - who had shown little-to-no response to several treatments prior to the study - were given varying doses of the drug in a major Phase I study. 72% of CLL subjects, 47% of iNHL subjects, and 40% of MCL subjects showed either complete or partial response to the treatment, while few side-effects were felt. These positive response rates give fuel to the belief that a key regulator of cancer growth has been identified, allowing for the development of a collection of treatments targeting this crucial component.

"Considering the high number of previous therapies that these patients had received, higher than we sometimes see in comparable studies, the efficacy of idelalisib that we observed was remarkable," said study author and renowned lymphoma expert Dr Ian Flinn, Director, Hematologic Malignancies Research Program, Sarah Cannon Research Institute, Nashville, Tennessee, USA.

More research, however, is required to determine how idelalisib might be best applied to the different blood cancers.



The benefits of being a mutant

"The peripheral blood was derived from only two active hematopoietic stem cells (in contrast to an estimated 1,300 simultaneously active stem cells), which were related to each other."

Dr Henne Holstege, VU University Medical Center, Amsterdam, the Netherlands

HEALTHY blood cells of a 115-year-old woman carried hundreds of mutations in her body throughout her life.

In 2005 a woman became the oldest person ever to donate her body to science. Researchers found that the deceased woman had over 400 genetic mutations in her healthy blood cells, but these lesions are harmless throughout a body's lifetime.

Little is known about the effects of mutations in healthy people, which are often the result of rapid stem cell division that is prone to DNA copying errors.

It is understood that hundreds of these mutations can cause blood cancers, however it is unknown whether mutations occur in healthy white blood cells.

To investigate this area, a research team led by Dr Henne Holstege, Department of Clinical Genetics, VU University Medical Center, Amsterdam, the Netherlands, carried out whole genome sequencing of healthy white blood cells from the 115-year-old woman. Data showed that the woman did not exhibit signs of hematological illness despite carrying 400 somatic mutations in white blood cells outside of the brain.

Dr Holstege commented: "To our great surprise we found that, at the time of her death, the peripheral blood was derived from only two active hematopoietic stem cells (in contrast to an estimated 1,300 simultaneously active stem cells), which were related to each other."

On top of this, the length of telomeres seems key in judging a stem cell's lifespan; white blood cell telomeres were found to be 17-times shorter than those of the brain. Dr Holstege said: "[The researchers] speculate that most hematopoietic stem cells may have died from 'stem cell exhaustion', reaching the upper limit of stem cell divisions."

More work is needed to determine whether stem cell exhaustion is a frequent cause of death in the very elderly.

Malaria hopes rekindled by spermicidal strategy

KILLING sperm in malaria-carrying mosquitoes could be the key to fighting one of the world's most devastating diseases.

Anopheles gambiae mosquitoes are chiefly responsible for the spread of malaria, which currently afflicts around 200 million people every year. The females, which reproduce just once in their whole lifetime, store sperm from this one moment of intercourse in an organ known as the spermatheca, drawing sperm from this organ to fertilise the eggs they lay.

Naturally, the female must ensure the sperm is kept healthy to ensure that it is ready for the fertilisation process; according to new research, the main component that is potentially responsible for preserving sperm is an enzyme known as HPX15. This enzyme partly protects sperm from damaging molecules called free radicals, particularly plentiful after a female takes a blood feed.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When HPX15 was disrupted in laboratory-held female *A. gambiae* mosquitoes, the females fertilised fewer eggs and, therefore, created fewer offspring. 3% of eggs laid by *A. gambiae* females do not develop into offspring usually; however, this number increased to 20% upon HPX15-inhibition.

Hope has swelled in the scientific community that a way to effectively fight malaria has been discovered; female fertility would be decimated by disabling HPX15, thus causing a significant reduction in the number of malaria-carrying mosquitoes.

Dr Robert Shaw, one of the lead authors of the study, Department of Life Sciences, Imperial College London, London, UK, said: "There is no single magic bullet for tackling malaria, but making mosquitoes less fertile could provide us with a valuable weapon against the disease."

A hormone called 20E, produced by the male mosquito, was also found. This is transferred to the female during mating and activates the HPX15 enzyme, thus providing another potential target for inhibition.

Further research is needed to better understand how to inhibit the activation of HPX15 and 20E to reduce female *A. gambiae* fertility.

Sticky cells, a living hell

"In future, it may be possible to measure BCR-ABL levels in individual cells in the clinic - this will help us identify the resistant high BCR-ABL cells."

Dr Richard Byers, University of Manchester, Manchester, UK

STICKY leukaemia cells may be hindering the effective treatment of chronic blood cancer.

Chronic myeloid leukaemia (CML) arises from a specific genetic mutation, caused by DNA on separate chromosomes breaking away and swapping places.

Genes at the breakpoint and re-joining point become disrupted as a result, including BCR-ABL, a gene which has proved to be a crucial target for the development of key CML treatment such as imatinib. Unfortunately, resistance to these drugs has been recorded in patients, signalling a need for awareness of the relevant biology behind this.

Scientists from The University of Manchester, part of the Manchester Cancer Research Centre have used a cell model to measure levels of BCR-ABL mutation. This involved the separation of these cells into two groups: those that stuck to plastic (known as 'sticky', or adherent, cells) and the non-adherent cells which showed no stickiness. Differences between the two groups were investigated, and a wide variation in BCR-ABL expression levels was noted. In particular, the sticky cells displayed greater levels of BCR-ABL expression and were also found to have superior resistance to imatinib.

"The small number of cells that show high levels of BCR-ABL may not be detectable through bulk analysis of large samples," said joint leader of the research Dr Richard Byers, Clinical Senior Lecturer, Institute of Cancer Sciences, University of Manchester, Manchester, UK.

"It looks like it is important to look at protein levels in single cells. In future, it may be possible to measure BCR-ABL levels in individual cells in the clinic - this will help us identify the resistant high BCR-ABL cells and better understand how patients develop resistance to imatinib treatment with the aim of combatting this resistance to make response more durable and the treatment more effective," Dr Byers continued.

Featured Suppliers

Hematology

With its headquarters in Connecticut, USA, Alexion is a global company with more than 1,800 employees worldwide helping patients in nearly 50 countries. Since 1992, Alexion has developed from a biotech start-up into one of the world's largest biopharmaceutical companies. The company is focused on developing life-altering therapies for patients with extremely rare, severe, and life-threatening diseases. Alexion's first commercial product was Soliris (eculizumab); the only approved terminal complement inhibitor currently available worldwide. Soliris is approved in USA, EU, and Japan, among others, as a treatment for patients with atypical haemolytic uraemic syndrome.

Bristol-Myers Squibb (BMS) is an established giant of the biopharmaceutical industry, having led research and development products for some of the most innovative medical products ever created. Across the world, the company's medicines have helped millions in their fight against raging diseases such as cancer, cardiovascular disease, hepatitis B, HIV/AIDS, and various psychiatric disorders. As part of its goal to provide patient access to healthcare worldwide, BMS has launched a ground-breaking $150m programme to help relieve HIV/AIDS in Africa, whilst in the USA, free medications are being provided to patients with financial hardship.

Pursuing a goal of delivering cutting-edge, life-altering therapies to patients in over 50 countries worldwide, Celgene seeks to establish itself as a major player in the global pharmaceuticals industry. A plethora of immune-inflammatory related conditions, including cancer, constitute the targets set by Celgene for the finding, development, and marketing of patented products. These are tested rigorously at key medical centres in over 300 clinical trials. Multiple myeloma, myelodysplastic syndromes, and chronic lymphocytic leukaemia are among the incurable haematological and solid tumour cancers currently being targeted with the development of investigational treatments.

Hexal develops, manufactures, and markets modern generic products and innovative pharmaceuticals. As a leading provider of off-patent medicines in Germany, Hexal aims to ensure that all patients get the medicines they need, offering high-quality medicines at a low cost in a range of major therapeutic areas. Hexal's portfolio includes products with more than 300 active pharmaceutical ingredients. It ranges from drugs with innovative systems for drug delivery to biosimilars, which are highly complex biotech 'drugs of the future'. Hexal belongs to the Sandoz Group and has about 4,300 employees in 6 locations in Germany.

Takeda is currently the largest pharmaceutical company in Japan, and is one of the main global healthcare players. The company has based its philosophy on the concept of 'Takeda-ism' (integrity, fairness, honesty, and perseverance), which has been developed over the company's 230-year lifetime. Following this, Takeda carries out its activities through the company slogan: "Strive towards better health for people worldwide through leading innovation in medicine." The Osaka-based firm has over 30,000 employees in more than 70 countries and regions worldwide. Takeda's pharmaceutical products have been marketed in around 100 countries.

Buyer's Guide

Exhibitors

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- SUNESIS PHARMACEUTICALS, INC.
- TAKEDA
- TEVA
- VIFOR PHARMA

35th World Congress of the International Society of Hematology (ISH) 2014

4th-7th September 2014

Beijing, China

World-renowned professors and healthcare professionals will congregate at this Congress to share their experience of hematological disorders within clinical and research fields. The scientific program will cover cutting-edge development in basic research, and the latest advances of clinical management in this promising field. Topics that will be covered include: acute/chronic leukaemia, bone marrow transplantation, bleeding disorders and melanoma.

3rd World Congress on Controversies in Hematology (COHEM) 2014

11th-13th September 2014

Istanbul, Turkey

This Congress will disseminate up-to-date information and provide a unique opportunity for world-class leaders in the field to debate vital and contentious issues in hematology. This event will address the most challenging current clinical and technological questions. These stimulating debates will provide clinicians with state-of-the-art recommendations regarding patient care. Topics that will be covered include myeloma, myleofibrosis and lymphoma.

2nd European Platelet Group Conference (EUPLAN) 2014

25th-26th September 2014

Bischoffsheim, France

This event aims to provide an international platform for information exchange and promotion of basic and clinical research on blood platelets and megakaryocytes. This is the perfect opportunity to network with a wide range of scientists and clinicians working in this specialised field of research. This event will build on the success of the first conference by incorporating a wide range of stimulating lectures, presentations and clinical cases.

2nd International Conference on Hematology & Blood Disorders 2014

29th September-1st October 2014

Baltimore, USA

This conference will be the pinnacle event for the interaction between distinguished researchers around the world in the core of hematological disorders. The anticipated audience will include hematologists, immunologists, pathologists, oncologists, and industrial professionals from biomedical and healthcare sectors. The conference will focus on the theme: "On the Path of Identifying Novel Therapeutics for Blood Disorders."

European School of Haematology (ESH) 6th International Conference on Myeloproliferative Neoplasms (MPN) 2014

23rd–25th October 2014

Estoril, Portugal

Over the past few years there has continued to be a flood of new insights into the classification, pathogenesis and management of MPN. This event will bring together scientists and clinicians with new data on the cellular and molecular biology of these disorders as well as their diagnosis and treatment. Data will be presented in the form of lectures, presentations, symposia, and courses. Topics to be covered include: genomics and Jak2 inhibitors.

World Congress on Controversies in Thrombosis and Hemostasis (CiTH) 2014

30th October-2nd November 2014

Berlin, Germany

This 'concept congress' will deal mainly with controversial issues in the format of debates and discussions, allowing for speaker-participant interaction. The ability to discuss topics with emphasis on clinical solutions in cases where no agreed-upon answers exist, provides clinicians with an insight and a take-home message that ameliorates treatment in the most difficult situations. Topics that will be covered include: thrombotic and bleeding disorders.

ESH 2nd International Conference on Multiple Myeloma

7th-9th November 2014

Athens, Greece

This educational meeting aims to address the latest developments in multiple myeloma as part of the quest for more effective and better-tolerated therapies and better understanding of the disease pathophysiology. A significant number of prestigious speakers will attend and deliver keynote lectures in the field. This meeting is expected to be a great success and there will also be plentiful opportunities for networking within the scientific community.

ESH International Conference on New Concepts in B Cell Malignancies 2014

14th-16th November 2014

Thessaloniki, Greece

This 'From Molecular Pathogenesis to Personalized Treatment' Conference will emphasise the principles and current developments of molecular pathogenesis of B cell disorders with in-depth discussions into the range of prognostic markers and evolution of treatment principles in B cell malignancies. The scientific programme will include a wide range of clinical conferences, symposia and presentations.