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INSIDE

Review of

EHA 2018

Stockholm, Sweden

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"We hope that you will enjoy the hand-picked content created for your benefit within this fantastic edition of EMJ Hematology."

Spencer Gore, CEO

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Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

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EMJ Hematol.

European Medical Journal 3.2

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VIEW ALL JOURNALS ←

Welcome

A very warm welcome to you as we embrace another spectacular year of haematological advancement and achievement. This 2018 edition of *EMJ Hematology* brings you the very best of the field's developments, with an emphasis on our unique review of the 23rd European Hematology Association (EHA) Congress, this year held in Stockholm, Sweden. Relive the best moments from the event before delving into peer-reviewed articles, abstract reviews straight from EHA 2018, and interviews with our valued *EMJ Hematology* Editorial Board.

The 23rd EHA Congress brought forward novel findings from across the globe to disseminate to its attendees, who were eager to learn and feast on the latest updates from the field. We bring you the best and most exciting highlights of these updates, with topics including Epstein–Barr virus, acute myeloid leukaemia, immune thrombocytopenia, and many more.

Continue your journey through the EHA Congress by stepping into our Abstract Review section. Presenters from the 4-day event leap from presentation to paper as they bring you short reviews of their research. Find information on polymorphisms in multidrug resistant chronic myeloid leukaemia patients; updates on lenalidomide, the treatment of choice for non-Hodgkin's lymphoma; and results surrounding studies for non-transfusion-dependent thalassaemia treatments.

Taking you further afield, *EMJ Hematology's* Interviews section allows you to get a glimpse of the lives of our esteemed Editorial Board members. Careers, inspirations, and future plans are all discussed, giving you a unique insight into the minds of some of the field's leading figures.

Our peer-reviewed articles bring you even more of the best information around and we are proud to present them to you. This year's Editor's Pick is a paper by Eskazan and Tiribelli. The authors discuss the use of allogeneic haematopoietic stem cell transplant and tyrosine kinase inhibitors as methods of treatment for chronic myeloid leukaemia. Though allogeneic haematopoietic stem cell transplants have long been an effective technique for curative treatment, tyrosine kinase inhibitors have been shown to improve prognosis for these patients. The authors investigate the indications of allogeneic haematopoietic stem cell transplant now that tyrosine kinase inhibitors are becoming increasingly available. Read this paper and others in the Articles section of the eJournal.

We hope that you will enjoy the hand-picked content created for your benefit within this fantastic edition of *EMJ Hematology*. Let us know your thoughts on our social media channels: Facebook, Twitter, LinkedIn, and Google+. Enjoy!

Spencer Gore

Chief Executive Officer, European Medical Group

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Abbreviations CML, chronic myeloid leukaemia; CP, chronic phase; ELN, European LeukaemiaNet; MMR, major molecular response, ≤0.1% BCR-ABL1 transcripts on the international scale with ≥3,000 ABL1 assessed. MR4.5, ≤0.0032% BCR-ABL1 transcripts on the international scale with ≥30,990 ABL1 assessed; Ph+, Philadelphia chromosome-postive; TEAE, treatment-emergent adverse event.

References **1.** Bower H, *et al. J Clin Oncol.* 2016;34(24):2851–7. **2.** Saussele S, *et al. Blood* 2015;126(1):42–9. **3.** Hehlmann R, *et al. Leukemia* 2017;31:2398–406. **4.** Cortes JE, *et al. J Clin Oncol.* 2018;36(3):231–7. **5.** Baccarani M, *et al. Blood* 2013;122(6):872–84. **6.** Pfizer Data on File, 2018. **7.** Cortes JE, *et al. J Clin Oncol* 2018;36:231–7. Supplementary materials: data. **8.** BOSULIF SmPC, 2018.

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Foreword

Dear colleagues,

It is a pleasure for me to introduce you to the latest issue of *EMJ Hematology*. In this edition there are several articles with huge importance for haematologists and everyone with an interest in the field. There are also relevant statistics and a review of the annual European Hematology Association (EHA) Congress.

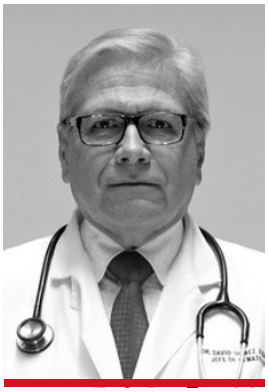
Focussing on some of the exciting articles in the journal, Gupta and Gadipudi have written about an ancient disease with a high impact around the world: anaemia in pregnancy. The article puts a spotlight on the disease's screening tools and therapeutic options, which vary significantly between high and low-income countries. In addition, Scarisbrick describes the latest clinical staging of mycosis fungoides and Sézary syndrome and asks the question: do we need new staging criteria based on current data? On the role of allogeneic stem cell transplantation (alloSCT) in chronic myeloid leukaemia after the introduction of tyrosine kinase inhibitors (TKI), Ezkazan and Tiribelli highlight that, even in the TKI era, we must remember that alloSCT has a powerful curative potential.

"In this edition there are several articles with huge importance for haematologists and everyone with an interest in the field. There are also relevant statistics and a review of the annual European Hematology Association (EHA) Congress."

This year's EHA Congress took place in Stockholm, Sweden on 14th–17th June and was an absolute party of research and knowledge, with >11,500 attendees from around the globe. Over 200 oral sessions and 1,300 posters were presented, embracing topics from basic science to new therapeutic options in clinical haematology, with immunotherapy being a hot topic. More information reviewing this latest EHA meeting awaits within this eJournal.

I have thoroughly enjoyed welcoming you to this new edition and want to thank everyone who collaborated to create this issue by providing fascinating, well-written articles and supporting the advancement of tomorrow's haematological practice.

Kind regards,



Dr David Gómez-Almaguer

Hospital Universitario Dr. Jose E. González UANL, México

Congress Review

Review of the 23rd European Hematology Association (EHA) Congress 2018

Location: Stockholm, Sweden – Stockholmsmässan

Date: 14.06.18–17.06.18

Citation: EMJ Hematol. 2018;6[1]:10-23. Congress Review.

A warm welcome to the *EMJ Hematology* review of the landmark 23rd annual congress of the European Hematology Association (EHA).

Moving north from the 22nd EHA Congress host city, Madrid, Spain, the 23rd annual EHA Congress arrived this year in the 'Venice of the North' and 'City of Islands', Stockholm, Sweden, for the first time in 5 years. Home to the Vasa Museum, the Royal Palace, and the world-famous ABBA museum, this June, Stockholm was host to >11,000 haematologists who were hotly anticipating the cornucopia of haematological updates, debates, and discussions that Europe's flagship haematology congress is renowned for.

The anticipation in the main hall at the Stockholmsmässan Centre was palpable, and that energy was translated into rapturous applause as EHA President Prof Pieter Sonneveld took to the stage. Speaking passionately about the field, Prof Sonneveld praised the work of the organising committee who had produced a scientific programme covering a plethora of haematological disorders, treatments, and hot topics. The programme attracted world-renowned experts to give presentations and lead the interactive plenary sessions, with the aim of combining novel findings and new developments with current care protocols to improve patient care.

The focus of the EHA Congress 2018 was not solely on established haematologists. The YoungEHA committee developed YoungEHA TRACK, a programme specifically for young haematologists to give them the opportunity to get hands-on with the field, meet experts, and attend the Young EHA Research Meeting. The YoungEHA TRACK will surely prove to be a tremendous success, aiding the next generation of field leaders to develop their fledgling careers.

Two new programmes were unveiled during the opening ceremony, the first of which focussing on the rapidly developing field of immunotherapy, which, despite all its

impressive potential, must still be scrutinised to ensure patient safety and best practice. The second programme is based on haemoglobinopathies, a broad spectrum of disorders that affect approximately 7% of the global population, with 300,000–400,000 babies born each year with sickle cell anaemia. The goal of the programme is ultimately to improve the curative therapies for these disorders. With the focus of improving research in mind, another new addition to the annual EHA Congress was the Topic-in-Focus session, the aim of which Prof Sonneveld described as: "To raise awareness, provide education, stimulate research, and build a network of experts together with us to improve the care of patients." A clear theme of this year's congress was not only the celebration of the field's achievements and the promotion of new research but also the improvement of patient care and therapies. Prof Sonneveld concluded his speech by stating: "By being here with so many colleagues, ready to share knowledge and ready to work in a sustainable manner, I am convinced that our journey together will lead to a cure for many blood disorders."

For those of you who were not able to attend this year's fantastic celebration of haematology, this Congress Review captures the highlights from the event, including the latest updates from a Phase III trial of a new single agent that could change the face of acute myeloid leukaemia (AML) therapeutics. Furthermore, novel results are discussed that show a new, off-the-shelf T cell immunotherapeutic with beneficial effects on post-transplantation lymphomas induced by Epstein–Barr virus. The EMJ reporting team take the history book down from the shelf and provide you with an update on two of the key themes of the congress, immunotherapy and haemoglobinopathies, and the winner does indeed take it all as the prestigious EHA Abstract Awards are celebrated below.

Preparations for the 24th EHA annual congress are already well underway, with the event due to be held from the 13th–16th June 2019 in Amsterdam, Netherlands. The EHA organising committee will be aiming to replicate and build upon their success from this year's event by attracting more haematologists than ever before. With an exciting year of haematological research and updates ahead of us, the EMJ team is looking forward to what the next 12 months will hold and cannot wait for next year's congress to celebrate the successes and put haematology back under the microscope.

New Hope for Epstein–Barr Virus-Associated Post-Transplant Lymphoproliferative Disorder Patients

HOPE is in sight for those patients with Epstein–Barr virus (EBV)-associated post-transplant lymphomas, as new results demonstrate a promising safety profile and durable remissions using the new T cell immunotherapy tab-cel™ (Atara Biotherapeutics, Inc., San Francisco, California, USA). Presented in a poster presentation at the 23rd EHA Congress and described in an Atara Biotherapeutics press release dated 15th June 2018, the results of two Phase II studies using tab-cel, also called tabelecleucel, were revealed.

It has long been established that EBV can cause the development of multiple lymphoproliferative disorders. The virus is present in human populations all around the world and asymptomatic infection can persist throughout a host's lifespan. However, when EBV is present in an immunocompromised host, such as patients receiving solid organ (SOT) or allogeneic haematopoietic cell transplants (HCT), the infection can quickly become life-threatening. This is known as an EBV-associated post-transplant lymphoproliferative disorder (EBV+PTLD).

On average, patients who develop EBV+PTLD following an HCT, who have failed rituximab-based first-line therapy, have a median survival of 16–56 days. In those patients following SOT, the average overall survival for those who have failed on rituximab at 1 and 2 years is 36% and 0%, respectively. Tab-cel offers a new alternative for patients who have failed first-line therapy, both for those with EBV+PTLD as well as other EBV-associated haematological and solid malignancies, such as nasopharyngeal carcinoma.

...no patient with EBV+PTLD who responded to tab-cel died as a result of their infection.

In the two Phase II studies, it was reported that no patient with EBV+PTLD who responded to tab-cel died as a result of their infection. The 1 and 3-year overall survival in EBV+PTLD patients treated with tab-cel who had failed rituximab treatment following HCT were 68% and 55%, respectively (n=35). In those following SOT, 1 and 3-year overall survival was 64% and 43%, respectively (n=14). Of all the patients who responded to tab-cel treatment, overall survival at 2 years was 83% in HCT patients (n=24) and 86% in SOT (n=7) patients.

These results are significant improvements on the previous overall survival statistics of patients following HCT or SOT. Phase III clinical trials of tab-cel are beginning to confirm the results from these two Phase II trials, but already this new therapy is looking hopeful for patients at risk of this life-threatening infection.

Ruxolitinib Associated with Better Outcomes for Rare Blood Cancer Patients

A PLETHORA of studies have been reported in a Novartis press release dated 15th June 2018, which suggest that ruxolitinib could lead to better outcomes for patients with polycythemia vera (PV) and myelofibrosis (MF) compared to the current best available therapy. With PV affecting up to 3 out of 100,000 people across the world annually[1] and this population being vulnerable to serious complications and bleeding,[2] these results were much anticipated.

Dr Alberto Alvarez-Larran, Hematology Department, Hospital Clinic, Barcelona, Spain, was the lead author of a comparison study assessing patients in the Phase III RESPONSE trial and the real-world Spanish GEMFINI patient registry, which demonstrated PV patients treated with ruxolitinib who were hydroxyurea-resistant or intolerant had a significantly reduced risk of thrombosis and death compared to those treated with the best available therapy.[3] He commented: "When you can complement clinical trial data with real-world experiences, it can provide valuable insight into how treatments affect patients in their day-to-day lives." He also explained how the results of these trials demonstrate the benefits of prescribing ruxolitinib for PV patients who are unable to tolerate hydroxyurea.

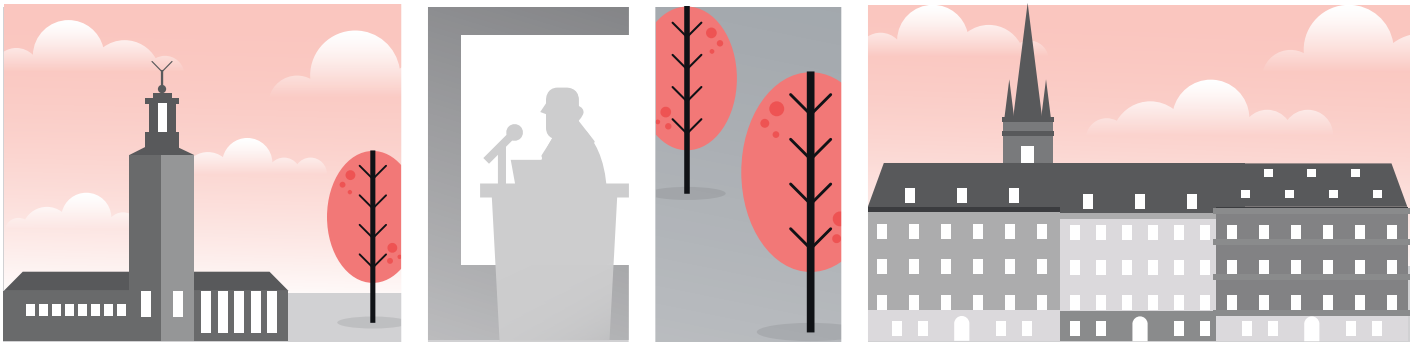
The data were presented at this year's EHA Congress alongside efficacy and safety analyses of the JUMP trial, which assessed MF patients treated with ruxolitinib. Efficacy analysis demonstrated that patients with lower-risk MF achieved reductions in spleen size following treatment; 82.1% of patients achieved ≥50% reduction at any time.[4,5] Additional analysis found that earlier treatment with ruxolitinib, and treatment with a higher dose, could improve spleen response in MF patients.[2]

Dr Samit Hirawat, Head of Novartis Oncology Global Drug Development, explained: "With limited treatment options, patients with myeloproliferative neoplasms often struggle to keep their disease under control." He added that these results for both PV and MF are vital for clarifying how ruxolitinib can improve the burden of the disease for patients.

References

1. Leukemia & Lymphoma Society. Polycythemia Vera facts. 2015. Available at: http://www.lls.org/content/nationalcontent/resourcecenter/freeeducationmaterials/mpd/pdf/polycythemiavera.pdf. Last accessed: 20 June 2018.
2. Vannuchi A et al. Results from 48-week follow-up of the EXPAND study: A phase 1b, open-label, dose-finding study of ruxolitinib in patients with myelofibrosis and low platelet counts (50-99 × 109/L) at baseline. Abstract PF611. European Hematology Association Congress, 14-17 June, 2018.
3. Alvarez-Larran A et al. Comparison of ruxolitinib real-world best available therapy in terms of overall survival and thrombosis in patients with polycythemia vera who are resistant or intolerant to hydroxyurea. Abstract PF628. European Hematology Association Congress, 14-17 June, 2018.
4. Guglielmelli P et al. Safety and efficacy of ruxolitinib (RUX) in patients (PTS) with DIPSS low-risk myelofibrosis (MF) in the Phase 3b expanded-access jump study. Abstract PF623. European Hematology Association Congress, 14-17 June, 2018.
5. Gupta V et al. Predictors of response to ruxolitinib (RUX) in patients (PTS) with myelofibrosis (MF) in the Phase 3b expanded-access jump study. Abstract PF616. European Hematology Association Congress, 14-17 June, 2018.

"When you can complement clinical trial data with real-world experiences, it can provide valuable insight into how treatments affect patients in their day-to-day lives."



QuANTUM-R Study Results: A New Hope for Acute Myeloid Leukaemia

ACUTE MYELOID LEUKAEMIA (AML) causes approximately 32% of all adult leukaemia cases, and AML patients have the lowest 5-year survival rate of all leukaemia patients, with only around 26% surviving for 5 years post diagnosis.[1,2] Mutations in *FLT3*, the gene that encodes the signal transducing fms-related tyrosine kinase 3, are commonly seen in AML patients. The *FLT3-1TD* mutation is the most common, affecting 1 in 4 patients; these patients have an increased chance of death and a higher risk of relapse compared with other AML patients. New results from the QuANTUM-R study, however, reported in a Daiichi Sankyo Co. Ltd. press release dated 16th June 2018, could provide a new hope for AML patients.

The aim of the Phase III, open-label, randomised QuANTUM-R trial was to identify whether quizartinib treatment resulted in longer overall survival compared to standard salvage chemotherapy. A total of 367 refractory or relapsed AML patients who were refractory to standard first-line therapy with or without haemopoietic stem cell transplantation or who had relapsed after ≤6 months, who expressed the *FLT3-1TD* mutation, were enrolled and divided in a 2:1 ratio to receive either the 60 mg (with a 30 mg lead-in) quizartinib treatment or salvage chemotherapy.

The results of the study showed that the patients receiving quizartinib had a 24% lower risk of death when compared with the patients receiving salvage chemotherapy therapy (hazard ratio: 0.76; p=0.0177; 95% confidence interval: 0.58–0.98). Furthermore, the median overall survival for the quizartinib group was higher than the salvage chemotherapy therapy patients, totalling 6.2 months versus 4.7 months, respectively. Extrapolation of these data provided researchers with the estimated survival probability at 1 year of 27% for the quizartinib patients, while the salvage chemotherapy patients had an estimated survival probability of 20%. "In relapsed/refractory AML with *FLT3-1TD* mutations, these findings represent the first reported clinical data demonstrating that a single agent can significantly improve overall survival, suggesting that quizartinib could potentially help these patients live longer", summarised Dr Jorge Cortes, Anderson Cancer Center, University of Texas, Houston, Texas, USA.

"In relapsed/refractory AML with FLT3-1TD mutations, these findings represent the first reported clinical data demonstrating that a single agent can significantly improve overall survival, suggesting that quizartinib could potentially help these patients live longer"

The median treatment duration in the two arms of the trial was four cycles of 28 days for quizartinib patients and one cycle of salvage chemotherapy for the control group. The occurrence of adverse events in both arms was comparable, with thrombocytopenia, anaemia, and neutropenia being the most common Grade ≥3 disorders. The results of the trial show great promise for the future use of the drug, which will soon be submitted to health authorities for review.

References

1. Cancer.net. Leukemia – Acute Myeloid – AML: Statistics. 2018. Available at: https://www.cancer.net/cancer-types/leukemia-acute-myeloid-aml/statistics. Last accessed: 25 June 2018.
2. Leukemia & Lymphoma Socitey. Facts 2016–2017. 2018. Available at: https://www.lls.org/sites/default/files/file_assets/PS80_Facts_2016-2017_FINAL.pdf. Last accessed: 25 June 2018.

Better Management in Line for Immune Thrombocytopenia Patients

PATIENTS with immune thrombocytopenia (ITP) may soon experience better management of their disease and symptoms thanks to a new tool developed to improve quality of life in this patient group. According to results presented at the EHA Congress 2018 and reported in a Novartis press release dated 15th June 2018, it is not just the bleeding symptoms of this disease that concern these individuals. The tool, named the ITP Life Quality Index (ILQI), consists of 10 questions that aim to quantify and monitor the quality of life of patients with ITP.

ITP is a rare blood disorder that results in the impaired production and increased destruction of platelets. Patients with the condition often present with bruising, red or purple dots on

the skin, bleeding from the gums, nosebleeds, and general bleeding that is difficult to stop. The main treatment goal for ITP patients is to maintain a safe platelet count that results in a minimal risk of bleeding; however, results from the ILQI survey show that patients are experiencing other symptoms that severely affect their quality of life, and that they would like to be treated.

The survey, which is still ongoing, is a cross-sectional online survey that began in January 2018 and has so far included 1,400 patients with ITP and 480 healthcare professionals who treat the disorder across 14 different countries, which were the UK, Spain, Japan, Italy, France, Colombia, Canada, China, Egypt, Germany, India, Norway, Turkey, and the USA. At the time that these results were presented, there were responses from >1,300 patients from 13 countries. There are plans for additional survey results to be released later this year.

The ILQI survey results revealed that for many patients, the factors of the disease that affected them the most were emotional wellbeing (36%) and symptoms that prevented them from working (28%). Another factor was fatigue, with 71% of patients revealing that this was their most severe symptom at diagnosis, and 64% saying this was still the case once they had completed the survey. Dr Nichola Cooper, Hammersmith Hospital, Imperial College London, London, UK, commented on the results: "Severe fatigue, in particular, was reported by many patients as the most difficult to manage symptom of ITP." She remarked: "This is an important message for healthcare providers treating patients with this rare disease; ITP is about more than bruising and risk of bleeding."

"Some patients only realise their fatigue has become such an issue in their daily lives after it is corrected by treatment," Dr Cooper said. She added: "The ILQI tool will help measure this correction more accurately and could also play a crucial role in monitoring disease impact on quality of life beyond just relying on the platelet count alone." These results will help to improve the standard of care for ITP patients, taking their specific needs into account, while also helping clinicians to better understand their patient's requirements and provide them with a more personalised style of treatment.

A Brief History of Immunotherapy

Immunotherapy has seen a meteoric rise to prominence across a wide range of medical disciplines for its exciting potential to fight cancer, as well as a myriad of other applications. As a result, it came as no surprise that this therapy featured prominently at the EHA Congress 2018, comprising one of the event's two main therapeutic focusses, alongside haemoglobinopathies.

This focus was made clear to delegates from the outset of the event, with a very special walkway just beyond the registration area. Taking the form of a tunnel with interactive screens on either side, the walkway's colourful floor display chronicled immunotherapy's development through the ages, allowing visitors to literally walk through the history of this exciting field.

Late 19th Century: The Journey Begins...

It is hard to pinpoint an exact date for the inception of immunotherapy; some consider this key event to be rooted in 1867, when German physician Wilhelm Busch noted that a patient's malignant tumour had disappeared following the contraction of erysipelas. The potential of the immune system to fight malignant cancers was slowly realised from this point on, helped a great deal by Emil von Behring's Nobel Prize-winning discovery of antibodies in 1890. One year later, in 1891, William Coley was building on the work of his predecessors, injecting heat-inactivated bacteria (so-called Coley's toxins) into patients with inoperable cancer, demonstrating a reduction in tumour size.

Turn of the Century: Adaptive Immunity is Conceptualised

The next major breakthrough came at the turn of the century (1897–1901), when Paul Ehrlich developed the theory of antibody specificity, for which he would receive a Nobel Prize. The concept of adaptive immunity was born. This idea would prove a significant lodestone in the development of immunotherapies, but it was not until the late 1950s that the field again began to advance at a blistering pace. Immune surveillance of tumours was postulated by Thomas and Burnet in 1959, a concept that would gradually evolve into the ideation of tumour immunoediting.

1960s–80s: Rapid Development and the Rise of Monoclonal Antibodies

The 1960s saw a flurry of immunotherapeutic activity: in 1965, the first allogenic bone marrow transplant was completed in a patient with acute lymphoblastic leukaemia; 1966 saw the discovery of B and T cells; and, in 1969, Wallace Clarke coined the term tumour-infiltrating lymphocytes, suggesting they comprise part of an immune response to melanoma.

Further discoveries blessed the 1970s, including the identification of dendritic cells, natural killer cells, and TNF, to name but a few. However, 1975 proved the real jewel in this decade's crown when Köhler, Milstein, and Jerne were awarded the Nobel Prize for their method of producing monoclonal antibodies. The next milestone noted the effectiveness of the Bacillus Calmette–Guérin vaccine for the treatment of bladder cancer and, in 1982, the T cell receptor was discovered.

1990s–2000s: First Cancer Treatments Approved

IL-2 was famously the first form of immunotherapy approved for cancer patients in 1992, and 5 years later, rituximab received approval from the U.S. Food and Drug Administration (FDA), becoming a prototype for a vast array of cancer treatments, many of which are still used today. Many more cancer drugs became available throughout the 2000s, notably thalidomide and lenalidomide.

2010–Present: What Does the Future Hold?

The more recent past holds some incredible breakthroughs of its own, with many seminal treatments developed. In 2010, the first autologous cell-based cancer vaccine for prostate cancer was discovered. This year also saw the first successful use of gene-edited T cells for the treatment of haematological malignancies. This decade has seen cancer more accurately defined, including its quintessential ability to evade immune destruction, as well as the advancement of immune checkpoint inhibitor treatment, with anti-CTLA-4 first approved in 2011. Finally, just last year in 2017, we saw chimeric antigen receptor T cell therapy approved for the treatment of paediatric acute lymphoblastic leukaemia; this is a treatment modality that is of growing importance for haemo-oncology and featured prominently in this year's EHA programme.

The future of immunotherapy is unquestionably bright, and the next breakthroughs are inevitably just around the corner. What those breakthroughs will be is unknown, but one thing is clear: the EHA Congress will champion their development, push them into the spotlight, and carry them into the future of haematological practice.

Haemoglobinopathies

Forming the second main theme of the EHA 2018 annual congress was the broad spectrum of haemoglobin disorders. In his opening speech, EHA President Prof Sonnerveld highlighted that approximately 7% of the world's population are carriers of the sickle cell trait, and that with 300,000–400,000 babies born worldwide each year with sickle cell anaemia, action is required to combat the growing number of cases of this long-neglected blood disorder. As a result, the EHA organised new Topic-in-Focus sessions, alongside the returning Meet the Expert opportunities that were devoted to an in-depth analysis of various haemoglobinopathies, including both thalassaemia and structural haemoglobin disorders.

There are a number of different diseases that are associated with the amino acid sequences of haemoglobin subunits (4α and 2β), including haemoglobin E, caused by a Glu26Lys mutation; haemoglobin C, caused by a Glu6Lys mutation; and, arguably the most famous, sickle cell anaemia. Thalassaemia can arise from partial or full deletions of the haemoglobin peptide chains, which gives rise to anaemia as well as bone and spleen disorders.

Sickle Cell Anaemia

First identified in 1900, sickle cell anaemia was primarily identified in Asian and African populations. However, since the dawn of the 20th century, the world has become a much smaller place and migration is commonplace; as such, sickle cell anaemia is becoming increasingly more common in northern and western European populations. Germany, in particular, has seen a growth in the number of sickle cell anaemia cases.

Initially described as peculiar, elongated sickle-shaped erythrocytes, it was not until the pioneering work of Dr Linus Pauling in 1949 that

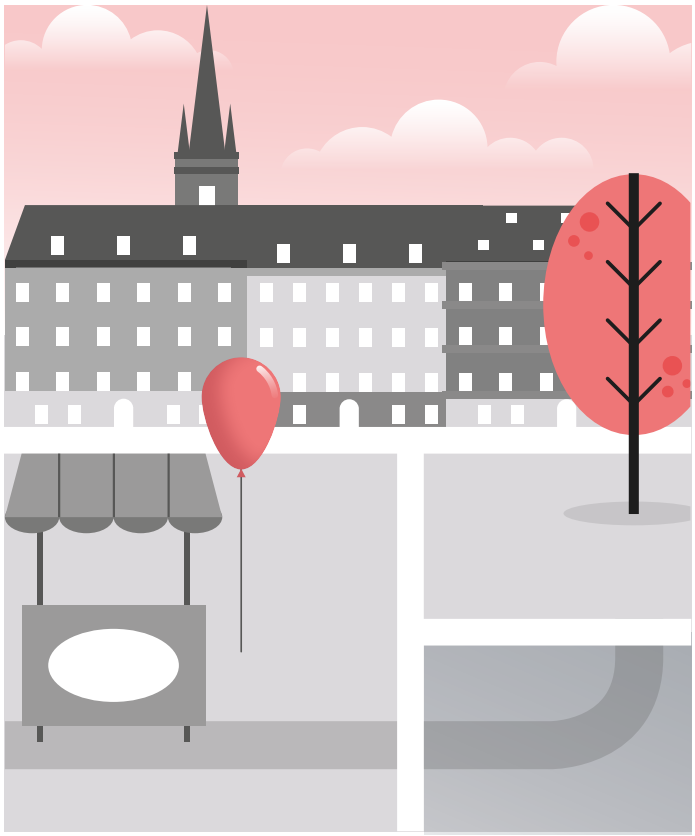
it was established that this disease is caused by abnormally shaped haemoglobin protein. Sickle cell anaemia was one of the first disorders to be understood at a biochemical level, but it was not until much later, in the 1970s, that disease therapeutics began to advance more rapidly. One such treatment was the prophylactic administration of penicillin.

A learning session during the EHA Congress was dedicated to furthering the medical community's current knowledge of anaemic disorders, with the aim of pushing the boundaries of medicine. A special focus was placed on genetics as Prof Douglas Higgs, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK, discussed gene expression regulations and highlighted how genome editing of haematopoietic stems cells can improve treatment. Prof Brigitte Ranque, Hôpital Européen Georges-Pompidou, Paris, France, looked at regional treatment options with a particular focus on sickle cell anaemia progress in sub-Saharan Africa. Dr Miguel Abboud, Department of Pediatrics and Adolescent Medicine, American University of Beirut Medical Center, Beirut, Lebanon, provided his take on the most recent advances detailing the results from the latest clinical trials. In an excellent Meet the Expert session, Dr Karina Yazdanbakhsh, Lindsley F. Kimball Research Institute, New York Blood Center, New York City, New York, USA, detailed her experiences with blood transfusion in patients with sickle cell disease.

Thalassaemia

Dr Thomas Cooley and Dr Pearl Lee, two American paediatricians, first identified Mediterranean anaemia in Italian children in 1925, 25 years after the discovery of sickle cell anaemia. Mediterranean anaemia was linked to an enlargement of internal organs, poor growth, and childhood death. Similar to its sickle counterpart, it was not until the 1940s that understanding of what we now call thalassaemia advanced. In 1943, Dr Ezio Silvestroni and Dr Ida Bianco, two Italian haematologists, identified a disorder they termed microcitaemia. Following the laws of Mendelian genetics and investigating the inheritance of the disorders, it was identified that children developed Mediterranean anaemia, or thalassaemia, if both their parents passed on their microcitaemia genes.

Thalassaemia arises from partial or complete deletions of the α or β haemoglobin chains, causing the body to destroy the red blood cells, resulting in anaemia. A knock-on effect of this response is the production of more red blood cells, inducing a positive feedback loop resulting in bone abnormalities and spleen enlargement.



In the 1960s, doctors first used monthly blood transfusions to ease the symptoms of thalassaemia and increase survival, and since then even more therapeutics have developed, such as folic acid supplementation and iron chelation therapy. With a view to the future, the EHA organised several thalassaemia presentations at this year's congress, including a panel discussion on clinical decision-making for patients with transfusion-dependent β-thalassaemia and the role of haematopoietic stem cell transplantation for these patients.

The focus of the EHA Congress 2018 was to further drive forward and improve the therapeutic options available to physicians, with the ultimate goal of improving patient outcomes. There were a number of sessions and poster presentations dedicated to the best practice of currently available therapeutic strategies, with a focus on the identification of the next therapeutic breakthrough that could improve patient outcomes many times over. With the ever-growing number of haemoglobinopathy cases, it is clear from presentations given at the EHA Congress that the medical community feels that there is plenty more to be done to combat this broad spectrum of disorders.

Abstract Awards

This year the EHA honoured researchers, physicians, and scientists at all levels for their outstanding contributions to the field of haematology with a range of awards and grants. In addition, the EHA proudly announced for promising young researchers in order to foster the next generation of haematologists. Herein, we summarise the most prestigious awards that were received at this year's congress and congratulates all the winners for their achievements.

José Carreras Award

The José Carreras Award recognises the very frontrunners of haematological research and is awarded to an investigator who is not only active and well-established in their research but has also made an important contribution to the field. This year, the winner was Prof Francesco Lo Coco, University Tor Vergata Rome, Rome, Italy, who was a driving force in the implementation of arsenic trioxide combined with retinoic acid as treatment of acute promyelocytic leukaemia. Prof Lo Coco joins a select group of researchers to have been recognised with this award since 1999.

David Grimwade Award

The David Grimwade Award is a brand-new award for 2018 and celebrates the outstanding work of basic and translational researchers in haematology. This year, the recipient was Dr Peter Campbell, The Wellcome Trust Sanger Institute, Hinxton, UK, for his work in the field of haematological cancer genetics. As a result of his dedication to this topic, a number of disease-specific driver genes in myeloid cancer, including *SF3B1* mutations in myelodysplastic syndrome with ringed sideroblasts, have been discovered, and the way in which tumours develop has been elucidated for the whole medical community. Dr Campbell received his award in the opening ceremony of the congress, where he also gave the David Grimwade honorary lecture.

Jean Bernard Lifetime Achievement Award

This year's Jean Bernard Lifetime Achievement Award, which was established in 2008 and honours an outstanding lifetime contribution to the advancement of haematology, was awarded to Prof Charlotte Niemeyer, University Medical Center of Freiburg, Freiburg, Germany. Prof Niemeyer is a paediatrician, oncologist, and haematologist and has dedicated her professional career to the study of paediatric myelodysplasia, bone marrow failure, and juvenile myelomonocytic leukaemia. Her translational research works have assisted in the elucidation of the biology and genetic origin of juvenile myelomonocytic leukaemia and have demonstrated the pivotal role of the RAS pathway, which has helped to improve the treatment options for this childhood disease.

Education and Mentoring Awards

The EHA is proud to have become well known for providing high-quality medical education to haematologists the world over. In order to recognise the people who make this education possible through their work contributing to and leading programmes and supporting committees, the EHA this year introduced another brand new award: EHA Education and Mentoring Awards. The first recipients of these exciting new awards were Prof Robin Foá, Sapienza University, Rome, Italy, and Prof Barbara Bain, Imperial College London, London, UK, for their outstanding contributions to the educational programmes of the EHA, as well as to the education of haematologists more broadly.

YoungEHA Best Abstract Award

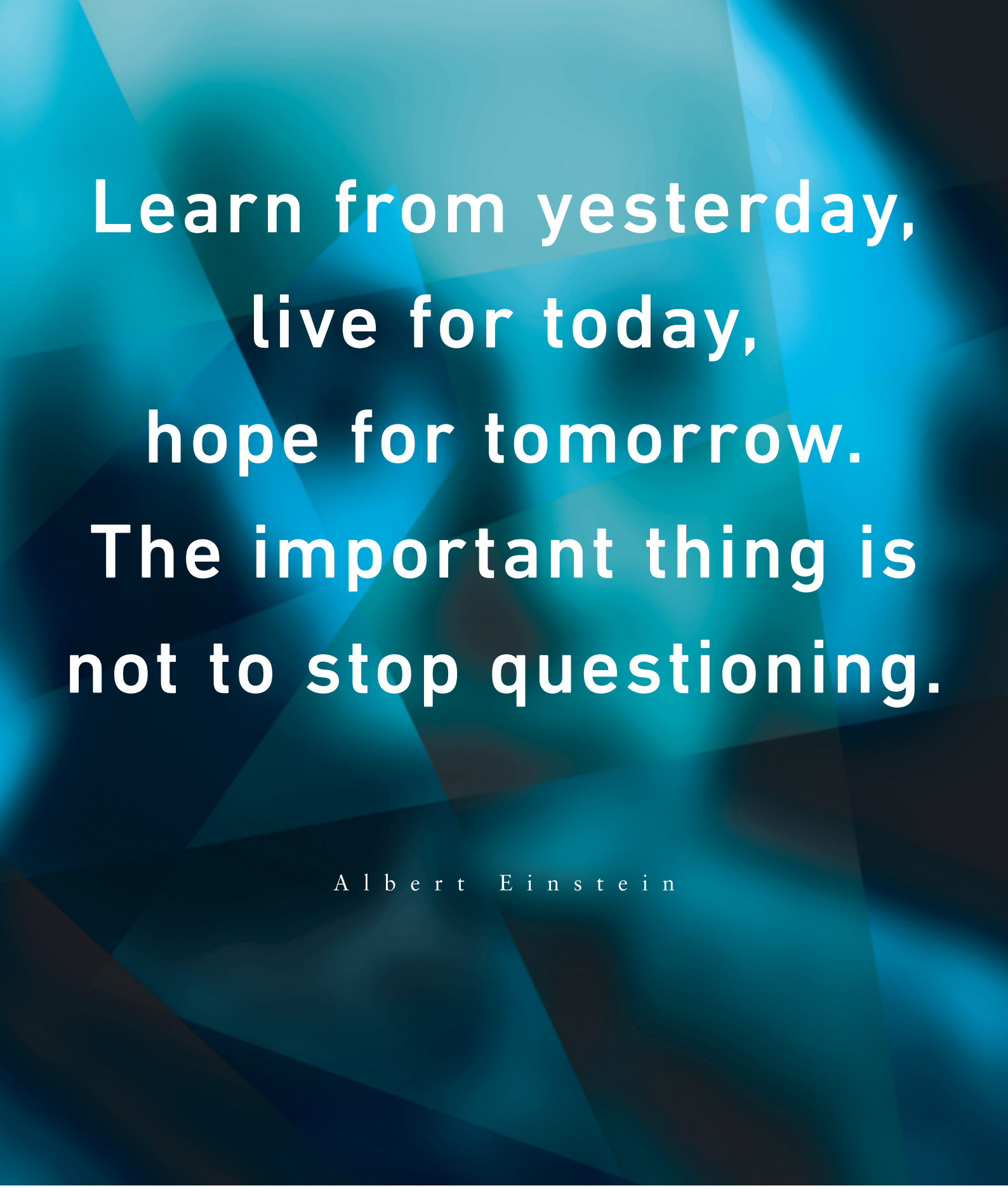
Young clinicians and researchers were also recognised by the EHA in the YoungEHA Best Abstract Award series. These prizes were awarded to abstract authors in four different categories: clinicians or medical students training for a PhD, PhD research students, postdoctoral fellows, and clinical haematologists. The awards were given at the opening ceremony and the EHA expressed their delight that such esteemed young researchers were presenting their work at the congress. The winners were as follows:

Clinical Trainee Award: R. Shouval, Israel

MD-PHD Award: L. Hinze, Germany

PHD Research Student Award:
F. Ribezzo, Germany

Postdoctoral Research Trainee Award:
A. Nai, Italy



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Interviews

Our esteemed *EMJ Hematology* Editorial Board have provided insights into haematology from across the UK, Armenia, and Spain

Featuring: Prof Smbat Dagbashyan, Dr Ciaren Graham, and Dr María-Victoria Mateos

Prof Smbat Dagbashyan

Yerevan State Medical University, Armenia

Firstly, who or what had the biggest influence on you when choosing to become a haematologist?

As I expected, the most difficult question came first. Asking the same to many of my students, it seems to me that we make the major decision to become a doctor when we are teenagers, and I was not an exception. Going back to the late 1970s, I remember my grandmother's health problems and my desire, and at the same time uselessness, to relieve her pain. She survived the Armenian genocide and had a large, happy family in Eastern Armenia. She was a kind person, full of love and empathy. I think she was the reason I selected medicine as my profession.

Haematology, however, is not my first specialisation. During a decade of clinical practice and teaching in internal medicine, I was involved in research on blood cell membranes at the Scientific Department at the Hematology Center after Prof R. Yeolyan (HC), Yerevan, Armenia. Soon after successfully completing my PhD defence, I was offered a position to manage the HC. I subspecialised in haematology to gain an understanding of the nuances of this rapidly developing field, where medicine and science come close together. I am indeed happy for the opportunity that opened such an incredible world for me.

You are Head of Hematology at Yerevan State Medical University, Director of the Hematology Center of the Republic of Armenia, and President of the Armenian Hematology Association (AHA); how do balance your time between these three demanding and diverse roles?

Without a doubt, the management of such an advancing field as haematology and transfusion medicine in Armenia requires a huge amount of time and effort, and I share some of the responsibilities with my colleagues. After the

dissolution of the Union of Soviet Socialist Republics (USSR), where the healthcare system was similar for every soviet country, we faced a huge problem in keeping the established healthcare system in Armenia. In the 1990s, our health system was worsened further by the Nagorno-Karabakh conflict, an earthquake in Gyumri, closed borders, and a lack of electricity. However, we created new relationships with Western partners, which opened avenues for further developments.

"One of the central problems for haematology in Armenia was the training of specialists, most of whom were previously trained in Moscow, Russia."

One of the central problems for haematology in Armenia was the training of specialists, most of whom were previously trained in Moscow, Russia. In 2004, I established and headed the Hematology Department at Yerevan State Medical University, which has now trained >25 haematology specialists. The establishment of medical associations became a requirement for the implementation of international guidelines. The AHA was created based on the HC in 2012 and one of its main missions is to adapt international haematology guidelines in Armenia. Alongside our well-trained specialists, with whom I can share some of my responsibilities, we have developed a newly renovated blood bank, the new haematology centre, a stem cell laboratory, a bone marrow transplantation unit, and an increased number of modern laboratory facilities.

As part of your role within the AHA, you have had to oversee the implementation of European haematology guidelines in Armenia. What has been the biggest challenge during the implementation of these practices and procedures?

The implementation of the best practice international guidelines is one of the main objectives of the Armenian healthcare system, particularly for the AHA and the HC. From 2014, when we first got involved in the Tempus educational project, we adopted an objective to harmonise the medical education of Armenia with that of Europe. In line with this, the need to implement international guidelines became apparent and in 2016 the Armenian Ministry of Health presented the programme of translation, adaptation, and implementation of international clinical guidelines for every field of medicine.

I can say that our team of haematologists, who are actively participating in this programme, could successfully accomplish the first part of the mission. During the first year of the programme, we translated >20 European Society of Medical Oncology (ESMO) clinical practice guidelines. The Ministry of Health has already adopted five clinical guidelines on haematological malignancies and five protocols on bone marrow transplantation. The challenge that we face during the guideline implementation is that many medications involved in the standard of care described in international guidelines are still not available in Armenia. We hope that, in the best interest of our patients, this challenge will be resolved in the near future. Meanwhile, we will continue to do our best to adapt the guidelines and recommendations based on available remedies.

How has medical treatment, practice, and funding changed in Armenia over the last 30 years?

After the dissolution of the USSR, before which treatment was totally free, we had to transfer to an alternative system of healthcare and, unfortunately, most patients now have to pay for their treatment. Although the Armenian government finances the treatment of blood cancer, the money provided is frequently not enough to cover all expenses of diagnostics and pharmacotherapy. Even with the help of fundraising and charity programmes, patients often face financial troubles during the course of their treatment. This remains a challenge to overcome for adult patients with blood diseases. However, the quality of the effective treatment has greatly improved over the last decade. Through the implementation of modern protocols, high-dose chemotherapy, and by continuous education of specialists in the leading European, American, and Russian clinics, we can continue to achieve better haematology care.

Improvements in haematology care have been achieved thanks to the help and generosity of our partners, such as The Max Foundation, which provided Imatinib for about 10 years; Novo Nordisk, Bagsværd, Denmark, which provided clotting factors for haemophilia patients and fellowships for haemophilia specialists; the World Federation of Hemophilia (WFH); the European Thrombosis and Haemostasis Alliance (ETHA); and the European Hematology Association (EHA). During these years, we have also established strong connections and received great support from the Institute of Hematology and Blood Transfusion (UHKT), University Medical Center Hamburg-Eppendorf, Russian oncology institutes, Centro Nazionale Sangue, and many other committed partners.

Thanks to the Disease Prevention and Control project funded by World Bank, we reopened the HC in 2016. Now the renovated building is enriched with modern devices and laboratories, and a transplantation unit.

With regard to paediatric haematology, I am also very proud to mention that currently the majority of Armenian children with blood diseases are being treated for free thanks to help from the charity fund Grant Life, headed by the First Lady of Armenia, and many other charity programmes. As described, we have had many achievements, but still there are many aspects of haematology care to work on.

You have recently stated that the number of unpaid volunteer blood donors in Armenia is very low and that most blood donations are from patients' relatives. What do you think can be done to increase the number of unpaid volunteer blood donors in Armenia?

Thirty years ago, blood donation was obligatory. However, after Armenia declared independence, there was a drop in the number of blood donations, which led us to make some important decisions. The law on blood donation was created in 2011 and other organisational activities were carried out, which led to the increase of blood donations in Armenia.

In 2004, Armenia became a member of the European Committee on Blood Transfusion (Steering Committee) (CD-P-TS). Soon we started to enhance blood service activities and donation criteria in accordance with European guidelines to ensure the quality and safety of tissues and cells. We also translated European Directives on safety and quality of blood and blood components (Directive 2002/98/EC, 2004/33/EC, 2005/61/EC, 2005/62EC). Nevertheless, these Directives are not yet adopted because of the absence of expensive devices and techniques, such as nucleic acid testing.

"Medicine is a humanitarian discipline and, in a perfect world, the fruit of the 'medicine tree' should be available for every patient."

Despite difficulties and limited resources, we have made some improvements in the quantity of blood donations as well as volunteer donations. Although the percentage of volunteer, non-remunerated donors is still low and does not correspond to World Health Organization (WHO) recommendations, the total reserves of blood components are sufficient to ensure adequate transfusion health support for every patient who needs a blood transfusion.

Armenian patients are always surrounded by relatives ready to support them. Many relatives willingly donate blood for the patient; in 2017, 50.0% of all blood donors were patient relatives, while 9.2% were volunteers. As a result, we developed a national volunteer blood donation programme, which focusses on the implementation of best clinical practice during volunteer blood donation and includes:

- Promotion of donation via educational materials used in the school and universities.
- Establishment of volunteer donor associations.
- Involvement and active participation of non-governmental organisations.

Nowadays we are on the way to fulfilling the aims of the programme, and I hope we will register better results in terms of the numbers of total donations and volunteer donations in the coming year.

In a recent abstract, you presented the report: Our Experience in the Treatment of Adult Lymphoblastic Leukemia (including Adult T Cell Leukemia/ Lymphoma) with German Multicenter ALL (GMALL) Protocol. Could you briefly summarise your findings and the implications of the study on the future of haematological research?

Haematology care is rapidly developing in Armenia and in 2016 we started to treat eligible patients with acute lymphoblastic leukaemia with the GMALL protocol. Eligibility depended on age, presence of comorbidities, and the financial status of the patient. Overall, eight patients were treated using this protocol and remission was achieved after the first induction phase in all patients; one patient died due to fungal sepsis after the first consolidation phase and two patients had a relapse after the reinduction phase, so we switched to another treatment regimen. The minimal residual disease (MRD) was measured via flow cytometry and the range of MRD was 0.034–1.400%. Four patients are still receiving their treatment and the MRD monitoring is being performed after each phase.

As mentioned, the choice of treatment protocol depended on the financial status of the patient. Although eight patients are not enough to evaluate protocol efficacy, we discussed the treatment options with our patients, trying to choose the most effective, tolerable, and affordable treatment protocol. Detailed comparisons have been described in other studies.

What do you think will be the biggest issues faced by haematologists across the world over the next 5 years?

Contemporary haematology is very different from what it was 10 years ago. The huge impacts of molecular biology, immunology, and epidemiology have created demand for a modern haematologist to be not only a clinician but also a researcher and a public health specialist. The discovery of new chemical molecules, biologics, and personalised medications has improved the outcomes of many fatal blood malignancies; however, with each new medication and protocol we face a new set of adverse reactions. Thus, I think soon we should learn to deal with these complications to improve the quality of life of those who overcome the disease.

The developed world has achieved enormous advances in the treatment of blood cancers that we could not have even imagined a couple of years ago. However, these treatments are available for <10% of world's population, and so making the care affordable for all is a major issue within the field. Medicine is a humanitarian discipline and, in a perfect world, the fruit of the 'medicine tree' should be available for every patient. This is another big challenge for the physicians of tomorrow; for example, Gilead Sciences, Inc., Foster City, California, USA, gave the patent of anti-hepatitis C treatment to Indian and Egyptian generic manufacturers and made Sofosbuvir, which initially cost >€50,000, available for <€1,000 for those in resource-limited countries. We need more of this kind of generosity.

"The field is complex and demanding, requiring multidisciplinary approaches, and the haematologist is elite in the medical field."

With regard to these challenges, how important is attending large haematological congresses, such as the EHA, to learn more about the most current advances in haematology treatments and therapies.

EHA is one of the leading haematology organisations in the world and provides comprehensive and evidence-based information for blood care. The meetings, congresses, and seminars are environments where information on best care of patients is shared between specialists. The society offers a huge opportunity for networking between Armenian and European haematologists, which will further help to improve healthcare in Armenia.

When it was created, the AHA had a vision to be integrated into EHA activities. The first big achievement took place in 2015 when an EHA

tutorial was conducted in Yerevan. This huge scientific event brought many leading experts of the field to Armenia and thus served as a basis for the establishment of co-operation and collaborations. In 2017, we again hosted an EHA tutorial, which was, as always, very informative and innovative.

EHA is also extremely important for next-generation specialists who are newly educated and interested; they are eager to capture any granule of new information and implement it into the practice, and EHA events are a good platform to achieve this goal.

Over the course of your medical career so far, what achievement are you most proud of?

When I started my position as Head of the HC, I promised myself to improve the haematology service in Armenia and bring it to the next level. I think the biggest achievement of mine so far is the establishment of the Department of Hematology at Yerevan State Medical University. It is now a well-recognised school for Armenian students who choose haematology as a specialisation. Year on year we see more enthusiastic and active students who are committed to the development of the Armenian haematology practice.

During the last decade, we have also been able to establish precise procedures of blood disease diagnostics in Armenia. Previously, we did not possess the necessary tools and equipment for the detailed diagnostics, and samples were sent abroad for immunohistochemistry, cytogenetics, and flow cytometry. We have achieved an enormous amount of success in the area of diagnostics by opening new laboratory departments in our centre equipped with modern machines run by trained specialists. We are now able to detect chromosomal and genetic shifts by karyotyping, fluorescence *in situ* hybridisation, and next-generation sequencing and we extensively use flow cytometry for diagnosis and monitoring of blood cancers. Electrophoresis is also used to detect abnormal haemoglobin chains in thalassaemia and our renovated immunology laboratory detects platelet and minor blood group antigens. Automated multiplexed systems in the blood bank enable early detection of blood-transmitted infections, thus serving a guarantee for safe transfusions. We have a modern laboratory for haemophilia, which serves all Armenian haemophilia patients, and is well equipped and able to detect a vast array of coagulation factors and thrombotic markers. Of course, I also must mention the stem cell laboratory dedicated to harvesting, storing, and supplying stem cells for further transplantations. This laboratory was built according to the international standards and is managed by high-level specialists. Finally, I would like to mention the bone marrow and stem cell transplantation unit; we started this project last year and, so far, five patients have undergone autologous stem cell transplantation. My goal is to develop the department to serve all Armenian patients requiring auto or allografting, so they will not need to seek the help abroad.

"During the last decade, we have also been able to establish precise procedures of blood disease diagnostics in Armenia."

Finally, if you could give one piece of advice to a young trainee doctor interested in specialising in haematology, what would it be?

Since the opening of the renovated HC, many young doctors have come to our hospital and expressed willingness to become a haematologist. The field is complex and demanding, requiring multidisciplinary approaches, and the haematologist is elite in the medical field. Therefore, this is a very hard mission and success is not possible without complete commitment and devotion.

Nowadays, many young specialists work in different departments of the HC and they are full of enthusiasm and desire to work. They are better than we were during our time, but still I have some advice to share for every young physician:

- Do not forget that the patient requires attention and that treatment must be patient-centred rather than protocol-centred.
- Learn foreign languages, at least two in addition to your native language.

- Be active in the international arena by attending conferences and meetings.
- Share your knowledge with senior and junior colleagues.
- Be active in research and publish research articles.
- Do not ignore the administrative work because success largely depends on healthcare management and organisation.
- Do not be restricted with statements and medical dogmas, be innovative and question every aspect of diagnosis and treatment.

Dr Ciaren Graham @ciaren_graham

Manchester Metropolitan University, UK

Your research focusses on the use of mass spectrometry for the investigation of myeloproliferative disorders and leukaemia. Briefly, how does mass spectrometry work and how do you apply the technique to your research?

I currently use mass spectrometry to understand the role of proteins in disease. Mass spectrometry allows you to identify and quantify changes in protein expression, on both a large scale with global proteomics or in a targeted manner with interactomes; this enables you to tease apart the molecular mechanisms of disease.

"The proteome is larger than either the genome or transcriptome and is ever-changing. Only by understanding the role gene dysregulation has on the proteome will we truly understand the mechanisms of disease..."

In your opinion, what is the most exciting prospect that this technique brings to haematological medicine?

Proteins are the functional product of the genome: the end readout, so to speak. The proteome is larger than either the genome or transcriptome and is ever-changing. Only by understanding the role gene dysregulation has on the proteome will we truly understand the mechanisms of disease, and ultimately this will lead to better treatments for haematological disorders.

"In the era of precision medicine, collaboration is essential."

What results would you like to see from your research in terms of future treatments for leukaemia and myeloproliferative disorders?

In my own research, I use proteomics to understand how blood disorders arise and hope this research will identify new drug targets. I am currently using a new mass spectrometry technique called sequential window acquisition of all theoretical mass spectra (SWATH), which generates digital proteomic maps from patients with myeloproliferative disorders and compares them to healthy controls. This will facilitate the discovery of the key molecular events that lead to myeloproliferative disorder development and progression.

How important do you feel collaborations between different institutions are for the advancement of scientific research?

In the era of precision medicine, collaboration is essential. By bringing together basic scientists and clinicians, we can push the field of haematology forward and have better outcomes for patients.

You have dedicated a significant amount of your professional life to teaching students, including trainee clinical and biomedical scientists. What drew you to teaching and what is your favourite aspect of your life as a lecturer?

I have always enjoyed teaching and the buzz of a classroom. I enjoy the balance that my academic position allows; it enables me to teach the next generation of scientists whilst carrying out my research.

What is your opinion on the current state of haematological education? Do you think the next generation of haematologists are adequately supported?

I feel it is a good time for haematological education in the UK; there is excellent synergy between clinicians and scientists, especially in haematology, which enables students to gain the most from their education. However, as always, funding constraints are a concern, especially how they will impact a student wishing to enter the discipline.

How important do you feel international congresses are for current and trainee medical professionals? Do you have a favourite congress that you like to attend? If so, why?

I attend the British Society of Haematology (BSH) annual meeting; this society is the professional society of haematological specialists in the UK, from research scientists and clinicians to biomedical scientists and haematological nurses. So, at this conference, all aspects of the discipline are covered, with the programme featuring the latest research and best practice.

In your opinion, what is currently the most challenging haematological disorder and why? How would you like to see this disorder managed or treated?

Acute myeloid leukaemia is a complex and heterogeneous disorder that is difficult to manage due to relapse and poor survival rates; this is despite an increase in the understanding of the disorder. However, I do believe that this understanding coupled with the role of omics technologies, such as genomics and proteomics in precision medicine, will translate into new therapies and biomarkers that will lead to improved patient outcomes over the next decade.

What do you think the biggest challenge to face the field of haematology will be in the next 10 years?

Realising the potential of precision medicine; for this new field to revolutionise healthcare we need to think about medicine differently and make teams truly interdisciplinary on a scale we are currently not doing. Scientists and informaticians need to fully integrate into the patient pathway alongside clinical haematologists.

Dr María-Victoria Mateos @mvmateos

University Hospital of Salamanca, Spain

Firstly, what or who inspired you to pursue a career in haematology?

There was not a concrete thing or person that inspired me to choose haematology as a career. When I was studying medicine I realised that I liked the clinic and the direct relationship with the patient but also the laboratory. I did a short period of training in haematology and realised that the haematologist was working both with patients and in the laboratory, and, in addition, in most cases they were able to do the diagnosis

of their patients. They could also look at the peripheral blood or bone marrow and analyse and investigate tumour cells. The combination of the clinic and laboratory was the key reason I chose haematology.

You are responsible for co-ordinating the Clinical Trials Unit at Salamanca University Hospital's Haematology Department. What do you believe is the most interesting and pivotal clinical trial to have been conducted at the hospital?

When I read this question, I thought of the first patient with myeloma included in the Phase III clinical trial APEX, who received bortezomib. She was the first patient in Spain to receive this new agent and this occurred at the beginning of the 21st century. It was amazing. Bortezomib was the first new agent we had for the treatment of patients with myeloma and I had the opportunity to treat and follow-up many patients that came to the clinic's myeloma days on Mondays and Thursdays because of the administration scheme for bortezomib. Indeed, we had to change the clinic's days for myeloma based on this. But the most exciting thing was to see how the monoclonal component of the patients' myelomas was reducing since the beginning of treatment, how the patients were excited about the treatment, and how they interacted with each other in the clinic, sharing their experiences in the clinical trials.

That was amazing, but I also remember with great clarity the first Phase I/II trial that I was involved in from design to publication. It was the first time bortezomib was combined with melphalan and prednisone in elderly patients with newly diagnosed myeloma. This combination resulted in a 30% complete response rate compared to a 2% complete response rate with melphalan and prednisone alone; therefore, these were highly relevant results and the combination of bortezomib, melphalan, and prednisone has become one of the standard care regimens for this population.

"...the main objective of the EHA Congress is to bring excellence in patient care, research, and education in haematology..."

I could continue to describe more and more exciting clinical trials conducted at the hospital, but overall the participation in trials with new agents is always exciting because you are offering your patients the newest agents and you are intrigued to see how the efficacy and safety are affected. I usually explain to my patients that they are contributing to the prescribing information of the new agents.

You are a member of many associations and committees, including the European Hematology Association (EHA) and the American Society of Hematology (ASH) Scientific Committee on Plasma Cell Neoplasia. How important do you think organisations such as these are in advancing the field of haematology and medicine as a whole?

I am a member of EHA and ASH, with active participation in both societies, although I am more involved in EHA activities. Both societies, and all medical societies in general, are very valuable organisations with well-designed strategic plans to promote excellence in education, research, and patient care. They are organisations with integrity, transparency, and intellectual independency; these aspects are key to promote the excellence in education that these organisations disseminate among their members. They represent physicians and scientists of all the member states, and young physicians, minority populations, less developed countries, and patients are always their priority.

As a member of the EHA Scientific Program Committee (SPC) 23rd Congress, can you tell us about the main tasks of the committee and what your role entails?

It is a pleasure to be part of the SPC because this is my fifth or sixth year in the role, so I know my responsibilities well. My specialised area is plasma cell disorders and, alongside other experts in the same area, my main task is to build the part of the scientific programme for the congress that focusses on plasma cell disorders. We try to select different topics each year to avoid repetition, with excellent speakers included in the programme. It is important to

remind ourselves that the main objective of the EHA Congress is to bring excellence in patient care, research, and education in haematology to the delegates, and these are the pillars that the programme of the congress is built on each year. We review and evaluate all of the abstracts submitted; this year I reviewed 214 abstracts focussed on clinical aspects of plasma cell disorders and discussed the scores with the other reviewers to finally compile the list of accepted oral and poster sessions.

In 2015 you were elected as a councillor for the Board of the EHA; how does this role differ from your SPC position?

My role as a member of the SPC is connected to and included in my role as councillor for the EHA Board. SPC is one of the committees that makes up the EHA and, in fact, one of my first formal relationships with the EHA was when I was invited to be a part of the SPC, from 2012–2016. I become a member of the SPC again in 2018. During these years, I was gaining expertise and, due to my involvement, as well as with the support of many members of the EHA, I was nominated and later elected as a member of the EHA board. As part of the EHA board, I am involved in other EHA committees like European Union (EU) affairs, research, and fundraising to contribute to and promote excellence in education, research, and patient care in haematology. Over the last 2 years, I have also been involved as training director and programme director of the Clinical Research Training Program developed by the EHA and I enjoyed being involved in this initiative in which we were training the future key opinion leaders in haematology clinical research in Europe. I will continue to be part of the EHA Board in 2018 and 2019 and will become the chair of the EHA SPC in 2019.

You have co-ordinated numerous clinical trials focussing on smouldering myeloma. Could you tell us more about this disease and the current treatment options?

Smouldering myeloma is a myeloma without symptoms or myeloma-defining events. It can be diagnosed in a routine analysis and the main problem is that it can potentially evolve to active disease. In addition, it is a heterogeneous disease and there are smouldering myeloma patients with a low risk of progression to myeloma (1% per year), intermediate risk (3% per year), and high risk (10% per year). The first two groups of patients only need to be monitored, but the high-risk patients can potentially benefit from early treatment. We have been working on this for many years and focussed initially on the identification of markers predicting high-risk patients, later demonstrating that early treatment significantly delays progression to myeloma and patient survival is longer than when these patients wait until they develop any symptoms to receive treatment. Now we are involved in a more exciting trial with a curative intention for these patients. Preliminary results are promising but longer follow-up is required.

"One of the main challenges is the speed at which the scientific knowledge is advancing, especially in some specific areas of haematology."

Much of your myeloma research has focussed on elderly patients. How does the treatment of this demographic with newly diagnosed myeloma differ from that of the general population?

Myeloma usually affects the elderly population and the median age at diagnosis is >65 years. The main difference with the treatment of younger populations is that the approach of therapy does not include autologous stem cell transplantation for the elderly population.

In the past, a combination of melphalan and prednisone was the treatment for the elderly population and the results were disappointing, but there was not any other option. However, the introduction of novel agents in the myeloma field benefited all patients and the elderly population experienced a great benefit. The reason for this is because the median overall survival for elderly patients with myeloma was 2 years with melphalan and prednisone and is now >5 years. In addition, the novel drugs can be safely used in patients aged >65 years and these patients can achieve excellent responses;

even minimal residual disease-negative status is associated with excellent outcomes.

The advances in the management of elderly patients are also exciting because we are now incorporating geriatric scales to evaluate if the patient is fit and we are, for the first time, designing different clinical trials according to frailty score. I think that the outcome of fit elderly patients with newly diagnosed myeloma can improve more and more and, at the same time, we are going to maintain the quality of life of frail patients and try to minimise the side effects.

What do you believe are the biggest challenges facing the field of haematology? What can be done to overcome these challenges?

There are many challenges in the field of haematology, which is why it is good to continue working in order to overcome them. One of the main challenges is the speed at which the scientific knowledge is advancing, especially in some specific areas of haematology. We have neither the time nor capacity to integrate all this new information, and specialisation in specific areas is becoming more and more necessary. This is a challenge, especially for physicians working in small hospitals, although a great advantage for large academic hospitals where you can give more precise attention to your patients.

In line with this, education continues to be a necessary tool to disseminate all these advances to all haematologists, and national and international societies are doing a great job on this together with the pharmaceutical companies that are also involved in many educational events.

If you could see one haematological disease cured, what would it be? Do you think this is achievable in the near future?

There are, fortunately, some haematological diseases that are already curable and we see a significant proportion of patients with acute leukaemias, chronic myeloid leukaemia, Hodgkin's disease, and Burkitt lymphoma that are actually cured.

However, we have to investigate why this is not applicable to all patients nor all haematological diseases and, as far as myeloma is concerned, we are working on this with the goal of converting myeloma into a curable disease; this would require early treatment with the best combinations and achievement of deep responses with no detection of minimal residual disease.

"The advances in the management of elderly patients are also exciting because we are now incorporating geriatric scales to evaluate if the patient is fit..."

If you could give one piece of advice to an aspiring young haematologist, what would it be?

Haematology is a very complete speciality in medicine with some relevant features: the clinic and laboratory work together and you can obtain an accurate diagnosis if you evaluate the peripheral blood of patients coming to the emergency department in a few hours. If you have any doubt, you can easily go to the peripheral blood or bone marrow to analyse the tumour cells in depth. Because of these features, molecular markers were first identified in patients with haematologic diseases and these laboratory results are now considered in routine clinical decisions so the treatment can be individualised.

It is an exciting speciality in which the relationship with the patients is very close due to the nature of the disease. Haematologists usually spend a lot of time with their patients, but not only that, they also share their thoughts, experiences, and advice. I absolutely recommend haematology as a career to an upcoming physician.

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Planning Your Next Move in Philadelphia Chromosome Positive Leukaemias

This satellite symposium took place on 14th June 2018, as part of the European Hematology Association (EHA) Congress in Stockholm, Sweden

Chairperson: Henrik Hjorth-Hansen[1]

Speakers: Simona Soverini,[2] Thomas Lion,[3] Renato Bassan,[4] Christian Junghanß[5]

1. Department of Haematology, St Olavs Hospital and NTNU, Trondheim, Norway
2. Institute of Hematology 'L e A. Seragnoli', Bologna University, Bologna, Italy
3. CCRI/LabDia Labordiagnostik GmbH, Vienna, Austria
4. UOC Ematologia, Ospedale dell'Angelo, Venice, Italy
5. Klinik III (Hämatologie, Onkologie, Palliativmedizin) Universitätsmedizin Rostock, Rostock, Germany

Disclosure: Prof Hjorth-Hansen has received lecture fees from Pfizer and Incyte; research support for the Nordic CML Study Group from Pfizer, Bristol-Myers Squibb, and Merck. Dr Soverini has acted as a consultant for Bristol-Myers Squibb, Incyte, and Novartis. Prof Lion has received honoraria for lectures and advisory board participation from Amgen, Angelini, Bristol-Myers Squibb, Incyte, Novartis, and Pfizer; and has received research support from Novartis. Dr Bassan has received travel and accommodation expenses and fees for advisory board participation and speeches given at congresses and symposia from Amgen, Incyte, Pfizer, and Shire. Prof Dr Junghanß has acted as a speaker and participated in advisory boards for Amgen, Bristol-Myers Squibb, Incyte, Novartis, Pfizer, and Roche.

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Meeting Summary

The meeting was arranged as a series of conversations between experts, following a question and answer format with two speakers in each presentation. In the first presentation, Dr Soverini and Prof Lion discussed the importance of the timing and depth of response with respect to clinical outcomes in Philadelphia chromosome positive (Ph+) leukaemias. They showed how sensitive and reproducible measurements of molecular response (MR) and the proper interpretation of laboratory data are critical to correctly inform therapeutic decisions in patients with chronic myeloid leukaemia (CML) and Ph+ acute lymphoblastic leukaemias (ALL). Detection of BCR-ABL mutations can establish the need for treatment change and, in some cases, indicate which tyrosine-kinase inhibitor (TKI) is most likely to be effective. The speakers addressed the need for more sensitive and accurate methods to monitor minimal residual disease (MRD) and detect mutations that drive resistance to TKI therapy. They explored two distinct patterns of mutation observed in patients with >1 mutation (polyclonal and compound mutations) and how in addition to selecting the most appropriate TKI it is also important to consider the most appropriate dose.

In the second presentation, Dr Bassan and Prof Dr Junghanß discussed the evolving treatment landscape for Ph+ ALL, including the role of TKI, chemotherapy, and allogenic stem cell transplantation (SCT). The advent of TKI has improved the prognosis for Ph+ ALL, allowing many more patients to achieve complete remission and be considered for allogeneic SCT. However, treatment-related mortality remains a significant issue after allogenic SCT affecting 20–33% of patients.

Studies show that early death rates are lower for patients receiving 'light' chemotherapy and TKI with steroids in place of chemotherapy. Furthermore, for patients achieving complete MR, in some studies there is no difference in outcome between those who undergo allogenic SCT and those who do not, provided that the latter subgroup was selected according to absence of residual disease by PCR analysis. Such data suggest that, in Ph+ ALL, novel therapeutic approaches may in some patients obviate the need for intensive chemotherapy and allogeneic SCT. Studies are now ongoing to explore whether Ph+ ALL patients can abstain from allogenic SCT through selection of the strongest TKI upfront and whether chemotherapy-free regimens might be an option.

Screening and Monitoring Approaches to Optimise Treatment in Philadelphia Chromosome Positive Leukaemias

It is well established in CML that achieving MR milestones at defined time points controls the trajectory towards optimal response and treatment free remission.[1]

The concept of early MR (EMR) is prognostically important, but, in recent years, the kinetics of BCR-ABL transcripts in the first 3 months have been shown to be a more reliable and accurate indicator of disease state than a single measurement at 3 months. An Australian study[2] demonstrated that BCR-ABL value from baseline halving time of <76 days in patients on first-line imatinib predicted progression-free survival and overall survival. Similarly, a German study showed that reduction of BCR-ABL transcripts of half a log or more within the first 3 months is also associated with better survival outcomes.[3-5]

Although studies suggest EMR kinetics offer prognostic information, this has not yet been incorporated into the recommendations published by European LeukemiaNet (ELN) or the National Comprehensive Cancer Network (NCCN), because precise cut-off levels have not yet been defined and technical issues (e.g., the need for a different control gene) still hamper prompt application of this approach.

The time taken to achieve MR is important, with CML patients that achieve major MR at 3 months having a higher cumulative incidence of achieving MR4.5 after 8 years of receiving imatinib than those achieving major MR at 6, 12, or 18 months.[6] Patients demonstrating good imatinib responses can take 5 years or more to reach MR4.5, the deep MR required for treatment free remission in most studies. Second generation TKI can achieve these levels of deep MR much more rapidly.[4]

Provisional criteria for selecting the best candidates for TKI discontinuation proposed by the NCCN include stable MR (MR4.0; BCR-ABL ≤0.01% IS) for ≥2 years documented on at least 4 tests performed at least 3 months apart.[7] Results for a range of TKI in CML show that relapse-free survival with at least major molecular remission was achieved for 33–68% of patients after 0.5–7.0 years of treatment.[8]

Detection and monitoring of MR requires reliable diagnostics. The importance of selecting reliable laboratories is underlined by a EUTOS laboratories review showing that around 17% were unable to reliably score MR4.5 levels in 2017 (unpublished data).

Monitoring of BCR-ABL transcript levels in Ph+ ALL is also considered valuable. Monitoring MRD helps determine who should receive allogeneic SCT and who should receive post-transplant therapies. Furthermore, MRD positivity predicts haematological relapse after allogeneic SCT, even with TKI therapy.[9]

Monitoring of MR with real-time quantitative PCR of BCR-ABL levels is the method of choice for Ph+ ALL management. Precise cut-offs for MR levels have not yet been defined,[10] although the European Working Group for

Adult ALL (EWALL) and the European Study Group-MRD-ALL consortium are looking to standardise methodologies, reduce variability, and optimise procedures.

The most common currently known mechanism behind TKI resistance is BCR-ABL mutations.[11] ELN and NCCN guidelines recommend BCR-ABL kinase domain mutation screening should be undertaken in chronic phase CML patients failing to reach response milestones, at any sign of loss of response, on disease progression, and for patients presenting in accelerated or blast phase.[12] The ELN currently recommends Sanger sequencing to detect *BCR-ABL* mutations, although a EUTOS study[13] showed Sanger sequencing only detects clones representing 20% of the entire leukaemic load.

Two distinct patterns have been observed for patients with >1 mutation: polyclonal mutations (mutations existing separately in different clones) and compound mutations (different mutations found in the same BCR-ABL protein)[14] (Figure 1). Data show compound mutations are harboured by 3% of chronic phase CML patients, 30% of accelerated phase or blast phase CML patients, and 35% with Ph+ ALL who are positive for mutations (Soverini S, personal communications).

Published methodologies for detecting polyclonal and compound mutations include next-generation sequencing (NGS) of short overlapping fragments (where four overlapping fragments were used to cover the kinase domain),[15] long range NGS (allowing the kinase domain to be covered in a single read; not commercially available any more),[16] and NGS on the PacBio® platform (Pacific Biosciences, Menlo Park, California, USA), allowing reading of longer stretches.[17] TK domain mutations detected by NGS sequencing allow a more accurate picture of BCR-ABL mutation status, enabling better TKI selection and mutations to be detected earlier than by Sanger sequencing.[15,18-21]

An Australian study[22] demonstrated that the number of low burden mutations was inversely associated with failure free survival. This finding was supported by a study showing patients without any mutations as assessed by NGS had significantly longer progression-free survival compared to those with mutations (p=0.041).[23] Such data suggests patients with complete cytogenetic response (but not with major MR) should be screened regularly for mutations.

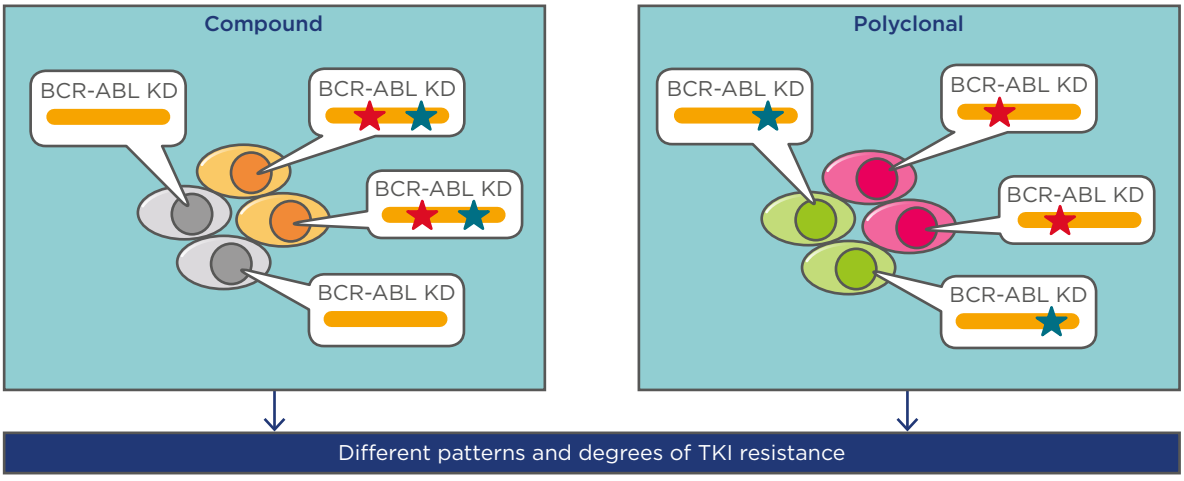

Figure 1: The presence of more than one BCR-ABL mutation in Philadelphia positive leukaemias: compound and polyclonal mutations.

BCR-ABL compound mutants present with two mutations within the same BCR-ABL molecule, whereas the mutations are in separate clones in the case of polyclonal mutations. Mutations are shown by the red and green stars.

KD: kinase domains; TKI: tyrosine kinase inhibitors.

Adapted from Khorashad et al.[14]

In Ph+ ALL, the complexity of mutant subclones has been shown to be higher than in CML, with genetic instability of the *BCR-ABL* gene in Ph+ ALL leading to early accumulation of point mutations.[24] At diagnosis, low burden mutations are detected in >20% of Ph+ ALL patients.[25-28]

The swift emergence of mutations and greater genetic complexity of Ph+ ALL poses clinical and diagnostic challenges with NGS sequencing of *BCR-ABL* mutations providing the basis for improved outcomes. Screening can identify different mutant subclones, the percentage of affected cells, and presence of compound mutations. Digital PCR permits absolute quantification of BCR-ABL molecules and facilitates assessment of the size of specific mutant subclones, but the currently available spectrum of mutations detectable on this platform is limited.

NCCN guidelines provide treatment recommendations for 12 of the most commonly occurring BCR-ABL1 mutations.[7] In the absence of guidelines, use of IC_{50} heat maps to select second-line TKI should be viewed with caution because different heat maps produce conflicting results.[29,30] TKI doses can also have a major influence, with doses achieving higher plasma concentrations being more likely to be effective against certain mutations.[30-32]

Compound mutations were thought to be broadly resistant to TKI, but a recent study suggests that there are three categories of compound mutations and the efficacy of ponatinib is different for each.[33]

- Those with an IC_{50} considered achievable even with the lowest dose of ponatinib.
- Those with very high IC_{50} values not achievable with any clinically feasible dose of ponatinib (particularly compound mutations including T315I or F317L).
- Those with IC_{50} values not achievable with lower doses but achievable with higher doses of ponatinib.

Such data underline that, in addition to selecting the most appropriate TKI, it may also be important to consider appropriate dosing regimens.

Evolving Strategies for The Management of Philadelphia Chromosome Positive with Acute Lymphoblastic Leukaemia

In the era of TKI, clinical trials demonstrate significant improvements in Ph+ ALL survival. Studies show adding any TKI to chemotherapy gives a 5-year survival rate of 35–50%, compared to pre-TKI studies showing survival rates around 20%.[34-38]

Before the introduction of TKI, patients with Ph+ ALL disease had much worse outcomes than those with Ph negative (-) ALL. A population based study (using USA Surveillance, Epidemiology, and End Results [SEER] data in the TKI era) showed no survival difference between Ph+ ALL and Ph- ALL, in the 18–39 year age group (p=0.46); however, older patients (>40 years) with Ph+ ALL had a slight but significant survival advantage over Ph- ALL patients (p=0.037).[39] Explanations for improvements in Ph+ ALL survival include addition of TKI to standard treatment increasing rates of complete remission, allowing allogeneic SCT to take place.[40]

Data from the European Society for Blood and Marrow Transplantation (EBMT) showed ALL accounted for 16% of allogenic SCT in 2015, and that ALL transplants across Europe increased from around 1,000 per year in 1998 to 2,500 per year in 2014. The rise can be attributed to marked increases in unrelated donors (due to tissue typing improvements), which increased donor availability (Figure 2).[41]

To allow cure by allogeneic SCT it is important to achieve complete remission with TKI prior to transplant. Regarding TKI availability, currently only imatinib is licensed in Europe for frontline ALL treatment, with dasatinib allowed in imatinib resistant or intolerant patients and ponatinib licensed after dasatinib failure or for patients with T315I mutations. Nilotinib, bosutinib, and ponatinib are available through clinical trials.

Important concepts for allogenic SCT include obtaining complete remission rates close to 100% and MRD remission ($<10^{-4}$), avoiding loss of response, MRD positivity, and therapy related mortality. Few relapsed/refractory ALL patients survive for >1 year, even with new therapies such as ponatinib, inotuzumab ozogamicin, and blinatumomab.[42-44]

Figure 2: Data from the European Group for Blood and Marrow Transplantation Activity Survey 2015 showing information on stem cell transplantation.

ALL: acute lymphoblastic leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; HSCT: haematopoietic stem cell transplant; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasms; NMD: non-malignant disorder; PCD: plasma cell disorder; SCT: stem cell transplantation; ST: solid tumour.

Adapted from Baldomero and Passweg.[41]

Clinical trials show TKI (imatinib, nilotinib, dasatinib, or ponatinib) achieve similar complete remission in ALL (90% plus).[45] However, higher rates of complete MR can be achieved for nilotinib (MR5: 86%)[46] and ponatinib (MR5: 78%)[47-48] compared to dasatinib (MR5: 24%).[27,49,50]

Studies demonstrate that achieving complete MR prior to allogenic SCT reduces risk of relapse. A representative study analysing Ph+ ALL transplant patients receiving imatinib showed cumulative incidence of relapse was 86.1% for patients achieving poor MR compared to 5.1% for EMR, 6.1% for late MR, and 16.9% for intermediate MR.[51] Clinicians need to take comorbidities into consideration, making use of the Haematopoietic Cell Transplantation Specific Comorbidity Index to score factors such as cardiac conditions, inflammatory bowel disease, diabetes, and obesity.[52] The index can be used to discuss transplant related mortality risks. Studies have shown treatment related mortality after allogenic-SCT affects 20–33% of patients,[53,54] creating the challenge of identifying the patients for whom transplant should be omitted.

TKI can be used to prevent relapse after allogeneic SCT. In the only randomised study using imatinib following allogeneic SCT in Ph+ ALL, there was no difference in 5-year survival (p=0.84) or event free survival (p=0.89) between SCT patients receiving prophylactic imatinib or treatment following detection of MRD.[55] However, the study showed molecular relapse was 69% in the MRD triggered approach group versus 40% in the prophylactic group. Both approaches have advantages and disadvantages, with the MRD triggered approach involving less toxicity and the prophylactic approach less molecular relapse.

In 2016, the EBMT's Acute Leukemia Working Party published a position statement on TKI use according to pre and post-transplant MRD status.[56] Recommendations included:

- Patients who are MRD and preallogenic SCT that had become negative should receive prophylactic TKI according to pre-transplant mutation status or observation and TKI when MRD positive.
- That patients who are MRD positive, preallogenic SCT, and remain MRD positive (or MRD negative preallogenic SCT and become positive) should be checked for BCR-ABL kinase domain mutations and receive TKI according to mutation status.
- Patients who are MRD negative before transplantation and remain MRD negative after transplantation should receive prophylactic imatinib or be observed to screen for becoming MRD positive and receive TKI according to mutation status.

A clear message from trials exploring chemotherapy combinations is that light chemotherapy regimens avoid induction mortality. Studies show early death rates for patients receiving intensive chemotherapy in combination with TKI are 4.0–8.8%;[34,46,57] for non-intensive chemotherapy plus TKI, these rates are 0.0–4.2%; [27,34,54] and for TKI (with steroids) without chemotherapy they are 0.0%.[50,58,59]

Recent studies using nilotinib or ponatinib in newly diagnosed Ph+ ALL showed no difference in overall survival between patients receiving and not receiving chemotherapy, raising the possibility of patients avoiding chemotherapy.[47,46,59-61] Furthermore, a poster[60] was presented at the European Hematology Association (EHA) 2018 Congress that found no difference in outcomes for Ph+ ALL patients with complete MR who underwent transplant and those who did not undergo transplantation. Such data suggest allogenic SCT might be avoidable in patients with complete and durable MR.

A number of trials are currently exploring whether Ph+ ALL patients can abstain from allogenic SCT through use of dual therapies, including the MDACC trial and the D-ALBA Frontline Sequential Dasatinib and Blinatumomab in Adult Philadelphia Positive Acute Lymphoblastic Leukemia study, combining second or third-generation TKI with a monoclonal antibody alone or with corticosteroids, respectively. Additionally, the EWALL03 study in Ph+ ALL patients above the age of 55 years with no transplant options (due to age) will be comparing deintensified chemotherapy plus first and third-generation TKI. Furthermore, in frontline Ph+ ALL, a Takeda study across all age groups is comparing different TKI regimens. In conclusion, TKI have been shown to confer clear benefits on Ph+ ALL patients, with new possibilities to reduce risk of mortality by reducing chemotherapy and allogenic SCT.

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https://goo.gl/rCmwv5

References

1. Baccarani M, Soverini S. Molecular response in CML: Where is the bar? Blood. 2014;124(4):469-71.
2. Branford S et al. Prognosis for patients with CML and >10% *BCR-ABL* after 3 months of imatinib depends on rate of *BCR-ABL1* decline. Blood. 2014;124(4):511-8.
3. Hanfstein B et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988-92.
4. Iriyama N et al. Shorter halving time of BCR-ABL1 transcripts is a novel predictor for achievement of molecular responses in newly diagnosed chronic-phase chronic myeloid leukemia treated with dasatinib: Results of the D-first study of Kanto CML study group. Am J Hematol. 2015,90(4):282-7.
5. El Missiry M et al. Early *BCR-ABL1* transcript decline after 1 month of tyrosine kinase inhibitor therapy as an indicator for treatment response in chronic myeloid leukemia. PLoS One. 2017;12(1):e0171041.
6. Branford S et al. Early molecular response and female sex strongly predict stable undetectable *BCR-ABL1*, the criteria for imatinib discontinuation in patients with CML. Blood. 2013;121(19):3818-24.
7. National Comprehensive Cancer Network. Guidelines v4. 2018 Chronic Myeloid Leukemia. 2018. Available at: https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Last accessed: 10 July 2018.
8. Khoury HJ et al. Chronic myeloid leukemia: What every practitioner needs to know in 2017. Am Soc Clin Oncol Educ Book. 2017;37:468-79.
9. Leonard JT, Stock W. The persistence of minimal residual disease in Philadelphia chromosome-positive acute lymphoblastic leukemia: We know it's bad, now what? Biol Blood Marrow Transplant. 2016;22(11):1913-4.
10. Chiaretti S, Foà R. Management of adult Ph-positive acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015:406-13.
11. Soverini S et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: Recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-15.
12. National Comprehensive Cancer Network. Guidelines v2. 2018. Available at: https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Last accessed: 10 July 2018.
13. Ernst et al. Harmonized testing for BCR-ABL kinase domain mutations in CML: Results of a survey and first control round within 28 national laboratories in Europe. Blood (ASH Annual Meeting Abstracts). 2010;116:625.
14. Khorashad JS et al. *BCR-ABL1* compound mutations in tyrosine kinase inhibitor-resistant CML: Frequency and clonal relationships. Blood. 2013;121(3):489-98.
15. Soverini S et al. Unravelling the complexity of tyrosine kinase inhibitor-resistant populations by ultra-deep sequencing of *BCR-ABL* kinase domains. Blood. 2013;122(9):1634-48.
16. Kastner et al. Rapid identification of compound mutations in patients with Philadelphia-positive leukaemias by long-range next generation sequencing. Eur J Cancer. 2014;50(4):793-800.
17. Cavelier S et al. Clonal distribution of *BCR-ABL1* mutations and splice isoforms by single-molecule long-read RNA sequencing. BMC Cancer. 2015;15:45.
18. Machova Polakova K et al. Next-generation deep sequencing improves detection of *BCR-ABL1* kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. J Cancer Res Clin Oncol. 2015;141(5):887-99.
19. Baer C et al. Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia. Haematologica. 2016;101(7):830-8.
20. Szankasi P et al. Detection of *BCR-ABL1* mutations that confer tyrosine kinase inhibitor resistance using massively parallel, next generation sequencing. Ann Hematol. 2016;95(2):201-10.
21. Soverini S et al. Next-generation sequencing for sensitive detection of BCR-ABL1 mutations relevant to tyrosine kinase inhibitor choice in imatinib-resistant patients. Oncotarget. 2016;7(16):21982-90.
22. Parker WT et al. Sensitive detection of BCR-ABL1 mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. J Clin Oncol. 2011;29(32):4250-9.
23. Kizilors A et al. Impact of finding low level kinase domain mutations using ultra deep next generation sequencing in patients with chronic phase CML. Abstract 347. ASH Annual Meeting, 5–8 December, 2015.
24. Soverini S et al. Philadelphia-positive acute lymphoblastic leukemia patients already harbor BCR-ABL kinase domain mutations at low levels at the time of diagnosis. Haematologica. 2011;96(4):552-7.
25. Pfeifer H et al. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood. 2007;110(2):727-34.
26. Pfeifer H et al. Prevalence and dynamics of BCR-ABL kinase domain mutations during imatinib treatment differ in patients with newly diagnosed and recurrent BCR-ABL positive acute lymphoblastic leukemia. Leukemia. 2012;26(7):1475-81.
27. Rousselot P et al. Dasatinib and low-intensity chemotherapy in elderly patients with Philadelphia chromosome-positive ALL. Blood. 2016;128(6):774-82.
28. Soverini S et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: Recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-15.
29. Radaelli S et al. Three novel patient-derived BCR/ABL mutants show different sensitivity to second and third generation tyrosine kinase inhibitors. Am J Haematol. 2012;87(11):E125-8.
30. Gozgit JM et al. Comprehensive analysis of the in vitro potency of ponatinib and all other approved BCR-ABL TKIs against a panel of clinically-relevant single and compound BCR-ABL mutants. Blood. 2013;122(21):3992.
31. Byrgazov K et al. BCR-ABL1 compound mutants display differential and dose-dependent responses to ponatinib. Haematologica. 2018;103(1):e10-2.
32. Byrgazov K et al. Transposon-mediated generation of BCR-ABL1-expressing transgenic cell lines for unbiased sensitivity testing of tyrosine kinase inhibitors. Oncotarget. 2016;7(47):78083-94.
33. Byrgazov et al. BCR-ABL1 compound mutants display differential and dose-dependent responses to ponatinib. Haematologica. 2018;103(1):e10-2.
34. Bassan R et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. J Clin Oncol. 2010;28(22):3644-52.
35. Fielding AK et al. UKALLXII/

ECOG2993: Addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood. 2014;123(6):843-50.

36. Chiaretti S et al. A sequential approach with imatinib, chemotherapy and transplant for adult Ph+ acute lymphoblastic leukemia: Final results of the GIMEMA LAL 0904 study. Haematologica. 2016;101(12):1544-52.

37. Daver N et al. Final report of a Phase II study of imatinib mesylate with hyper-CVAD for the front-line treatment of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Haematologica. 2015;100(5):653-61.

38. Chalandon Y et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood. 2015:125(24):3711-9.

39. Igwe IJ et al. The presence of Philadelphia chromosome does not confer poor prognosis in adult pre-B acute lymphoblastic leukaemia in the tyrosine kinase inhibitor era – A surveillance, epidemiology and end results database analysis. J Haematol. 2017;179(4):618-26.

40. Leoni V, Biondi A. Tyrosine kinase inhibitors in BCR-ABL positive acute lymphoblastic leukemia. Haematologica. 2015;100(3):295-9.

41. Baldomero H, Passweg JR. Transplant activity survey. 2016. Available at: https://www.ebmt.org/registry/transplant-activity-survey. Last accessed: 10 July 2018.

42. Cortes JE et al. Ponatinib efficacy and safety in Philadelphia chromosome-positive leukemia: Final 5-year results of the phase 2 PACE trial. Blood. 2018. [Epub ahead of print].

43. Kantarjian HM et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016;375(8):740-53.

44. Martinelli G et al. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: Results from a Phase II, single-arm, multicentre study. J Clin Oncol. 2017;35(16):1795-802.

45. Yilmaz M et al. Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: Current treatments and future perspective. Clin Adv Hematol Oncol. 2018;16(3):216-23.

46. Kim DY et al. Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. Blood. 2015;126(6):746-56.

47. Jabbour E et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: A single-centre, Phase 2 study. Lancet Oncol. 2015;16(15):1547-55.

48. Martinelli G et al. First report of the Gimema LAL1811 Phase II prospective study of the combination of steroids with ponatinib as frontline therapy of elderly or unfit patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2017;130(Suppl 1):99.

49. Ravandi F et al. First report of Phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia. Blood. 2010;116(12):2070-7.

50. Foà R et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome positive (PH+) acute lymphoblastic leukemia. Blood. 2011;118(2):6521-8.

51. Lee S et al. Impact of minimal residual disease kinetics during imatinib-based treatment on transplantation outcome in Philadelphia chromosome-positive acute lymphoblastic leukemia. Leukemia. 2012;26(11):2367-74.

52. Sorror ML et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: A new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-9.

53. Lussana F et al. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Impact on relapse and long-term outcome. Biol Blood Bone Marrow Transpl. 2016;22(11):1983-7.

54. Chalandon Y et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood. 2015;125(24):3711-9.

55. Pfeifer H et al. Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for *BCR-ABL1*-positive acute lymphoblastic leukemia. Leukemia. 2013;27(6):1254-62.

56. Giebel S et al. Use of tyrosine kinase inhibitors to prevent relapse after allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: A position statement of the acute leukemia working party of the European society for blood and marrow transplantation. Cancer. 2016;122(19):2941-51.

57. Ravandi F et al. First report of Phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia. Blood. 2010;116(12):2070-7.

58. Vignetti M et al. Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: Results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. Blood. 2007;109(9):3676-8.

59. Martinelli G et al. First report of the Gimema LAL1811 Phase II prospective study of the combination of steroids with ponatinib as frontline therapy of elderly or unfit patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2017;130(Suppl 1):99.

60. Ribera JM et al. Incidence and outcome of relapses in young adults (18-60 yr) with PH--positive alltreated with imatinib, chemotherapy and allogenic hematopoietic stem cell transplantation (PETHEMA ALLPH08 Trial). Abstract PS927. EHA Congress, 14-17 June, 2018.

61. Short NJ et al. Frontline hyper-CVAD plus ponatinib for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Updated results of a Phase II study. Abstract 7013. ASCO Annual Meeting, 5 June, 2017.

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Abstract Reviews

Take a look at these abstract summaries, written by their respective presenters from the EHA Congress 2018

Polymorphisms in Multidrug Resistance Transporter Genes Affect the Duration of Molecular Response to Nilotinib in Chronic Myeloid Leukaemia Patients

Authors: *Federica Loscocco,[1] Giuseppe Visani,[1] Sara Galimberti,[2] Annamaria Ruzzo,[3] Irene Bagaloni,[3] Fabio Stagno,[4] Patrizia Pregno,[5] Mario Annunziata,[6] Antonella Gozzini,[7] Sara Barulli,[1] Mauro Magnani,[3] Alessandro Isidori[1]

1. Hematology and Stem Cell Transplant Center, AORMN, Pesaro, Italy
2. Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
3. Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
4. AOU Policlinico Vittorio Emanuele, Divisione Clinicizzata di Ematologia con Trapianto di Midollo Osseo, Catania, Italy
5. Hematology, AOU Città della Scienza e della Salute di Torino, Torino, Italy
6. Hematology, Cardarelli Hospital, Napoli, Italy
7. Unità Funzionale di Ematologia, AOU Careggi, Firenze, Italy

*Correspondence to federica.loscocco@gmail.com

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Keywords: ABC multidrug transporters, chronic myeloid leukaemia, molecular response, nilotinib, single nucleotide polymorphisms (SNP).

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Despite the high efficacy and improved clinical response of nilotinib in chronic myeloid leukaemia (CML), there is still a significant proportion of patients who fail to obtain or maintain a major molecular response (MR), defined as ≥3 log reduction from the standardised baseline.[1] For these patients, treatment-free remission once in deep molecular response (DMR), which is one of the goals of nilotinib therapy, will never be an achievable endpoint. Although *BCR/ABL* mutations are the major contributory factor for the loss of response to tyrosine kinase inhibitors (TKI), the reduced bioavailability of TKI in leukaemic stem cells is also an important pharmacokinetic factor.[2]

Table 1: Characteristics of patients.

Clinical characteristic	CML patients
Sex	
Male	40
Female	31
Age at diagnosis	
Median age (range)	50 (18–75)
18–50 years	37
≥50–74 years	34
Nilotinib treatment	
First-line	34
Second-line	37
Sokal score	
Low	37
Intermediate	28
High	6
Hasford score	
Low	37
Intermediate	28
High	6
EUTOS score	
0	1
1	66
2	4
Best molecular response to nilotinib	
MR3	90%
MR4	59%
MR4.5	48%

CML: chronic myeloid leukaemia; MR: molecular response.

In this regard, the presence of polymorphisms in drug transports may contribute to mechanisms of drug resistance, disease progression, or loss of MR.

Single nucleotide polymorphisms (SNP) affecting gene expression or function, in both normal and cancerous cells, can cause inherent inter-individual differences in the metabolism and disposition of TKI. Genetic polymorphisms in ABC transporter genes are likely to influence intracellular drug delivery, and, therefore, the effectiveness of TKI.[3] It has been documented that ABC multidrug transporter (MDR-ABC proteins) overexpression contributes to imatinib and novel agent asciminib resistance.[4] However, less is known about how genetic variants in ABC genes may modify pharmacological properties and affect the response to second-generation TKI.

This study examined five SNP in three ABC transporter genes: *ABCC1* 5463T>A, *ABCC2* 3972C>T, *ABCC2* rs4148386, *ABCC2* 1549G>A, and *ABCB1* 3435C>T, to determine the achievement and loss of molecular responses in CML patients treated with nilotinib. A total of 71 CML patients (40 male and 31 female) with

a median age of 50 years were enrolled in the study (Table 1). The genotypes were analysed by PCR-HRM (high resolution melting) assay and PCR-pyrosequencing assay, as previously reported in Visani et al.[5] It was noted whether the polymorphisms showed any deviations from the Hardy–Weinberg equilibrium. Differences in genotype and allele distributions among the CML patients and the associations between genotypes with good response, resistance, or loss of response to nilotinib was assessed by Fisher's exact test, the Kaplan–Meier method, and log-rank test (SPSS, IBM, Chicago, USA; SNPStats package, Bioconductor, Buffalo, New York, USA).

It was found that the *ABCC2* 3972C>T (rs3740066) SNP significantly impacted the loss of MR3 in dominant, codominant, and recessive models. Moreover, different genotypes and allele frequencies of rs3740066 in *ABCC2* were found in patients who maintain the MR3 and in those who lose it. Patients with the T/T genotype in *ABCC2* gene lost the MR3 more frequently than patients with C/C or C/T genotypes in dominant and codominant model (p=0.02; odds ratio [OR]: 11.56 (95% confidence interval: 1.70–78.46); p=0.01, OR: 5.61 (95% confidence interval 1.36–23.09), respectively).

In conclusion, the findings of this study show that the *ABCC2* (rs3740066) SNP is associated with a higher incidence of MR3 loss in CML patients treated with nilotinib. A number of previous studies have suggested that individual genetic differences in ABC transporters play an important role in the efficacy and adverse effects produced by drugs. In particular, it has been hypothesised that genetic variants in ABC transporters may alter the pharmacokinetics of TKI and, consequently, may modify the therapeutic response.[6,7] Now we are analysing a larger series of CML patients treated with nilotinib and we have extended the genotype analyses to also include SNP in *ABCG2*, to try to find a possible association between genetic variants and molecular response in CML patients treated with nilotinib.

References

1. Nath A et al. Pharmacogenetics and pharmacogenomics of targeted therapeutics in chronic myeloid leukemia. Mol Diagn Ther. 2017;21(6):621-31.
2. Ankathil R et al. Pharmacogenetics and the treatment of chronic myeloid leukemia: How relevant clinically? An update. Pharmacogenomics. 2018;19(5):475-93.
3. Hegedùs C et al. Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: Implications for altered anti-cancer effects and pharmacological properties. Br J Pharmacol. 2009;158(4):1153-64.
4. Eadie LN et al. The new allosteric inhibitor asciminib is susceptible to resistance mediated by ABCB1 and ABCG2 overexpression *in vitro*. Oncotarget. 2018;9(17):13423-37.
5. Visani G et al. MTHFR, TS and XRCC1 genetic variants may affect survival in patients with myelodysplastic syndromes treated with supportive care or azacitidine. Pharmacogenomics J. 2018;18(3):444-9.
6. Galimberti S et al. The *hOCT1* and *ABCB1* polymorphisms do not influence the pharmacodynamics of nilotinib in chronic myeloid leukemia. Oncotarget. 2017;8(50): 88021-33.
7. Wang YJ et al. Repositioning of tyrosine kinase inhibitors as antagonists of ATP-binding cassette transporters in anticancer drug resistance. Cancers (Basel). 2014;6(4):1925-52.

Inherited Platelet Disorders Caused by Thrombopoietin Mutation

Authors: *Federica Melazzini, Patrizia Noris

IRCCS Policlinico San Matteo Foundation, University of Pavia, Pavia, Italy

*Correspondence to federica.melazzini@gmail.com

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Keywords: Bone marrow failure, inherited platelet disorders, thrombopoietin (THPO).

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Thrombopoietin (THPO) and the interaction with its receptor c-Mpl have a nonredundant role in platelet biogenesis and maintenance of the haematopoietic stem cell compartment in postnatal haematopoiesis.

Mutations of *c-MPL* can either result in loss-of-function and inherited thrombocytopenia (IT) or in gain-of-function and hereditary thrombocythaemia. Similarly, heterozygous gain-of-function mutations in *THPO* cause hereditary thrombocythaemia, a chronic myeloproliferative syndrome characterised by elevated numbers of circulating platelets, thrombotic or haemorrhagic episodes, and occasional leukaemic transformation. On the other hand, homozygous *THPO* loss-of-function mutations result in thrombocytopenia evolving in bi-tri lineage bone marrow aplasia of varying degree.

Until recently, loss-of-function mutations in *c-MPL* were the only known cause of congenital amegakaryocytic thrombocytopenia (CAMT), a disorder characterised by congenital hypomegakaryocytic thrombocytopenia and the development of further cytopenias during childhood until progression to generalised bone marrow aplasia. This severe aplasia is fatal unless children are treated with haematopoietic stem cell transplantation (HSCT).[1] Of note, a proportion of patients with the clinical presentation of CAMT do not carry mutations in *c-MPL*, suggesting that the disease is genetically heterogeneous. It has been hypothesised that the patients with wild-type *c-MPL* have alterations in genes playing a role upstream or downstream of the receptor.[1,2]

Recent research showed that homozygous loss-of-function variants in *THPO* induce a phenotype similar to that caused by *c-MPL* mutations.[3-5] In fact, patients present with hypomegakaryocytic thrombocytopenia with or without anaemia or neutropenia, which progresses to trilineage bone marrow aplasia. The only difference with patients affected with CAMT due to *c-MPL* mutation was that in these *THPO*-mutated subjects the HSCT was constantly unsuccessful.

Since the endogenous THPO is cleared from circulation by megakaryocytes and platelets, the serum or plasma THPO levels are markedly increased in all forms of bone marrow aplasia or hypoplasia, including CAMT due to *c-MPL* mutations.[6-8] In contrast, the serum THPO concentration was not increased in patients with *THPO*-variants. These observations suggest that measurement of the serum THPO level could be useful to discriminate CAMT patients with *c-MPL* mutations from those with *THPO*-variants or other bone marrow failure syndromes.

THPO-mimetic drugs represent an appealing therapeutic option for the cytopenias caused by *THPO* mutations.[9] Consistently, romiplostim was effective at increasing platelet count in all at-risk patients,[4,5] as well as at increasing haemoglobin concentration and neutrophil count in subjects presenting with anaemia and/or neutropenia. Besides the haematological response, romiplostim induced remission of spontaneous bleeding and transfusion independence. Thus, recognising *THPO*-variants is essential for correct management and avoiding the use of invasive and unnecessary treatments, such as HSCT and immunosuppressive drugs.

Additionally, Noris et al.[10] recently described a new, autosomal dominant form of IT caused by a *THPO* heterozygous variant leading to a truncated THPO protein, characterised by normal or slightly increased platelet size; affected subjects had no bleeding tendency and their thrombocytopenia was discovered incidentally. Unlike the homozygous *THPO*-variant, this innocuous disease has to be distinguished from the more severe autosomal dominant IT with normal platelet size deriving from mutations in *ETV6*, *ANKRD26*, and *RUNX1*, which predispose individuals to the development of haematological malignancies.

References

1. Ballmaier M, Germeshausen M. Congenital amegakaryocytic thrombocytopenia: Clinical presentation, diagnosis, and treatment. Semin Thromb Hemost. 2011;37(6):673-81.
2. Geddis AE. Congenital amegakaryocytic thrombocytopenia. Pediatr Blood Cancer. 2011;57(2):199-203.
3. Dasouki MJ et al. Exome sequencing reveals a thrombopoietin ligand mutation in a Micronesian family with autosomic recessive aplastic anemia. Blood. 2013;122(20):3440-9.
4. Seo A et al. Bone marrow failure unresponsive to bone marrow transplantation is caused by mutation in thrombopoietin. Blood. 2017;130(7):875-80.
5. Pecci A et al. Thrombopoietin mutation in congenital amegakaryocytic thrombocytopenia treatable with romiplostim. EMBO Mol Med. 2018;10(1):63-75.
6. Ballmaier M, Germeshausen M. Advances in the understanding of congenital amegakaryocytic thrombocytopenia. Br H Haematol. 2009;146(1):3-16.
7. Olnes MJ et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. N Engl J Med. 2012;367(1):11-9.

8. Ballmaier M et al. Flow cytometric detection of MPL (CD110) as a diagnostic tool for differentiation of congenital thrombocytopenias. Haematologica. 2015;100(9):e341-4.
9. Basciano PA, Bussel JB. Thrombopoietin-receptor agonist. Curr Opin Hematol. 2012;19(5):392-8.
10. Noris P et al. A new form of inherited thrombocytopenia due to monoallelic loss of function mutation in the thrombopoietin gene. Br J Haematol. 2018;181(5):698-701.

Bacterial Contamination of Platelet Transfusion Units Affects Platelet Haemostatic Function and Immunological Activity

Authors: Joels Wilson-Nieuwenhuis, Kathryn A. Whitehead, *Nina C. Dempsey-Hibbert

School of Healthcare Science, Manchester Metropolitan University, Manchester, UK
*Correspondence to n.dempsey-hibbert@mmu.ac.uk

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Keywords: Bacteria, biofilm, platelet, RANTES, sepsis, transfusion.

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Transfusion-associated bacterial infection is a major risk of platelet transfusion and can be fatal.[1] The storage requirements of platelet concentrates (PC) pre-transfusion allow for bacterial proliferation and biofilm formation on the inner surface of the blood bag.[2] Biofilms are particularly problematic, exhibiting increased virulence, antimicrobial resistance,[3] and reduced respiration rates of the bacteria, leading to decreased detection by bacterial recognition systems and the resultant transfusion of contaminated units.

Platelets are key players in inflammation, secreting cytokines and chemokines that orchestrate immune responses. Platelets express all 10 toll-like receptor transcripts, allowing direct recognition of pathogen-associated molecular patterns (PAMP) on invading micro-organisms.[4] One of the many chemokines released by activated platelets during inflammation is CCL5 (RANTES).[5] RANTES has well-established roles in the homing and migration of several leukocyte subsets during inflammation, thereby promoting an immune response. However, elevated RANTES is reported in early infection and sepsis, correlating with infection severity, tissue damage, and mortality.[6-8] This work aimed to determine the effects of planktonic bacteria and biofilms on platelet function, establishing whether contaminated PC have altered haemostatic efficacy and/or heightened immunological activity.

Platelet-rich plasma (PRP) was isolated from healthy participants after ethical approval and informed consent; the PRP was incubated with *Staphylococcus epidermidis* or *Serratia marcescens* (two species commonly implicated in PC contamination)[9,10] in either planktonic or biofilm forms prior to stimulation with ADP (10 µM) and analysis of activation markers (CD62P and PAC1 binding). The concentrations of bacteria used for inoculation were reflective of those found in contaminated PC. ADP-stimulated aggregation was assessed using light transmission aggregometry after these treatments and RANTES release was determined by cytometric bead array.

Platelet aggregation following incubation with biofilms (grown for 5 or 7 days prior to culture with PRP), but not with planktonic cells, significantly inhibited platelet aggregation.

However, no significant changes in CD62P or PAC1 binding were determined following culture. RANTES released by platelets (ADP-stimulated or not) was significantly increased when platelets were incubated with a 7-day biofilm of either bacterial species.

This work demonstrated the inhibitory effects of biofilms formed from common PC contaminants on platelet haemostatic potential, indicating that contaminated PC may lose their therapeutic efficacy. This investigation further indicated that recipients of contaminated PC will experience high concentrations of soluble RANTES. Although this may be initially beneficial in the recruitment of leukocytes to eliminate infection in the host, it could itself initiate acute immunological reactions following transfusion and contribute to transfusion-associated sepsis.

References

1. Brecher ME, Hay SN. Bacterial contamination of blood components. Clin Microbiol Rev. 2005;18(1):195-204.
2. Wilson-Nieuwenhuis JST et al. Surface modification of platelet concentrate bags to reduce biofilm formation and transfusion sepsis. Colloids and Surf B Biointerfaces. 2017;160:126-35.
3. Rutherford ST, Bassler BL. Bacterial quorum sensing: Its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med. 2012;2(11):a012427.
4. Koupenova M et al. Sex differences in platelet toll-like receptors and their association with cardiovascular risk factors. Arterioscler Thromb Vasc Biol. 2015;35(4):1030-7.
5. von Hundelshausen P, Weber C. Platelets as immune cells: Bridging inflammation and cardiovascular disease. Cir Res. 2007;100(1):27-40.
6. Cavaillon JM et al. Cytokine cascade in sepsis. Scand J Infect Dis. 2003;35(9):535-44.
7. Stojewska M et al. Evaluation of serum chemokine RANTES concentration as a biomarker in the diagnosis of early-onset severe infections in neonates. Postepy Hig Med Dosw (Online). 2016;70:272-9.
8. Ness TL et al. CCR1 and CC chemokine ligand 5 interactions exacerbate innate immune responses during sepsis. J Immunol. 2004;173(11):6938-48.
9. Ali H et al. Characterization of the growth dynamics and biofilm formation of Staphylococcus epidermidis strains isolated from contaminated platelet units. J Med Microbiol. 2014;63(Pt 6):884-91.
10. Greco-Stewart VS et al. Serratia marcescens strains implicated in adverse transfusion reactions form biofilms in platelet concentrates and demonstrate reduced detection by automated culture. Vox Sang. 2012;102(3):212-20.

Lenalidomide: The Gift That Keeps on Giving

Authors: *Abdulraheem Yacoub

The University of Kansas Medical Center, Kansas City, Kansas, USA
*Correspondence to ayacoub@kumc.edu

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Keywords: Lenalidomide, maintenance, non-Hodgkin's lymphoma, rituximab-refractory disease.

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First-line therapy for indolent non-Hodgkin's lymphoma usually consists of a combination of rituximab-based chemoimmunotherapy. Historically, first-line therapies have been associated with deep responses that are often durable; however, therapy failure due to primary refractory disease or relapse is inevitable. A particularly concerning subgroup encompasses patients who exhibit refractoriness to rituximab, refractoriness to chemotherapy, or early relapses. This group of patients has been associated with a worse prognosis, increased mortality, more aggressive disease biology, and likely refractoriness to additional classic chemoimmunotherapy interventions.

Lenalidomide is a second-generation immunomodulatory agent that has a mechanism of action distinct from classic chemotherapy regimens. It has a well-recognised activity in multiple myeloma, chronic lymphocytic leukaemia, and subtypes of myelodysplastic syndromes. It has also shown significant activity in various non-Hodgkin's lymphoma subtypes as a single agent and in combination.

The MAGNIFY trial was designed as a Phase III international effort to investigate the combination of lenalidomide and rituximab as initial induction therapy followed by randomised maintenance with rituximab with or without

lenalidomide in patients with relapsed and/or refractory follicular lymphoma, marginal zone lymphoma, or mantle cell lymphoma.[1] Enrolled subjects had a median of two prior systemic therapies. Given the nature of the inclusion criteria, 41% of subjects met the criteria for rituximab-refractory disease. The clinical trial is still open and the results are not yet mature enough to analyse the primary endpoint of progression-free survival. Nonetheless, the study has been presented to demonstrate preliminary efficacy and safety data.

The preliminary results of the study showed significant activity of the combination during the initial therapy with an overall response rate of 75% and a complete response rate of 47% in rituximab-sensitive patients (n=110). Patients who met the criteria for rituximab refractoriness (n=77) also had a high response rate, with an overall response rate of 58% and complete response rate of 47%. The results will be updated at the EHA Congress with a larger number of patients and longer follow-up. These preliminary responses are significantly better than the historic outcomes of these patients. Follow-up is still ongoing for the analysis of progression-free survival.

Treatment-emergent adverse events were consistent with the known adverse events of lenalidomide and rituximab treatment, with haematologic toxicities being the most common events.

Although this is only a preliminary analysis, it shows significant activity and deep responses in patients with particularly high-risk diseases and historically poor outcomes. Importantly, it was able to restore rituximab sensitivity in patients with prior suboptimal response or early relapse after rituximab-based therapy.

Lenalidomide is being investigated in multiple non-Hodgkin's lymphomas both in upfront and relapsed/refractory settings. In addition to the indications in myeloma and myelodysplasia, lenalidomide is approved by the U.S. Food and Drug Administration (FDA) as a single agent for relapsed mantle cell lymphoma after two lines of therapy. This trial will hopefully pave the way for the use of this chemotherapy-free combination as a second-line therapy in a broader range of indolent non-Hodgkin's lymphomas. This is particularly relevant in patients with a suboptimum response to classic chemoimmunotherapy given the novel mechanism of action of lenalidomide.

References

1. Celgene. Updated data from Phase IIIb MAGNIFY study of REVLIMID (lenalidomide) and rituximab combination (R2) show clinical activity and responses in relapsed/refractory follicular and marginal zone lymphoma. 2017. Available at: http://ir.celgene.com/releasedetail.cfm?releaseid=1030542. Last accessed: 28 June 2018.

A Targeted Next-Generation Sequencing-Based Diagnosis for Hereditary Anaemias

Authors: *Roberta Russo,[1,2] Immacolata Andolfo,[1,2] Francesco Manna,[2] Antonella Gambale,[1,2] Roberta Marra,[1,2] Barbara Eleni Rosato,[1,2] Paola Caforio,[1,2] Valeria Pinto,[3] Piero Pignataro,[1] Kottayam Radhakrishnan,[4] Sule Unal,[5] Giovanna Tomaiuolo,[6] Gian Luca Forni,[3] Achille Iolascon[1,2]

1. Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Napoli, Italy
2. CEINGE Biotecnologie Avanzate, Napoli, Italy
3. Centro della Microcitemia e Anemie Congenite, Ospedale Galliera, Genova, Italy
4. Department of Haematology, Monash Medical Centre, Paediatric Haematology - Oncology, Children's Cancer Centre, Monash Children's Hospital, Melbourne, Australia
5. Division of Pediatric Hematology, Hacettepe University, Ankara, Turkey

6. Dipartimento di Ingegneria Chimica, dei Materiali e della Prod. Indus., University Federico II, Napoli, Italy
*Correspondence to roberta.russo@unina.it

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Keywords: Differential diagnosis, hereditary anaemias (HA), targeted next-generation sequencing (t-NGS).

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Anaemia affects 1.6 billion people worldwide, with roughly 10% of these individuals affected by rare anaemias, of which 80% are hereditary.[1] Hereditary anaemias (HA) encompass a highly heterogeneous group of disorders characterised by anaemia of variable degrees and by complex genotype–phenotype correlations. Differential diagnosis, classification, and patient stratification among HA is often very difficult.

To date, the major current application of next-generation sequencing (NGS) in diagnostics is through disease-targeted tests, for which multiple causal genes are known. Some studies have already demonstrated the utility of a targeted NGS (t-NGS) approach in the study of specific subtypes of HA patients. In this study, we described the diagnostic workflow based on t-NGS that we developed for the diagnosis of patients affected by HA. Within this wide group of disorders, we included a) hyporegenerative anaemias, such as congenital dyserythropoietic anaemias (CDA); b) haemolytic anaemias due to red cell membrane defects, such as hereditary spherocytosis (HS) and stomatocytosis (HSt); and c) haemolytic anaemias due to enzymatic defects, such as pyruvate kinase (PK) deficiency.[2-5]

We generated two consecutive versions of the same custom gene panel: the first included 34 genes, the second 71 genes. The probe design was performed by SureDesign (Agilent Technologies, Santa Clara, California, USA). Sample preparation was obtained by HaloPlex Target Enrichment kit for Illumina Sequencing (Agilent Technologies), and high-throughput sequencing was performed by Illumina NextSeq 500 (Illumina Inc., San Diego, California, USA). For bioinformatic analyses, we used Agilent SureCall software (v 3.0.3.1, Agilent Technologies). The pathogenicity of each variant was evaluated according to the guidelines of the American College of Medical Genetics and Genomics (ACMG).[6,7]

We investigated 74 probands with clinical suspicion of HA. Our approach revealed a diagnostic yield of 64.9% of analysed patients. Genetic data by t-NGS analysis confirmed the clinical suspicion in 54.2% of patients. Of note, most of these patients were originally suspected to have red cell membrane disorders (HSt or HS).

Conversely, t-NGS analysis modified the original diagnosis in 45.8% of patients; 81.8% of these patients were clinically suspected to have CDA. Of note, among the 22 patients originally classified as CDA, we identified 45.5% of cases with a conclusive genetic diagnosis of congenital haemolytic anaemias due to enzymatic defects. Indeed, we diagnosed one case with biallelic mutations in *GPI*, the causative gene of haemolytic non-spherocytic anaemia due to glucose phosphate isomerase deficiency; another case due to mutations in *AK1*, the causative locus of haemolytic anaemia due to adenylate kinase deficiency; and eight cases due to mutations in *PKLR*, the causative gene of PK deficiency.[7]

Our observation regarding congenital haemolytic anaemia patients misdiagnosed as CDA is highly relevant; it underlines how t-NGS analysis is valuable not only for achieving a correct and conclusive diagnosis but also for guiding possible treatment of HA patients. This is mainly true for the treatment of PK deficient-patients, for whom there is an allosteric activator of PK enzyme available that can increase the enzymatic activity of patient erythrocytes treated *ex vivo*.[8]

References

1. Hertz L et al. Is increased intracellular calcium in red blood cells a common component in the molecular mechanism causing anemia? Front Physiol. 2017;8:673.
2. Gambale A et al. Diagnosis and management of

congenital dyserythropoietic anemias. Expert Rev Hematol. 2016;9(3):283-96.
3. Andolfo I et al. New insights on hereditary erythrocyte membrane defects. Haematologica. 2016;101(11):1284-94.
4. Andolfo I et al. Hereditary stomatocytosis: An underdiagnosed condition. Am J Hematol. 2018;93(1): 107-21.
5. Canu G et al. Red blood cell PK deficiency: An update of PK-LR gene mutation database. Blood Cells Mol Dis. 2016;57:100-9.
6. Al-Riyami AZ et al. Targeted next generation sequencing identifies a novel β-spectrin gene mutation A2059P in two Omani children with hereditary pyropoikilocytosis. Am J Hematol. 2017;92(10):E607-9.
7. Russo R et al. Multi-gene panel testing improves diagnosis and management of patients with hereditary anemias. Am J Hematol. 2018;93(5):672-82.
8. Kung C et al. AG-348 enhances pyruvate kinase activity in red blood cells from patients with pyruvate kinase deficiency. Blood. 2017;130(11):1347-56.

Genes and Pathways Dysregulated by Nilotinib Treatment in Bone Marrow CD34+/Lin- Cells of Patients with Chronic-Phase Chronic Myeloid Leukaemia

Authors: *Alessandra Trojani,[1] Ester Pungolino,[1] Alessandra Dal Molin,[2] Milena Lodola,[1] Gabriella De Canal,[3] Giuseppe Rossi,[4] Mariella D'Adda,[4] Alessandra Perego,[5] Ester Orlandi,[6] Malato Simona,[7] Mauro Turrini,[8] Lorenza Borin,[9] Alessandra Iurlo,[10] Maria Luisa Latargia,[11] Maria Cristina Carraro,[12] Francesco Spina,[13] Salvatore Artale,[14] Michela Anghilieri,[15] Francesco Lanza,[16] Enrica Morra,[17] Roberto Cairoli[1]

1. Division of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy
2. Department of Information Engineering, University of Padova, Italy
3. Pathology Department, Cytogenetics, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy
4. Department of Hematology, ASST Spedali Civili, Brescia, Italy
5. Internal Medicine-Haematology, Desio Hospital, Desio, Italy
6. Hematology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
7. Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milano, Italy
8. Division of Hematology, Department of Internal Medicine, Valduce Hospital, Como, Italy
9. Hematology Division, San Gerardo Hospital, Monza, Italy
10. Oncohematology Division, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation, Milano, Italy
11. ASST Valle Olona Ospedale di Circolo, Busto Arsizio, Italy
12. Hematology & Transfusion Medicine, Sacco Hospital, Milano, Italy
13. Division of Hematology - Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy
14. ASST Valle Olona Sant'Antonio Abate, Gallarate, Italy
15. ASST Lecco, Lecco, Italy
16. Division of Hematology, Hospital of Cremona, Cremona, Italy
17. Executive Committee, Rete Ematologica Lombarda, Milano, Italy

*Correspondence to alessandra.trojani@ospedaleniguarda.it

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Keywords: Bone marrow CD34+/Lin- cells, chronic myeloid leukaemia (CML), genes and pathways, nilotinib.

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BACKGROUND

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder with heterogeneous biological and clinical features. Specifically, it presents a reciprocal association between chromosomes 9 and 22, yielding the BCR-ABL fusion protein with overacting tyrosine kinase activity.[1] CML is treated with tyrosine kinase

inhibitors (TKI), which have dramatically improved the long-term survival of CML patients to approximately 80%.[2] Among TKI, nilotinib is very effective for the treatment of imatinib-resistant patients in the clinic.[3] However, despite the remarkable success of TKI in controlling CML in the chronic phase, some patients develop drug resistance. The incapability of TKI to eradicate the disease completely is best explained by intrinsic and acquired drug resistance in leukaemic stem cells.[4] The main research focus in the CML field is therefore to identify pathways and genes that contribute to the survival of leukaemic stem cells.

AIMS

We performed gene expression profiling (GEP) of selected bone marrow (BM) CD34+/Lin- cells of patients with chronic phase (CP)-CML at diagnosis versus 12 months of nilotinib treatment to gain new insights into the molecular mechanisms of nilotinib treatment in CML.

METHODS

We selected and counted BM CD34+/Lin- cells of 30 CP-CML patients at diagnosis and during 3, 6, and 12 months of first-line nilotinib treatment. BM CD34+/Lin- cells were isolated by immunomagnetic separation technology and tested by standard fluorescence *in situ* hybridisation (FISH) for all 30 patients at diagnosis and after 12 months of nilotinib treatment. GEP analyses of selected BM CD34+/Lin- cells of the CP-CML patients at diagnosis and after 12 months of nilotinib treatment were performed by Affymetrix™ (Thermo Fisher Scientific Ltd., Waltham, Massachusetts, USA) microarray analysis. Data were preprocessed using the ComBat method to adjust for batch effects and quantile normalisation.[5] The significance analysis of microarrays (SAM) test was used for expression analysis at diagnosis versus 12 months of treatment.[6] Selection was performed using the R statistical computing software. False discovery rate-adjusted p values <5% were considered significant.[7]

RESULTS AND CONCLUSION

We demonstrated that the number of BM CD34+/Lin- cells dramatically decreased after 3, 6, and 12 months of nilotinib treatment (Figure 1). FISH analysis detected CD34+/Lin- Ph+ cells in 30 CP-CML patients at diagnosis, while no Ph+ nuclei were detected in CD34+/Lin- cells after 12 months of nilotinib.

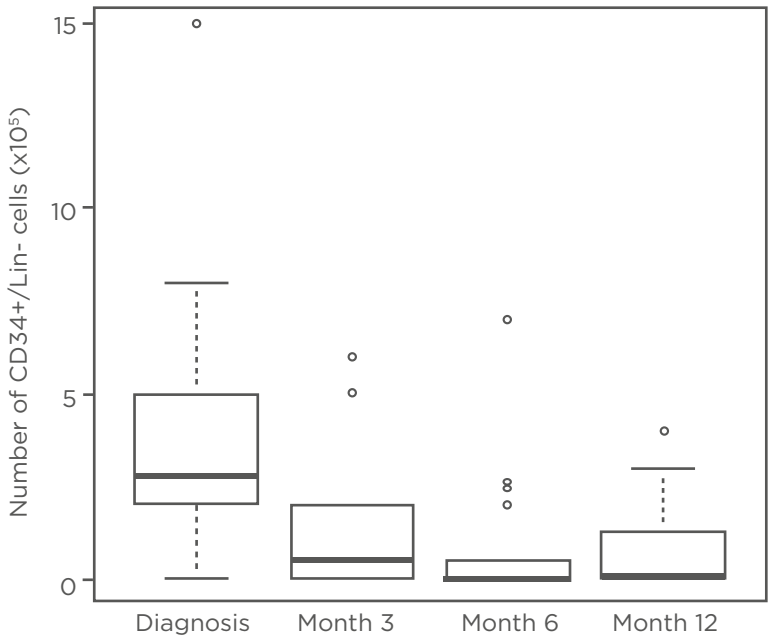

Figure 1: The number of bone marrow CD34+/Lin- cells at diagnosis and after 3, 6, and 12 months of nilotinib treatment in 30 chronic myeloid leukaemia patients.

GEP detected 264 statistically significant differentially expressed genes at diagnosis versus 12 months of nilotinib treatment. Functional enrichment analysis showed that groups of genes belonging to 14 pathways were significantly dysregulated in CP-CML patients after 12 months of nilotinib. Lipid, glucose, and sphingolipid metabolism, insulin resistance, complement and coagulation, platelet activation, the cytoskeleton, cell adhesion, cellular transport, B cell differentiation, the RAS signalling pathway, proliferation, growth factors, and apoptosis were all shown to be significantly altered after 12 months of nilotinib in comparison with diagnosis in CP-CML patients.[8,9]

References

1. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. Nat Rev Cancer. 2007;7(6)441-53.
2. Jabbour E et al. Long-term outcome of patients with chronic myeloid leukemia treated with second-generation tyrosine kinase inhibitors after imatinib failure is predicted by the in vitro sensitivity of BCR-ABL kinase domain mutations. Blood. 2009;114(10):2037-43.
3. Kantarjian H et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354(24):2542-51.
4. Jiang X et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia. 2007;21(5):926-35.
5. Johnson WE et al. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007;8(1):118-27.
6. Tusher VG et al. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2011;98(9):5116-21.
7. Storey JD. A direct approach to false discovery rates. J R Stat Soc. 2002;64(3)479-98.
8. Trojani A et al. Wide-transcriptome analysis and cellularity of bone marrow CD34+/lin- cells of patients with chronic-phase chronic myeloid leukemia at diagnosis vs. 12 months of first-line nilotinib treatment. Cancer Biomark. 2017;21(1):41-53.
9. Pungolino E et al. Nilotinib induced bone marrow CD34+/lin-Ph+ cells early clearance in newly diagnosed CP-chronic myeloid leukemia. Am J Hematol. 2018. [Epub ahead of print].

A High Pretreatment Bone Marrow CD34+/CD38- Cell Burden in Patients with Myelodysplastic Syndrome is a Prognostic Factor for Disease Progression After Allogeneic Stem Cell Transplantation

Authors: *Madlen Jentzsch, Ulrike Geus, Juliane Grimm, Stefanie Beinicke, Janine Häntschel, Vladan Vucinic, Wolfram Pönisch, Gerhard Behre, Georg-Nikolaus Franke, Dietger Niederwieser, Sebastian Schwind

Department of Hematology and Clinical Oncology, University Hospital Leipzig, Leipzig, Germany

*Correspondence to Madlen.Jentzsch@medizin.uni-leipzig.de

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Keywords: Allogeneic stem cell transplantation, leukaemia stem cells, myelodysplastic syndrome (MDS), prognosis.

Citation: EMJ Hematol. 2018;6[1]:54-56. Abstract Review No. AR7.

BACKGROUND

Myelodysplastic syndromes (MDS) are highly heterogeneous clonal haematopoietic disorders.[1] Allogeneic haematopoietic stem cell transplantation (HSCT) remains the only curative treatment for MDS and is of special interest in patients with a high risk of progression to acute

myeloid leukaemia (AML).[2] It has been shown that, in MDS, CD34+/CD38- cells possess MDS stem cell potential and that clones of secondary AML originate from the MDS disease stage.[3,4] However, no study has evaluated the prognostic impact of the MDS stem cell-containing cell population burden in MDS patients prior to therapy.

AIM

The aim of the study was to analyse the prognostic impact of CD34+/CD38- cell burden in MDS patients receiving allogeneic HSCT.

METHODS

We retrospectively analysed 124 patients diagnosed with MDS (n=105) or myelodysplastic/myeloproliferative neoplasm (n=19) receiving HSCT at our institution. The median age at HSCT was 61.3 years (range: 22.2–74.4). The conditioning regimens used were reduced intensity (44%, fludarabine with busulfan or treosulfan) or non-myeloablative (56%, fludarabine with 2Gy or 3Gy total body irradiation). Prior to HSCT, 59% of patients received cytoreductive therapy with hypomethylating agents (25%), AML chemotherapy (24%), or both (10%). Median follow-up after HSCT was 4.3 years. Karyotype analyses were performed centrally at our institution. Risk values according to the Revised International Prognostic Scoring System (IPSS-R) were 7% low, 28% intermediate, 21% high, 36% very high, and 7% unknown. The CD34+/CD38- cell burden was evaluated by flow cytometry in untreated bone marrow material. Using R's OptimalCutpoint package, a 1% CD34+/CD38- cell cut-off was determined and divided the cohort into patients with a high (34%) or low (66%) CD34+/CD38- cell burden.

RESULTS

A high pretreatment bone marrow CD34+/CD38- cell burden was associated with a higher bone marrow mononuclear cell expression of CD13 (p<0.001), CD33 (p<0.001), and CD117 (p<0.001), an excess of blasts (p<0.001), and worse IPSS-R risk group (p=0.03). Patients with a high CD34+/CD38- cell burden had worse IPSS-R genetic risk (p=0.02); were more likely to have an abnormal (p=0.04), complex (p=0.002), or monosomal (p=0.004) karyotype; and more often received cytoreductive treatment prior to HSCT (p=0.008).

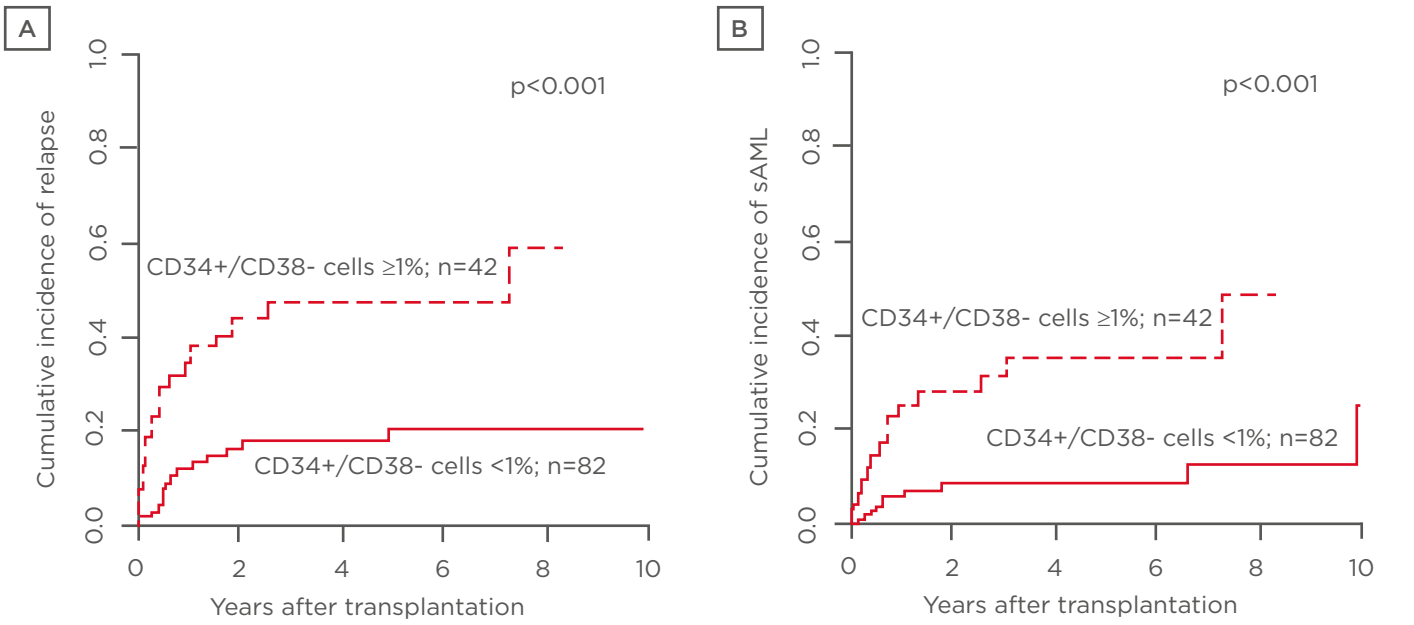

Figure 1: A) Cumulative incidence of relapse and B) cumulative incidence of secondary acute myeloid leukaemia.

sAML: secondary acute myeloid leukaemia.

Patients with a high CD34+/CD38- cell burden had a significantly higher cumulative incidence of relapse or progression (CIR; p<0.001; Figure 1A), a higher cumulative incidence of secondary AML (CIsAML; p<0.001; Figure 1B), and a shorter overall survival (OS; p=0.12) by trend. In multivariate analyses, a high CD34+/CD38- cell burden remained an independent prognostic factor for higher CIR (hazard ratio: 2.88; p=0.005) after adjustment for IPSS-R risk, for higher CIsAML (hazard ratio: 3.13; p=0.02) after adjustment for IPSS-R risk, age at HSCT and human leukocyte antigen match, and for shorter OS (odds ratio: 0.47; p=0.01) after adjustment for pre-HSCT bone marrow blast count, human leukocyte antigen match, and donor type. Analysing IPSS-R low or intermediate and high or very high risk MDS patients separately, a high CD34+/CD38- cell burden indicated patients with higher CIR (p<0.001), higher CIsAML (p=0.003), and shorter OS (p=0.12) by trend irrespective of the IPSS-R risk group.

CONCLUSION

In conclusion, a high pretreatment CD34+/CD38- cell burden was associated with higher CIR, higher CIsAML, and a trend for shorter OS after HSCT. Despite the correlation with high-risk disease, a high CD34+/CD38- cell burden provided independent prognostic information for all endpoints in the multivariable analyses and in separate analyses for IPSS-R low or intermediate and high or very high-risk patients. The observed prognostic impact is likely mediated by MDS stem cells within the CD34+/CD38- cell population, initiating MDS relapse or progression to AML.

References

1. Malcovati L et al.; European Leukemia Net. Diagnosis and treatment of primary myelodysplastic syndromes in adults: Recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943-64.
2. Kröger N. Allogeneic stem cell transplantation for elderly patients with myelodysplastic syndrome. Blood. 2012;119(24):5632-9.
3. Walter MJ et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366(12):1090-8.
4. Nilsson L et al. Involvement and functional impairment of the CD34(+)CD38(-)Thy-1(+) hematopoietic stem cell pool in myelodysplastic syndromes with trisomy 8. Blood. 2002;100(1):259-67.

Longitudinal Trend Analysis of Serum Transferrin Receptor-1 Level in a Cohort of 104 Patients Affected by Non-Transfusion-Dependent Thalassaemia

Authors: *Paolo Ricchi,[1] Massimiliano Ammirabile,[2] Biagio Gargiulo,[1] Silvia Costantini,[1] Aldo Filosa[1]

1. UOSD Malattie Rare Del Globulo Rosso, Azienda Ospedaliera di Rilievo Nazionale "Antonio Cardarelli", Napoli, Italy
2. Laboratory of Clinical Chemistry and Microbiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

*Correspondence to pabloricchi@libero.it

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Keywords: Biomarkers, blood transfusion, hydroxyurea, non-transfusion-dependent thalassemia (NTDT), serum transferrin receptor.

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The proper management of patients with non-transfusion-dependent thalassaemia (NTDT) has been a challenging mission. In fact, NTDT encompasses a great variety of genetic syndromes mixed in terms of their molecular background, clinical course, and severity. Despite the availability of recent guidelines

from the Thalassaemia International Federation (TIF), the strength of several treatments and recommended strategies for follow-up should be confirmed on an individual basis.[1,2] Furthermore, in clinical practice, most recommendations are difficult to apply to the wide-spectrum of phenotypes, and the lack of biomarkers and/or predictive factors for the course of the disease in individual patients is evident.[1] Biomarkers are of increasing importance in medicine, particularly in the area of personalised medicine. They are helpful in predicting prognosis and could be particularly useful in detecting therapeutic and adverse responses for patients with NTDT. Previous retrospective and cross-sectional studies have shown that morbidity in patients with NTDT is directly proportional to the severity of the disease, which in turn depends on the variable degree of ineffective erythropoiesis, iron loading, and peripheral haemolysis. Thus, biomarkers reflecting these primary pathogenetic parameters, such as growth differentiation factor-15, fetal haemoglobin, and liver iron concentration, have been previously used to score the clinical severity of NTDT patients, but are not all used for current management of the disease.[3-5] In previous retrospective and cross-sectional studies, we showed that the soluble transferrin receptor 1 (sTfR1) level, which fully reflects the marrow erythropoietic activity, had a very high diagnostic accuracy in predicting the risk of extramedullary haematopoiesis, scoring disease severity, and patient stratification.[6-8] Recent retrospective data also highlighted a relationship between sTfR1 level and some fundamental events in the management of patients with NTDT, such as age at diagnosis, age at first transfusion, age at splenectomy, and age when beginning chelation therapy.[9]

The purpose of this study was to further validate the use of the sTfR1 level as a biomarker in a longer-term prospective assessment. In a cohort of 104 patients, who have been undergoing measurement of their respective sTfR1 level approximately twice a year since 2007, we explored the longitudinal trend in sTfR1 level. Our data showed that, during the observation time, most of the patients (n=76; 73%) did not require any treatment (controls) affecting erythropoiesis and therefore maintained a steady sTfR1 level (Table 1). In contrast, 16 (15%) patients were started on regular blood transfusion therapy and 12 (12%) on hydroxyurea, because of the treatment and the prevention of anaemia and/or several complications such as extramedullary haematopoiesis, cardiomyopathy, and fatigue. Both populations of treated patients had a significantly higher level than controls at baseline (Table 1). Conversely, following the start of treatments, we observed a statistically significant reduction in sTfr1 level in both populations (p=0.00023 and p=0.005 in BT and HU group, respectively; data not shown).

Table 1: Characteristics of the evaluated population.

	p value (BT versus controls)	BT (n=16)	Controls (n=76)	HU (n=12)	p value (HU versus controls)
Male/Female (%)	0.577	5/11 (31.2/68.7)%	32/44 (42.1/57.9)%	8/4 (66.7/33.3)%	0.131
Median age (years) (range)	0.005	48.30 (35.0–73.8)	38.20 (11.0–82.7)	44.20 (31.0–60.1)	0.200
Median observation time (years)	0.240	4.34 (0.97–8.56)	5.780 (0.07–11.45)	6.99 (2.19–8.31)	0.330
Median sTfR1 (mg/L) (baseline)	0.000	8.68 mg/L (6.39-18.10)	5.085 mg/L (1.61–14.00)	11.80 mg/L (7.87–14.50)	0.000
Median sTfR1 (mg/L) (pre-treatment)	0.001	8.12 mg/L (5.42–15.00)	4.875 mg/L (1.63–15.40)	11.85 mg/L (8.39–12.40)	0.000
Median sTfR1 (mg/L) (post-treatment)	N.A.	5.86 mg/L (3.22–10.90)	N.A.	8.35 mg/L (4.84–16.40)	N.A.

BT: blood transfusion; HU: hydroxyurea; N.A.: not applicable; sTfR1: soluble transferrin receptor 1.

Biomarkers are essential tools for tailoring treatment to the individual, enabling personalised medicine; in the field of NTDT, they could allow more efficient management and interference with the natural history of the disease. Overall, these prospective data further reinforce the need for testing the sTfR1 level in patients with NTDT following first diagnosis, as its increased level may provide an early indicator of the need for treatments to reduce and/or prevent several NTDT complications linked to anaemia and/or expanded erythropoiesis.

References

1. Ricchi P et al. Non-transfusion-dependent thalassemia: A complex mix of genetic entities yet to be fully discovered. Biomed Res Int. 2015; 2015:161434.
2. Taher A et al.; Weatherall D (Ed.). Guidelines for the clinical management of non-transfusion dependent thalassaemia (NTDT) [Internet]. 2013.
3. Musallam KM et al. Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia. Haematologica. 2011;96(11): 1605-12.
4. Musallam KM et al. Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with β thalassemia intermedia. Blood Cells Mol Dis. 2011;47(7):232-4.
5. Musallam KM et al. Fetal hemoglobin levels and morbidity in untransfused patients with β-thalassemia intermedia. Blood. 2012;119(2):364-7.
6. Ricchi P et al. Soluble form of transferrin receptor as a biomarker of overall morbidity in patients with non-transfusion-dependent thalassaemia: A cross-sectional study. Blood Transfus. 2016;14(6):538-40.
7. Ricchi P et al. A useful relationship between the presence of extramedullary erythropoeisis and the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassemia intermedia: A prospective study. Ann Hematol. 2012;91(6):905-9.
8. Ricchi P et al. Extramedullary haematopoiesis correlates with genotype and absence of cardiac iron overload in polytransfused adults with thalassaemia. Blood Transfus. 2014;12(Suppl 1):s124-30.
9. Ricchi P et al. Soluble form of transferrin receptor-1 level is associated with the age at first diagnosis and the risk of therapeutic intervention and iron overloading in patients with non-transfusion-dependent thalassemia. Ann Hematol. 2017;96(9):1541-6.

Combined Oral Administration of Analgesia and Anxiolysis for Pain Associated with Bone Marrow Aspiration and Biopsy

Authors: *Claudio Cerchione

Department of Clinical Medicine and Surgery, AOU Federico II, Napoli, Italy
*Correspondence to claudiocerc@hotmail.com

Disclosure: The author has declared no conflicts of interest.

Keywords: Analgesia, anxiolysis, bone marrow aspiration and biopsy (BMAB), fentanyl citrate, haematological malignancies, pain, quality of life, randomised trial.

Citation: EMJ Hematol. 2018;6[1]:58-60. Abstract Review No. AR9.

BACKGROUND

Bone marrow aspiration and biopsy (BMAB) is a painful procedure. The commonly adopted local infiltration anaesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalled thereafter. As there are no formal guidelines for adding a sedoanalgesic premedication before beginning BMAB, many combinations have been adopted by several authors.[1-8]

AIMS

Our randomised and patient-blinded trial aimed to evaluate the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who underwent BMAB for haematological malignancies, which was the primary endpoint of the trial. The secondary endpoints were firstly, to define if patients who had undergone BMAB without LIA preferred sedoanalgesia and secondly, to demonstrate if

sedoanalgesia could influence the quality of the biological specimen harvested.

METHODS

Patients were randomly assigned into two arms, receiving either placebo plus LIA (standard group: 48.6%) or oral fentanyl citrate 200 µg plus oral midazolam 5 mg in addition to LIA (combo group: 51.4%) during BMAB. Preprocedural anxiety and procedural pain were assessed according to the Numeric Rating Scale (0–10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of the procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later. A total number of 116 patients were enrolled in the study. Nine patients did not meet the inclusion criteria and were excluded. Fifty-two patients were randomised and assigned to the standard group and 55 to the combo group (Figure 1).

RESULTS

At T2b and T3 (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower ($p<0.05$) perception of pain in the patients who received sedoanalgesia (combo group) compared to those who did not (standard group). Moreover, 100% of the patients in the combo group who had previously undergone this procedure without premedication reported that they would prefer sedoanalgesia for the subsequent procedures, thus confirming the effectiveness of this combination in relieving anticipatory anxiety. Finally, the histological specimen was found to be high in quality, as defined by International Council for Standardization in Haematology (ICSH) standards.[9]

CONCLUSION

Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in the outpatient setting; sedoanalgesia is very effective in reducing pain during the biopsy and it diminishes the anticipatory anxiety related to a painful procedure. Patients should have the option to choose between local anaesthesia alone or sedoanalgesia plus local anaesthesia.

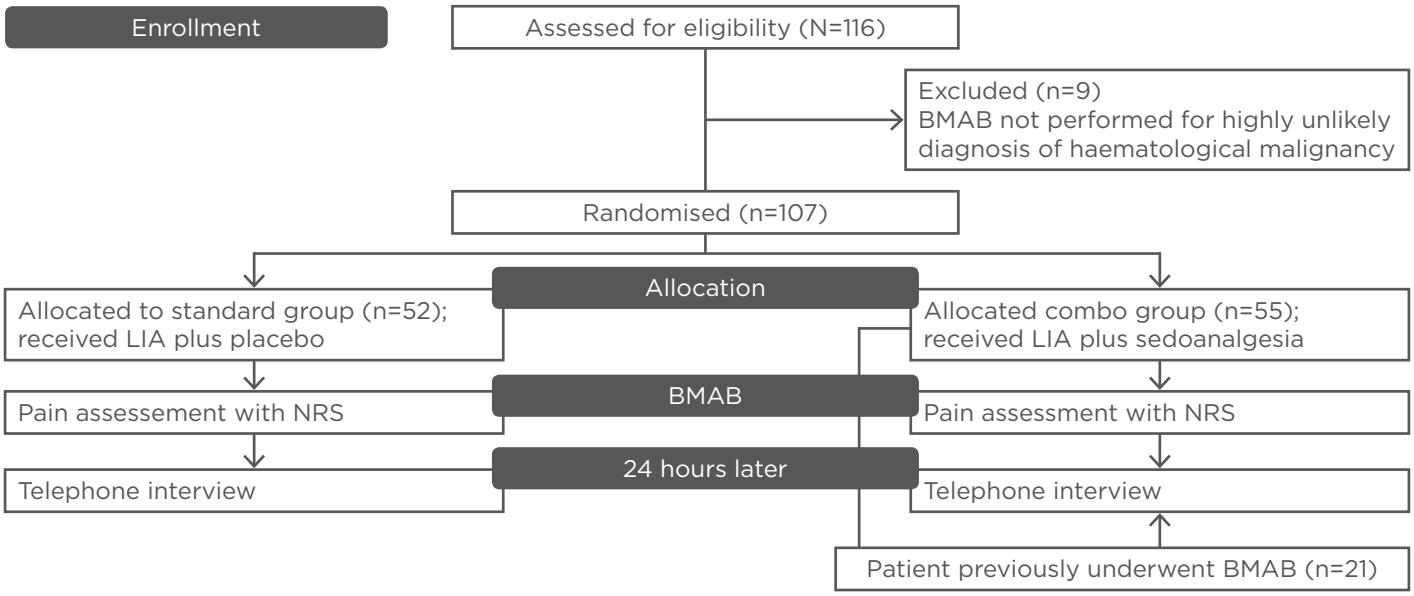

Figure 1: Flow chart of the trial.

BMAB: Bone marrow aspiration and biopsy; NRS: Numeric Rating Scale; LIA: local infiltration anaesthesia.

References

1. Cerchione C et al. Combined oral administration of analgesia and anxiolysis for pain associated with bone marrow aspiration and biopsy. EHA Congress, 14-17 June, 2018.
2. Talamo G et al. Oral administration of analgesia and anxiolysis for pain associated with bone marrow biopsy. Support Care Cancer. 2010;18(3):301-5.
3. Mystakidou K et al. Oral transmucosal fentanyl citrate: Overview of pharmacological and clinical characteristics. Drug Deliv. 2006;13(4):269-76.
4. Farrar JT et al. Oral transmucosal fentanyl citrate: randomized, double-blinded, placebo-controlled trial for treatment of breakthrough pain in cancer patients. J Natl Cancer Inst. 1998;90(8):611-6.
5. Coluzzi PH et al. Breakthrough cancer pain: A randomized trial comparing oral transmucosal fentanyl citrate (OTFC) and morphine sulfate immediate release (MSIR). Pain. 2001;91(1-2):123-30.
6. Talamo G et al. Perceived levels of pain associated with bone marrow aspirates and biopsies. J Support Oncol. 2012;10(4):166-70
7. Hjortholm N et al. Strategies of pain reduction during the bone marrow biopsy. Ann Hematol. 2013;92(2):145-9.
8. Park SH et al. A randomized double-blind placebo-controlled study of low-dose intravenous Lorazepam to reduce procedural pain during bone marrow aspiration and biopsy. Pain Med. 2008;9(2):249-52.
9. Lee SJ et al; International Council for Standardization in Hematology. ICSH guidelines for the standardization of bone marrow specimens and reports. Int J Lab Hematol. 2008;30(5):349-64.

Novel Agents for the Management of Relapsed Multiple Myeloma

Authors: *Claudio Cerchione

Department of Clinical Medicine and Surgery, AOU Federico II, Napoli, Italy
*Correspondence to claudiocerc@hotmail.com

Disclosure: The author has declared no conflicts of interest.

Keywords: Multiple myeloma (MM), real life, refractory, relapse.

Citation: EMJ Hematol. 2018;6[1]:60-61. Abstract Review No. AR10.

Multiple myeloma (MM) is a malignant neoplasm of plasma cells that accumulate in bone marrow, leading to bone destruction and marrow failure. MM accounts for approximately 1.8% of all haematologic and solid cancers; moreover, MM accounts for >15% of haematologic malignancies in the USA. MM is typically sensitive to different classes of cytotoxic drugs, both as front-line treatment and as treatment for relapsed disease. Unfortunately, even if responses are typically durable, nowadays MM is not considered curable with current approaches. However, treatment of MM has been rapidly evolving due to the introduction of new classes of drugs, such as proteasome inhibitors, immunomodulatory drugs, histone deacetylase inhibitors, and monoclonal antibodies, alongside new indications for old classes of drugs, such as alkylating agents. Furthermore, there is increasing understanding of MM tumour biology, creating the rationale for new combinations of drugs and the development of new therapies. Discovery of the associated cytogenetic abnormalities confirm the hypothesis that MM is a heterogeneous disease, suggesting that risk-adapted therapies and individualising treatment will further help to improve patient management.

With the aforementioned in mind, the aim of this study was to evaluate, in relapsed and refractory MM, the retrospective experience of several treatments, including old and novel agents, in order to compare real-world experience with the results of clinical trials.

During the EHA Congress 2018, four retrospective, observational studies were presented. They aimed to evaluate, in a real-life setting, a cohort of heavily pretreated patients affected by relapsing/refractory MM. Efficacy and safety data were evaluated (Table 1). Data on efficacy and safety of these real-life experiences seem to be highly comparable to those of major trials adopting the same regimen in the same clinical setting, demonstrating how these therapies can be considered feasible salvage therapeutic options, even in heavily pretreated patients.

Table 1: Novel agents for management of relapsed and refractory multiple myeloma: Real-life experiences in AOU Federico II, Naples, Italy.

	Number of patients	Age diagnosed, years (range)	Age treatment started, years (range)	Number of previous therapies (range)	ORR: (≥PR)	ORR: (≥SD)	OS: since diagnosis (range)	OS: since start of treatment (range)	TTR (range)
Pom-Dexa Cerchione et al.,[1] 2018	26 (14 M/12 F)	69.0 (52–84)	73.0 (56–87)	6 (2–9)	42.0%	73.0%	87.0 (21–228)	8.0 (1–14)	2.0 (1–6)
KRD Cerchione et al.,[2] 2018	27 (16 M/11 F)	63.0 (47–79)	66.0 (53–83)	3 (2–11)	66.7%	76.1%	51.0 (9–170)	5.0 (1–13)	2.0 (1–3)
Bort-Len-Dexa Cerchione et al.,[3] 2018	29 (19 M/10 F)	56.0 (38–74)	64.0 (38–79)	3 (1–6)	62.0%	79.3%	56.0 (12–221)	26.0 (6–48)	3.0 (1–6)
BVD Cerchione,[4] 2018	56 (31 M/25 F)	57.3 (36–82)	61.8 (37–83)	6 (2–11)	64.0%	85.7%	62.7 (6–151)	9.8 (2–36)	1.2 (1–3)

Bort-Len-Dexa: bortezomib-lenalidomide-dexamethasone; BVD: bendamustine-bortezomib-dexamethasone; F: females; KRD: carfilzomib-lenalidomide-dexamethasone; M: males; Pom-Dexa: pomalidomide-dexamethasone; PR: partial response rate; ORR: overall response rate; OS: overall survival; SD: standard deviation; TTR: time to response.

In particular,

- Pomalidomide-dexamethasone has shown significant efficacy and a very good compliance, thanks to oral administration, in a particularly severe group of heavily pretreated patients relapsed and refractory to all available therapeutic resources.[1,5]
- Carfilzomib-lenalidomide-dexamethasone has shown significant efficacy in a particularly severe group of patients relapsed and refractory to all available therapeutic resources and lenalidomide. In particular cases, it could be considered as a bridge to a second autologous or allogenic stem cell transplant.[2,6]
- Bortezomib-lenalidomide-dexamethasone triplet, thanks to a notable proven synergistic mechanism of action between bortezomib and lenalidomide, has shown significant efficacy in a severe group of heavily pretreated patients relapsed and refractory to bortezomib and lenalidomide.[3,7]
- The triplet bendamustine-bortezomib-dexamethasone has shown significant efficacy in a particularly severe group of patients relapsed and refractory to all available therapeutic resources. In particular cases, it could be considered as a bridge to a second autologous or allogenic stem cell transplant.[4,8]

References

1. Cerchione C et al. Pomalidomide-dexamethasone in the management of heavily pretreated multiple myeloma. Abstract number: PB2249. EHA Congress, 14-17 June, 2018.
2. Cerchione C et al. Carfilzomib-lenalidomide-dexamethasone in the management of lenalidomide-refractory multiple myeloma. Abstract number: PB2223. EHA Congress, 14-17 June, 2018.
3. Cerchione C et al. Bortezomib, lenalidomide and dexamethasone in the management of relapsed and refractory multiple myeloma: a real-life experience. Abstract number: PB2243. EHA Congress, 14-17 June, 2018.
4. Cerchione C. Bendamustine-Bortezomib-Dexamethasone (BVD) in heavily pretreated Multiple Myeloma. Abstract number: PB2203. EHA Congress, 14-17 June, 2018.
5. Miguel JS et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): A randomised, open-label, Phase 3 trial. Lancet Oncol. 2013;14(11):1055-66.
6. Stewart AK et al.; ASPIRE Investigators. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med. 2015;372(2):142-52.
7. Richardson PG et al. A phase 2 trial of lenalidomide, bortezomib, and dexamethasone in patients with relapsed and relapsed/refractory myeloma. Blood. 2014;123(10):1461-9.
8. Offidani M et al. Efficacy and tolerability of bendamustine, bortezomib and dexamethasone in patients with relapsed-refractory multiple myeloma: A phase II study. Blood Cancer J. 2013;3:e162.

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Allogeneic Haematopoietic Stem Cell Transplantation for Chronic Myeloid Leukaemia in the Era of Tyrosine Kinase Inhibitors

EDITOR'S PICK

Allogeneic haematopoietic stem cell transplant (alloSCT) is an effective therapeutic choice for chronic myeloid leukaemia and remained the only curative option for many years. However, the introduction of targeted drugs against the *BCR-ABL1* tyrosine kinase has changed the therapeutic approach for this disease. In this article, the authors describe the current indications for alloSCT during the tyrosine kinase inhibitor era and explore the role of these drugs in multiple situations, including before and after transplant.

Dr David Gómez-Almaguer

Hospital Universitario Dr. Jose E. González UANL, México

Authors: *Ahmet Emre Eskazan,[1] Mario Tiribelli[2]

1. Division of Hematology, Department of Internal Medicine, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey
2. Division of Hematology and BMT, Department of Medical Area, University of Udine, Udine, Italy

*Correspondence to emreeskazan@hotmail.com

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Abstract

The introduction of tyrosine kinase inhibitors (TKI) has dramatically improved the prognosis of chronic myeloid leukaemia (CML) patients and, therefore, changed the therapeutic scenario of this disease. Before the advent of the first TKI imatinib, allogeneic haematopoietic stem cell transplantation (alloSCT) was the only curative approach for CML, and all patients deemed eligible for transplant were referred to a centre for transplant where possible. Nowadays, with the wide availability of five different TKI, indications to alloSCT have been reduced to only include patients in the advanced

phase of CML and those with multiple TKI treatment failures. Nonetheless, even in the TKI era, alloSCT retains its curative potential. Herein, the authors give an overview of the indications to allogeneic transplant for CML and the management of TKI in the pre and post-transplant settings.

INTRODUCTION

BCR-ABL1 tyrosine kinase inhibitor (TKI) therapy is the current standard of care for patients with chronic myeloid leukaemia (CML) in the chronic phase (CML-CP). Nowadays, these patients usually have near-normal life spans and survival has reached approximately 80–90%.[1,2] The percentage of patients progressing to advanced-phase disease (particularly to blast crisis [BC]) is smaller when compared to the pre-TKI era. In CML-CP patients receiving upfront second-generation TKI (2G-TKI), this rate is even lower when compared to imatinib, a first-generation TKI.[3,4] Prior to the era of targeted therapy with TKI, early treatment modalities for patients with CML-CP included arsenic, busulfan, hydroxyurea, and IFN-α with or without cytosine arabinoside, but, in general, none of these treatments can induce a long-term survival benefit. During the 1980s, allogeneic haematopoietic stem cell transplantation (alloSCT) became the only curative therapy for CML.[5] However, not all patients could undergo alloSCT because there were (and still are) some challenges, including age and donor availability problems that physicians and patients face during and after alloSCT, and this procedure can be associated with significant early and late transplant-related morbidities and even mortality. After the year 2000, with the introduction of imatinib, the number of transplants performed for CML-CP substantially decreased and, although it remains an important therapeutic option for eligible patients, the place of alloSCT in the management of CML has become limited.

ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKAEMIA PRIOR TO THE TYROSINE KINASE INHIBITOR ERA

The first documented disappearance of a Philadelphia chromosome-positive (Ph+) clone following alloSCT was performed in syngeneic twins,[6] which was then followed by transplantation using human leukocyte antigen (HLA)-matched sibling donors and later unrelated donors.[7] Eventually, in the 1990s, CML was the most frequent indication for alloSCT. CML was a testing ground for the use of alloSCT,[8] and this disease provided the first example for risk assessment with the European Group for Blood and Marrow Transplantation (EBMT) risk score,[9] showing that disease stage was more important than the bulk of the disease; this score is still the most powerful predictor of transplant outcome for haematological malignancies. Patients with the lowest risk score have been shown to have a transplant-related mortality of 20% and a 5-year overall survival (OS) of 72%, whereas those with the highest score have been shown to have a transplant-related mortality of 72% with a 5-year OS of 22%.[10] CML was also the first disease for which a consistent graft-versus-leukaemia (GvL) effect was demonstrated.[11] Relapse risk was very high after T cell depletion and, conversely, donor lymphocyte infusion (DLI) was proven to be very effective in CML, especially with the additional role of pre-emptive DLI use in patients receiving reduced-intensity conditioning regimens. Although myeloablative conditioning remains the preferred approach for the majority of transplant-eligible CML patients, the understanding of transplant immunology resulted in the development of reduced-intensity conditioning regimens to extend access to alloSCT to those who are unfit for the myeloablative conditioning regimens.

THE PLACE OF ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE ERA OF TYROSINE KINASE INHIBITORS

Indications for Allogeneic Haematopoietic Stem Cell Transplantation

The data derived from the Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis of the

Worldwide Network for Blood and Marrow Transplantation (WBMT) policy clearly showed that the number of patients with CML undergoing alloSCT was extensively reduced, with only 1,059 allotransplants (only 3.3% of the total number of transplants) performed globally in 2012.[12] In a study by Özen et al.,[13] prior to the introduction of imatinib in Turkey, the percentage of patients receiving alloSCT for CML was 40%, whereas this percentage was 11% and 5% between 2002 and 2006 and after 2007, respectively. Supporting this finding, at the EBMT 2018 meeting, there were only five abstracts regarding SCT in CML out of a total of 1,083.

Most patients presenting with CML-CP receive upfront treatment with imatinib and approximately 70% achieve complete cytogenetic response (CCyR) after 12 months of therapy; however, during long-term follow-up, about 40% of these patients switched to a second-line TKI therapy due to resistance and/or intolerance.[14] Nearly half of patients who fail frontline imatinib may achieve durable responses with 2G-TKI (dasatinib, nilotinib, and bosutinib).[15-17] Although it can be beneficial, after failing two lines of TKI treatment the chance of achieving optimal responses with third-line 2G-TKI is relatively low.[18] In patients with CML-CP receiving upfront dasatinib or nilotinib, by 24 months of TKI therapy, only 77% and 76% of the cases were still on 2G-TKI treatment, respectively.[19,20] Also, in CML-CP patients who harbour a Thr315Ile mutation, the third-generation TKI ponatinib can be a reasonable option,[21] and can be used in patients with CML-CP who fail two lines of TKI treatment. A paper reporting data within the era of TKI showed that when alloSCT was performed among cases in first CP with a low EBMT risk score (0–2), using standard myeloablative conditioning with standard graft-versus-host disease (GvHD) prophylaxis and an optimal stem cell source (e.g., HLA-identical sibling), the low transplant-related mortality results were remarkable.[22]

In the most recent European LeukemiaNet (ELN) recommendations, CML-CP patients and siblings should undergo HLA typing at diagnosis only in cases of baseline warning signs.[23] In cases of first-line imatinib failure, it is also recommended to search for a sibling donor. In addition, in patients with CML-CP who failed upfront 2G-TKI (nilotinib and dasatinib), searching for an unrelated stem cell donor is recommended in case an HLA-identical sibling donor is unavailable.[23] In patients harbouring a Thr315Ile mutation, the search for a sibling or unrelated stem cell donor is highly advised at any line of TKI therapy.

In a recently published paper, it was shown that patients with newly diagnosed high-risk CML and non-responders to first-line TKI can benefit from an early low-risk alloSCT with improved long-term survival, shorter time of treatment, a higher rate of molecular remissions, and lower healthcare costs,[24] which were consistent with another previous study performed in the imatinib era.[25] In that study, patients undergoing early low-risk alloSCT had no early additional mortality but a significantly higher rate of molecular remissions compared to those with imatinib.[25]

While proceeding to alloSCT, one should balance the potential risks of the transplant against the risk of disease progression. The indications for alloSCT in patients with CML-CP include failing to achieve durable responses with two lines of TKI therapy and cases with a Thr315Ile mutation. Even eligible patients with a low transplant score who fail initial TKI therapy might be considered for an early alloSCT rather than salvage TKI therapy. In patients with CML-CP who have a Thr315Ile mutation, alloSCT can be curative; however, in cases with advanced disease phases (accelerated phase [AP] or BC), transplantation should be preserved for patients who return to second CP after anti-CML therapy.[26]

In cases with AP or BC, eligible patients should undergo alloSCT,[27-29] and among patients with BC harbouring a Thr315Ile mutation, the outcomes are better with alloSCT than those with ponatinib.[21] The current evidence suggests that alloSCT may be the best chance of cure in BC patients. In these patients, alloSCT should be performed following a treatment with a suitable TKI selected according to mutation profile in combination with chemotherapy in order to achieve a second CP.[30] Nowadays, more CML patients are transplanted in second CP or advanced phases than in first CP.

Although long-term TKI treatment effectively controls the disease in most patients, this has resulted in a significant economic impact for patients and healthcare systems worldwide,

especially for those in low-income countries.[31] In the developing world, early alloSCT can be considered in the management of selected cases before disease transformation, and this treatment modality may be, sometimes unavoidably, due to economic issues, chosen over 2G-TKI.[24] As generic imatinib becomes available, this would decrease the therapy-related expenses, thus increasing the accessibility of TKI therapy, which might further minimise the rate of CML progression and the need for alloSCT.[32,33]

The Impact of Prior Tyrosine Kinase Inhibitor Use on Allogeneic Hematopoietic Stem Cell Transplantation Outcomes

Several previous studies have demonstrated that the use of TKI prior to transplant does not seem to have a negative impact on the outcome of alloSCT in CML.[34-40] In a more recent study by Kondo et al.,[41] the authors included patients receiving one, two, or three TKI before alloSCT (153, 49, and 35 patients, respectively). They clearly showed that, in addition to conventional risk factors, using three TKI prior to transplantation was associated with an adverse outcome. Non-relapse mortality rate was higher in patients with three TKI than those in patients treated with one or two TKI, and the authors concluded that alloSCT could be considered for young patients with CML-CP who had resistance to second-line TKI therapy and who had an appropriate donor.[41]

The Type of Transplant: Myeloablative Versus Reduced-Intensity Conditioning

Transplantation should be performed with an HLA-identical sibling or HLA-matched unrelated donor; if unavailable, a haploidentical donor can be used, bearing in mind that haploidentical transplants in CML are rare.[42] Although the number of patients undergoing haploidentical alloSCT continues to increase, in a recent activity survey report of EBMT, it was demonstrated that 398 transplants were performed for CML and, of these, only 26 were haploidentical.[43] For years, myeloablative conditioning regimens were used in CML and are still in use among fit patients. It remains the standard of care for those who tolerate the regimen and includes total body irradiation and cyclophosphamide or busulfan and cyclophosphamide.[27] For GvHD prevention, the combination of cyclosporine and short-term methotrexate is commonly used. Since long-term remission is usually dependent on the GvL effect, reducing the intensity of the conditioning and reinforcing the GvL effect with pre-emptive DLI enables transplantation of elderly patients and those with comorbidities.[44-47] Although T cell depletion is associated with reduced severity and frequency of GvHD, the risks of relapse[48] and infection[49] are both increased.

Source of Haematopoietic Stem Cells

Generally, over the last decades there has been a shift from bone marrow to peripheral blood as a source for stem cells. However, the use of peripheral blood stem cells for CML-CP has been associated with an increased risk of non-relapse mortality and chronic GvHD.[50,51] The issue of increased GvHD risk is of particular interest in CML due to the frequent need for DLI to treat molecular relapse after transplant. If the eventual goal is to limit the risks on GvHD, as may be the case in CML-CP, a prudent approach would favour the use of bone marrow-derived haematopoietic stem cells.

Prevention of Relapse after Allogeneic Haematopoietic Stem Cell Transplantation

Relapse after alloSCT still represents an important cause of failure of transplant procedures, mainly in high-risk disease cases which make up the majority of CML patients undergoing alloSCT. Though the real value of the use of TKI after a successful alloSCT is still unknown, mainly because most CML patients receive transplant after failure of multiple TKI, the possible role of prophylactic TKI therapy to prevent disease relapse remains attractive.

In the last 10 years, various investigations have tested the safety and efficacy of imatinib and, later, 2G-TKI after alloSCT. Carpenter et al.[52] reported on 22 patients with Ph+ leukaemias (15 with acute lymphoblastic leukaemia [ALL] and 7 with CML) prospectively treated with imatinib 400 mg from engraftment to Day 365, proving the overall safety of imatinib administration despite a high incidence of nausea, vomiting, and transaminase increase. A Japanese group[53] compared 20 patients (18 with Ph+ ALL and 2 with CML) receiving

imatinib for the prevention of disease relapse for at least 3 months after alloSCT with 76 patients (33 with Ph+ ALL and 43 with CML) who did not receive imatinib. Imatinib, started at 400 mg and administered within 100 days of alloSCT, was associated with a reduced incidence and severity of chronic GvHD in most patients, but this study failed to assess the prophylactic impact of TKI therapy on the incidences of leukaemia relapse due to a small number of patients and its retrospective nature.[53] In 2015, Shimoni at al.[54] reported on a Phase I/II study of nilotinib prophylactic maintenance in 16 patients with advanced CML or Ph+ ALL undergoing alloSCT, started after engraftment and continued until progression or toxicity. Nilotinib's maximal tolerated dose was determined to be 200 mg twice a day. The median duration of therapy was 20 months and 6 patients stopped nilotinib due to toxicities (hepatic in 3, haematological in 1, allergic in 1, and late cerebrovascular in 1). Among the 11 patients who achieved a complete molecular response with alloSCT with or without nilotinib, only 1 progressed on nilotinib maintenance, with an overall 2-year survival rate of 55% and a 2-year relapse risk of 23%, lower than what is expected in such a high-risk population. The same group subsequently studied the immune function of 12 patients receiving nilotinib for at least 90 days after transplant, demonstrating a rapid reconstitution of NK cells and CD8+ T cells; moreover, T cell response was not inhibited by nilotinib administration.[55] DeFilipp et al.[56] published a monocentric experience of 26 patients (17 Ph+ ALL and 9 AP/BC CML) receiving maintenance post-alloSCT therapy with different TKI, including dasatinib (n=14), nilotinib (n=1), and ponatinib (n=1). The TKI was chosen according to pre-alloSCT response, tolerability, and *ABL* mutation; imatinib was started at 400 mg daily and other TKI at 50% of the pretransplant dose. The 9 CML patients were transplanted in second or third CP and started TKI (7 with dasatinib) while in molecular remission. The 5-year OS for CML and Ph+ ALL in second complete remission (reported together) was 79%. Recently, a multicentric study[57] enrolled 40 patients who received nilotinib (n=11) or imatinib followed by nilotinib by Day 81 (n=29) after myeloablative alloSCT for Ph+ leukaemias; 17 patients who consented to enter the study before alloSCT were not eligible to start TKI prophylaxis at engraftment. Despite various causes of discontinuation of treatment that produced a failure rate of 77% (44 out of 57) of the starting intention-to-treat population, all 13 patients who completed nilotinib therapy were alive and in remission.[57]

In summary, there is no definitive evidence that prophylactic use of TKI after alloSCT may significantly reduce the risk of CML recurrence. On the other hand, there are no concerns of TKI safety in the post-alloSCT setting and the National Comprehensive Cancer Network (NCCN) 2018 guidelines[58] recommend considering 1 year of standard TKI therapy after a successful transplant.

Treatment of Relapse

In patients relapsing after alloSCT, CML recurrence can occur quickly or many years after transplant,[59] and is generally preceded, at least in patients receiving alloSCT in CP, by a molecularly detectable *BCR-ABL1* transcript. As previously stated, prior to the era of TKI, treatment of CML relapse relied on DLI, exploiting the well-known GvL effect observed in CML.[60] However, due to the potential complications of DLI use in terms of GvHD or myelosuppression, TKI have emerged as an alternative. The MD Anderson Cancer Center (MDACC) group reported in 2002 its experience of 28 CML patients (5 in CP and 23 in AP/BC) receiving imatinib 400–1,000 mg daily for relapse occurring at a median of 9 months after alloSCT. Though it was not reported if patients had received imatinib prior to alloSCT, responses were promising, particularly in CP or AP, with a CCyR rate of 63%.[61] One year later, a larger study of 128 CML patients relapsing after alloSCT was published by the EBMT group.[62] The study included patients in all phases of the disease (51 at CP, 31 at AP, and 46 at BC) and, among CP cases, there were few cytogenetic or molecular relapses; 50 patients had failed DLI before imatinib treatment. Beyond confirming the MDACC experience (CCyR was 58% for CP, 48% for AP, and 22% for BC, and 2-year survival for CP and AP patients was 100% and 86%, respectively), it was found that imatinib therapy was able to restore full donor chimerism in 57% patients.[62] Other subsequent studies also confirmed the efficacy of imatinib in inducing durable cytogenetic and molecular responses

and in restoring complete donor chimerism,[63,64] as well as the possible synergic activity of imatinib and DLI, which, when used in combination, induced rapid and sustained molecular responses in patients relapsing in advanced-phase CML and that some patients maintained response even after imatinib was stopped.[65] In all these studies, post-transplant imatinib was generally well tolerated and haematological toxicity was manageable with dose adjustments and brief discontinuations.

Fewer data are available on the efficacy and safety of 2G-TKI for the treatment of CML relapse after alloSCT, since most of the published experiences are single cases or very small series of patients. The MDACC group reported at the 2006 American Society of Hematology (ASH) meeting on 11 patients (9 with CML and 2 with ALL) treated with dasatinib as salvage therapy after alloSCT; all patients had failed high-dose imatinib before transplant. Nine patients (82%) responded, including three with molecular remission, one with CCyR, and two with a partial cytogenetic response. Dasatinib was well-tolerated at a dose of 100 mg daily, while higher doses led invariably to drug discontinuation.[66] Another study of nine patients with advanced CML receiving dasatinib 100–140 mg daily in the post-alloSCT period reported a lower response rate (four out of nine) and negligible toxicity, with only one patient interrupting dasatinib because of thrombocytopenia-related gastrointestinal bleeding.[67] Overall, the data on 2G-TKI for the post-transplant relapse are too scarce to draw any firm conclusions.

A main limit to TKI use in the post-alloSCT relapse scenario is that most CML patients receiving transplant have often received multiple lines of treatment before alloSCT, experiencing either resistance or intolerance that had led to the ultimate transplant decision. Three studies compared DLI with TKI therapy (mainly imatinib) at first CML relapse after allo-SCT.[68-70] Despite the small numbers (31, 46, and 40 patients, respectively), all studies found TKI to be at least not inferior to DLI in terms of efficacy and superior in terms of safety, even if outcomes were poor in advanced-phase relapse, confirming a possible role of combination therapy. However, future trials are needed to compare different TKI, with and without DLI, to determine the most effective and safe treatment modality.[71]

CONCLUSION

With the development of TKI, alloSCT has become a salvage therapy for a minority of CML patients, mostly those in CP. The current indication of alloSCT in CP is limited to cases failing ≥2 lines of TKI therapy. Also, for patients with advanced disease and cases harbouring a Thr315Ile mutation, alloSCT is a reasonable treatment option. Transplantation should be performed with an HLA-identical sibling or HLA-matched unrelated donor, or alternatively a haploidentical donor. Myeloablative conditioning is generally used in fit patients, while reduced-intensity conditioning and DLI enable transplantation in elderly patients and those with comorbidities. As a result, alloSCT remains the only proven curative approach in CML and, though limited, its use must be considered by physicians treating CML patients.

References

1. Radich J. When to consider allogeneic transplantation in CML. Clin Lymphoma Myeloma Leuk. 2016;Suppl:S93-5.
2. Gambacorti-Passerini C et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. J Natl Cancer Inst. 2011;103(7):553-61.
3. Saglio G et al.; ENESTnd Investigators. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251-9.
4. Kantarjian H et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260-70.
5. Innes AJ et al. Allogeneic transplantation for CML in the TKI era: Striking the right balance. Nat Rev Clin Oncol. 2016;13(2):79-91.
6. Fefer A et al. Disappearance of Ph1-positive cells in four patients with chronic granulocytic leukemia after chemotherapy, irradiation and marrow transplantation from an identical twin. N Engl J Med. 1979;300(7):333-7.
7. Pavlů J, Apperley JF. Allogeneic stem cell transplantation for chronic myeloid leukemia. Curr Hematol Malig Rep. 2013;8(1):43-51.
8. Gratwohl A et al. The role of hematopoietic stem cell transplantation in chronic myeloid leukemia. Ann Hematol. 2015;94(Suppl 2):S177-86.

9. Gratwohl A et al. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Lancet. 1998;352(9134):1087-92.
10. Gratwohl A et al.; European Group for Blood and Marrow Transplantation and the European Leukemia Net. Risk score for outcome after allogeneic hematopoietic stem cell transplantation: A retrospective analysis. Cancer. 2009;115(20):4715-26.
11. Butturini A, Gale RP. The role of T-cells in preventing relapse in chronic myelogenous leukemia. Bone Marrow Transplant. 1987;2(4):351-4.
12. Niederwieser D et al. Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey. Bone Marrow Transplant. 2016;51(6):778-85.
13. Özen M et al. Allogeneic transplantation in chronic myeloid leukemia and the effect of tyrosine kinase inhibitors on survival: A quasi-experimental study. Turk J Haematol. 2017;34(1):16-26.
14. de Lavallade H et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: Incidence of sustained responses in an intention-to-treat analysis. J Clin Oncol. 2008;26(20):3358-63.
15. Milojkovic D et al. Responses to second-line tyrosine kinase inhibitors are durable: An intention-to-treat analysis in chronic myeloid leukemia patients. Blood. 2012;119(8):1838-43.
16. Tiribelli M et al. Excellent outcomes of 2G-TKI therapy after imatinib failure in chronic phase CML patients. Oncotarget. 2018;9(18):14219-27.
17. Cortes JE et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011;118(17):4567-76.
18. Ongoren S et al. Third-line treatment with second-generation tyrosine kinase inhibitors (dasatinib or nilotinib) in patients with chronic myeloid leukemia after two prior TKIs: Real-life data on a single center experience along with the review of the literature. Hematology. 2018;23(4):212-20.
19. Kantarjian HM et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the Phase 3 randomised ENESTnd trial. Lancet Oncol. 2011;12(9):841-51.
20. Kantarjian HM et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized Phase 3 trial (DASISION). Blood. 2012;119(5):1123-9.
21. Nicolini FE et al. Overall survival with ponatinib versus allogeneic stem cell transplantation in Philadelphia chromosome-positive leukemias with the T315I mutation. Cancer. 2017;123(15):2875-80.
22. Koenecke C et al. Outcome of patients with chronic myeloid leukemia and a low-risk score: Allogeneic hematopoietic stem cell transplantation in the era of targeted therapy. A report from the EBMT Chronic Malignancies Working Party. Bone Marrow Transplant. 2016;51(9):1259-61.
23. Baccarani M et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-84.
24. Gratwohl A et al.; SAKK; German CML Study Group. Long-term outcome of patients with newly diagnosed chronic myeloid leukemia: A randomized comparison of stem cell transplantation with drug treatment. Leukemia. 2016;30(3):562-9.
25. Saussele S et al.; German CML Study Group. Allogeneic hematopoietic stem cell transplantation (allo SCT) for chronic myeloid leukemia in the imatinib era: Evaluation of its impact within a subgroup of the randomized German CML Study IV. Blood. 2010;115(10):1880-5.
26. Xu LP et al. Allogeneic stem cell transplantation for patients with T315I BCR-ABL mutated chronic myeloid leukemia. Biol Blood Marrow Transplant. 2016;22(6):1080-6.
27. Nair AP et al. Allogeneic hematopoietic stem cell transplantation is an effective salvage therapy for patients with chronic myeloid leukemia presenting with advanced disease or failing treatment with tyrosine kinase inhibitors. Biol Blood Marrow Transplant. 2015;21(8):1437-44.
28. Mukherjee S, Kalaycio M. Accelerated phase CML: Outcomes in newly diagnosed vs. progression from chronic phase. Curr Hematol Malig Rep. 2016;11(2):86-93.
29. Hehlmann R et al. Management of CML-blast crisis. Best Pract Res Clin Haematol. 2016;29(3):295-307.
30. Jain P et al. Prognostic factors and survival outcomes in patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era: Cohort study of 477 patients. Cancer. 2017;123(22):4391-402.
31. Gómez-Almaguer D et al. The treatment of CML at an environment with limited resources. Hematology. 2016;21(10):576-82.
32. Soysal T et al. Generics in chronic myeloid leukemia: Current arguments for and against and the established evidence. Expert Rev Hematol. 2014;7(6):697-9.
33. Eskazan AE. The issue of financial toxicity in the management of chronic myeloid leukemia with blast crisis. J Med Econ. 2018;21(7):709-11.
34. Deininger M et al. The effect of prior exposure to imatinib on transplant-related mortality. Haematologica. 2006;91(4):452-9.
35. Oehler VG et al. The effects of imatinib mesylate treatment before allogeneic transplantation for chronic myeloid leukemia. Blood. 2007;109(4):1782-9.
36. Jabbour E et al. Novel tyrosine kinase inhibitor therapy before allogeneic stem cell transplantation in patients with chronic myeloid leukemia: No evidence for increased transplant-related toxicity. Cancer. 2007;110(2):340-4.
37. Lee SJ et al. Impact of prior imatinib mesylate on the outcome of hematopoietic cell transplantation for chronic myeloid leukemia. Blood. 2008;112(8):3500-7.
38. Shimoni A et al. Prior treatment with the tyrosine kinase inhibitors dasatinib and nilotinib allows stem cell transplantation (SCT) in a less advanced disease phase and does not increase SCT toxicity in patients with chronic myelogenous leukemia and Philadelphia positive acute lymphoblastic leukemia. Leukemia. 2009;23(1):190-4.
39. Breccia M et al. Second-generation tyrosine kinase inhibitors before allogeneic stem cell transplantation in patients with chronic myeloid leukemia resistant to imatinib. Leuk Res. 2010;34(2):143-7.
40. Piekarska A et al. Pretransplantation use of the second-generation tyrosine kinase inhibitors has no negative impact on the HCT outcome. Ann Hematol. 2015;94(11):1891-7.
41. Kondo T et al. Clinical impact of pretransplant use of multiple tyrosine kinase inhibitors on the outcome of allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia. Am J Hematol. 2017;92(9):902-8.
42. Ma YR et al. Transplantation from haploidentical donor is not inferior to that from identical sibling donor for patients with chronic myeloid leukemia in blast crisis or chronic phase from blast crisis. Clin Transplant. 2016;30(9):994-1001.
43. Passweg JR et al. Use of haploidentical stem cell transplantation continues to increase: The 2015 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant.

2017;52(6):811-7.

44. Or R et al. Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. Blood. 2003;101(2):441-5.
45. Crawley C et al.; Chronic Leukemia Working Party of the EBMT. Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: An analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. Blood. 2005;106(9):2969-76.
46. Kebriaei P et al. Long-term follow-up of allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning for patients with chronic myeloid leukemia. Blood. 2007;110(9):3456-62.
47. Warlick E et al. Reduced intensity conditioning is superior to nonmyeloablative conditioning for older chronic myelogenous leukemia patients undergoing hematopoietic cell transplant during the tyrosine kinase inhibitor era. Blood. 2012;119(17):4083-90.
48. Martin PJ et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood. 1985;66(3):664-72.
49. Soiffer RJ, Martin P, "T-cell depletion of allogenetic hematopoietic stem cell grafts," Atkinson K et al. (eds.), Clinical bone marrow and blood stem cell transplantation (2004), Cambridge, UK: Cambridge University Press, pp. 416-25.
50. Eapen M et al. Long-term survival after transplantation of unrelated donor peripheral blood or bone marrow hematopoietic cells for hematologic malignancy. Biol Blood Marrow Transplant. 2015;21(1):55-9.
51. Anasetti C et al.; Blood and Marrow Transplant Clinical Trials Network. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012;367(16):1487-96.
52. Carpenter PA et al. Prophylactic administration of imatinib after hematopoietic cell transplantation for high-risk Philadelphia chromosome-positive leukemia. Blood. 2007;109(7):2791-3.
53. Nakasone H et al.; Kanto Study Group for Cell Therapy. Prophylactic impact of imatinib administration after allogeneic stem cell transplantation on the incidence and severity of chronic graft versus host disease in patients with Philadelphia chromosome-positive leukemia. Leukemia. 2010;24(6):1236-9.
54. Shimoni A et al. Phase 1/2 study of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. Cancer. 2015;121(6):863-71.
55. Varda-Bloom N et al. Immunological effects of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. Oncotarget. 2017;8(1):418-29.
56. DeFilipp Z et al. Does post-transplant maintenance therapy with tyrosine kinase inhibitors improve outcomes of patients with high-risk Philadelphia chromosome-positive leukemia? Clin Lymphoma Myeloma Leuk. 2016;16(8):466-71.
57. Carpenter PA et al. Posttransplant feasibility study of nilotinib prophylaxis for high-risk Philadelphia chromosome positive leukemia. Blood. 2017;130(9):1170-2.
58. National Comprehensive Cancer Network. NCCN guidelines for patients. Chronic myeloid leukemia. 2018. Available at: https://www.nccn.org/patients/guidelines/cml/files/assets/common/downloads/files/cml.pdf. Last accessed: 21 March 2018.
59. Goldman JM et al. Relapse and late mortality in 5-year survivors of myeloablative allogeneic hematopoietic cell transplantation for chronic myeloid leukemia in first chronic phase. J Clin Oncol. 2010;28(11):1888-95.
60. Dazzi F et al. Donor lymphocyte infusions for relapse of chronic myeloid leukemia after allogeneic stem cell transplant: Where we now stand. Exp Hematol. 1999; 27(10):1477-86.
61. Kantarjian HM et al. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. Blood. 2002;100(5):1590-5.
62. Olavarria E et al.; Chronic Leukaemia Working Party of the European Group of Bone and Marrow Transplantation (EBMT). Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Leukemia. 2003;17(9):1707-12.
63. DeAngelo DJ et al. Extended follow-up of patients treated with imatinib mesylate (gleevec) for chronic myelogenous leukemia relapse after allogeneic transplantation: Durable cytogenetic remission and conversion to complete donor chimerism without graft-versus-host disease. Clin Cancer Res. 2004;10(15):5065-71.
64. Hess G et al. Sustained complete molecular remissions after treatment with imatinib-mesylate in patients with failure after allogeneic stem cell transplantation for chronic myelogenous leukemia: Results of a prospective Phase II open-label multicenter study. J Clin Oncol. 2005;23(30):7583-93.
65. Savani BN et al. Imatinib synergizes with donor lymphocyte infusions to achieve rapid molecular remission of CML relapsing after allogeneic stem cell transplantation. Bone Marrow Transplant. 2005;36(11):1009-15.
66. Atallah E et al. The role of dasatinib in patients with Philadelphia (Ph) positive acute lymphocytic leukemia (ALL) and chronic myeloid leukemia (CML) relapsing after stem cell transplantation (SCT). Blood. 2006;108:4520.
67. Klyuchnikov E et al. Second-generation tyrosine kinase inhibitors in the post-transplant period in patients with chronic myeloid leukemia or Philadelphia-positive acute lymphoblastic leukemia. Acta Haematol. 2009;122(1):6-10.
68. Weisser M et al. A comparison of donor lymphocyte infusions or imatinib mesylate for patients with chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation. Haematologica. 2006;91(5):663-6.
69. Shanavas M et al. A comparison of long-term outcomes of donor lymphocyte infusions and tyrosine kinase inhibitors in patients with relapsed CML after allogeneic hematopoietic cell transplantation. Clin Lymphoma Myeloma Leuk. 2014;14(1):87-92.
70. Zeidner JF et al. The evolution of treatment strategies for patients with chronic myeloid leukemia relapsing after allogeneic bone marrow transplant: Can tyrosine kinase inhibitors replace donor lymphocyte infusions? Leuk Lymphoma. 2015;56(1):128-34.
71. Eskazan AE et al. Relapse after allogeneic hematopoietic stem cell transplant in patients with chronic myeloid leukemia: Tyrosine kinase inhibitors, donor lymphocyte infusions or both? Leuk Lymphoma. 2015;56(10):2995-6.

Therapeutic Options in Myelodysplastic Syndromes: Established and Emerging Therapies

Authors: Nina Kim, *Shyamala Navada

Icahn School of Medicine at Mount Sinai, New York City, New York, USA
*Correspondence to shyamala.navada@mssm.edu

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Abstract

Although hypomethylating agents (HMA) have revolutionised the treatment of myelodysplastic syndromes (MDS), a significant proportion of patients either fail to respond to HMA or their disease progresses after an initial response. Established therapeutic options for these patients remain limited. Fortunately, recent advancements in the knowledge of MDS pathogenesis have allowed for the development of many targeted therapies, including epigenetic regulators, signal transduction regulators, immune checkpoint inhibitors, cell apoptosis regulators, and novel cytotoxic agents. These novel therapeutics have shown varying degrees of promise in clinical trials. Epigenetic regulators, such as second-generation HMA and isocitrate dehydrogenase inhibitors, have shown modest efficacy in early studies, while histone deacetylase inhibitors have, thus far, failed to show significant clinical benefit. Signal transduction modulators, such as transforming growth factor (TGF)-β inhibitors and toll-like receptor inhibitors, appear to alleviate anaemia symptoms, but further studies are needed to determine their effect on survival. Rigosertib, a multikinase inhibitor, improved survival in a small subset of patients with very high-risk MDS. Immune checkpoint inhibitors have shown mixed results. Agents that have recently been approved for use in specific types of high-risk acute myeloid leukaemia, including FMS-like tyrosine receptor kinase 3 inhibitors and CPX-351, are also being studied for use in MDS, with early studies suggesting efficacy. Several other agents are also under investigation with results pending. These novel agents represent potential therapeutic options for patients who have failed HMA and for whom no currently established therapies are available.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders resulting from the clonal expansion of a haematopoietic progenitor, leading to bone marrow dysplasia, ineffective haematopoiesis, and increased risk of transformation to acute myeloid leukaemia (AML). Risk factors include older age, male sex, and prior exposure to radiation or chemotherapy.[1] Treatment selection is generally based on calculated risk. The International Prognostic Scoring System (IPSS), which stratifies patients with MDS into four risk categories (low, intermediate-1 [INT-1], intermediate-2 [INT-2], and high-risk) based on blast percentage, number of cytopenias, and cytogenetic profile, is the most commonly used risk stratification tool for treatment selection (Table 1). Of note, an

updated scoring system, the IPSS-R, is available (Table 2); however, all currently established therapies were approved using the older IPSS system.[2] This paper will review both established and emerging therapeutic options for both lower and higher-risk MDS, with an emphasis on novel therapies.

ESTABLISHED THERAPIES FOR LOWER-RISK MYELODYSPLASTIC SYNDROMES

For patients with lower-risk MDS (INT-1), the decision to treat is often based on the degree of anaemia and transfusion dependence. Patients who are transfusion independent may simply be observed; however, for those who become transfusion dependent, therapy is often warranted. Standard options include erythropoiesis-stimulating agents (ESA), lenalidomide, hypomethylating agents (HMA), and immunosuppressants (Table 3). Of these options, ESA, such as recombinant erythropoietin and darbepoietin, are generally considered first-line therapy in lower-risk MDS, having been shown to alleviate anaemia and possibly overall survival (OS) in this population.[3-5] Granulocyte-colony stimulating factor improves the efficacy of ESA through a synergistic effect without increasing the risk of leukaemic transformation; as a result, granulocyte-colony stimulating factor has gained wide clinical acceptance in most countries.[5-7] Lenalidomide is considered first-line therapy for those with del(5q), having been shown in clinical trials to produce haematologic improvement (HI) in 56.0% of lower-risk MDS patients, transfusion independence (TI) in 46.0%, and partial or complete cytogenetic response in 71.6%, with best response in those with del(5q).[8,9]

HMA have shown efficacy in lower-risk MDS, particularly after ESA and/or lenalidomide failure. In a Phase II clinical trial, low-dose 5-AZA and decitabine showed good overall response rates (ORR) (49% and 70%, respectively), cytogenetic response rates (61% and 25%, respectively), and achievement of TI (32% and 16%, respectively) in this lower-risk population.[10] However, further studies are needed to determine whether HMA alter the natural history of lower-risk MDS. Immunosuppressants, including antithymocyte globulin, cyclosporine, and steroids, are also used in lower-risk MDS and have been associated with haematologic response in clinical studies, though no survival benefit has been found (Table 3).[11]

Table 1: International prognostic scoring system for the stratification of myelodysplastic syndromes.

		Value	IPSS Score
Blasts (%)		<5	0
		5–10	0.5
		11–20	1.5
		21–30	2.0
Cytogenetics	› Normal › -Y only › Del(5q) only › Del(20q) only	Good	0.0
	› Abnormalities other than good or poor	Intermediate	0.5
	› Complex › 3 or more abnormalities › Abnormal chromosome 7	Poor	1.0
Cytopenias	› Haemoglobin <10 g/dL › Absolute neutrophil count <1,500 cells/µL › Platelet count <100,000/µL Each cytopenia counts as a value of 1	0 or 1	0.0
		2 or 3	0.5

IPSS risk score interpretation: low risk: 0; intermediate-1 risk: 0.5–1.0; intermediate-2 risk: 1.5–2.0; high risk: ≥2.5.
Del: deleted; IPSS: International Prognostic Scoring System.

Table 2: Revised international prognostic scoring system for the stratification of myelodysplastic syndromes.

		Value	IPSS-R Score
Blasts (%)		≤2	0.0
		>2–<5	1.0
		5–10	2.0
		>10	3.0
Cytogenetics	› -Y › Del(11q)	Very good	0.0
	› Normal › Del(5q) › Del(12q) › Del(20q) › Double including del(5q)	Good	1.0
	› Del(7q) › +8 › +19 › i(17q) › Any other single or double independent clone	Intermediate	2.0
	› -7 › Inv(3)/+(3q)/del(3q) › Double including -7/del(7q) › Complex: 3 abnormalities	Poor	3.0
	› Complex: >3 abnormalities	Very poor	4.0
Cytopenias	Haemoglobin (g/dL)	≥10.0	0.0
		8.0–>10.0	1.0
		<8.0	1.5
	Absolute neutrophil count (x10^9/L of blood)	≥0.8	0.0
		<0.8	0.5
	Platelet count (x10^9/L of blood [µL])	≥100.0	0.0
		50–<100	0.5
		<50	1.0

IPSS-R risk score interpretation: very low risk: ≤1.5; low risk: >1.5 to 3.0; intermediate risk: >3.0–4.5; high risk: >4.5–6.0; very high risk: >6.

Del: deleted; IPSS-R: International Prognostic Scoring System-Revised

ESTABLISHED THERAPIES FOR HIGHER-RISK MYELODYSPLASTIC SYNDROMES

For patients with higher-risk MDS (INT-2), HMA are considered the standard of care. 5-AZA has been particularly well-studied in this population by two multicentre randomised trials. In the CALGB 9221 trial,[12] 191 patients with higher-risk MDS were randomised to 5-AZA or best supportive care. Treatment with 5-AZA was associated with 7% complete response, 16% partial response, longer median time to leukaemic transformation (21 months versus 13 months; p=0.007), and improved survival (18 months versus 11 months). Similarly, the AZA-001 trial[13] showed that, compared to supportive care and low-dose cytarabine (araC), 5-AZA treatment is associated with longer median survival (24.5 months versus 15.0 months; p=0.0001), delayed progression to AML, decreased transfusion requirements, and decreased rate of infections. Decitabine has also demonstrated a modest clinical benefit in clinical trials, with a multicentre Phase II trial (ADOPT)[14] showing an ORR of 32%, with 17 patients having complete responses. However, unlike 5-AZA, decitabine has not demonstrated significant survival benefit in a randomised trial.[15]

Table 3: Established and emerging therapies for myelodysplastic syndromes.

Mechanism of action	Agents
Immunosuppressants	› Antithymocyte globulin › Cyclosporine › Steroids
Epigenetic regulators	Hypomethylating agents › Azacitidine (5-AZA), administered via IV, SC, PO › Decitabine, administered via IV, SC, PO › Guadecitabine, administered via SC Histone deacetylase inhibitors › Sodium phenylbutyrate › Valproic acid › Romidepsin › Vorinostat › Mocetinostat › Panobinostat › 4SC-202 Mutant IDH1 and IDH2 inhibitors › Enasidenib › Ivosidenib PDH inhibitors › CPI613 BET inhibitors › CPI-0610
Signal transduction regulators	Growth factors › Erythropoiesis-stimulating agents › Granulocyte-colony stimulating factor › Thrombopoietin agonists - Romiplostim - Eltrombopag TGF-β signalling modulators › Luspatercept (ACE-536) › Sotatercept (ACE-011) › Galunisertib Toll-like receptor inhibitors › OPN-305 Multi-kinase inhibitors › Rigosertib FLT-3 inhibitors › Midostaurin › Sorafenib
Immune checkpoint inhibitors	PD-1 inhibitors › Nivolumab › Pembrolizumab › Durvalumab CTLA-4 inhibitors › Ipilimumab
Cell death inhibitors	Proteasome inhibitors › Bortezomib BCL-2 inhibitors › Venetoclax
RNA splicing modulators	H3B-8800
Cytotoxic agents	› Standard anthracycline-araC-based chemotherapy › Low-dose clofarabine › CPX-351

BCL: B cell lymphoma; BET: bromodomain and extraterminal domain; FLT: FMS-like tyrosine receptor kinase; IDH: isocitrate dehydrogenase; IV: intravenous; PD: programmed cell death protein; PDH: pyruvate dehydrogenase; PO: per os (oral administration); SC: subcutaneous.

Standard AML-based chemotherapy (classically, an anthracycline-araC combination) can also be used in higher-risk MDS, but this therapeutic strategy is associated with significant morbidity, due to prolonged cytopenias and poor long-term survival.[16] Therefore, its use is generally limited to younger patients with a favourable karyotype.[2,16] Allogeneic stem cell transplantation remains the only curative treatment for higher-risk MDS, and has been associated with prolonged disease-free survival in about 30–50% of patients.[17] Although allogeneic stem cell transplantation use has historically been limited to younger patients due to significant toxicities and poor tolerability in older patients,[18,19] the emergence of reduced-intensity conditioning allogeneic stem cell transplantation has improved its availability to older patients in recent years.

EMERGING THERAPIES FOR MYELODYSPLASTIC SYNDROMES

Despite the aforementioned advancements, there remain limitations to established therapies. ESA can improve symptoms in low-risk patients with MDS, but do not provide definitive treatment. Lenalidomide has shown efficacy in low-risk MDS with del(5q), but no clear survival benefit has been found. Although HMA have revolutionised the treatment of MDS, only about half of patients treated with HMA achieve objective responses, and most responders eventually lose response within 1–2 years.[20] Unfortunately, there remains a lack of therapeutic options for those who fail HMA. Use of conventional chemotherapy in the elderly MDS population is limited by toxicities. Allogeneic stem cell transplantation remains the only curative option, but is limited by donor availability and tolerability. As a result, prognosis for patients with MDS who fail HMA remains poor. For patients with lower-risk MDS who have failed HMA, survival is estimated to be between 14 and 17 months.[21] Those with higher-risk MDS who have failed HMA have an even poorer prognosis, with an estimated survival of 4–6 months.[22] Fortunately, several novel therapeutics are now being investigated for use in MDS, either as first-line or salvage treatments after HMA failure.

DRUGS TARGETING EPIGENETIC DYSREGULATION

Oral Hypomethylating Agents

Oral azacitidine and decitabine are being explored as alternatives to the more conventional intravenous and subcutaneous administration formulation HMA in ongoing Phase III clinical trials.[23] Benefits of an oral formulation include increased patient convenience and the feasibility of an extended drug dosing schedule. Oral azacitidine has already been shown in Phase I and II trials to be bioavailable, clinically active, and well-tolerated, with an ORR, defined as complete response, HI, or TI, of 35% in previously treated patients and 73% in previously untreated patients.[24] An extended dosing schedule has also been found to be beneficial, with improved ORR after a 14-day dosing schedule (ORR: 36%) versus a 21-day dosing schedule (ORR: 41%) in those with lower-risk MDS.[24]

Oral decitabine is the subject of clinical trials as part of a combination agent called ASTX727, which includes a novel cytidine deaminase inhibitor, cedazuridine (E7727). Cedazuridine inhibits cytidine deaminase, thereby inhibiting decitabine degradation in the gut and liver. Initial results from a Phase II study[25] of ASTX727 in intermediate and higher-risk MDS were presented at the American Society of Hematology (ASH) Congress 2017, and reported a clinical benefit in 31 out of 50 patients (62%), with 8 (16%) patients in complete remission (CR), 14 (28%) patients with marrow CR, and 9 (18%) individuals with HI.[25] A Phase III randomised, open-label, crossover pharmacokinetic study of ASTX727 versus intravenous decitabine is underway to demonstrate comparable exposures of decitabine.

Guadecitabine

Guadecitabine (SGI-110) is a next-generation, subcutaneous, small volume dinucleotide of decitabine and deoxyguanosine currently in clinical trials for higher-risk MDS and AML. Unlike decitabine, guadecitabine is resistant to deamination by cytidine deaminase, allowing prolonged *in vivo* exposure to the active ingredient decitabine.[26-29] A Phase I study has confirmed that a dose of 60 mg/m^2 guadecitabine for 5 days is clinically active and well-tolerated in

patients with relapsed or refractory MDS or AML that failed HMA.[26] Preliminary results of Phase II trials in various MDS cohorts have demonstrated efficacy of guadecitabine as both an initial therapy in higher-risk HMA-naïve MDS patients (ORR: 50–60%)[27,29] and as a salvage therapy in higher-risk MDS patients who have failed prior therapy with 5-AZA (ORR: 16%).[28] A Phase III randomised open-label study comparing guadecitabine to standard therapy in patients with MDS and chronic myelomonocytic leukaemia after HMA failure is currently underway.[30]

Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) are similar to HMA in that they target epigenetic dysregulation, albeit through a different mechanism. HDACi modulate gene expression by inhibiting the deacetylation of histone lysine tails, relaxing chromatin structure. Secondary mechanisms of action include direct acetylation of nonhistone proteins, alterations to the NFκB signalling pathway, and upregulation of the death receptor pathway.[31] Several different HDACi, including sodium phenylbutyrate, valproic acid, romidepsin, vorinostat, mocetinostat, panobinostat, and 4SC-202, have been tested in clinical trials, with many trials still ongoing. Despite robust preclinical data suggesting benefits, Phase I and II clinical trials of HDACi have shown only a modest effect at best.[31,32] Notably, none of these agents used alone or in combination with other therapeutic agents have been shown to be superior to HMA monotherapy.[33-35]

Mutant Isocitrate Dehydrogenase 1 and 2 Inhibitors

Isocitrate dehydrogenase (IDH)1 and IDH2 are enzymes involved in histone demethylation. Recurrent mutations are detected in *IDH1/2* genes in about 5% of MDS patients and 20% of AML patients. These are typically gain-of-function mutations that result in increased enzymatic activity, leading to DNA and histone hypermethylation and a blockade of haematopoietic cellular differentiation.[36] Several mutant IDH (mIDH) inhibitors are currently being investigated in clinical trials (most of which are still in Phase I or II) for use in patients with relapsed or refractory MDS or AML. The most promising results thus far have been seen with enasidenib (a mIDH2 inhibitor) and ivosidenib (a mIDH1 inhibitor).[36] In a recent Phase I and II trial of enasidenib in relapsed and refractory AML, the ORR was 40.3%, with 19.3% of patients achieving CR.[37] A significant survival benefit was also demonstrated in those who achieved CR (median OS: 19.7 months in those who achieved CR versus 9.3 months in others).[37] Preliminary results of a Phase I and II study, including 17 patients with *IDH2*-mutated MDS, suggested that enasidenib also has activity in MDS, with a reported OSS of 59%.[38] Similarly, ivosidenib has been suggested to have efficacy in patients with advanced haematologic malignancies, including MDS, with 36% ORR in a Phase I study.[39]

Pyruvate Dehydrogenase and Bromodomain and Extraterminal Domain Inhibitors

Pyruvate dehydrogenase inhibitors (e.g., CPI613)[40] and bromodomain and extraterminal domain inhibitors (e.g., CPI-0610) also target epigenetic dysregulation. These inhibitors are currently being studied in Phase I clinical trials with results pending.[41]

DRUGS TARGETING ABNORMAL SIGNAL TRANSDUCTION

Thrombopoietin Agonists

Thrombopoietin agonists have also been explored for use in lower-risk MDS patients with thrombocytopenia and have shown some benefit. Unfortunately, widespread use has been limited by concern for increased risk of leukaemogenesis. This fear is based on early studies showing a transient increase in blast percentage in 15% of patients treated with high-dose romiplostim, despite a recently published follow-up study reporting no difference in the risk of transformation to AML or mortality with romiplostim versus placebo at 5 years.[42] Eltrombopag is an alternative thrombopoietin agonist that has shown efficacy in treating aplastic anaemia and, unlike romiplostim, is not associated with an increase in blast percentage. A recently published Phase II clinical trial (ASPIRE) showed that eltrombopag improved platelet counts and reduced clinically relevant thrombocytopenic events compared to placebo

in high-risk MDS and AML patients with thrombocytopenia who are ineligible for other treatment or not receiving disease-modifying treatment.[43] Clinical trials to investigate the efficacy of eltrombopag in lower-risk MDS are ongoing; however, preliminary results show efficacy in improving thrombocytopenia and reducing bleeding events in this population as well.[44]

Transforming Growth Factor-β Signalling Modulators

TGF-β signalling modulators have been developed for treatment of MDS with the aim of inhibiting TGF-β-meditated myelosuppression,[45] thereby promoting maturation of haematopoietic progenitors in bone marrow. Luspatercept (ACE-536) is a selective activin receptor ligand that traps GDF11, blocking TGF-β signalling and promoting late-stage erythropoietic maturation. It has shown promising activity in preliminary results of an ongoing Phase II multicentre study in patients with lower risk MDS with anaemia (PACE-MDS): 53% of patients showed erythroid response and 38% of patients achieved TI.[46] In this study, particularly high response rates were observed among patients with refractory anaemia with ring sideroblasts, *SF3B1* mutation, and/or low transfusion burden, leading to the development of a randomised trial of luspatercept for patients with refractory anaemia with ring sideroblasts.[47] Sotatercept (ACE-011), which functions through a very similar mechanism, has also shown promising evidence of clinical activity in ESA-refractory lower-risk MDS patients in an ongoing Phase II study, with 49% of patients showing improvement in anaemia.[48] Galunisertib, an agent that inhibits the kinase activity of TGF-β receptor Type 1, also known as ALK5, is also currently in Phase II and III clinical trials, with preliminary results showing HI in 21% of the 38 lower-risk MDS patients studied.[49]

Toll-Like Receptor Inhibitors

TLR signalling is abnormally activated in MDS, especially after HMA therapy, leading to activation of the NF-κB pathway and inhibition of haematopoiesis.[50] OPN-305 is a fully humanised IgG4κ monoclonal antibody against TLR2 that is currently being investigated in Phase I and II clinical trials for the treatment of low or INT-1 risk MDS after HMA failure.[51] Preliminary results in 21 patients have shown haematologic improvement in 53% of patients, with 20% achieving TI.[52] Further studies are necessary to confirm this response and evaluate effect on survival.

Rigosertib

Rigosertib is a multikinase inhibitor that induces mitotic arrest and apoptosis in neoplastic cells while sparing normal cells. It has been explored for use in patients with refractory MDS with excess blasts or treatment-related MDS who have failed HMA.[53] In a Phase III study (ONTIME)[54] comparing rigosertib to best supportive care with or without low-dose araC in this population, rigosertib was not shown to improve OS. However, in a post-hoc analysis, patients with very high-risk IPSS-R scores and monosomy 7 or trisomy 8 were found to have significant improvement in survival with rigosertib compared to supportive care (median OS: 7.6 months versus 3.2 months, p=0.015).[54] A second Phase III study in this smaller cohort of very high-risk patients is now underway to further evaluate this effect.[55]

FMS-like Tyrosine Receptor Kinase Inhibitors

Midostaurin is a FMS-like tyrosine receptor kinase (FLT) 3 inhibitor that was recently approved for use in combination with 7+3 chemotherapy for patients with newly diagnosed *FLT3* mutation-positive AML, based on clinical trial data showing improved OS and event-free survival in patients treated with midostaurin.[56] Though not formally approved for use in MDS as of yet, FLT3 inhibitors (i.e., midostaurin, sorafenib) have also demonstrated efficacy in Phase I and II clinical trials when used in combination with HMA (i.e., 5-AZA, decitabine) in patients with *FLT3*-mutated MDS.[57,58] Notably, although *FLT3* mutations are seen in <1% of patients with newly diagnosed MDS, they have been observed in up to 5% of patients at the time of MDS transformation to AML and are associated with much poorer outcomes.[59] The hope is that mutation-targeted therapy will improve these outcomes.

OTHER NOVEL THERAPEUTICS

Immune Checkpoint Inhibitors

Several monoclonal antibodies against immune checkpoint regulators, both alone and in combination with HMA, are being tested in clinical trials. These include nivolumab (a programmed cell death protein [PD-1] inhibitor), ipilimumab (a CTLA-4 inhibitor), pembrolizumab (a PD-1 inhibitor), and durvalumab (blocks interaction of PD ligand-1 with PD-1 and CD80 molecules). Early results of these studies have been mixed. Preliminary results from a Phase Ib study of pembrolizumab in 27 patients with MDS after HMA failure (KEYNOTE-013) show only modest response to therapy, with no CR, 1 partial remission, 3 marrow CR, and 3 HI.[60] However, 2-year survival was reportedly 57% in those treated with pembrolizumab, which is considerably superior to that typically expected in MDS patients after HMA failure.[60] A Phase II study evaluating nivolumab and ipilimumab as single agents or in combination with AZA for patients with MDS (as initial or salvage therapy) is also ongoing, with preliminary results in 39 patients showing an ORR of 69% in the AZA plus nivolumab cohort, 22% in the ipilimumab monotherapy cohort, and no response in the nivolumab monotherapy cohort.[61] Further follow-up is needed to clarify efficacy and safety.

Proteasome Inhibitors

Proteasome inhibitors inhibit cell death by preventing degradation of proapoptotic proteins. The proteasome inhibitor, bortezomib, has recently been explored as a therapeutic option in MDS. In a limited study of 15 patients with low or INT-1 MDS after HMA failure, bortezomib showed only modest effect, with HI observed in 20% of patients.[62] However, an unexpected significant reduction of ring sideroblasts was seen in 70% of patients treated with bortezomib in this study, suggesting potential benefit specifically in MDS patients with ring sideroblasts.[62] Further studies are necessary to better evaluate this effect.

B Cell Lymphoma-2 Inhibitors

Antiapoptotic resistance due to B cell lymphoma (BCL)-2 overexpression has been reported in higher-risk MDS and may contribute to disease pathogenesis. As a result, BCL-2 inhibitors have been explored as a therapeutic option in higher-risk MDS after HMA failure and in relapsed and refractory AML. Venetoclax is the best studied BCL-2 inhibitor thus far, and has shown modest efficacy (ORR: 19%) as monotherapy in patients with high-risk relapsed or refractory AML.[63] Venetoclax has also shown significant efficacy in combination with araC for patients with treatment-naïve AML (including those with previously treated MDS) in a Phase I trial of 20 patients, with 14 out of 20 patients (70%) achieving CR or CR with incomplete marrow recovery.[64] The presence of mutations in chromatin-RNA splicing genes (*ASXL1*, *EZH2*) or *NPM1* correlated with a higher likelihood of response in a separate analysis.[65] A Phase I clinical trial evaluating venetoclax alone and in combination with azacitidine in higher-risk MDS after HMA failure is ongoing.[66]

RNA Splicing Modulators

Genes encoding various spliceosome components (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*) are frequently mutated in patients with MDS.[67,68] While the precise mechanism by which abnormal RNA splicing leads to the development of MDS is unknown, the association is clear. Based on this knowledge, H3B-8800, a potent and orally bioavailable SF3b complex modulator, was developed and is now being studied in a Phase I clinical trial for use in patients with MDS and AML with splicing mutations, with results pending.[69,70]

Cytotoxic Agents

Although many patients with MDS cannot tolerate standard AML-based chemotherapy, less intensive cytotoxic agents can be considered. Cytotoxic agents that have been explored for use in MDS patients include low-dose clofaribine and CPX-351. Low-dose clofarabine is currently under investigation for both higher-risk MDS and AML, with promising results. A Phase II study evaluating the safety and efficacy of low-dose clofaribine in combination with araC in patients with MDS who have failed HMA has reported an ORR of 44%, median OS of 10 months overall, and median OS of 22 months in responding patients (versus 4 months in non-responding patients).[71] CPX-351, a liposomal formulation of araC and daunorubicin in a 5:1

molar ratio, was recently U.S. Food and Drug Administration (FDA) approved as front-line therapy for patients with therapy-related AML and AML with MDS-related changes based on a Phase III randomised trial reporting a survival advantage compared to standard 7+3 chemotherapy (OS: 9.6 months versus 5.9 months).[72] However a subsequent Phase II randomised trial testing attenuated doses of CPX-351 in a small cohort of less fit adults with untreated AML or MDS with 10% or more blasts in peripheral blood or bone marrow did not show much efficacy.[73]

CONCLUSION

Although HMA have changed the landscape of MDS therapy in the past decade, they have limitations and are not curative. There remains a significant proportion of patients who simply do not respond to HMA, or who develop resistance to HMA after initial response. Unfortunately, established therapeutic options for patients with MDS who have failed HMA therapy are limited, and these patients have very poor prognosis. Thus, multiple novel agents are being developed in clinical trials with the aim of improving symptoms and OS in patients with MDS.

References

1. Rollison DE et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008;112(1):45-52.
2. Montalban-Bravo G, Garcia-Manero G. Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management. Am J Hematol. 2018;93(1):129-47.
3. Fenaux P, Adès L. How we treat lower-risk myelodysplastic syndromes. Blood. 2013;121(21):4280-6.
4. Moyo V et al. Erythropoiesis-stimulating agents in the treatment of anemia in myelodysplastic syndromes: A meta-analysis. Ann Hematol. 2008;87(7):527-36.
5. Jädersten M et al. Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. J Clin Oncol. 2008;26(21):3607-13.
6. Hellström-Lindberg E et al. Scandinavian MDS Group; A validated decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: Significant effects on quality of life. Br J Haematol. 2003;120(6):1037-46.
7. Santini V. Treatment of low-risk myelodysplastic syndrome: Hematopoietic growth factors erythropoietins and thrombopoietins. Semin Hematol. 2012;49(4):295-303.
8. List A et al. Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med. 2005;352(6):549-57.
9. List A et al. Extended survival and reduced risk of AML progression in erythroid-responsive lenalidomide-treated patients with lower-risk del(5q) MDS. Leukemia. 2014;28(5):1033-40.
10. Jabbour E et al. Randomized Phase 2 study of low-dose decitabine vs low-dose azacitidine in lower-risk MDS and MDS/MPN. Blood. 2017;130(13):1514–22.
11. Passweg JR et al. Immunosuppressive therapy for patients with myelodysplastic syndrome: A prospective randomized multicenter Phase III trial comparing antithymocyte globulin plus cyclosporine with best supportive care - SAKK 33/99. J Clin Oncol. 2011;29(3):303-9.
12. Silverman LR et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: A study of the cancer and leukemia group B. J Clin Oncol. 2002;20(10):2429-40.
13. Fenaux P et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: A randomised, open-label, Phase III study. Lancet Oncol. 2009;10(3):223-32.
14. Steensma DP et al. Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: The alternative dosing for outpatient treatment (ADOPT) trial. J Clin Oncol. 2009;27(23):3842-8.
15. Lübbert M et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: Final results of the randomized Phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol. 2011;29(15):1987-96.
16. Wattel E et al. Long-term follow-up of de novo myelodysplastic syndromes treated with intensive chemotherapy: Incidence of long-term survivors and outcome of partial responders. Br J Haematol. 1997;98(4):983-91.
17. Chang C et al. Hematopoietic cell transplantation in patients with myelodysplastic syndrome or acute myeloid leukemia arising from myelodysplastic syndrome: Similar outcomes in patients with de novo disease and disease following prior therapy or antecedent hematologic. Blood. 2007;110(4):1379-88.
18. Koreth J et al. Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with de novo myelodysplastic syndromes: An international collaborative decision analysis. J Clin Oncol. 2013;31(21):2662-70.
19. Cutler CS et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: Delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood. 2004;104(2):579-85.
20. Zeidan AM et al. Beyond hypomethylating agents failure in patients with myelodysplastic syndromes. Curr Opin Hematol. 2014;21(2):123-30.
21. Jabbour E et al. Outcome of patients with low-risk and intermediate-1-risk myelodysplastic syndrome after hypomethylating agent failure a report on behalf of the MDS Clinical Research Consortium. Cancer. 2015;121(6):876-82.

22. Harel S et al. Outcome of patients with high risk myelodysplastic syndrome (MDS) and advanced chronic myelomonocytic leukemia (CMML) treated with decitabine after azacitidine failure. Leuk Res. 2015;39(5):501-4.

23. Celgene. The efficacy and safety of oral azacitidine plus best supportive care versus placebo and best supportive care in subjects with red blood cell (RBC) transfusion-dependent anemia and thrombocytopenia due to International Prognostic Scoring System (IPSS) low risk myelodysplastic syndrome (MDS). NCT01566695. https://clinicaltrials.gov/ct2/show/NCT01566695.

24. Garcia-Manero G et al. Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. J Clin Oncol. 2011;29(18):2521-7.

25. Garcia-Manero G et al. A Phase 2 dose-confirmation study of oral ASTX727, a combination of oral decitabine with a cytidine deaminase inhibitor (CDAi) cedazuridine (E7727), in subjects with myelodysplastic syndromes (MDS). Abstract 4274. ASH 2017, 9-12 December, 2017.

26. Issa JJ et al. Safety and tolerability of guadecitabine (SGI-110) in patients with myelodysplastic syndrome and acute myeloid leukaemia: A multicentre, randomized, dose-escalation Phase 1 study. Lancet Oncol. 2015;16(9):1099-110.

27. Montalban-Bravo G et al. Initial results of a Phase 2 study of guadecitabine (SGI-110), a novel subcutaneous (sc) hypomethylating agent, for patients with previously untreated intermediate-2 or high risk myelodysplastic syndromes (MDS) or chronic myelomonocytic leukemia (CMML). Blood. 2016;128(22):346.

28. Sebert M et al. Results of a Phase II study of guadecitabine (SGI-110) in higher risk MDS, CMML or low blast count AML patients refractory to or relapsing after azacitidine (AZA) treatment. Blood. 2016;128(22):347.

29. Kantarjian HM et al. Guadecitabine (SGI-110) in treatment-naive patients with acute myeloid leukaemia: Phase 2 results from a multicentre, randomised, Phase 1/2 trial. Lancet Oncol. 2017;18(10):1317-26.

30. Astex Pharmaceuticals. Guadecitabine (SGI-110) vs treatment choice in adults with MDS or CMML previously treated with HMAs. NCT02907359. https://clinicaltrials.gov/ct2/show/NCT02907359.

31. Griffiths E et al. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Semin Hematol. 2008;45(1):23-30.

32. Stahl M et al. Lost in translation? Ten years of development of histone deacetylase inhibitors in acute myeloid leukemia and myelodysplastic syndromes acute myeloid leukemia and myelodysplastic syndromes. Expert Opin Investig Drugs. 2016;25(3):307-17.

33. Prebet T et al.; Groupe Francophone des Myélodysplasies. Addition of suberoylanilide hydroxamic acid (Vorinostat) to azacitidine for patients with higher risk myelodysplastic syndromes and azacitidine failure: A Phase II add-on study from the Groupe Francophone des Myelodysplasies. Br J Haematol. 2016;180(5):735-7.

34. Sekeres MA et al. Randomized Phase II study of azacitidine alone or in combination with lenalidomide or with vorinostat in higher-risk myelodysplastic syndromes and chronic myelomonocytic leukemia: North American Intergroup Study SWOG S1117. J Clin Oncol. 2017;35(24):2745-53.

35. Garcia-Manero G et al. Phase 2, randomized, double-blind study of pracinostat in combination with azacitidine in patients with untreated, higher-risk myelodysplastic syndromes. Cancer. 2017;123(6):994-1002.

36. Medeiros BC et al. Isocitrate dehydrogenase mutations in myeloid malignancies. Leukemia. 2017;31(2):272-81.

37. Stein EM et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. Blood. 2017;130(6):722-31.

38. Stein E et al. Enasidenib (AG-221), a potent oral inhibitor of mutant isocitrate dehydrogenase 2 (IDH2) enzyme, induces hematologic responses in patients with myelodysplastic syndromes (MDS). Abstract 343. Present 58th ASH Annu Meet Expo San Diego, 2016.

39. DiNardo C et al. Molecular profiling and relationship with clinical response in patients with IDH1 mutation-positive hematologic malignancies receiving AG-120, a first-in-class potent inhibitor of mutant IDH1, in addition to data from the completed dose escalation portion. Abstract 1306. 57th Annual Meeting & Exposition, Orlando, 5-8 December, 2015.

40. Pardee T et al. A Phase I study of the safety, efficacy and pharmacokinetics of the first-in-class anti-mitochondrial metabolism agent, CPI-613, in patients with advanced hematologic malignancies. Clin Cancer Res. 2013;122(21):486.

41. Chaidos A et al. Inhibition of bromodomain and extra-terminal proteins (BET) as a potential therapeutic approach in haematological malignancies: Emerging preclinical and clinical evidence. Ther Adv Hematol. 2015;6(3):128-41.

42. Kantarjian HM et al. Long-term follow-up for up to 5 years on the risk of leukaemic progression in thrombocytopenic patients with lower-risk myelodysplastic syndromes treated with romiplostim or placebo in a randomised double-blind trial. Lancet Hematol. 2018;5(3):e117-26.

43. Mittelman M et al. Eltrombopag for advanced myelodysplastic syndromes or acute myeloid leukaemia and severe thrombocytopenia (ASPIRE): A randomised, placebo-controlled, Phase 2 trial. Lancet Haematol. 2018;5(1):e34-43.

44. Oliva E et al. Eltrombopag versus placebo for lowrisk myelodysplastic syndromes with thrombocytopenia (EQoL-MDS): Phase 1 results of a single-blind, randomised, controlled, Phase 2 superiority trial. Lancet Hematol. 2017;4(3):e127-36.

45. Zhou L et al. Inhibition of the TGF-beta receptor I kinase promotes hematopoiesis in MDS. Blood. 2008;112(8):3434-44.

46. Platzbecker U et al. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): A multicentre, open-label Phase 2 dose-finding study with long-term extension study. Lancet Oncol. 2017;18(10):1338-47.

47. Celgene. A study of luspatercept (ace-536) to treat anemia due to very low, low, or intermediate risk myelodysplastic syndromes (medalist). NCT02631070. https://clinicaltrials.gov/ct2/show/NCT02631070.

48. Komrokji R et al. A Phase 2, dose-fiding study of sotatercept (ACE-011) in patients with lower risk myelodysplastic syndromes (MDS) or non-proliferative chronic myelomonocytic leukemai (CMML) and anemia requiring transfusion. Leuk Res. 2015;39(Suppl 1):S5-6.

49. Valcarcel D et al. Phase 2 study of monotherapy galunisertib (LY2157299 Monohydrate) in very low-, low-, and intermediate-risk patients with myelodysplastic syndromes. Blood. 2015;126(23):1669.

50. Wei Y et al. Toll-like receptor alterations in myelodysplastic syndrome. Leukemia. 2013;27(9):1832-40.

51. Opsona Therapeutics Ltd. A Phase I/II study of opn-305 in second-line lower risk myelodysplastic syndrome. NCT02363491. https://clinicaltrials.gov/ct2/show/NCT02363491.

52. Garcia-Manero G et al. Phase II Study OPN-305, a (TLR-2) Antibody, in patients with lower risk

myelodysplastic syndromes with prior hypomethylating agent (HMA) therapy. Abstract P317. EHA22 Friday 23, 2017.

53. Silverman LR et al. Clinical activity and safety of the dual pathway inhibitor rigosertib for higher risk myelodysplastic syndromes following DNA methyltransferase inhibitor therapy. Hematol Oncol. 2015;33(2):57-66.
54. Garcia-Manero G et al. Rigosertib versus best supportive care for patients with high-risk myelodysplastic syndromes after failure of hypomethylating drugs (ONTIME): A randomised, controlled, Phase 3 trial. Lancet Oncol. 2016;17(4):496-508.
55. Onconova Therapeutics, Inc. Controlled study of rigosertib versus physician's choice of treatment in MDS patients after failure of an HMA (INSPIRE). NCT02562443. https://clinicaltrials.gov/ct2/show/NCT02562443.
56. Stone RM et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med. 2017;377(5):454-64.
57. Strati P et al. Phase I/II Trial of the combination of midostaurin (PKC412) and 5-azacytidine for patients with acute myeloid leukemia and myelodysplastic syndrome. Am J Hematol. 2015;90(4):276-81.
58. Ravandi F et al. Phase 2 study of azacytidine plus sorafenib in patients with acute myeloid leukemia and FLT-3 internal tandem duplication mutation. Blood. 2013;121(23):4655-62.
59. Badar T et al. Detectable FLT3-ITD or RAS mutation at the time of transformation from MDS to AML predicts for very poor outcomes. Leuk Res. 2015;39(12):1367-74.
60. Garcia-Manero G et al. Pembrolizumab, a PD-1 inhibitor, in patients with myelodysplastic syndrome (MDS) after failure of hypomethylating agent treatment. Blood. 2016;128(22):345.
61. Garcia-Manero G et al. A Phase II study evaluating the combination of nivolumab (Nivo) or ipilimumab (Ipi) with azacitidine in Pts with previously treated or untreated myelodysplastic syndromes (MDS). Blood. 2016;128(22):344.
62. Daher M et al. An exploratory clinical trial of bortezomib in patients with lower risk myelodysplastic syndromes. Am J Hematol. 2017;92(7):674-82.
63. Konopleva M et al. Efficacy and biological correlates of response in a Phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. Cancer Discov. 2016;6(10):1106-17.
64. Wei A et al. Safety and efficacy of venetoclax plus low-dose cytarabine in treatment-naive patients aged ≥65 years with acute myeloid leukemia. Blood. 2016;128(22):102.
65. Chyla B et al. Correlative biomarkers of response to venetoclax in combination with chemotherapy or hypomethylating agents in elderly untreated patients with acute myeloid leukemia. Blood. 2016;128(22):1709.
66. AbbVie. A study evaluating venetoclax alone and in combination with azacitidine in subjects with higher-risk myelodysplastic syndromes (mds) after hypomethylating agent-failure. NCT02966782. https://clinicaltrials.gov/ct2/show/NCT02966782.
67. Papaemmanuil E et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365(15):1384-95.
68. Yoshida K et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64-9.
69. Seiler M et al. H3B-8800, an orally bioavailable modulator of the SF3b complex, shows efficacy in spliceosome-mutant myeloid malignancies. Blood. 2018;131(24):497-504.
70. H3 Biomedicine Inc. Phase 1 trial to evaluate the safety, pharmacokinetics and pharmacodynamics of splicing modulator h3b-8800 for subjects with myelodysplastic syndromes, acute myeloid leukemia, and chronic myelomonocytic leukemia. NCT02841540. https://clinicaltrials.gov/ct2/show/NCT02841540.
71. Jabbour E et al. Phase 2 study of low-dose clofarabine plus cytarabine for patients with higher-risk myelodysplastic syndrome who have relapsed or are refractory to hypomethylating agents. Cancer. 2017;123(4):629-37.
72. Talati C, Lancet JE. CPX-351: Changing the landscape of treatment for patients with secondary acute myeloid leukemia. Future Oncol. 2018;14(12):1147-54.
73. Walter RB et al. Unsatisfactory efficacy in randomized study of reduced-dose CPX-351 for medically less fit adults with newly diagnosed acute myeloid leukemia or other high-grade myeloid neoplasm. Haematologica. 2018;103(3):e106-9.

Making Rituximab Directly Cytotoxic for Substantial Improvement in Therapeutic Efficacy

Authors: *Xinjian Chen

Department of Pathology, University of Utah, Salt Lake City, Utah, USA
*Correspondence to xinjian.chen@path.utah.edu

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Abstract

The humanised anti-CD20 antibody (Ab) rituximab (RTX) has significantly improved the prognosis of B cell non-Hodgkin's lymphomas (BNHL). However, major challenges remain: a) RTX is often used with toxic chemotherapy that not only causes serious side effects but may also compromise RTX activity and host antitumour immunity, predisposing patients to relapse; b) indolent low-grade BNHL remain largely incurable; c) a significant percentage of aggressive BNHL do not respond to RTX-based therapy; and d) a significant number of responders may eventually relapse in long-term follow-up. The data suggest that the limit in the efficacy may result from the inability of RTX to directly kill lymphoma cells. RTX primarily relies on indirect mechanisms to attack lymphoma cells, which include complement-dependent cytotoxicity, Ab-dependent cellular cytotoxicity, induction of apoptosis, and immune activation. These mechanisms could be readily compromised by various situations, such as chemotherapy. The new generation of anti-CD20 Ab have not been found to be directly cytotoxic. Cytotoxic radioactive isotope-conjugated anti-CD20 Ab appeared to be highly effective, but serious radiotoxicity prohibited their clinical application. Increasing Ab valency augments activity; a recent study has demonstrated drastic improvement in activity by non-covalently associating RTX with nanomaterial graphene oxide (GO). The multivalent Ab product RTX/GO is highly cytotoxic, capable of directly killing BNHL cells *in vitro* and rapidly eliminating established xenograft lymphoma *in vivo* in the absence of toxic chemo-agents. While further studies are needed to determine the mechanism of activity and clinical efficacy, the current data suggest a significant possibility that RTX/GO might constitute nontoxic but effective therapy for BNHL.

INTRODUCTION

The non-Hodgkin's lymphomas (NHL) are the most common haematological malignancies in adults, with approximately 85% of NHL of B cell origin expressing the specific B cell marker CD20. CD20 is a transmembrane protein of undefined function[1] and its expression is B cell maturation-regulated. The prototype therapeutic anti-CD20 antibody (Ab) is rituximab (RTX), a chimeric Ab composed of a human IgG1 heavy-chain constant region and murine Ig variable region that is specific for CD20. The first therapeutic monoclonal Ab (mAb) to receive U.S. Food and Drug Administration (FDA)

approval in 1997, RTX has been routinely used for treatment of almost all types of B cell NHL, whether indolent or aggressive. Addition of RTX to standard chemotherapy substantially enhances response to therapy and improves overall outcomes, which makes RTX therapy the most noticeable advance in lymphoma treatment over the past decades. Despite this remarkable success, major challenges remain: a) a significant percentage of patients with aggressive NHL are refractory to RTX-containing regimens;[2] b) the long-term survival of patients with aggressive lymphomas is still limited (with 10-year progression-free survival approximately 35% in high-risk patients;[3,4] c) most indolent lymphomas remain incurable;[5] and d) RTX relies heavily on chemotherapy to achieve optimal therapeutic outcome, but chemotherapy is often associated with toxic adverse effects, which may compromise RTX activity and antitumour immunity, as discussed below.[6] Continuous efforts have been made in an attempt to generate new anti-CD20 Ab with efficacy superior to RTX, but the new Ab do not seem to be fundamentally improving therapeutic outcome yet. The main reason for this appears to be that all anti-CD20 Ab are not directly cytotoxic to lymphoma cells but rely primarily on indirect effector mechanisms to attack lymphoma cells. In settings where the indirect effector mechanisms are absent, consumed, or compromised, such as in patients receiving toxic chemotherapy, the activity of anti-CD20 Ab can be disrupted.

Making anti-CD20 Ab directly cytotoxic to lymphoma cells may circumvent the dependence on indirect mechanisms and fundamentally enhance and sustain Ab activity. In this article, the mechanisms of RTX action and resistance, the features of newer generations of anti-CD20 mAb, and the therapeutic advantages of directly cytotoxic antibodies are reviewed.[7]

MECHANISMS OF RITUXIMAB ACTION AND RESISTANCE

The mechanism of RTX action is the subject of active study. While controversies remain, all the data demonstrate that RTX targets lymphoma cells through multiple mechanisms. Despite the multiple activities, the therapeutic capacity of RTX as a monotherapy appears rather limited.[8] The reason for this appears to be, as discussed below, that RTX targets lymphoma cells mostly via indirect mechanisms that could readily be compromised by various situations, including chemotherapy.

Complement-Dependent Cytotoxicity

RTX can activate the complement system by binding C1q to the Fc region of the Ab, generating a membrane attack complex to disrupt the plasma membrane and kill target cells. Components of the activated complement system can also act as opsonins by binding receptors on phagocytes and natural killer (NK) cells to activate Ab-dependent cell-mediated cytotoxicity (ADCC). While *in vitro* experiments have demonstrated complement-dependent cytotoxicity (CDC) in RTX activity,[9,10] controversies remain whether *in vivo* CDC is required, sufficient, or unnecessary to mediate therapeutic effects of RTX.[11,12] In patients with chronic lymphocytic leukaemia (CLL), RTX infusion results in rapid and profound depletion of complement components,[13] suggesting that complement depletion may contribute to observed RTX treatment failure.[14] Alternatively, CDC does not appear to be similarly active in follicular NHL, which is nodal, as opposed to CLL, which is predominantly bone marrow and peripheral blood-based. Genetic polymorphisms in the gene coding for C1q have been linked to variations in RTX efficacy,[15] and Ab-resistant cells surviving RTX therapy have been reported to express high levels of complement-regulatory proteins (mCRP), which inhibit complement action.[16-18] Therefore, the activity of RTX-induced CDC varies depending on patients, lymphoma type, and treatment duration, and can be compromised under various circumstances. CD20 downregulation is also reported as a mechanism of acquired RTX resistance. The primary mechanism for CD20 downregulation is believed to result from the shaving or stripping of CD20 from the cell surface by macrophages.[18] This phenomenon is particularly relevant during the binding of RTX to CD20, and it does not eliminate lymphoma cells because of absence or exhaustion of the host effector mechanisms such as CDC and ADCC. Making RTX directly cytotoxic might overcome this resistance mechanism.

Antibody-Dependent Cellular Cytotoxicity

RTX can also activate ADCC attack on CD20+ cells through Fcγ receptor-bearing effector cells, such as NK cells, granulocytes, and macrophages. Activation of ADCC by RTX has been established by *in vitro* experiments.[19] In murine models, depletion of normal B cells by RTX was reported to be dependent on FcγRI and FcγRIII; B cell depletion did not occur in FcγR-deficient mice, supporting an *in vivo* role for ADCC.[12] In humans, single nucleotide polymorphisms (SNP) in *FCGR3A* (low affinity immunoglobulin gamma Fc region receptor III-A) with substitution of either a valine (V) or phenylalanine (F) residue at position 158 of the FCγRIIIa receptor can substantially impact ADCC. Cells bearing Fc receptor homozygous for V (158V/V) have a higher *in vitro* affinity for IgG1 compared to cells with the 158V/F or 158F/F receptor,[20] showing higher response rates to RTX in NHL patients with the 158V/V receptor as compared to patients with 158V/F or 158F/F receptor.[21-23] The polymorphisms have no prognostic significance in patients treated with chemotherapy alone,[24] suggesting ADCC as an effector mechanism for anti-CD20 therapy. However, studies have not found an impact of the *FCGR3A* genotype on outcomes in B cell CLL treated with RTX,[25] suggesting that the clinical contribution of ADCC depends on the characteristics of the underlying malignant cells.

In addition to genetic variations and lymphoma types, other conditions affect ADCC. For instance, activated complement components, such as C3b, inhibit NK cell-mediated ADCC.[26] A common mechanism to disrupt ADCC is chemotherapy, which causes cytopenia with loss of effector cells of both myeloid and lymphoid lineages, including NK cells, granulocytes, macrophages, and T cells. Given that the FcγR-bearing cells are required for ADCC, loss of these effector cells in patients with cytopenia can be expected to halt RTX-mediated ADCC.

Induction of Apoptosis

RTX has very limited, if any, capacity to directly kill target cells in culture.[7] While studies have reported induction of a degree of apoptosis to some malignant B cell lines in culture, this often requires plate-coated RTX or the presence of a second Ab crosslink Ab or FcγR to crosslink with RTX.[27-29] Even with plate-immobilised RTX, the most vigorous form of crosslinking, the extent of cell death is limited, especially with cells of aggressive lymphomas, e.g., diffuse large B cell lymphoma (DLBCL) line SU-DHL-4.[29] While studies have reported CLL cell apoptosis in patients receiving RTX treatment, it is unclear whether indirect mechanisms are involved in the induction of apoptosis.[30] Discrepancies also remain regarding the mechanism of RTX-induced apoptosis. Some studies have suggested a central role of caspase in the induction of apoptosis,[30] while others have shown caspase-independent apoptosis.[31-33]

It is noteworthy that while brief exposure to RTX downregulates B cell lymphoma (BCL)-2 expression, sensitising cells to apoptosis and chemotherapy,[34] prolonged RTX exposure inhibits expression of the pro-apoptotic BCL-2 family proteins BAX and BAK, leading to resistance not only to apoptosis but also to multiple antineoplastic agents.[35,36] RTX is typically administered over a number of weeks in the induction therapy, followed by multiple cycles of therapy every 8 weeks for up to 12 doses. While the intensive therapy protocol might play an important role in improving the response and durability of RTX treatment, it may also contribute to induction of resistance, as indicated in the long-term follow-up studies.[3]

Immune Activation

In vitro experiments have shown that RTX facilitates the uptake and cross-presentation of apoptotic Daudi cell antigen to cytotoxic T lymphocytes (CTL).[37] Whether RTX therapy similarly promotes antigen presentation and activation of specific CTL *in vivo* in patients remains to be established. It is unclear whether antitumour immunity in patients is generally compromised as a result of the intensive chemotherapy administered along with RTX. Antitumour immunity is now known to play a critical role in the prognosis of cancers, including lymphomas.[38-40]

The Adverse Impact of Chemotherapy on Rituximab Activity

Despite the proposed multiple antilymphoma mechanisms of RTX, the therapeutic capacity of RTX as a monotherapy appears limited.[8] Patients treated with RTX alone showed very low response rates, especially for aggressive lymphoma, which is often fatal in months if untreated effectively. As a result, RTX has generally been used as adjuvant therapy in combination with chemotherapy consisting of multiple toxic chemotherapeutic agents for optimal therapeutic outcome, such as in CHOP (cyclophosphamide, doxorubicin [hydroxydaunomycin], vincristine [Oncovin®, Cellpharm GmbH, Hanover, Germany], and prednisolone), the most commonly used regimen.[8] While intensive CHOP substantially increases response rate to RTX, it is often associated with toxic side effects, including life-threatening cytopenia.[41] Cytopenia not only predisposes patients to high infection risk but can also disrupt RTX activity. For instance, ADCC might be compromised due to lack of effector cells. Induction of apoptosis might be impaired due to loss of FcγR-bearing cells that are required for RTX crosslinking. Lymphopenia can also seriously weaken immune activation function of RTX because of T cell deficiency.[6] Lymphopenia could have significant long-term adverse sequela because T cell recovery could be very slow, especially in adult patients, due to thymic involution.[42] A number of studies have demonstrated close relationships between lymphopenia and high incidence of relapse of lymphomas, including DLBCL,[6,43] implicating the role of antitumour immunity in preventing recurrence. Therefore, implementation of toxic chemotherapy to RTX therapy might not only compromise RTX's original activity but also the antitumour immunity of the hosts, predisposing patients to delayed lymphoma relapse. Novel strategies are needed to improve RTX efficacy in the absence of toxic chemotherapy.

THE NEWER GENERATIONS OF ANTI-CD20 ANTIBODIES

There are several new-generation anti-CD20 mAb engineered to provide advantages over RTX, including ofatumumab, ocrelizumab, veltuzumab, ocaratuzumab, and ublituximab. These mAb induce potent CDC but relatively weak ADCC, similar to RTX, and are categorised as type 1 Ab. Ibritumomab tiuxetan and obinutuzumab (OBZ) are regarded as type 2 Ab because of stronger ADCC relative to CDC. Compartmentalisation and redistribution of CD20 into lipid rafts is believed to be the mechanism behind Type 1 Ab induction of intensive strong Fc clustering and complement activation.[44] The Type 2 Ab do not redistribute CD20 into lipid rafts.

Ofatumumab is at the most advanced stage of clinical development. Ofatumumab binds to a unique CD20 epitope, giving rise to a slow off-rate and a high capacity for complement activation. Ofatumumab-induced CDC in RTX-resistant CLL cells and lymphoma cell lines that express high levels of complement defence proteins and/or low levels of CD20.[45] In clinical trials, ofatumumab showed improved response rates and progression-free survival, but benefits are still limited. For treatment of follicular and DLBCL, chemotherapy is still required.[46,47] If enhancing CDC does not improve anti-CD20 mAb efficacy and RTX resistance is largely dictated by a failure of immune effector cell function, it would seem unlikely that these novel mAb will result in substantial improvements over RTX.

OBZ has a glycoengineered Fc region that includes nonfucosylated oligosaccharides that interact with FcR, particularly FcRIIIa, which enhances ADCC.[48] It has features of a Type 2 anti-CD20 mAb in evoking nonapoptotic programmed cell death in addition to not having a strong ability to translocate CD20 into lipid rafts for complement activation.[49] Studies reported superior tumour growth inhibition compared with RTX in lymphoma xenograft models,[50] and greater B cell depletion than RTX in nonhuman primates and hCD20 transgenic mice. OBZ is approved for CLL and follicular lymphoma. Despite reports of potent capacity to induce strong ADCC and nonapoptotic programmed cell death, OBZ relies on chemotherapy for optimal outcome. While OBZ-based immunochemotherapy and maintenance therapy resulted in longer progression-free survival than RTX-based therapy in follicular lymphoma, the improvement appeared limited.[51] The dependence on

chemotherapy might compromise OBZ-mediated ADCC. If enhancing ADCC does not improve anti-CD20 mAb efficacy because of chemotherapy-induced failure of effector cells, the importance of having cytotoxic anti-CD20 Ab capable of eliminating lymphoma cells in the absence of chemotherapy will be heightened.

Radioisotope-Labelled Anti-CD20 Antibodies

Radioisotope-labelled anti-CD20 Ab had the potential to be good examples of directly cytotoxic anti-CD20 Ab. Two such Ab have been produced: ^{90}Y-labelled ibritumomab tiuxetan and ^{131}I-labelled tositumomab.[52,53] Both Ab were FDA-approved for the treatment of relapsed or refractory follicular lymphoma or transformed B cell NHL. These Ab were highly effective even when used as monotherapy during the treatment of lymphomas refractory to salvage immunochemotherapy at relapse, demonstrating the unmatched potency of cytotoxic anti-CD20 Ab. However, neither Ab is in clinical application because of serious radiation-derived side effects, including bone marrow suppression, severe and prolonged cytopenia, and the sequelae of cytopenia.[54,55] Tositumomab and I^{131} tositumomab were discontinued by the manufacturer in February 2014 and are no longer available.

OTHER ANTI-CD20 MODIFICATION APPROACHES

Other anti-CD20 modifications have been comprehensively reviewed in recent literature.[56] Bispecific Ab, such as anti-CD20/CD3 or anti-CD20/NKGD2L, act through nonspecific activation of conventional or gamma/delta T cells initiating an attack on lymphoma cells. Toxin-combined anti-CD20 can directly attack,[57] but because of utilisation of only one CD20-specific single-chain, the avidity of the bispecific Ab for CD20 might be substantially diminished. Various approaches have been taken to generate hypervalent anti-CD20 Ab without using second crosslinking Ab, FcγR, or plate immobilisation. Tetravalent anti-CD20 Ab are generated by genetic engineering.[58] The dock-and-lock method has been used to produce hexavalent anti-CD20 antibodies.[59] More recently, a two-step method using pretargeting component (anti-CD20 Fab' conjugated with an oligonucleotide-1) and a subsequent crosslinking component (N-[2-hydroxypropyl] methacrylamide [HPMA] grafted with multiple complementary oligonucleotide-2) has also been reported to generate multivalent anti-CD20 Ab.[60] All these different forms of hypervalent anti-CD20 Ab demonstrate enhanced direct antilymphoma activity in culture in the absence of cross-linking antibodies as compared to the native Ab counterparts, which is manifested as enhanced antiproliferative and apoptosis-inducing activity. However, direct cytotoxicity is not reported. In animal experiments using severe combined immunodeficiency mice, both the dock-and-lock and two-step pretargeting components generated multivalent anti-CD20 Ab and inhibited lymphoma progression in the absence of chemotherapy.

ANTIBODY–GRAPHENE OXIDE COMPLEX

More recent studies have reported drastic enhancement of RTX as well anti-HER2 Ab trastuzumab activity by non-covalently associating Ab with nanomaterial graphene oxide (GO).[7,61]

Noncovalent Binding Between Rituximab and Graphene Oxide Generates Stable Rituximab/Graphene Oxide Complex with Marked High Avidity for CD20

GO is the thinnest nanomaterial available. Composed of a single-atom-thick nanosheet, GO has recently attracted intense interest within research investigating drug delivery. GO is nontoxic at low concentrations, but can cause oxidative stress, rupture of liposomes, disrupt the integrity of bacterial cell membranes, and kill cancer stem cells at high concentrations. When mixed in low salt solution, RTX and GO form a stable complex at 37°C, which no longer dissociates in physiological solutions such as phosphate-buffered saline and serum.[7] Despite the fact that the RTX forms a complex with GO through a stochastic process, the random interaction between the two does not interfere with RTX reactivity with CD20. The RTX/GO

complex demonstrated substantially enhanced CD20-binding capacity as compared to free RTX. While the mechanism behind the substantial increase in binding capacity is not yet fully understood, the data suggest that RTX interacts with GO through the Fc region with the Fab region uninterrupted, and the multivalent nature of RTX/GO gives rise to high avidity.[7]

Rituximab/Graphene Oxide Complex is Directly Cytotoxic to Malignant B Cells

When Raji cells, which are known to be resistant to apoptosis, were cultured with RTX/GO they underwent rapid cell death, determined by trypan blue and microscopic cell counting, Cell Count Kit, LIVE/DEAD stain, and electron microscopy. Annexin V staining of RTX/GO-killed cells did not detect significant staining, and DNA electrophoresis did not identify apoptotic DNA fragmentation, indicating nonapoptotic cell death (necroptosis, as determined in unpublished experiments). In line with these findings, the pan-caspase inhibitor Z-VAD-FMK could not rescue RTX/GO-treated cells. In contrast, RTX/GO-killed cells show rapid loss of plasma membrane integrity.[7] Complement is not required for RTX/GO-mediated cytotoxicity, indicating RTX/GO directly kills lymphoma cells. Similarly, potent cytotoxicity was also demonstrated on other CD20+ cells, including Daudi (Burkitt lymphoma), SUDHL-4 and SUDHL-8/9 (DLBCL), primary lymphoma cells from a patient with CLL, as well as primary B lymphocytes from healthy donors. When the capacity of RTX/GO to activate complement was examined, RTX/GO had much weaker activity to activate complement as compared to free RTX. As complement activation is believed to be responsible for infusion-related side effects,[62] the weak complement activation function of RTX/GO might be beneficial. Therefore, association of RTX with GO confers potent, direct cytotoxicity to CD20+ lymphoma cells, which is not observed for any of the previously reported anti-CD20 Ab formulations.

The Mechanism of Rituximab/Graphene Oxide Complex-Mediated Cytotoxicity

It remains incompletely understood how RTX/GO kills target cells. The results show that the highest valence of RTX in RTX/GO gives rise to the strongest cytotoxicity.[7] As CD20 crosslinking causes CD20 capping, and reorganisation of the actin network is required for capping,[63] the actin polymerisation inhibitor, latrunculin B (LatB), was tested in a RTX/GO-cytotoxicity assay. LatB completely abrogated RTX/GO-induced cell death, indicating that the actin network is involved in the cytotoxicity.[7] Anti-major histocompatibility complex Class I (HLA-A/B/C) and Class II (HLA-DR) mAb W6/32 and L243 were also used to generate mAb/GO complexes. HLA-DR is a lipid raft-associated protein whereas HLA-A/B/C are located outside of lipid rafts.[64] W6/32/GO induced limited Raji cell death; on the other hand, L243/GO killed the target cells as extensively as RTX/GO.[7] These results suggest that lipid raft-associated proteins are involved in RTX/GO-mediated activity. Given the previous report that the actin network plays a role in controlling location and function of lipid raft proteins, the protective activity of LatB might result from interruption of relocation of lipid-raft proteins. Calcium (Ca) influx is required for RTX/GO-mediated killing as Ca^{2+} chelator alleviates the cytotoxicity. RTX/GO also induces strong reactive oxygen species in the target cells and blocking reactive oxygen species production prevents the cell death (unpublished data). All the results indicate that potent cytotoxicity of RTX/GO results from a combined action of CD20-crosslinking-induced intracellular activity and biological activity of GO.

Rituximab/Graphene Oxide Complex Rapidly Eliminates Established Burkitt Lymphoma *in Vivo*

The therapeutic potential of RTX/GO *in vivo* was studied in immunodeficient NODrag$^{ko}\gamma^{ko}$ (NRG) mice that were intravenously transplanted (Burkitt lymphoma) with Raji cells. As NRG mice are deficient in T, B, and NK cells along with a defective complement system and macrophage activity,[65] they constitute an ideal animal model for the evaluation of RTX/GO therapeutic activity in the absence of host effector mechanisms such as CDC and ADCC. To determine whether RTX/GO could eliminate established lymphoma, the treatment was not initiated until the 8th day of Raji cell transplantation, when extensive lymphoma infiltrates were identified in the bone marrow

and liver.[7] The mice were treated intravenously every 2–3 days for a total of four treatments. When analysed 3 days after the last treatment, extensive lymphoma infiltrates were identified in the bone marrow of the PBS, GO, and RTX-treated animals, but not in RTX/GO-treated mice. The Raji cell counts in the bone marrow of GO and RTX-treated mice were similar to that of PBS-treated mice. Infiltrating lymphoma was identified in all the PBS, GO, and RTX-treated mice but not in any of the RTX/GO-treated mice. No pathological abnormalities nor any evidence of morbidity was identified in the RTX/GO-treated mice.[7] The *in vivo* results demonstrate that RTX/GO has the capacity to diffuse out of the blood circulation, penetrate through the tissue to reach target cells, and rapidly eliminate established lymphomas in the absence of host effector mechanisms.

DISCUSSION AND CONCLUSION

The findings with RTX/GO demonstrate a new strategy to substantially improve therapeutic activity of RTX. While CD20-crosslinking-induced cell death has been reported to be apoptotic, RTX/GO kills by necroptosis. Necroptosis might provide a therapeutic advantage because faulty apoptotic pathways often contribute to therapy resistance. The serum half-life of RTX/GO may be shortened compared to that of free RTX, according to previous reports;[66,67] however, an extended serum half-life may not be necessary for the RTX/GO therapeutic effect, as RTX/GO appears to eliminate lymphoma rapidly. Elimination of established lymphoma in the NRG hosts is unique to RTX/GO therapy. The results suggest effectiveness of RTX/GO therapy in patients with compromised host effector mechanisms. More importantly, the independence on toxic chemo-agents of RTX/GO therapy implicates protection of the host immune cells that are critical in antitumour immunity and maintenance of durable remission. Clearly, much is yet to be understood regarding RTX/GO, including the mechanism of cytotoxicity, pharmacological kinetics, side effects, and clinical efficacy. In a more recent study, GO-associated anti-HER2 Ab complex, trastuzumab/GO, demonstrated the ability to directly kill *HER2^{lo}* cancer cells,[61] similar to RTX/GO. Therefore, the data taken together indicate that noncovalent association of antitumour Ab with GO might constitute a highly effective methodology to enhance efficacy.

References

1. Li H et al. Store-operated cation entry mediated by CD20 in membrane rafts. J Biol Chem. 2003;278(43):42427-34.
2. Nowakowski GS et al. Beyond RCHOP: A blueprint for diffuse large B cell lymphoma research. J Natl Cancer Inst. 2016;108(12).
3. Coiffier B et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: A study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040-5.
4. Horvat M et al. Diffuse large B-cell lymphoma: 10 years' real-world clinical experience with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone. Oncol Lett. 2018;15(3):3602-09.
5. Cabanillas F. Curability of advanced indolent or low-grade follicular lymphomas: Time for a new paradigm? J Clin Oncol. 2013;31(1): 14-6.
6. Porrata LF et al. Lymphopenia assessed during routine follow-up after immunochemotherapy (R-CHOP) is a risk factor for predicting relapse in patients with diffuse large B-cell lymphoma. Leukemia. 2010;24(7):1343-9.
7. Luo C et al. Association of rituximab with graphene oxide confers direct cytotoxicity for CD20-positive lymphoma cells. Oncotarget. 2016;7(11):12806-22.
8. Cheson BD, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N Engl J Med. 2008;359(6):613-26.
9. Reff ME et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994;83(2):435-45.
10. Czuczman MS et al. Acquirement of rituximab resistance in lymphoma cell lines is associated with both global CD20 gene and protein down-regulation regulated at the pretranscriptional and posttranscriptional levels. Clin Cancer Res. 2008;14(5):1561-70.
11. Di Gaetano N et al. Complement activation determines the therapeutic activity of rituximab in vivo. J Immunol. 2003;171(3):1581-7.
12. Uchida J et al. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med. 2004;199(12):1659-69.
13. Kennedy AD et al. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. J Immunol. 2004;172(5):3280-8.
14. Klepfish A et al. Enhancing the action of rituximab in chronic lymphocytic leukemia by adding fresh frozen plasma: Complement/rituximab interactions & clinical results in refractory CLL. Ann N Y Acad Sci. 2009;1173:865-73.
15. Racila E et al. A polymorphism in the complement component C1qA

correlates with prolonged response following rituximab therapy of follicular lymphoma. Clin Cancer Res. 2008;14(20):6697-703.

16. Bannerji R et al. Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: Relationship to in vivo rituximab resistance. J Clin Oncol. 2003;21(8):1466-71.
17. Fishelson Z et al. Obstacles to cancer immunotherapy: Expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol. 2003;40(2-4):109-23.
18. Takei K et al. Analysis of changes in CD20, CD55, and CD59 expression on established rituximab-resistant B-lymphoma cell lines. Leuk Res. 2006;30(5):625-31.
19. Flieger D et al. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expressing lymphoma cell lines. Cell Immunol. 2000;204(1):55-63.
20. Koene HR et al. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood. 1997;90(3):1109-14.
21. Kim DH et al. FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. Blood. 2006;108(8):2720-5.
22. Cartron G et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood. 2002;99(3):754-8.
23. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol. 2003;21(21):3940-7.
24. Weng WK et al. Immunoglobulin G Fc receptor polymorphisms do not correlate with response to chemotherapy or clinical course in patients with follicular lymphoma. Leuk Lymphoma. 2009;50(9): 1494-500.
25. Farag SS et al. Fc gamma RIIIa and Fc gamma RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. Blood. 2004;103(4):1472-4.
26. Wang SY et al. Depletion of the C3 component of complement enhances the ability of rituximab-coated target cells to activate human NK cells and improves the efficacy of monoclonal antibody therapy in an in vivo model. Blood. 2009;114(26):5322-30.
27. Hofmeister JK et al. Clustered CD20 induced apoptosis: Src-family kinase, the proximal regulator of tyrosine phosphorylation, calcium influx, and caspase 3-dependent apoptosis. Blood Cells Mol Dis. 2000;26(2): 133-43.
28. Shan D et al. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood. 1998;91(5):1644-52.
29. Mathas S et al. Anti-CD20- and B-cell receptor-mediated apoptosis: Evidence for shared intracellular signaling pathways. Cancer Res. 2000;60(24):7170-6.
30. Byrd JC et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: Evidence of caspase activation and apoptosis induction. Blood. 2002;99(3):1038-43.
31. Chan HT et al. CD20-induced lymphoma cell death is independent of both caspases and its redistribution into triton X-100 insoluble membrane rafts. Cancer Res. 2003;63(17):5480-9.
32. van der Kolk LE et al. CD20-induced B cell death can bypass mitochondria and caspase activation. Leukemia. 2002;16(9):1735-44.
33. Shan D et al. Signaling events involved in anti-CD20-induced apoptosis of malignant human B cells. Cancer Immunol Immunother. 2000;48(12):673-83.
34. Vega MI et al. Rituximab-mediated cell signaling and chemo/immuno-sensitization of drug-resistant B-NHL is independent of its Fc functions. Clin Cancer Res. 2009;15(21):6582-94.
35. Jazirehi AR et al. Development of rituximab-resistant lymphoma clones with altered cell signaling and cross-resistance to chemotherapy. Cancer Res. 2007;67(3):1270-81.
36. Olejniczak SH et al. Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Bax and Bak expression. Clin Cancer Res. 2008;14(5):1550-60.
37. Selenko N et al. CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of CD8+ cytotoxic T cells. Leukemia. 2001;15(10):1619-26.
38. Mlecnik B et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol. 2011;29(6):610-8.
39. Grossman SA et al. Survival in patients with severe lymphopenia following treatment with radiation and chemotherapy for newly diagnosed solid tumors. J Natl Compr Canc Netw. 2015;13(10):1225-31.
40. Fridman WH et al. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol. 2017;14(12):717-34.
41. Cunningham D et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: A Phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet. 2013;381(9880):1817-26.
42. de Gast GC et al. Reinfusion of autologous lymphocytes with granulocyte-macrophage colony-stimulating factor induces rapid recovery of CD4+ and CD8+ T cells after high-dose chemotherapy for metastatic breast cancer. J Clin Oncol. 2002;20(1):58-64.
43. Kim YR et al. Lymphopenia is an important prognostic factor in peripheral T-cell lymphoma (NOS) treated with anthracycline-containing chemotherapy. J Hematol Oncol. 2011;4:34.
44. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood. 2004;103(7):2738-43.
45. Teeling JL et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006;177(1):362-71.
46. Czuczman MS et al. Chemoimmunotherapy with ofatumumab in combination with CHOP in previously untreated follicular lymphoma. Br J Haematol. 2012;157(4):438-45.
47. Peyrade F et al. Combination of ofatumumab and reduced-dose CHOP for diffuse large B-cell lymphomas in patients aged 80 years or older: An open-label, multicentre, single-arm, Phase 2 trial from the LYSA group. Lancet Haematol. 2017;4(1):e46-e55.
48. Niwa R et al. Enhancement of the antibody-dependent cellular cytotoxicity of low-fucose IgG1 Is independent of FcgammaRIIIa functional polymorphism. Clin Cancer Res. 2004;10(18 Pt 1):6248-55.
49. Alduaij W et al. Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies. Blood. 2011;117(17):4519-29.
50. Mossner E et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood. 2010;115(22):4393-402.
51. Marcus R et al. Obinutuzumab for the first-line treatment of follicular lymphoma. N Engl J Med. 2017;377(14):1331-44.
52. Alcindor T, Witzig TE. Radioimmunotherapy with yttrium-90 ibritumomab tiuxetan for patients

with relapsed CD20+ B-cell non-Hodgkin's lymphoma. Curr Treat Options Oncol. 2002;3(4):275-82.

53. Davies AJ et al. Tositumomab and iodine I 131 tositumomab for recurrent indolent and transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol. 2004;22(8):1469-79.
54. Rizzieri D. Zevalin(®) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned? Crit Rev Oncol Hematol. 2016;105:5-17.
55. Srinivasan A, Mukherji SK. Tositumomab and iodine I 131 tositumomab (Bexaar). AJNR Am J Neuroradiol. 2011;32(4):637-8.
56. Marshall MJE et al. Therapeutic antibodies: What have we learnt from targeting CD20 and where are we going? Front Immunol. 2017;8:1245.
57. Huang S et al. The CD20-specific engineered toxin antibody MT-3724 exhibits lethal effects against mantle cell lymphoma. Blood Cancer J. 2018;8(3):33.
58. Li B et al. Development of novel tetravalent anti-CD20 antibodies with potent antitumor activity. Cancer Res. 2008;68(7):2400-8.
59. Rossi EA et al. Novel designs of multivalent anti-CD20 humanized antibodies as improved lymphoma therapeutics. Cancer Res. 2008;68(20):8384-92.
60. Chu TW et al. A two-step pretargeted nanotherapy for CD20 crosslinking may achieve superior anti-lymphoma efficacy to rituximab. Theranostics. 2015;5(8):834-46.
61. Li L et al. Association of anti-HER2 antibody with graphene oxide for curative treatment of osteosarcoma. Nanomedicine. 2018;14(2):581-93.
62. van der Kolk LE et al. Complement activation plays a key role in the side-effects of rituximab treatment. Br J Haematol. 2001;115(4):807-11.
63. Bourguignon LY, Singer SJ. Transmembrane interactions and the mechanism of capping of surface receptors by their specific ligands. Proc Natl Acad Sci U S A. 1977;74(11):5031-5.
64. Goebel J et al. Lipid rafts, major histocompatibility complex molecules, and immune regulation. Hum Immunol. 2002;63(10):813-20.
65. Shultz LD et al. Humanized mice for immune system investigation: Progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786-98.
66. Zhang X et al. Distribution and biocompatibility studies of graphene oxide in mice after intravenous administration. Carbon. 2010;49(3):986-95.
67. Regazzi MB et al. Pharmacokinetic behavior of rituximab: A study of different schedules of administration for heterogeneous clinical settings. Ther Drug Monit. 2005;27(6):785-92.

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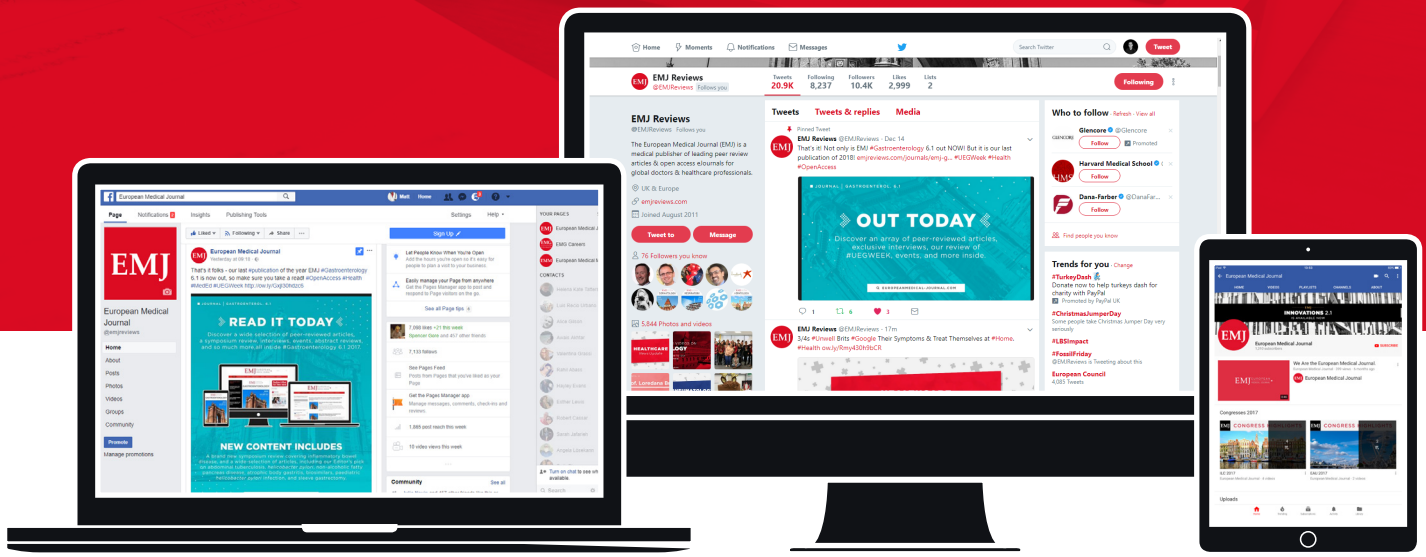

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Staging of Mycosis Fungoides and Sézary Syndrome: Time for an Update?

Authors: *Julia Scarisbrick

University Hospital Birmingham, Birmingham, UK
*Correspondence to julia.scarisbrick@uhb.nhs.uk

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Abstract

Mycosis fungoides (MF) is the most common variant of cutaneous T cell lymphoma and frequently presents as early-stage disease with skin patches and plaques with an indolent course, but patients experience significant morbidity from itch and disfigurement. Around 30% of patients with MF present in the advance stages with skin tumours, erythroderma, and extensive nodal or visceral involvement. Sézary syndrome (SS) is the leukaemic cutaneous T cell lymphoma variant. The staging of MF or SS was revised in 2007 to include skin, nodal, visceral, and blood (tumour-node-metastasis-blood classification) to determine nine stages (IA–IVB). While most patients with early disease (Stages IA–IIA) have a good prognosis, 25% progress to advanced disease, with a poor life expectancy of around 3 years; however, some patients do survive for ≥10 years. Accurate staging is crucial since management strategies are stage-based, with skin-directed therapy recommended in early-stage disease and with no curative therapeutic options to improve symptoms and reduce skin tumour burden. In contrast, advanced-stage patients mostly require systemic therapy. Most treatments have only partial response rates, around 40%, and allogeneic bone marrow transplant may provide a more long-lasting therapeutic option for advanced patients.

Relevant prognostic factors within the tumour-node-metastasis-blood classification are discussed in this review and their relevance to overall IA–IVB staging and outcomes are debated. Several important prognostic features have been identified that may be used alongside staging to give further prognostic information. These prognostic features include age >60 years at diagnosis, large cell transformation of the skin, and raised serum lactate dehydrogenase levels, which could be developed into a prognostic index to identify patients at risk of progression and requiring more aggressive therapy. The PROCLIPI study, a prospective cutaneous lymphoma international study, has been ongoing since 2015 to collect such data, with the aim of developing a prognostic index for MF and SS.

INTRODUCTION

Mycosis fungoides (MF) is the most common primary cutaneous T cell lymphoma (CTCL), which comprise a heterogeneous group of non-Hodgkin's lymphoma.[1] Sézary syndrome (SS) is the leukaemic form of CTCL. The original staging system for CTCL was based on the tumour, lymph node, metastasis (TNM) system devised by Bunn and Lamberg in 1979.[2]

The TNM system, which was used to stage a wide range of malignancies, was revised jointly in 2007 by the International Society for Cutaneous Lymphoma (ISCL) and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer (EORTC) for MF and SS to include a blood stage, with an overall stage determined from the tumour-node-metastasis-blood (TNMB) classification, which stratifies patients into nine stages (IA–IVB) (Table 1).

Table 1: Tumour-node-metastasis-blood (TNMB) classification for staging in mycosis fungoides and Sézary syndrome.

Stage	T	N	M	B
IA	T1: patches and plaques over <10% of BSA T1a: patches only T1b: plaques only	N0: no palpable nodes or histological evidence of MF N0a: clone-negative N0b: clone-positive	M0: no visceral involvement	B0: <5% peripheral blood lymphocytes atypical B0a: clone-negative B0b: clone-positive B1: >5% of lymphocytes atypical but <1,000/µL B1a: clone-negative B1b: clone-positive
IB	T2: patches and plaques over >10% of BSA T2a: patches only T2b: plaques only	N0	M0	B0–1
IIA	T1 or T2	N1: no histological evidence of MF (dermatopathic) N1a: clone-negative N1b: clone-positive N2: early involvement with MF, aggregates of atypical cells with preservation of nodal architecture N2a: clone-negative N2b: clone-positive	M0	B0–1
IIB	T3: tumours; lesions >1 cm diameter with deep infiltration	N0–2	M0	B0–1
IIIA	T4: erythroderma >80% BSA involved	N0–2	M0	B0
IIIB	T4: erythroderma	N0-2	M0	B1: >5% of lymphocytes atypical but <1,000/µl
IVA1	T1–T4	N0–2	M0	B2: >1,000/µl circulating atypical lymphocytes (Sézary cells)
IVA2	T1–T4	N3: lymph nodes involved with loss of normal architecture	M0	B0–2
IVB	T1–T4	N0–N3	M1: metastasis	B0–2

B: blood; BSA: body surface area; M: metastasis; MF: mycosis fungoides; N: node; T: tumour.

The early and advanced stages of MF are IA–IIA and IIB–VB, respectively,[3] whereas SS is always an advanced disease from diagnosis and can be Stage IVA1–IVB. Management of MF and SS is stage-dependant, so accurate staging is essential for best management.[4] Non-MF and cutaneous B cell lymphoma patients have a separate TNM staging system with no B category.[5]

For a staging system to be clinically meaningful, it should have prognostic significance. While increasing stage classification often correlates with a worse survival rate, there are discrepancies, with some early-stage patients having rapidly progressive disease and others with advanced stages living >10 years.[6-8] For example, those with Stage IIB disease may have a worse prognosis (median survival: 2.9 years) than those with Stage III disease (median survival: 3.6–4.6 years).[6,8] Furthermore, patients with the folliculotropic variant of MF (FMF) Stage IB disease have a worse disease-specific survival rate at 10 years than those with Stage IIB disease.[9] A large international study that included 1,275 MF and SS patients, staged according to the TNMB classification, found that, at diagnosis, raised serum lactate dehydrogenase (LDH) levels, large cell transformation (LCT) of the skin, and age >60 years were all significant factors for poor survival, independent of stage at diagnosis.[8] This narrative review describes TNMB classification and discusses the recent advances in the identification of prognostic factors and development of prognostic indices for MF and SS.

TUMOUR-NODE-METASTASIS-BLOOD STAGING

The ISCL/EORTC TNMB staging system confirmed the general concept of the previous Bunn and Lamberg TNM staging system,[2] but incorporated recent advances in tumour biology and newly developed diagnostic techniques. This included addition of a blood class (B), which splits erythrodermic patients according to blood tumour burden (B0–2) into Stage IIIA, IIIB, and IVA1. From 10 TNM categories there are now 21 TNMB categories: 7 skin classes (T1a–T4[3]), 7 nodal classes (N0–N3), 5 blood classes (B0a–B2), and 2 metastatic classes (M0 and M1). These classes are used to categorise patients into one of the nine stages from IA–IVB, as shown in Table 1.[3] Although new T, N, and M subcategories were added to record the presence or absence of plaques ($T1_{a/b}$ and $T2_{a/b}$) and T cell clonality ($N1/2_{a/b}$ and $B0/1_{a/b}$), these are not used to determine disease stage.[3]

Tumour Classification

Skin involvement in MF can involve different cutaneous lesions defined as a) patch: any size lesion without induration or significant elevation above the surrounding uninvolved skin; b) plaque: any size lesion that is elevated or indurated; or c) tumours: any solid or nodular lesion >1 cm in diameter with evidence of deep infiltration in the skin and/or vertical growth. Erythroderma is defined as confluent erythema covering >80% of the body surface area (BSA). The type of skin involvement in MF and the amount of BSA involved are used together to define the T class from T1–4(3), where T1 is <10% BSA patches and plaques, T2 is ≥10% BSA patches or plaques, T3 designates tumours, T4 designates erythroderma, and T4(3) identifies erythroderma with tumours. Classes T1–2 are considered early skin lesions and T3–4 are advanced. The presence of patches only is denoted by $T1_a$ or $T2_a$, and if plaques are present with or without patches, then this is denoted by $T1_b$ or $T2_b$. Evidence of poikiloderma, follicular lesions, or ulceration should also be recorded. Ulcerative lesions are those with a significant loss of superficial skin, including the entire epidermis and some portion of the upper dermis, and may be plaque or tumour.

Additional information on skin tumour burden in MF or SS may be recorded using the modified severity weighted assessment tool (MSWAT), which is the preferred method for measuring skin tumour burden and is scored from 0–400.[10] This skin scoring system provides a more comparative measure of skin tumour burden than T class, and it may be used to track skin involvement, notably during treatment. The BSA involved with patches, plaques, and tumours is calculated, usually using the palmar method where the patient's palm equates to 0.5% BSA.[11] Multiplication of patch x1, plaque x2, and tumour x4 produces a numerical value for MSWAT out of 400. Erythrodermic patients can similarly be scored by summation of the BSA involved with macular erythema (patch) and erythema with induration or oedema (plaque),

while maintaining the ability to simultaneously track any tumours present.[10] However, the prognostic significance of MSWAT has not yet been determined.

The PROCLIPI[12] study is an observational study that has recruited nearly 1,000 patients from 44 specialist centres worldwide. The centres have collected prospective data at diagnosis, annually, and at stage progression, and these will be used to determine the prognostic significance of MSWAT, alongside other potentially important factors, with the aim of developing a prognostic index (PI) to preselect patients with a worse prognosis who require more aggressive therapies.[12,13]

Node Classification

Clinically abnormal peripheral lymph nodes (LN) are defined as ≥15 mm in any diameter or a firm, irregular, clustered, or fixed node regardless of size.[3] Clinically enlarged or abnormal nodes should be corroborated by radiological imaging, with CT as the preferred technique. The largest peripheral LN or one that shows intense uptake on a fludeoxyglucose-positron emission tomography scan should be selected for biopsy and, if there are multiple nodes, cervical node biopsy should be selected above axillary and inguinal nodes. This hierarchical approach is used because cervical nodes have a higher chance of showing lymphomatous involvement.[14-16]

MF and SS LN status is denoted by N0–3, where N0 represents no clinically abnormal LN with no biopsy required. Clinically abnormal LN should be excised to determine the N class; an excisional LN biopsy is necessary to evaluate abnormal lymph nodes because core biopsy or fine needle aspiration do not provide adequate information on nodal architecture to define N3. Excisional LN biopsies are associated with morbidity typically from infection and in some instances may be deemed unnecessary by the treating physician; for example, this may be the case in patients with recurrent sepsis, previous LN biopsy, or when management will not be altered from the result. Clinically abnormal LN that have not been biopsied are classed as Nx. Excised LN histologically showing either no atypical lymphocytes or occasional or isolated atypical lymphocytes in clusters <7 cells are considered dermatopathic lymphadenopathy. LN classed as N1 (Dutch Grade 1 or National Cancer Institute Lymph Node [NCI LN] Grade 0–2) have early MF involvement that is defined by the presence of cerebriform nuclei >7.5 µm or aggregates of ≥7 atypical lymphocytes, and N2 defines LN with the nodal architecture preserved (Dutch Grade 2 or NCI LN Grade 3). Partial or complete loss of nodal architecture by atypical lymphocytes or neoplastic cells is scored N3 (Dutch Grade 3–4 or NCI LN Grade 4).[3]

It is advised that T cell receptor (*TCR*) gene analysis studies are performed on the excised LN. Clone-positive nodes are recorded as $N1_b$ or $N2_b$ and, correspondingly, clone-negative nodes are $N1_a$ or $N2_a$. The use of 'a' or 'b' to record clonality is different to the T class, where 'a' denotes skin patches only and 'b' the presence of plaques. It must be appreciated that the N class only relates to the histology of the excised LN and does not give a measure of total LN burden. The PROCLIPI[12] study involves prospectively recording the number of nodal sites (total nodal score) with enlarged LN at six peripheral sites and two central sites to score the patients LN burden from 0–8. The total nodal score is correlated with survival and N class may provide further prognostic information.[12,13,15]

Metastasis Classification

Visceral involvement with MF and SS is a well-documented, independently significant prognostic factor.[8,17-20] Visceral disease is recorded as M1 and, independent of TNB class, is always categorised within the most advanced stage, Stage IVB.[3] Visceral involvement tends to occur in late disease and <5% of patients present with Stage IVB.[8,17-20] During the disease course, MF and SS may involve virtually all organ systems. More common extracutaneous systems are the liver, spleen, lungs, and the central nervous system, with the lungs being the most common.[17] Visceral involvement is exceedingly rare in the absence of node or blood involvement and should therefore be questioned in these cases.[3]

Splenomegaly should be recorded as visceral disease, even without biopsy confirmation, when it is present on physical examination or documented radiographically (enlargement or multiple focal defects that are neither cystic nor vascular).[3] A biopsy is not required because

splenomegaly is rare in healthy persons and a spleen biopsy carries risks of bleeding. However, if lung abnormalities or other suggestions of extracutaneous lymphomatous involvement besides splenomegaly are seen on imaging, biopsy confirmation is usually recommended before categorising this as visceral involvement of MF or SS. Liver involvement may be suggested by clinical hepatomegaly, abnormal liver function tests, or radiologic tests (CT, fludeoxyglucose-positron emission tomography, and liver or spleen scan) and should be confirmed by liver biopsy.[3] Cerebral lesions may occur and may not be amenable to biopsy. Visceral abnormalities could be from MF or SS, but infection or another unrelated cancer is possible since second malignancies are frequent in MF and SS.[21-23]

Blood Classification

The revised staging in 2007[3] introduced a blood classification (B0-2), in which blood involvement is determined using a manual Sézary cell count (on a peripheral blood smear) and scored as B0 (absence of significant blood involvement, ≤5% of peripheral blood lymphocytes are morphologically Sézary cells), B1 (low blood tumour burden: >5% of peripheral blood lymphocytes are atypical Sézary cells but does not meet the criteria of B2), and B2 (high blood tumour burden: ≥1,000/µL Sézary cells with positive T clone identical to the skin clone [relevant clone]). TCR clonality in the blood should be recorded as 'a' or 'b' alongside blood class in B0 and B1, but clonality does not alter the overall stage. For T cell clonality to be relevant it must be identical to the skin T cell clone (index clone) because blood clones may occur in the elderly population with unknown significance.[3]

The number of centres performing manual Sézary cell counts has declined in recent years because the process is highly subjective and requires considerable experience. Flow cytometry has therefore become the most popular alternative for the measurement of blood involvement in MF and SS.[24,25] The 2007 staging paper acknowledges this decline and states that if Sézary cells are not able to be used to determine tumour burden for B2, then one of the following modified ISCL criteria may be used instead.[3] The ISCL criteria state that B2 may be defined by flow cytometry in patients with a relevant T cell clone in blood as either a) expanded CD4+/CD3+ cells with a CD4:CD8 ratio of ≥10, or b) expanded CD4+ cells with abnormal immunophenotype including loss of CD7/CD26 (≥40% CD4+/CD7- or ≥30% CD4+/CD26-).[26] However, no definition of expanded CD4+ cells was given. Furthermore, using the percentage of CD4+/CD7- or CD4+/CD26- cells, or the CD4:CD8 ratio, as opposed to absolute values, to define B2 has a disadvantage in that it detects patients with skewed CD populations but not necessarily a raised or high blood burden. In a paper by Olsen et al.,[10] it was suggested that 1,600/µL can be used as an upper limit of normal for CD4 cells in the blood and an absolute count <250/µL CD4+/CD26- or CD4+/CD7- cells to define B0.[10] A series of studies demonstrated that a very high blood tumour burden with >10,000/mm^3 absolute Sézary cell count (H4 according to the suggested British classification) was associated with a worse poor prognosis.[27-29] Recently, the EORTC Cutaneous Lymphoma Task Force published recommendations for B0–2 to be defined using absolute counts of either CD4+/CD7- or CD4+/CD26- by flow cytometry, where B0 is <250/µL (250 SI units), B1 is 250–<1,000/µL (250–1,000 SI units), and B2 is ≥1,000/µL (1,000 SI units), plus a relevant blood clone.[24]

Overall Stage IA–IVB

The MF or SS stage (Table 1) is the primary prognostic indicator and, although cases of MF or SS may relapse or remit with time and treatment, the stage cannot improve. Similarly, at stage progression, despite any subsequent improvement in TNMB, the stage cannot decrease. Therefore, the ISCL and EORTC recommend that, in addition to stage at diagnosis of MF or SS, the TNMB classification should be used to track tumour burden at any given timepoint to indicate the current and maximum tumour burden for an individual patient.[3]

DISCUSSION

The management strategy of MF or SS is decided according to clinical stage.[4,30] Patients with Stages IA–IIA are deemed to have early-stage disease and are recommended for

skin-directed therapy. Most of these patients have an excellent outcome and survive for 12–20 years or more.[6,7,17-20] However, early-stage patients with FMF have been shown to have a poorer prognosis, more similar to tumour stage MF (Stage IIB).[9,18] In addition, some patients with early-stage disease become refractory to skin-directed therapy and require systemic treatment, and around 25% of these patients progress to the advanced stages (Stages IIB–IVB).[6] The advanced stages of MF and SS tend to have a poor prognosis and survival <4 years.[6,8,17-20] Although some patients with advanced disease survive >10 years,[6] Stage IVA2–IVB patient survival is almost universally poor and is commonly <12 months.[8,17,18] Patients with advanced disease may require immunotherapy or chemotherapy and typically sequential treatments are given when a treatment response can no longer be measured. Treatment is frequently palliative in advanced MF or SS patients and decided on an individual patient basis, dependent on the presence of poor prognostic factors in addition to staging, but no algorithm exists and management of these patients varies between centres.[4,30] Patients in remission may be offered an allogeneic stem cell transplant, but careful consideration is required because transplant-related mortality at Year 1 is significant (15–20%) and relapse rates are 39–51%; despite this, good responses to transplant and durable remissions may occur.[31-33]

Although T subcategories have been added to capture the different clinical presentations between patches and plaques ($T1_{a/b}$ and $T2_{a/b}$), these are not used to determine the stage. However, an improved survival with patches versus plaques has been reported previously,[18,20,34] while thick plaques are associated with a worse prognosis.[35,36] Furthermore, the percentage of BSA involvement of the skin is only captured as <10% (T1; patches or plaques), ≥10% (T2; patches or plaques), or >80% (T4; erythroderma). The extent of skin lesions or skin tumour burden may be accurately tracked using the objective MSWAT scale, scored from 0–400. In time, the PROCLIPI study[12] will determine the significance of patch versus plaque disease and skin tumour burden in MF and SS. TCR studies should be performed on skin to identify the index clone, which can then be compared to the blood TCR (and nodal if performed) to identify identical relevant T cell clones in the blood (or node) that are recorded as B0b or B1b (or N1b or N2b); however, T cell clonality in the skin is not included in the T class, which records plaques as T1b or T2b.

Skin tumours are always associated with advanced disease (Stages IIB–IVB) and there are conflicting results in the literature as to the survival differences between tumour stage (IIB) and erythrodermic MF (IIIA/B); some papers have showed a worse prognosis for tumour stage[37] and others for erythrodermic patients,[38] while similar survival rates for both have also been shown.[18-20,36] The Italian Group of Cutaneous Lymphomas reported retrospective data on 1,422 MF patients; the only prognostic parameters selected by the multivariate analysis were the TNMB classification at first diagnosis and stage progression.[19]

Patients with early-stage skin lesions (patches with or without plaques) with either clinically abnormal nodes without a biopsy (Nx), dermatopathic nodes (N1), or early involvement of MF, but with preservation of nodal architecture (N2), are Stage IIA and considered early-stage disease. However, the prognosis is considerably worse than Stage I (Table 1), with N1 being associated with a higher relative risk of death compared to N0, and N2 being worse and more similar to N3 survival (Stage IVA2).[18,20] Node subcategories were included to record clonality ($N1_{a/b}$ and $N2_{a/b}$), where 'a' represents negative and 'b' represents positive clonality, following reports of a worse outcome in dermatopathic nodes with evidence of a relevant T cell clone, but do not alter stage.[3]

Visceral involvement or metastasis (M1) is rare at presentation of MF and SS and occurs in <5% of cases. It is almost universally associated with aggressive disease, which is preterminal and associated with a survival rate of <1 year. Apart from splenomegaly, histologic evidence of organ involvement is recommended for staging because the incidence of second malignancies and infection is common and may be treatable.[21-23]

Blood classification is recommended for all stages of MF and SS and was originally defined according to Sézary cell counts performed on

peripheral blood smears;[3] however, due to the expertise required for reporting Sézary counts and subjective results, it is not performed in all centres.[24,25] Flow cytometry is now more commonplace and available at all expert centres in Europe, but it is frequently restricted to the advanced stages of disease and only 35% of centres perform flow cytometry on all disease stages.[24] This leads to many early stages not receiving a blood class. Previous publications have used different definitions of blood class according to flow, and recommendations from the EORTC suggest that, for consistency and until further prognostic information is known, B0 is <250/µL, B1 is 250–<1,000/µL, and B2 is ≥1,000/µL. A very high peripheral blood involvement >10,000/µL has been shown to have further prognostic information and poorer survival than B2.[28,29]

Low-level blood involvement, as detected by a relevant T cell blood clone, should be recorded alongside blood class as polyclonal or clonal, but again this is frequently restricted to the advanced stages and performed at all disease stages in <50% of expert centres.[24] This makes interpretation of the prognostic importance of blood class and clonality difficult and may bias results to association with later stage and worse prognosis.

The clinical relevance of a Stage $B0_{a/b}$/$B1_{a/b}$ has not yet been proven, and the results of the PROCLIPI study[12] will determine whether these are independent prognostic factors for survival. Recently, it has been shown by a European group that changes in blood tumour burden as determined by flow cytometry do not correlate with skin tumour burden.[39] Staging of erythrodermic patients is affected by blood classification; those with B2 blood involvement with N1/2 and M0 are classed as Stage IVA1 or Sézary, as opposed to erythrodermic MF Stage IIIB (B1) or IIIA (B0), with different treatment recommendations.[4,30] However, a recent large study of 1,275 advanced-stage patients found no significant difference in survival between Stages IIIA/IIIB and IVA1.[8]

Specific prognostic factors in MF outside staging have been reported over the past three decades, including improved prognosis with poikiloderma, association with lymphomatoid papulosis, and juvenile age of onset; on the other hand, age >60 years, FMF, and the histological feature of LCT have been found to convey a worse prognosis.[40] The ISCL and EORTC recommend tracking patients with FMF or LCT to determine if either warrants a different staging system from classical MF and SS. A staging system that relates to prognosis is vital because this dictates the treatment for MF and SS.[4,30]

By combining clinicopathological features affecting survival, a PI may be developed to identify high-risk patients with diseases that have a wide range of survival rates. The development of a PI for aggressive non-Hodgkin's lymphoma in 1993 has been widely used to stratify patients for treatment.[41] Early attempts to develop a PI in MF and SS were not ratified in multicentre international trials.[37,42-44] A cutaneous lymphoma PI for early-stage (Stage IA–IIA) and late stage (Stage IIB–IVB) disease from London, UK, included male sex, age >60 years, presence of plaques, FMF, and Nx/1 for early-stage disease, and male sex, age >60 years, N2/3, B1/B2, and M1 for late-stage disease.[45] The predicted 10-year overall survival in the early-stage model was 90.3% (low risk) and 48.9% (high risk), and for the late-stage model was 53.2% (low risk) and 15.0% (high risk).[45] A recent large multicentre study of 1,275 advanced-stage patients from 29 international centres identified four independent prognostic markers associated with a poor survival: Stage IV, an age at diagnosis >60 years, LCT, and raised LDH.[8] Using these four parameters together in a prognostic model identified three risk groups across Stages IIB–IVB, with significantly different 5-year survival rates: low risk (68%), intermediate risk (44%), and high risk (28%).[8] PROCLIPI[12] has 956 patients currently enrolled from 46 international sites over five continents and has confirmed a male predominance of 1.6:1.0. This includes 680 early-stage (Stages IA–IIA) and 276 advanced-stage (Stages IIB–IV) patients and has found the median age at diagnosis of advanced disease is significantly older than early-stage patients, at 65 years and 57 years, respectively (p<0.0001). Furthermore, this large prospective study found the median time of MF-like lesions prior to diagnosis was 36 months in both early and advanced-stage disease, confirming diagnostic delay

and suggesting that patients presenting with advanced disease are not undiagnosed early-stage patients. LCT has been recorded in 20 of the 680 (3%) early-stage patients and in 69 of the 276 (25%) advanced-stage patients ($p<0.001$). A total of 9% of early-stage and 30% of advanced-stage patients exhibited raised serum LDH at diagnosis ($p<0.001$).[12]

CONCLUSION

Staging systems are used to predict the likely outcome of disease and select appropriate treatments. Therefore, for a staging system to be clinically meaningful it must relate to likelihood of survival and response to similar treatments. The ISCL/EORTC staging of MF and SS provides useful prognostic information; however, some discrepancies exist and some patients with the early stages have a poor prognosis, while, conversely, others with advanced disease may live for 10 years or more. Also, Stage IIB may have a worse outcome than Stage III. By identifying patients at a high risk of progression, management may be tailored with the hope of improving survival, although no relevant biomarkers of disease activity or novel genetic alterations have been validated. However, several important independent prognostic factors are now well established in MF and SS, such as patch versus plaques, large cell transformation, FMF, raised serum LDH, and increased age, which may be developed into a PI to be used alongside staging to give further insights into the likely survival and help guide treatment choices. This is highly relevant because there are no curative therapies for MF and SS, and patients with a likely poor outcome should be selected for allogeneic stem cell transplantation, including some early-stage patients.

The PROCLIPI study[12] is, and has been, collecting prognostic information prospectively at international centres in both early and advanced disease since 2015, but the data are not yet fully matured. At present there are not adequate prospective data on prognostic factors to deliver a PI for global use and there are not sufficient data to recommend a staging update, but it is anticipated this will be possible in the near future.

References

1. Willemze R et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105(10):3768-85
2. Bunn PA, Lamberg SI. Report of the committee on staging and classification of cutaneous T-cell lymphomas. Cancer Treat Rep. 1979;63(4):725-8.
3. Olsen E et al. Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: A proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110(6):1713-22.
4. Trautinger F et al. European Organisation for Research and Treatment of Cancer consensus recommendations for the treatment of mycosis fungoides/Sézary syndrome– Update 2017. Eur J Cancer. 2017;77:57-74.
5. Kim YH et al.; ISCL and the EORTC. TNM-classification system for primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome: A proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110(2):479-84.
6. Whittaker SJ et al.; British Association of Dermatologists (BAD); UK Cutaneous Lymphoma Group. Joint British Association of Dermatologists and U.K. Cutaneous Lymphoma Group guidelines for the management of primary cutaneous T-cell lymphomas. Br J Dermatol. 2003;149(6):1095–107.
7. Wernham AG et al. Stage I mycosis fungoides: Frequent association with a favourable prognosis but disease progression and disease-specific mortality may occur. Br J Dermatol. 2015;173(5):1295-7.
8. Scarisbrick JJ et al. Cutaneous Lymphoma International Consortium (CLIC) study of outcome in advanced stages of mycosis fungoides & Sézary syndrome: Effect of specific prognostic markers on survival and development of a prognostic model. J Clin Oncology. 2015;33(32):3766-73.
9. van Doorn R et al. Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: A clinicopathologic and follow-up study of 51 patients. Arch Dermatol. 2002;138(2):191-8.
10. Olsen EA et al.; International Society for Cutaneous Lymphomas; United States Cutaneous Lymphoma Consortium; Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: A consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. J Clin Oncol. 2011;29(18):2598-607.
11. Scarisbrick JJ, Morris SJ. How big is your hand and should you use it to score skin in cutaneous T-cell lymphoma? Br J Dermatol. 2013;169(2):260-5.
12. Scarisbrick J et al. 516 Global collaboration for establishment of a prognostic index in mycosis

fungoides & Sezary Syndrome. J Invest Dermatol. 2018;138(5):S88.

13. Scarisbrick JJ. Prognostic factors in mycosis fungoides: International advances in the validation of prognostic indices. Br J Dermatol. 2017;176(5):1129-30.
14. Vonderheid EC et al. Lymph node histopathologic findings in cutaneous T-cell lymphoma: A prognostic classification system based on morphologic assessment. Am J Clin Pathol. 1992;97(1):121-9.
15. Haththotuwa R et al. Biopsy correlation of surface area vs. single-axis measurements on computed tomography scan of lymph nodes in patients with erythrodermic mycosis fungoides and Sézary syndrome. Br J Dermatol. 2017;177(3):877-8.
16. Spencer A et al. Computed tomography scanning in mycosis fungoides: Optimizing the balance between benefit and harm. Br J Dermatol. 2018;178(2):563-4
17. Kim YH et al. Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome: Clinical prognostic factors and risk for disease progression. Arch Dermatol. 2003;139(7):857-66.
18. Agar NS et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: Validation of the revised International Society for Cutaneous Lymphomas/ European Organisation for Research and Treatment of Cancer staging proposal. J Clin Oncol. 2010;28(31):4730-9.
19. Quaglino P et al. Time course, clinical pathways, and long-term hazards risk trends of disease progression in patients with classic mycosis fungoides: A multicenter, retrospective follow-up study from the Italian Group of Cutaneous Lymphomas. Cancer. 2012;118(23):5830-9.
20. Talpur R et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sézary syndrome from 1982 to 2009. Clin Cancer Res. 2012;18(18):5051-60.
21. Amber KT et al. Second primary malignancies in CTCL patients from 1992 to 2011: A SEER-based, population-based study evaluating time from CTCL diagnosis, age, sex, stage, and CD30+ subtype. Am J Clin Dermatol. 2016;17(1):71-7.
22. Huang KP et al. Second lymphomas and other malignant neoplasms in patients with mycosis fungoides and Sézary syndrome: Evidence from population-based and clinical cohorts. Arch Dermatol. 2007;143(1):45-50.
23. Scarisbrick JJ et al. Secondary malignancy in 71 patients with Sézary syndrome. Arch Dermatol. 1999;135(11):1381-5.
24. Scarisbrick J et al. Blood classification and blood response criteria in mycosis fungoides and Sézary syndrome using flow cytometry: Recommendations from the EORTC cutaneous lymphoma task force. Eur J Cancer. 2018;93:47-56.
25. Gibson JF et al. Cutaneous T-cell lymphoma (CTCL): Current practices in blood assessment and the utility of T-cell receptor (TCR)-Vb chain restriction. J Am Acad Dermatol. 2016;74(5):870e7.
26. Vonderheid EC et al. Update on erythrodermic cutaneous T-cell lymphoma: Report of the International Society for Cutaneous Lymphomas. J Am Acad Dermatol. 2002;46(1):95-106.
27. Vonderheid EC et al. Sézary cell counts in erythrodermic cutaneous T-cell lymphoma: Implications for prognosis and staging. Leuk Lymphoma. 2006;47(9):1841-56.
28. Scarisbrick JJ et al. Prognostic significance of tumor burden in the blood of patients with erythrodermic primary cutaneous T-cell lymphoma. Blood. 2001;97(3):624-30.
29. Vidulich KA et al. Overall survival in erythrodermic cutaneous T-cell lymphoma: An analysis of prognostic factors in a cohort of patients with erythrodermic cutaneous T-cell lymphoma. Int J Dermatol. 2009;48(3):243-52.
30. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Available at: https://www.nccn.org/professionals/physician_gls/default.aspx. Last accessed: 7 June 2018.
31. de Masson A et al. Allogeneic stem cell transplantation for advanced cutaneous T-cell lymphomas: A study from the French Society of Bone Marrow Transplantation and French Study Group on Cutaneous Lymphomas. Haematologica. 2014;99(3):527-34.
32. Duvic M et al. Total skin electron beam and non-myeloablative allogeneic hematopoietic stem-cell transplantation in advanced mycosis fungoides and Sézary syndrome. J Clin Oncol. 2010;28(14):2365-72.
33. Duarte RF et al. Allogeneic hematopoietic cell transplantation for patients with mycosis fungoides and Sézary syndrome: A retrospective analysis of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2010;28(29):4492-9.
34. Kashani-Sabet M et al. A modified staging classification for cutaneous T-cell lymphoma. J Am Acad Dermatol. 2001;45(5):700-6.
35. Marti RM et al. Prognostic clinicopathologic factors in cutaneous T-cell lymphoma. Arch Dermatol. 1991;127(10):1511-6.
36. Zackheim HS et al. Prognosis in cutaneous T-cell lymphoma by skin stage: Long-term survival in 489 patients. J Am Acad Dermatol. 1999;40(3):418-25.
37. Diamandidou E et al. Prognostic factor analysis in mycosis fungoides/ Sézary syndrome. J Am Acad Dermatol. 1999;40(6 Pt 1):914-24.
38. Toro JR et al. Prognostic factors and evaluation of mycosis fungoides and Sézary syndrome. J Am Acad Dermatol. 1997;37(1):58-67.
39. Wernham A et al. 542 Changes in blood involvement in Sézary syndrome positively correlate with skin severity but not in mycosis fungoides. J Inv Dermatol. 2018;5:S92.
40. Scarisbrick JJ et al. Prognostic factors, prognostic indices and staging in mycosis fungoides and Sézary syndrome: Where are we now? Br J Dermatol. 2014;170(6):1226-36.
41. Shipp MA. Prognostic factors in aggressive non-Hodgkin's lymphoma: Who has "high-risk" disease? Blood. 1994;83(5):1165-73.
42. Dippel E et al. Cutaneous T-cell lymphoma severity index and T-cell gene rearrangement. Lancet. 1997;350(9093):1776-7.
43. Klemke CD et al. Prognostic factors and prediction of prognosis by the CTCL Severity Index in mycosis fungoides and Sézary syndrome. Br J Dermatol. 2005;153(1):118-24.
44. Evans KG et al. Validity assessment of the cutaneous T-cell lymphoma severity index to predict prognosis in advanced mycosis fungoides/Sézary syndrome. J Am Acad Dermatol. 2011;64(4):682-9.
45. Benton EC et al. A cutaneous lymphoma international prognostic index (CLIPi) for mycosis fungoides and Sézary syndrome. Eur J Cancer. 2013;49(13);2859-68.

Iron Deficiency Anaemia in Pregnancy: Developed Versus Developing Countries

Authors: *Avantika Gupta, Avanthi Gadipudi

Department of Obstetrics and Gynaecology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India
*Correspondence to dravantikagupta@gmail.com

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Abstract

Anaemia is the most widespread of the haematological disorders, affecting about one-third of the global population. Despite decades of public health interventions, anaemia in pregnancy remains a major health problem worldwide, with an estimated 41.8% of pregnant women being diagnosed with anaemia at some point in their gestation. At least half of the cases of anaemia in pregnant women are assumed to be due to iron deficiency, with folate or vitamin B12 deficiency, chronic inflammatory disorders, parasitic infections like malaria, and certain inherited disorders accounting for the remaining cases. A considerable variation has been observed in the incidence and aetiology of iron deficiency anaemia among developed and developing nations, warranting differences in the screening protocols and management strategies used by clinicians in these countries. This article highlights the differences in the management of iron deficiency anaemia among low and high-income countries, with a detailed review of the policies followed in India.

DEFINING ANAEMIA IN PREGNANCY

The definition of iron deficiency anaemia (IDA) in pregnancy is imprecise as a result of pregnancy-induced changes in plasma volume and haematocrit, differences in haemoglobin (Hb) concentration through the trimesters, differences in diagnostic tests, and ethnic variation. According to the World Health Organization (WHO), a pregnant woman is considered to be anaemic if her Hb concentration is <11 g/dL,[1] whereas Centers for Disease Control and Prevention (CDC) guidelines define anaemia as Hb <11 g/dL in the first trimester and <10 g/dL in the second or third trimester.[2] Based on the finding of typically lower Hb and haematocrit levels among black adults, the Institute of Medicine (IOM) recommends lowering the Hb cut-off level by 0.8 g/dL in this population.[3] The British Committee for Standards in Haematology (BCSH)[4] defines anaemia as a Hb value <2 standard deviations below the mean value for a healthy matched population, which amounts to Hb levels <11.0 g/dL in the first trimester, <10.5 g/dL in the second to third trimesters, and <10.0 g/dL in the postpartum period.

Around three-quarters of anaemia-related maternal mortality cases in south Asia occur in India, and hence the Indian Council of Medical Research (ICMR) further classifies anaemia

in pregnancy according to grades of severity to aid in the triage of women requiring early intervention:[5] mild anaemia with Hb of 10.0–10.9 g/dL,moderate anaemia with Hb of 9.9–7.0 g/dL, severe anaemia with Hb of 6.9–4.0 g/dL, and very severe anaemia with Hb of <4.0 g/dL.

PATHOPHYSIOLOGY OF IRON DEFICIENCY ANAEMIA IN PREGNANCY

Physiological or dilutional anaemia of pregnancy is observed in healthy pregnant women as a result of a relatively greater expansion of plasma volume by 30–40% in comparison to a 20–25% raise in Hb mass and erythrocyte volume. This results in a modest decrease in Hb levels, creating a low viscosity state, which promotes oxygen transport to the placenta and fetus. Pregnancy significantly increases the demand for iron to balance the physiological requirements of increased haematocrit, developing the fetoplacental unit, and for losses during delivery and lactation. The Institute of Medicine (IOM) estimated that the total iron loss associated with pregnancy and lactation is approximately 1,000 mg and has recommended a daily dietary allowance for iron in pregnancy of 27 mg instead of the 8 mg required for the nonpregnant adult population.[6] If this recommended daily allowance of iron in pregnancy is not met, it results in women with depleted iron stores developing IDA.

Recent research into iron metabolism in humans has paved the way for the discovery of a novel peptide hormone, hepcidin, that acts as a homeostatic regulator of systemic iron concentration by controlling iron efflux into the plasma. Hepcidin levels in pregnant women are, in general, lower than in nonpregnant healthy women, and decrease as pregnancy advances, with the lowest levels of hepcidin observed in the third trimester.[7] With decreasing expression of hepcidin, there is an increased absorption of dietary iron and increased mobilisation of iron from body stores modulated by ferroportin. Inflammatory states, including pre-eclampsia, malaria infection, and obesity, have been associated with higher hepcidin during pregnancy compared to healthy controls, suggesting that maternal and fetal iron bioavailability could be compromised in these conditions.

INCIDENCE OF IRON DEFICIENCY ANAEMIA IN DEVELOPED VERSUS DEVELOPING COUNTRIES

IDA remains the most common nutritional deficiency globally, with about 32 million pregnant women categorised as anaemic and about 0.75 million pregnant women categorised as severely anaemic.[8]

An analysis of National Health and Nutrition Examination Survey (NHANES) epidemiological data from the USA from 1999–2006 demonstrated an overall prevalence of IDA of nearly 18.0%. Iron deficiency was shown to increase from 6.9% to 14.3% to 28.4% across the three trimesters during pregnancy.[9] A multicentre cross-sectional study in the UK estimated a 24.4% prevalence of maternal anaemia at some stage of the antenatal period.[10]

A systematic analysis of population-representative data of Hb concentration and the prevalence of total and severe anaemia for 1995–2011 reported the prevalence of anaemia in pregnancy as 14.0% in high-income regions and 23.0% in central and eastern Europe.[11] In contrast to these developed countries, about 53.0% of pregnant women in south Asia were diagnosed with anaemia, of whom 3.8% were found to be severely anaemic. Iron-amenable anaemia represented >70.0% of these cases.[11] Insufficient quantity of iron-rich foods, poor environmental sanitation, unsafe drinking water, iron loss due to parasite load (e.g., malaria or intestinal worms), and adolescent anaemia, along with teenage pregnancies and repeated pregnancies in low resource countries, are the predominant causes for the disproportionately increased prevalence of IDA in pregnancy in these nations.

India is one of the countries with the highest prevalence of anaemia in the world. According to the Indian National Family Health Survey, the prevalence of IDA in pregnancy ranges from 23.6–61.4%.[12] The incidence of IDA in India was estimated at 60.0% in the urban population and 69.0% in the rural population, and IDA resulted in approximately 326,000 maternal deaths with an associated disability-adjusted life years of 12,497,000.[13] Diverse cultures, religions, food habits, lifestyles, and traditions pose a challenge to the implementation of various government health programmes in India.

The situation is no different in other Asian countries, like Pakistan, where in 2008 the prevalence of anaemia among pregnant women was estimated at 90.5%; of these women, 75.0% had mild anaemia and 14.8% had moderate anaemia.[14] In the year 2000, a community-based sample of 336 pregnant women in the plains of Nepal showed that 72.6% of pregnant women were anaemic and 88.0% of cases of anaemia were associated with iron deficiency.[15]

MATERNAL AND FETAL EFFECTS OF IRON-DEPENDENT ANAEMIA IN PREGNANCY

The existing literature shows that failure to meet the increased iron requirements in pregnancy may result in adverse maternal and fetal consequences. This is also supported by a comparative quantification of health risks by the WHO, which estimated that about 591,000 perinatal deaths and 115,000 maternal deaths worldwide can be attributed to IDA, either directly or indirectly.[16] Furthermore, it was shown that correcting IDA of any severity reduces the risk of maternal death by about 20% for each 1 g/dL increase in Hb. In light of these findings, newer health policies need to focus on mild-to-moderate anaemia in pregnancy, in addition to severe anaemia, for a greater public health impact.

Iron deficiency in pregnancy causes maternal morbidity with increased risk of abortion, increased susceptibility to infection due to defective macrophage phagocytosis and lymphocyte replication, physical weakness, pre-eclampsia, preterm labour, heart failure, increased risk of postpartum haemorrhage due to impaired myometrial contractility resulting from hypoxia-induced enzymatic and cellular dysfunction, puerperal sepsis, and postnatal depression. According to a study by Lone et al.,[17] anaemia in pregnancy increases the risk of preterm birth 4.0-fold, low birth weight babies 2.2-fold, and low Apgar score in newborn babies 1.8-fold in comparison to nonanaemic women. Maternal iron depletion also reduces fetal iron stores and increases the risk of neonatal anaemia and perinatal morbidity. Correction of iron deficiency has proved to have beneficial effects on both the mother and the fetus. A meta-analysis by Haider et al.[18] demonstrated a dose–response relationship between increasing doses of iron supplements and reduction in the incidence of low birth weight babies.

SCREENING IN DEVELOPED COUNTRIES VERSUS DEVELOPING COUNTRIES

Strategies for anaemia screening may include a routine screening of all expectant mothers or targeted screening based on established risk factors, diagnostic tests, or risk assessment instruments. Though the need for antenatal screening is well established, countries differ from one another in their screening policies and criteria.

The U.S. Department of Veterans Affairs/Department of Defence (VA/DoD) and CDC[2] recommend that all pregnant women should be screened for anaemia at some point during pregnancy, irrespective of their risk stratification. The VA/DoD recommends screening during the first antenatal visit and is against routine repeat screening in asymptomatic pregnant women.[19] In comparison, the American College of Obstetricians and Gynaecologists (ACOG) recommends routine screening of all pregnant women for anaemia and implementing iron therapy if IDA is confirmed.[20]

Australian guidelines recommend the screening of all pregnant women for anaemia at the time of booking and at 28 weeks.[21,22] If the pregnant woman is at risk of thalassaemia or haemoglobinopathy, she will require additional investigations, such as Hb variant analysis and iron studies.

The IOM recommends that screening for anaemia should be reserved for high-risk pregnant women only, who need to be followed up during each trimester and at 4–6 weeks postpartum.[3] The U.S. Preventive Services Task Force concluded that the current evidence is inadequate to assess the balance of benefits and harms of screening for IDA in pregnant women to prevent adverse maternal and fetal outcomes. It also stated that although there is insufficient evidence to prove the superiority of any screening tool, measurement of serum Hb or haematocrit levels may often be considered

as the first step used in primary care practice. The Canadian Task Force on Preventive Health Care, on the other hand, does not have a current recommendation for this topic.[23,24]

With >80% of antenatal women diagnosed with IDA, routine screening of all pregnant women is standard practice in India. Indian National Rural Health Mission (NRHM) guidelines recommend a compulsory Hb estimation for all pregnant women by the cyanmethaemoglobin method or by photocalorimeter at 14–16 weeks, followed by at 20–24 weeks, 26–30 weeks, and 30–34 weeks of pregnancy (a minimum of four Hb estimations). The interval between the Hb estimations should be a minimum of 4 weeks. The trigger point for referral to a more specialised institution would be a Hb level of ≤7 g/dL at 14 weeks, 20–24 weeks, and 26–30 weeks, or a Hb level of ≤9 g/dL at 30–34 weeks.[25]

WORK-UP

Clinical Symptoms and Signs

Symptoms and signs of IDA in pregnancy are usually nonspecific and mimic normal pregnancy changes, unless the anaemia is severe. Fatigue is the most common symptom, followed by varying degrees of pallor, lassitude, headache, palpitations, dizziness, dyspnoea, lack of concentration, and irritability. Pica develops in rare cases.

Full Blood Count, Blood Film, and Red Cell Indices

A complete blood count is usually the primary step in the diagnosis of IDA. It is simple, rapid to perform, inexpensive, and helpful in the early prediction of IDA. A full blood count provides a complete blood picture, showing low Hb, mean cell Hb (MCH), mean cell volume (MCV), and mean cell Hb concentration (MCHC); a peripheral smear with presence of microcytic hypochromic red cells and characteristic 'pencil cells' or anisopoikilocytosis characterises IDA.

Red Cell Distribution Width

Increased red cell distribution width implies variance in the red blood cell (RBC) volume distribution, similar to a peripheral blood smear anisocytosis, and helps in differentiating IDA from thalassaemia and other haemoglobinopathies. The sensitivity and specificity, respectively, of red cell distribution width in the diagnosis of IDA in pregnancy were reported by Sultana et al.[26] as 97.4% and 83.2% and by Tiwari et al.[27] as 72.8% and 82.4%.

Serum Ferritin

Measurement of serum ferritin accurately reflects iron stores and is commonly the first laboratory test to become abnormal as iron stores decrease. Serum ferritin values are not affected by recent iron ingestion and are generally considered the best parameter to assess deficient iron stores in pregnancy. A concentration <15 µg/L indicates iron depletion in all stages of pregnancy. Assessment of serum ferritin in pregnancy takes precedence over other investigations in women with suspected thalassaemia or haemoglobinopathy, in women whose anaemia fails to respond to a 2-week trial of oral iron, in women with multifactorial anaemia, and before any parenteral iron replacement.

Serum Iron, Total Iron Binding Capacity, and Transferrin Saturation

Serum iron is an unreliable indicator of the iron available at the tissue level because of wide fluctuations in serum iron levels with recent ingestion of iron, infection, and diurnal rhythm. Total iron binding capacity is increased with iron deficiency and is an indirect measure of obtainability of iron-binding sites and transferrin levels.

Zinc Protoporphyrin

Zinc protoporphyrin levels increase when iron availability decreases as zinc is incorporated into the protoporphyrin ring instead of iron. Serum zinc protoporphyrin has the advantage of not being influenced by the plasma dilution and hence has greater sensitivity and specificity for iron depletion; however, this test is rarely performed because it is not readily available.

Bone Marrow Iron

A bone marrow sample stained for iron with Prussian blue has been considered the gold standard for assessment of marrow iron stores. However, the invasive nature of this test restricts

its use to the most complicated cases, in which the underlying cause of anaemia cannot be identified by simpler means.

A Trial of Iron Therapy

A trial of iron therapy has simultaneous diagnostic and therapeutic applications in IDA. Microcytic hypochromic anaemia can be assumed to be due to iron deficiency until proven otherwise. A rise in Hb level, if demonstrable by 2 weeks, confirms iron deficiency and is both cost and time-effective. If the patient is at high risk of haemoglobinopathy and the status is unknown, they can be evaluated with ferritin and iron therapy can be started while screening is being performed. If there has been no improvement in Hb by 2 weeks, referral should be made to more specialised centres to consider other causes of anaemia.

WHO and CDC technical guidance is that, in the absence of infection, measuring serum ferritin or serum transferrin receptor in association with Hb provides the best assessment of iron status in the general population.[28] However, in low-resource settings like India, the majority of these tests are either not easily affordable or not available. Therefore, the RBC indices hold great value for primary diagnosis, which can reduce unnecessary investigative costs. Of all the available indices, the Meltzer index (MCV:RBC ratio) has been shown to be the most reliable indicator with the highest sensitivity.[29]

According to Indian National Rural Health Mission guidelines,[25] management of IDA in pregnancy includes:

- Hb estimation by cyanmethemoglobin method using a semiautoanalyser or photocalorimeter, which is mandatory in all institutions.
- Peripheral smear, MCV:RBC ratio, serum iron binding capacity, and Hb electrophoresis performed in medical colleges, District Headquarter hospitals, and other secondary care institutions with facilities for these tests.
- Urine assessment for albumin, sugar, and deposits, and a urine culture if pus cells are detected, to rule out refractory anaemia.

Supplementation and Prophylaxis

All antenatal women must be given advice for dietary modification to improve iron content and its absorption. Iron-rich food items include meat from cattle, fish, and poultry, and legumes and green leafy vegetables. The recommended dietary intake of iron in the second half of pregnancy is 30 mg. Absorption of iron increases 3-fold by the third trimester and the requirement increases from 1–2 mg to 6 mg per day.[30] This increase cannot be taken care of by dietary modification alone and hence results in anaemia during pregnancy in many women. The WHO strongly recommends daily oral iron and folic acid supplementation as a part of antenatal care to reduce the risk of low birth weight babies, maternal anaemia, and iron deficiency. The International Nutritional Anemia Consultative Group (INACG), as well as the WHO, recommends that 60 mg elemental iron has to be given as prophylaxis to all antenatal women in countries where the prevalence of anaemia is >40%.[1]

In the UK and Australia, routine administration of iron supplementation to all pregnant women is not recommended. However, in India, where prevalence is as high as 58%, the Ministry of Health and Family Welfare (MOHFW) recommends iron supplementation in the form of 100 mg elemental iron for 100 days along with 500 µg of folic acid starting from 14–16 weeks. However, the problem with 100 mg elemental iron compared to 60 mg is increased incidence of gastrointestinal side effects like nausea, vomiting, and constipation. For nonanaemic patients with iron deficiency, the dose can be as low as 20–60 mg.

According to a recent Cochrane review,[31] iron supplementation reduces the risk of anaemia in full-term mothers by 70% and the risk of iron deficiency by 57%. However, there was no significant effect on preterm birth and neonatal death. The fetus is relatively protected from the effects of iron deficiency by upregulation of placental iron transport proteins.[31] To improve acceptability of adherence to supplementation, a behavioural change communication strategy should be implemented to communicate the benefits of oral iron supplementation. This is consolidated by the provision of an information leaflet in the patient's language in the UK.

Table 1: Elemental iron content and dose per tablet of oral iron preparations.

Iron salt	Dose per tablet	Elemental iron
Ferrous sulphate	300 mg	60 mg
Ferrous sulphate (dried)	200 mg	65 mg
Ferrous fumarate	322 mg	100 mg
Ferrous gluconate	300 mg	35 mg
Ferrous succinate	100 mg	35 mg

In India, nutrition counselling is provided at antenatal check-ups during the monthly village health and nutrition day. Accredited social health workers are given incentives for providing iron folic acid supplements to patients at their doorstep.

The available ferrous salts include ferrous fumarate, ferrous sulphate, ferrous gluconate, and succinate. The amount of elemental iron varies in each salt and hence the number of tablets to be taken daily varies; for instance, if a woman takes ferrous gluconate she needs to take two tablets per day, as compared to ferrous sulphate or ferrous fumarate (one tablet per day), as shown in Table 1.

Oral iron supplementation should be taken on an empty stomach along with vitamin C-containing food items and women should be informed not to have tea, coffee, or antacids when taking iron tablets.

Supplementation with oral iron is continued for 3 months, and a therapeutic dose of iron is started if the anaemia is not corrected. In populations with endemic hookworm (a prevalence of ≥20–30%), antihelminthic therapy should be given to any patient with severe anaemia because treatment is safer and cheaper than diagnosing a hookworm infection. After the first trimester, mebendazole or albendazole can be safety administered to pregnant women. In India, all pregnant women are given one tablet of 400 mg albendazole at 14–16 weeks. In malaria endemic areas, provision of iron folic acid supplementation should be implemented in addition to measures for prevention, diagnosis, and treatment of malaria.[32]

TREATMENT

National Institute for Health and Care Excellence (NICE), British Society of Haematology (BSH), and Australian Guidelines all recommend a trial of oral iron for 2 weeks in women diagnosed with anaemia during the antenatal period.[4] This treatment can be started at the community level and an adequate rise in Hb is considered to be diagnostic of IDA.[21,22,34] If the haemoglobinopathy state is unknown, iron therapy should be started and a haemoglobinopathy screen should be organised simultaneously. Duration of therapy should be the time until the patient's Hb rises to normal and then a further 3 months or until 6 weeks postpartum in order to replenish stores. If Hb is <10 g/dL in the postpartum period, 100–200 mg elemental iron should be given for a further 3 months. Nonanaemic iron-deficient women should be given 65 mg elemental iron and Hb test should be repeated after 8 weeks.

Supplementation compliance has to be ensured in patients. For example, women should be asked about the colour of their stools, which should be black while on oral iron treatment, or they can be asked to return the empty supplement packaging. Gastrointestinal side effects can be as high as 40% with oral iron therapy, and include nausea and vomiting, constipation, and diarrhoea. Particularly if the mother is near term, the indications of referral to secondary care in the UK are:

- No rise in Hb during 2 weeks of treatment.
- Hb <7 g/dL.
- Symptoms of iron supplementation.
- >34 weeks into pregnancy.

Absorption of oral iron can be increased by taking a source of vitamin C along with the supplement on an empty stomach. Parenteral iron should be considered from the second treatment month onwards in patients with:

- Severe gastrointestinal side effects.
- Intolerance to oral iron malabsorption.
- IDA unresponsive to oral iron.
- Absolute noncompliance, particularly if mother is near term.

Among the various parenteral iron preparations, iron carboxymaltose is the most preferred drug, and there is no need to give a test dose because it is rarely associated with anaphylaxis. This drug can be given as total drug infusion and is available at the concentration of 50 mg/mL of elemental iron. The dose is calculated on the basis of pre-pregnancy or booking weight, aiming for a target Hb of 11 g/dL.

Infused over 15 minutes, 1,000 mg in 20 mL is diluted in 250 mL of 0.9% sodium chloride. The patient is observed for 30 minutes after administration and oral iron is avoided for the next 5 days. Hb testing is repeated after 2–3 weeks and a second dose can be planned if required. A single dose should not exceed 1,000 mg of iron per week. A general practitioner has to be notified if continued iron therapy is needed.

In India, oral iron therapy is initially trialled in all women with Hb ≤9 g/dL. In patients with Hb 7–9 g/dL, parental iron should be considered after 32–34 weeks for an early rise, ensuring 100% compliance. In patients with Hb <7 g/dL, parenteral iron should be considered. The government of India recommends the use of intravenous iron sucrose since it is available readily and cheaper than alternatives.[26] It is available at a concentration of 20 mg/mL and is given as a slow intravenous injection of 100 mg or infusion of 200 mg. The U.S. Food and Drug Administration (FDA) approves a maximum dose of parenteral iron of 600 mg per week. Unlike ferric carboxymaltose, it cannot be given as a total dose infusion. Contraindications for parenteral iron are hypersensitivity to iron or any cause other than IDA.

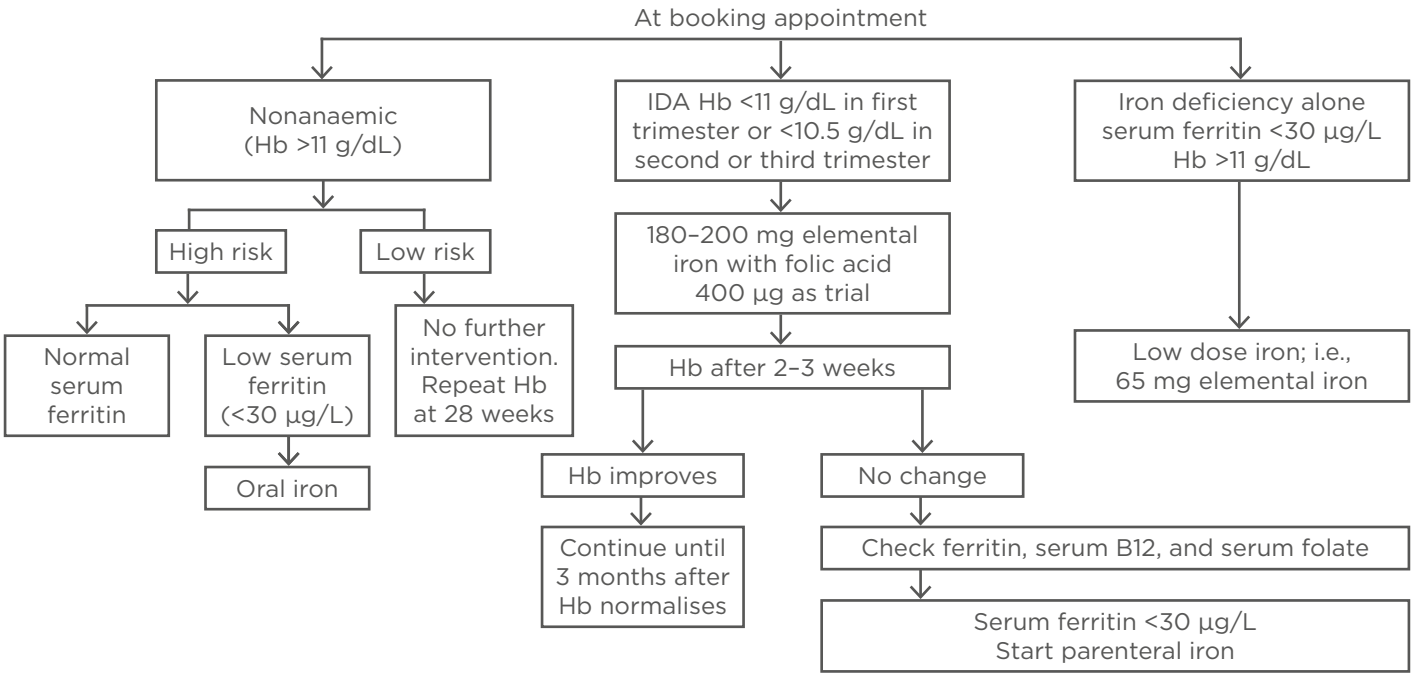

Figure 1: Management of iron deficiency anaemia in pregnancy in accordance to Royal College of Obstetricians and Gynaecologists (RCOG) Green-top Guidelines.

Hb: haemoglobin; IDA: iron deficiency anaemia.

Adapted from Blood Transfusion in Obstetrics: Green-top Guideline No. 47[31]

Among intramuscular preparations, the only one available in the UK is low molecular weight iron dextran. In India, the most popular iron preparation is iron sorbitol citrate complex; however, these injections tend to be painful and cause permanent skin staining. Their use is therefore generally discouraged and no longer recommended in the UK. If one is to be given, the Z-track injection technique should be used.

Women who are still anaemic at the time of birth may require additional precautions, such as:

- Birth in a hospital setting.
- Group and save of blood.
- Active management of the third stage.
- A plan to deal with postpartum haemorrhage.

The need for blood transfusion can arise in the following situations:

- Severe anaemia in the last trimester for immediate improvement in Hb status.
- Severe anaemia with signs of cardiac failure or hypoxia.
- Haemoglobinopathy or bone marrow failure syndromes.
- Acute haemorrhage: if Hb <6 g/dL or if the patient becomes haemodynamically unstable due to ongoing haemorrhage.

The recent Royal College of Obstetricians and Gynaecologists (RCOG) (Figure 1) blood transfusion guideline[33] recommends blood transfusion in labour or the immediate postpartum period if Hb is <7 g/dL. In Western countries, provision of cell salvage should be considered at the time of caesarean section.

CONCLUSION

IDA during pregnancy continues to be a major health problem in the developing world. This warrants certain steps at the individual and community levels, such as education of pregnant women about anaemia, its causes, and health implications. Nutritional education, with a special emphasis on strategies based on locally available foodstuffs, administration of appropriate iron and folate supplements while ensuring maximum compliance, treatment of chronic disease like malaria deworming, and universal antenatal care to pregnant women will help in combatting this serious health hazard. Long-term governmental policies should be directed towards formulation of effective plans to eradicate anaemia in children and adolescent girls. Owing to the significant heterogeneity in screening, diagnosis, and treatment of IDA across nations, more research is needed to understand the clinical effects of routine screening, the ideal screening tools, and the most effective treatment of IDA during pregnancy.

References

1. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. 2011. Available at: http://www.who.int/vmnis/indicators/haemoglobin/en/. Last accessed: 14 June 2018.
2. Centers for Disease Control (CDC). CDC criteria for anemia in children and childbearing-aged women. MMWR Morb Mortal Wkly Rep. 1989;38(22):400-4.
3. Institute of Medicine (US) Committee on the Prevention, Detection, and Management of Iron Deficiency Anemia Among U.S. Children and Women of Childbearing Age, "Iron deficiency anemia: Recommended guidelines for the prevention, detection, and management among US children and women of childbearing age," (1994) Earl R, Woteki CE (eds.), Washington (DC): National Academies Press.
4. Pavord S et al.; British Committee for Standards in Haematology. UK guidelines on the management of iron deficiency in pregnancy. Br J Haematol. 2012;156(5):588-600.
5. Ezzati M et al. Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. The Lancet. 2002;360(9343):1347-60.
6. Trumbo P et al. Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc. 2001;101(3):294-301.
7. Valore EV, Ganz T. Posttranslational processing of hepcidin in human hepatocytes is mediated by the prohormone convertase furin. Blood Cells Mol Dis. 2008;40(1):132-8.
8. Stevens GA et al.; Nutrition Impact Model Study Group (Anaemia). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: A systematic analysis of population-representative data. Lancet Glob Health. 2013;1(1):e16-25.
9. Mei Z et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. Am J Clin Nutr. 2011;93(6):1312-20.
10. Barroso F et al. Prevalence of maternal anaemia and its predictors:

A multi-centre study. Eur J Obstet Gynecol Reprod Biol. 2011;159(1): 99-105.

11. Balarajan Y et al. Anaemia in low-income and middle-income countries. Lancet. 2011;378(9809):2123-35.
12. Lokare PO. A study of prevalence of anemia and sociodemographic factors associated with anemia among pregnant women in Aurangabad city, India. Annals of Nigerian Medicine. 2012;6(1):30-4.
13. Toteja GS et al. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. Food Nutr Bull. 2006;27(4):311-5.
14. Baig-Ansari N et al. Anemia prevalence and risk factors in pregnant women in an urban area of Pakistan. Food Nutr Bull. 2008;29(2):132-9.
15. Dreyfuss ML et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr. 2000;130(10):2527-36.
16. Stoltzfus RJ et al., "Iron deficiency anaemia," Ezzati et al. (eds.), Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors: Volume 1 (2004), Geneva: World Health Organisation.
17. Lone FW et al. Maternal anaemia and its impact on perinatal outcome. Trop Med Int Health. 2004;9(4):486-90.
18. Haider BA et al; Nutrition Impact Model Study Group (anaemia). Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: Systematic review and meta-analysis. BMJ. 2013;346:f3443.
19. O'Brien KO, Ru Y. Iron status of North American pregnant women: An update on longitudinal data and gaps in knowledge from the United States and Canada. Am J Clin Nutr. 2017;106(Suppl 6):1647S-54S.
20. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 95: Anemia in pregnancy. Obstet Gynecol. 2008;112(1):201-7.
21. RANZCOG 2009. Prepregnancy counselling. 2017. Available at: https://www.ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women%27s%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Pre-pregnancy-Counselling-(C-Obs-3a)-review-July-2017.pdf?ext=.pdf. Last accessed: 9 July 2018.
22. RANZCOG 2009. Routine antenatal assessment in the absence of pregnancy complications. 2012. Available at: http://www.testingportal.ashm.org.au/resources/C-Obs_3_(b)_Routine_Antenatal_Assessment_in_the_Absence_of_Pregnancy_Complications_NEW_Mar_13.pdf. Last accessed: 9 July 2018.
23. International Nutritional Anemia Consultative Group (INACG), World Health Organization (WHO), United Nations Childrens Fund (UNICEF), "Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia," Stoltzfus RJ, Dreyfuss ML (eds.), (1998), Washington, DC: Ilsi Press.
24. Siu AL; Preventive Services Task Force. Screening for iron deficiency anemia and iron supplementation in pregnant women to improve maternal health and birth outcomes: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2015;163(7):529-36.
25. Kapil U, Bhadoria AS. National iron-plus initiative guidelines for control of iron deficiency anaemia in India, 2013. Natl Med J India. 2014;27(1):27-9.
26. Sultana GS et al. Value of red cell distribution width (RDW) and RBC indices in the detection of iron deficiency anemia. Mymensingh Med J. 2013;22(2):370-6.
27. Tiwari M et al. Correlation of haemoglobin and red cell indices with serum ferritin in Indian women in second and third trimester of pregnancy. Med J Armed Forces India. 2013;69(1):31-6.
28. World Health Organization (WHO), "Assessing the iron status of populations: Including literature reviews: Report of a joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the assessment of iron status at the population level, Geneva, Switzerland," (2004) 2nd edition.
29. Urrechaga E, Hoffmann JJML. Critical appraisal of discriminant formulas for distinguishing thalassemia from iron deficiency in patients with microcytic anemia. Clin Chem Lab Med. 2017;55(10):1582-91.
30. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr. 2000;72(1 Suppl):257S-64S.
31. Peña-Rosas JP et al. Daily oral iron supplementation during pregnancy. Cochrane Database Syst Rev. 2015;(7):CD004736.
32. Orton LC, Omari AA. Drugs for treating uncomplicated malaria in pregnant women. Cochrane Database Syst Rev. 2008;(4):CD004912.
33. Royal College of Obstetricians and Gynaecologists. Blood Transfusion in Obstetrics: Green-top Guideline No. 47. 2015. Available at: https://www.rcog.org.uk/globalassets/documents/guidelines/gtg-47.pdf. Last accessed: 21 June 2018.
34. NICE. Antenatal care for uncomplicated pregnancies. 2017. Available at: https://www.nice.org.uk/Guidance/cg62. Last accessed: 9 July 2018.

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