VOL 1
ISSUE 1
2022

ISSN 2816-5152 (PRINT)
ISSN 2816-5160 (ONLINE)

# CANADIAN HEMATOLOGY TODAY

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# WELCOME

Dear Canadian Hematology Community,

It is with great pleasure that we welcome and introduce you to the inaugural issue of *Canadian Hematology Today*. As disease management becomes more complex and as we have more therapies in our arsenal, it is becoming even more important to communicate and share best practices and techniques across the clinical community.

We are tremendously proud of the content in this issue and the forthcoming issues in 2022. We are also incredibly grateful to all the authors who have contributed to this journal. Of course, we would be remiss if we didn't also thank all the advertising partners for their support in helping us launch this exciting peer-to-peer initiative.

As the journal continues to grow, we welcome new ideas, new topics and new submissions which can be sent directly to info@catalytichealth.com. The journal's aim is to provide practical and pragmatic real-world content that helps to inform disease management for Canadian clinicians.

We do sincerely hope you enjoy this first issue, and we look forward to your readership and your ideas for future articles as we grow and expand the reach of this publication!

Best wishes,

Peter Anglin, MD  Laurie H. Sehn, MD  Julie Stakiw, MD  Darrell White, MD



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Dr. Kennedy is a Malignant Hematologist at Sunnybrook Health Sciences Centre and Assistant Professor at the University of Toronto. He completed the combined MD/PhD program in Toronto, where his graduate research focused on developing experimental models of hematologic malignancies. Building on this background, he subsequently completed residency training in Internal Medicine and Hematology, also at the University of Toronto, then enrolled in a combined clinical/research malignant hematology fellowship, splitting time between the Princess Margaret Cancer Centre and Brigham & Women's Hospital / Harvard Medical School. From a research standpoint, James is interested in understanding the genetic events that drive the leukemogenic process and how these can be targeted therapeutically. His clinical focus at Sunnybrook Health Sciences Centre encompasses acute leukemia, bone marrow failure and chronic myeloid malignancies.

# NEXT GENERATION SEQUENCING FOR MYELOID MALIGNANCIES – PROGRESS AND PRACTICAL APPLICATIONS

Over the past two decades, next-generation sequencing (NGS) has revolutionized our understanding of the pathogenesis of myeloid neoplasms (MNs) and their clinical management. While traditional Sanger sequencing allows for the interrogation of single loci, NGS enables the parallel sequencing of multiple genomic locations, ranging from targeted sets of genes to the entire genome. Initially, NGS was used predominantly in research, where the ability to interrogate large regions of the genome facilitated the discovery of genes recurrently mutated in myeloid malignancies. Soon thereafter, NGS entered the clinical realm where it is now routinely utilized in diagnosis, prognostication and treatment decision-making. However, the broad availability of clinical NGS comes with a unique set of challenges. Hematologists must interpret complex molecular reports and appropriately apply the provided mutational information to their patients' care in real-time. Consequently, a systematic approach to interpreting NGS reports is crucial; the following will outline one such framework.

*1) Understand the range of genetic alterations detectable by your panel*

A detailed understanding of the mutational landscape of MNs has emerged over the past 20 years. Whole genome and exome sequencing of patient samples has led to the discovery of a set of ~40 genes recurrently mutated in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN). Importantly, these genes can be organized into a limited number of biological categories, highlighting the key cellular processes whose deregulation drives pathologic myelopoiesis: RNA splicing, epigenetic regulation, the cohesin complex, transcription factors, the DNA damage response, and signal transduction (**Table 1**).[1,2]

By focusing on these recurrently mutated genes, targeted 'myeloid' NGS panels have been developed. The Association of Molecular Pathology has proposed a minimum gene list for chronic myeloid neoplasms (**Table 1, bold genes**).[3] However, the content of myeloid panels can vary with respect to the specific genes included as well as their covered regions (i.e.: hotspot vs. complete coding sequence). Early generation myeloid panels may not contain genes whose relevance to MNs has emerged more recently, such as *PPM1D* (implicated in therapy-related MNs)[4] and *DDX41* (implicated in familial MDS/AML).[5] NGS platforms can also differ from a technical standpoint, influencing their sensitivity and the types of variants that can be detected. For example, by using RNA as a starting material, some panels can detect reciprocal gene rearrangements, such as *PML-RARA, RUNX1-RUNX1T1* and *CBFB-MYH11*, which have previously required standalone RT-PCR based assays or cytogenetics/FISH for their detection.[6]

To assist clinicians, NGS reports contain a wealth of information including the genomic regions being interrogated, the assay technology, the bioinformatic pipeline, as well as the types of genetic alterations that can be detected with their associated

| Molecular category | Genes |
|---|---|
| Splicing factors | ***SF3B1, SRSF2, U2AF1, ZRSR2*** |
| Epigenetic regulation | |
| DNA methylation | ***DNMT3A, TET2, IDH1/2*** |
| Histone methylation | ***ASXL1, EZH2, BCOR, BCORL1,*** *KMT2A,* ***SETBP1*** |
| Cohesin subunits | ***STAG2, RAD21, SMC1A, SMC3*** |
| Transcription factors | ***RUNX1, ETV6, CEBPA,*** *CUX1, GATA2,* ***PHF6*** |
| Signal transduction | |
| JAK-STAT | ***JAK2, CALR, MPL, CSF3R*** |
| RAS | ***KRAS, NRAS, PTPN11, CBL, NF1,*** *GNAS, BRAF* |
| Other | ***FLT3, KIT*** |
| DNA repair | ***TP53, PPM1D*** |
| Miscellaneous | ***NPM1,*** *DDX41, ETNK1* |

***Table 1.*** *Recurrently mutated genes in myeloid malignancies. Bolded genes are part of the Association of Molecular Pathology recommended minimum gene list for chronic myeloid malignancies; adapted from McClure et al, 2018*

sensitivity limits. Familiarity with these technical details is important for clinicians in order to fully appreciate the strengths and limitations of the NGS platform in use, and how this may impact the variants that are ultimately reported.

*2) Review the reported variants and evidence supporting their pathogenicity*

Though practices vary, molecular labs follow general guidelines for the reporting of mutations.[7] Genetic variants are listed using Human Genome Variation Society (HGVS) nomenclature (**Table 2**).[8] The detected variants can range from benign germline polymorphisms, to pathogenic driver mutations, to incidental passenger mutations lacking a discernible impact on leukemogenesis. Given this complexity, evidence-based variant annotation performed by molecular diagnostics specialists is a critical upstream analytical step.

For MNs, the ideal method to distinguish between tumor-associated alterations and germline changes is to compare mutation patterns in skin fibroblasts to those present in the blood. However, such analysis is usually limited to the investigation of inherited predisposition syndromes. Instead, probable germline polymorphisms are identified using data from large databases that have pooled genetic information from healthy populations, such as the Genome Aggregation Database (gnomAD).[9] In practice, variants with greater than 1% frequency in the general population are presumed to represent germline polymorphisms and are filtered out prior to clinical reporting. Certain variant allelic frequencies (VAF) can also be suggestive of a germline alterations (i.e.: 40-60% for assumed heterozygosity); however, VAF is not a fully reliable estimate of zygosity as it can be influenced by copy number as well as the relative proportion of the mutant cell clone.[11] Myeloid NGS panels, though primarily focused on the detection of somatic variants, do include genes such as *TP53, RUNX1, GATA2, CEBPA* and *DDX41* whose alteration in the germline can predispose to the development of MNs.[5,10] Identification of such variants in the blood of patients with a suggestive family/clinical history should prompt sequence analysis of skin fibroblasts for confirmation of germline status and genetic counselling.

A second challenge centers around evaluation of the pathogenicity of detected variants. In general, this is performed by pooling evidence from sources including large scale cancer databases (i.e.: The Catalogue of Somatic Mutations in Cancer, COSMIC)[12], healthy population databases (i.e.: gnomAD), clinically annotated mutation databases (i.e.: ClinVar), in silico tools that predict the functional consequences of a given mutation (i.e.: SIFT) and primary scientific literature.[11] An evidence-based tiered system for categorizing variants is in broad use (**Table 3**), and facilitates the identification of variants of clinical significance for hematologists.[7]

*3) Clinical application of the provided mutational data*

***Diagnosis***: The 2016 World Health Organization (WHO) MN diagnostic criteria rely heavily on CBC parameters, morphologic assessment of the bone marrow (BM) and cytogenetics, with a relatively smaller role for gene mutations.[13] Though a common cadre of genes are mutated in MNs, disease-defining genetic alterations are rare, with certain notable exceptions. Activating mutations in *JAK2* are present in ~99% of polycythemia vera cases, and the majority of patients with essential thrombocythemia and

| Variant Type | Example | Breakdown | Description |
|---|---|---|---|
| Substitution | JAK2 (NM_004972.3) c.1849G>T<br>p.(Val617Phe) | *Gene:* JAK2<br>*Transcript ID:* NM_004972.3<br>*cDNA change:* c.1849G>T<br>*Amino acid change:* p.(Val617Phe) | cDNA nucleotide 1849 (G) changed to T<br>Amino acid 617 (Val) changed to Phe |
| Nonsense | TET2 (NM_001127208.2) c.5298C>G<br>p.Tyr1766* | *Gene:* TET2<br>*Transcript ID:* NM_001127208.2<br>*cDNA change:* c.5298C>G<br>*Amino acid change:* p.(Tyr1766*) | cDNA nucleotide 5298 (C) changed to G<br>Amino acid 1766 (Tyr) changed to stop codon |
| Insertion - frameshift | CALR (NM_004343.3)<br>c.1154_1155insTTGTC<br>p.(Lys385Asnfs*47) | *Gene:* CALR<br>*Transcript ID:* NM_004343.3<br>*cDNA change:* c.1154_1155insTTGTC<br>*Amino acid change:* p.(Lys385Asnfs*47) | Insertion of TTGTC between cDNA positions 1154 & 1155<br>Lys385 changed to Asn & reading frame altered with a stop codon 47 amino acids later |
| Deletion - frameshift | EZH2 (NM_004456.4) c.928delA<br>p.Thr310Leufs*11 | *Gene:* EZH2<br>*Transcript ID:* NM_004456.4<br>*cDNA change:* c.928delA<br>*Amino acid change:* p.(Thr310Leufs*11) | Deletion of A at cDNA position 928<br>Thr310 changed to Leu & reading frame altered with a stop codon 11 amino acids later |
| Duplication – in-frame | SRSF2 (NM_001195427.1)<br>c.281_283dupGCC<br>p.(Arg94dup) | *Gene:* SRSF2<br>*Transcript ID:* NM_001195427.1<br>*cDNA change:* c.281_283dupGCC<br>*Amino acid change:* p.(Arg94dup) | Duplication of cDNA nucleotides 281-283 (GCC)<br>Duplication of amino acid 94 (Arg) |
| Deletion – in-frame | CALR (NM_004343.3) c.1191_1199del<br>p.(Glu398_Asp400del) | *Gene:* CALR<br>*Transcript ID:* NM_004343.3<br>*cDNA change:* c.1191_1199del<br>*Amino acid change:* p.(Glu398_Asp400del) | Deletion of 9 nucleotides between cDNA positions 1191 & 1199<br>Deletion of amino acids 398 to 400 |
| Splice site | CBL NM_005188.3 c.1096-1G>C | *Gene:* CBL<br>*Transcript ID:* NM_005188.3<br>*cDNA change:* c.1096-1G>C | cDNA nucleotide 1096 is the start of exon 8. In the corresponding genomic sequence, 1 nucleotide prior to nt 1096 (the -1 position) is part of the splice acceptor site (AG). Substitution of G to C disrupts the splice acceptor site, resulting in deletion of exon8.[43] |

***Table 2.*** *Variant nomenclature examples; adapted from den Dunnen et al, 2016*

| Tier | | Level of Evidence | Description |
|---|---|---|---|
| I | Variants of Strong Clinical Significance | A | Included in professional guidelines related to disease diagnosis, prognosis and/or therapy<br>Targeted by an FDA-approved therapy |
| | | B | Described in well-powered studies with consensus from experts in the field |
| II | Variants of Potential Clinical Significance | C | Described in multiple small, published studies with some consensus<br>Targeted by either FDA-approved therapies in different tumor types or investigational therapies |
| | | D | Described in preclinical trials or a few case reports without consensus |
| III | Variants of Unknown Clinical Significance | | Not observed at significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases<br>No convincing published evidence of cancer association |
| IV | Benign or Likely Benign Variants | | Observed at significant allele frequency in the general or specific subpopulation databases<br>No existing published evidence of cancer association |

***Table 3.*** *A four-tiered system to categorize somatic sequence variations – based on consensus recommendations from the Association for Molecular Pathology; adapted from Li et al, 2016*

myelofibrosis carry a driver mutation in one of *JAK2, CALR* or *MPL*.[14] In the pediatric realm, more than 90% of cases of juvenile myelomonocytic leukemia carry an activating mutation in genes involved in RAS pathway regulation (*KRAS, NRAS, PTPN11, CB*L or *NF1*), leading to their incorporation into its diagnostic criteria.[13] Lastly, mutations in the splicing factor *SF3B1* are enriched in MN patients with ringed sideroblasts (RS).[15] The specificity of this association is reflected in the WHO diagnostic criteria for MDS-RS, where, in the presence of cytopenias and dysplasia, detection of an *SF3B1* mutation can establish this diagnosis when RS comprise as few as 5% of all nucleated erythroid cells, compared to the traditional cutoff of 15%.[13]

Conversely, the majority of genes recurrently mutated in myeloid malignancies are not specific to a particular disease entity; for example, *TET2* mutations are prevalent in AML, MDS, MPNs and MPN/MDS overlap syndromes.[16] Complicating matters further, recurrent somatic mutations in MN-associated genes have been identified in the blood of individuals without hematologic disease.[17–19] These mutations are a strong independent predictor for the future development of MNs. However, the absolute risk of malignant transformation is low, approximately 0.5-1% per year, leading to this entity being termed "clonal hematopoiesis of indeterminate potential" (CHIP).[20] Thus, clonality, as defined by the presence of MN-associated somatic mutations, should not be considered as definitive evidence of a frank hematologic malignancy in the absence of supporting CBC alterations or BM pathology.

***Prognosis***: Given the central pathogenic role of gene mutations in MNs, it follows that they have the potential to provide insight into disease risk. The European Leukemia Net (ELN) has proposed a risk stratification schema for AML based on cytogenetic and NGS findings (**Table 4A**).[21] Biallelic mutations in *CEBPA* or *NPM1* alterations confer a favorable prognosis, whereas mutations in *ASXL1, RUNX1, TP53* and *FLT3-ITD* (particularly at a high allelic ratio), confer an adverse risk, prompting clinicians to consider consolidative allogeneic stem cell transplant (allo-SCT) in eligible patients.

| Risk category | Genetic abnormality |
|---|---|
| Favorable | t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*<br>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*<br>Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*$^{low*}$<br>Biallelic mutated *CEBPA* |
| Intermediate | Wildtype *NPM1* without *FLT3-ITD* or with *FLT3-ITD*$^{low**}$<br>Mutated *NPM1* with *FLT3-ITD*$^{high}$<br>t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*<br>Cytogenetic abnormalities not classified as favorable or adverse |
| Adverse | t(6;9)(q23;q34.1); *DEK-NUP214*<br>t(v;11q23.3); *KMT2A* rearranged<br>t(9;22)(q34.1;q11.2); *BCR-ABL1*<br>inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM (EVI1)*<br>-5 or del(5q); -7; -17/abn(17p)<br>Complex or monosomal karyotype§<br>Wildtype *NPM1* with *FLT3-ITD*$^{high}$<br>Mutated *RUNX1*¶<br>Mutated *ASXL1*¶<br>Mutated *TP53* |

***Table 4A.*** *ELN AML risk stratification; adapted from Döhner et al, 2016.*
** Allelic ratio, calculated as FLT3-ITD/FLT3-wildtype; low < 0.5; high ≥ 0.5*
*** without adverse-risk genetic lesions*
*§ Complex cytogenetics: 3 or more unrelated chromosomal abnormalities; Monosomal karyotype: 1 single monosomy in association with at least 1 additional monosomy or chromosomal abnormality*
*¶ Unless occur with favorable-risk AML subtypes*

NGS-based risk stratification is also emerging for chronic myeloid malignancies. Mutations in *ASXL1, EZH2, IDH1/2, SRSF2* and *U2AF1* (at Q157) define a high molecular risk group in myelofibrosis,[22,23] and are integrated alongside traditional risk factors in prognostic scoring systems such as the MIPSS70[24] and MIPSS70 plus version 2.0[25] (**Table 4B/C**) which strive to identify patients where allogeneic SCT should be considered. Similarly, for MDS, several groups have developed scoring systems that improve upon the traditional International Prognosis Scoring System (IPSS) and IPSS-R by integrating mutational data.[26–28] While the exact molecular features of these scoring systems vary, common themes have emerged. For example, a higher absolute number of mutated genes as well as TP53 alterations (particularly bi-allelic) confer negative prognostic impact,[29] while SF3B1 alterations are generally associated with lower risk disease, though this can be modulated by co-mutation.[28] A notable strength of these novel scoring systems is that instead of simply classifying patients into broad categories, personalized outcome predictions are generated for each patient, enabling a more refined estimate of disease risk.

***Therapy*:** The genetic profile of a MN can also provide key information regarding responsiveness to therapy. For example, IDH1/2 mutant AML blasts have an intrinsically lower apoptotic threshold, rendering them particularly sensitive to depletion of the anti-apoptotic protein, BCL2.[30] Consequently, IDH mutant AMLs are highly responsive to therapeutic regimens containing the BCL2 inhibitor venetoclax.[31,32] In MDS, there has been much interest in using molecular data to predict responsiveness to hypomethylating agents (HMA). In some studies, TET2 mutations have predicted a favorable treatment response, particularly among individuals where it is an early, clonal mutation.[33–35] However, in a recent study using a machine-learning approach, no single or combination of gene mutations predicted HMA responsiveness; instead, eight genomic combinations predicting HMA resistance were identified. While further validation is required, such analyses

| Risk factor | Score |
|---|---|
| Hemoglobin < 100 g/L | 1 |
| Leukocytes > 25 x $10^9$/L | 2 |
| Platelets < 100 x $10^9$/L | 2 |
| Circulating blasts ≥ 2% | 1 |
| Constitutional symptoms* | 1 |
| MF fibrosis grade ≥2 | 1 |
| HMR category§ | 1 |
| Absence of CALR type 1 mutation | 1 |
| 2 or more HMR mutations | 2 |

| Risk group | Overall score | Median OS |
|---|---|---|
| Low | 0-1 | 27.7 years |
| Intermediate | 2-4 | 7.1 years |
| High | ≥ 5 | 2.3 years |

***Table 4B.*** *Myelofibrosis - MIPSS70; adapted from Guglielmelli et al, 2018*
** Weight loss >10% of baseline in the year before diagnosis, unexplained fever or excessive sweats persisting for more than 1 month.*
*§ HMR category: mutation in any one of ASXL1, EZH2, SRSF2 or IDH1/2*

| Risk factor | Score |
|---|---|
| Anemia | |
| 80-99 g/L (women); 90-109 g/L (men) | 1 |
| < 80 g/L (women); < 90 g/L (men) | 2 |
| Circulating blasts ≥ 2% | 1 |
| Constitutional symptoms* | 2 |
| HMR category§ | 2 |
| Absence of CALR type 1 mutation | 2 |
| 2 or more HMR mutations | 3 |
| *Cytogenetics*¶ | |
| *Unfavorable* | 3 |
| Very high risk | 4 |

| Risk group | Overall score | Median OS |
|---|---|---|
| Very low | 0 | Not reached |
| Low | 1-2 | 16.4 years |
| Intermediate | 3-4 | 7.7 years |
| High | 5-8 | 4.1 years |
| Very high | ≥ 9 | 1.8 years |

***Table 4C.*** *Myelofibrosis - MIPSS70 Plus version 2.0; adapted from Tefferi et al, 2018*
** Weight loss >10% of baseline in the year before diagnosis, unexplained fever or excessive sweats persisting for more than 1 month*
*§ HMR category: mutation in any one of ASXL1, EZH2, SRSF2, IDH1/2 or U2AF1 at amino acid Q157*
*¶ Cytogenetic classification as per reference 44*
*- Favorable: Normal karyotype; Sole 20q−; Sole 13q−; Sole +9; Sole sex chromosome abnormality; Sole chromosome 1 translocation/ duplication*
*- Unfavorable: Sole +8; Sole 7q−; Sole translocations not involving chromosome 1; Two abnormalities not including a VHR abnormality; Single/multiple 5q− abnormalities; Complex karyotype without a VHR abnormality; Monosomal karyotype without a VHR abnormality; Sole abnormalities not otherwise classified*
*- Very high risk: Single/multiple monosomy 7; Single/multiple inv(3)/3q21 abnormalities; Single/multiple i(17q) abnormalities; Single/ multiple 12p−/12p11.2 abnormalities; Single/multiple 11q−/11q23 abnormalities; Single/multiple autosomal trisomies other than +8 or +9 (e.g., +21, +19)*

highlight the power of evaluating gene mutations, not in isolation, but in networks to discern their clinical relevance.

Uncovering the mutational landscape of MNs has also fueled the development of novel therapies that target specific gene mutations. The FLT3 inhibitors midostaurin and gilteritinib have emerged as efficacious therapies for newly diagnosed and relapse/ refractory (R/R) FLT3-mutant AML, respectively.[36,37] Similarly, ivosidenib and enasidenib have shown promising results for IDH1 and IDH2 mutant R/R AML.[38,39] Additional targeted therapies are currently at early stages of development. For example, eprenetapopt, a small molecule that restores wildtype p53 function to cells bearing TP53 mutations, is currently under study in TP53 mutant MNs.[40] The spliceosome inhibitor H3B-8800 is being evaluated in early phase clinical trials, hoping to exploit the inherent vulnerability of cells with heterozygous splicing factor mutations to further inhibition of the splicing machinery.[41,42] Together, these therapies portend an exciting future where NGS will inform personalized therapeutic approaches in patients with MNs.

## *Conclusion*

The advent of NGS technology has revolutionized our understanding of MN pathogenesis while offering significant potential for clinical application. As evidence continues to accumulate highlighting its utility, physicians must learn to integrate this information into routine practice. In addition to keeping abreast of the ever-expanding literature in this field, a working understanding of the technical and bioinformatic details pertaining to the sequencing platform in use is also required. As clinicians continue to gain familiarity with NGS, the future is extremely bright, as molecular profiling will be central to ongoing efforts to provide personalized care to patients through individualized predictions of disease risk and tailored therapeutic regimens.

*Acknowledgements:* The author would like to thank Dr. Marta Davidson for critical comments on the manuscript.

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## MANAGEMENT OF FOLLICULAR LYMPHOMA AT FIRST RELAPSE

Follicular lymphoma (FL) is the most common subtype of indolent B-cell non-Hodgkin's lymphoma (NHL). Histologically, it is subcategorized as grade 1, 2, 3A or 3B[1]. FL, grade 3B is considered an aggressive form of the disease and is managed similar to diffuse large B-cell lymphoma (DLBCL). The intent of this article is to discuss the management of FL at first relapse. However, the knowledge of upfront management strategy is crucial in planning treatment in the event of a relapse.

FL lymphoma is an incurable disease except for small subset patients with limited stage disease (Stage I/II); with local radiotherapy, these patients may attain a 50 to 70% chance of cure[2]. For those with advanced stage disease (Stage III/IV), upfront management strategies include a wait and watch (WW) approach[3–6], monotherapy with rituximab[7] or a combination of anti-CD20 monoclonal antibodies and systemic chemotherapy/oral agents. WW and monotherapy with rituximab are typically pursued for patients with stage III/IV (including extensive limited stage not amenable to radiation) who are asymptomatic and do not meet criteria for treatment.

For patients meeting the indications for upfront treatment, several options are available that combine anti-CD20 monoclonal antibodies with either systemic chemotherapy or oral agents (lenalidomide). R-CHOP (rituximab, cyclophosphamide, vincristine, prednisone)[8], R-CVP (rituximab, cyclophosphamide, vincristine, prednisone)[9] and R-FM (rituximab, fludarabine, mitoxantrone)[10] have been widely used for upfront management of FL. Both R-CHOP and R-FM have demonstrated similar outcomes but superior 3-year progression free survival (PFS) and time to treatment failure (TTF) compared to R-CVP[11]. Trials comparing bendamustine and rituximab (BR) to R-CHOP and/or R-CVP show superior PFS and lesser toxicities with the BR[12,13] regimen. Therefore, BR is, the most preferred choice for upfront treatment of patients with FL, grades 1 to 2. Some centers have extrapolated the results of the STiL and BRIGHT trials to include FL, grade 3A (who were excluded in both of these studies) whereas some offer R-CHOP therapy to this subset of patients. The current body of data does not support upfront stem cell transplantation (SCT) following induction chemo-immunotherapy[14]. Instead, following upfront systemic therapy, maintenance rituximab (MR) is pursued for patients who attain a complete response (CR) or partial (PR) response to induction therapy based on improved PFS[15]. It should be noted that there are currently no definitive studies demonstrating an OS benefit with MR and there is a paucity of prospective data to support the use of MR versus observation following BR, however several retrospective studies support MR following BR[16].

The management of patients with untreated FL continues to evolve. In a multicenter, international, phase 3 superiority trial to evaluate rituximab plus lenalidomide, as compared with rituximab plus chemotherapy, in patients with previously untreated follicular lymphoma, patients were randomly assigned to receive one of the two regimens, followed by

maintenance monotherapy with rituximab. Lenalidomide plus rituximab (R2) when compared to R-chemotherapy (BR, R-CHOP, R-CVP) showed similar 3-year PFS between the two groups with the interim 3-year rate of progression-free survival being 77% (95% CI, 72 to 80) and 78% (95% CI, 74 to 82), in the R2 group compared with the R-chemotherapy group[17] making a new chemo-free treatment option available for patients with FL, grades 1 to 3A (**Table 1)**. This R2 regimen has not been approved for frontline use as the trial was not powered to show non-inferiority.

A novel anti-CD20 monoclonal antibody, obinutuzumab (O), is now available in the first-line management of FL. A study from 2017 compared O-chemotherapy (BO, O-CHOP, O-CVP) followed by O-maintenance (MO) to R-chemotherapy (BR, R-CHOP, R-CVP) followed by MR in treatment of FL, grades 1-3A and demonstrated a significantly better 3-year PFS in the O-chemotherapy group with the estimated 3-year rate of progression-free survival at 80.0% in the O-chemotherapy group compared with 73.3% in the R-chemotherapy group (hazard ratio for progression, relapse, or death, 0.66; 95% confidence interval [CI], 0.51 to 0.85; P=0.001)[18]. Lenalidomide with obinutuzumab (GALEN) also appears to show efficacy in an upfront setting[19].

Treatment at first relapse is determined by numerous factors including the patient's age, performance status, evidence of histologic transformation, first-line approach, type of monoclonal antibody received, whether a maintenance regimen was pursued and the time to first relapse. Among these variables, age and performance status allow for an assessment of eligibility for high dose systemic therapy/ SCT following second-line treatment. The time to relapse is also a critical

| Variable | Rituximab-Lenalidomide Group (N= 513) | Rituximab-Chemotherapy Group (N=517) | Hazard Ratio (95%= CI) | P Value |
|---|---|---|---|---|
| **Response status at 120 weeks, as assessed by independent review committee** | | | | |
| Overall response - no. (% [95% CI]) | 312 (61 [56-65]) | 336 (65 [61-69]) | | |
| Confirmed or unconfirmed complete response - no. (%[95% CI]) | 247 (48 [44-53]) | 274 (53 [49-57]) | | 0.13 |
| Complete response, confirmed - no. (%) | 142(28) | 169 (33) | | |
| Complete response, unconfirmed - no. (%) | 105 (20) | 105 (20) | | |
| Partial response - no. (%) | 65 (13) | 62 (12) | | |
| Stable disease - no. (%) | 2(<1) | 0 | | |
| Progressive disease or death - no. (%)* | 87 (17) | 79 (15) | | |
| Not evaluated or data missing - no. (%) | 112 (22) | 102(20) | | |
| **Response status at 120 weeks, as assessed by investigator** | | | | |
| Overall response - no. (% [95% CI]) | 335 (65 [61-69]) | 353 (68 [64-72]) | | |
| Confirmed or unconfirmed complete response - no. (%[95% CI) | 283 (55 [51-60]) | 299 (58 [53-62]) | | 0.38 |
| Complete response, confirmed - no. (%) | 201 (39) | 242(47) | | |
| Complete response, unconfirmed - no. (%) | 82(16) | 57(11) | | |
| Partial response - no. (%) | 52(10) | 54(10) | | |
| Stable disease - no. (%) | 0 | 0 | | |
| Progressive disease or death - no. (%)* | 90(18) | 94(18) | | |
| Not evaluated or missing -no. (%) | 88(17) | 70(14) | | |
| Progression-free survival at 3 years | | | | |
| Rate, as assessed by independent review committee - %(95% CI) | 77(72-80) | 78(74-82) | 1.10 (0.85-1.43) | 0.48 |
| Rate, as assessed by investigator - % (95% CI) | 77 (72-80) | 78(74-81) | 0.94 (0.73-1.22) | 0.63 |
| **Overall survival rate at 3 years - % (95% CI)** | 94(91-96) | 94(91-96) | 1.16 (0.72-1.861 | |

***Table 1.*** *Efficacy (Intention-to-Treat Population); adapted from Morschhauser F, 2018.*

determinant as patients who relapse within 2 years of initial therapy tend to have poorer overall outcomes requiring the consideration of more aggressive salvage therapies[20,21].

Alternate combination chemotherapy is usually the treatment of choice at relapse. Bendamustine as a second-line treatment option for patients without prior exposure to bendamustine may be considered provided there is no histologic transformation. In a study from 2010, 161 patients were enrolled with a median of 2 previous chemotherapy regimens. Histologies included follicular (68%), small lymphocytic (20%), marginal zone (11%), and lymphoplasmacytic (1%) lymphoma. Sixty patients (34.1%) were refractory to their last chemotherapy, 53 (30.1%) were alkylating agent refractory. The overall response rate (ORR) was 76% with a median 10-month duration of response[22]. Considering monoclonal antibodies are widely available, bendamustine can be combined with rituximab (if not refractory) or obinituzumab (if refractory to rituximab). The use of BR in the treatment of patients with relapsed indolent or mantle cell lymphoma (excluding rituximab refractory patients) produced superior median PFS with BR compared to fludarabine-rituximab (FR) (54.5 months versus 22.9 months, respectively, p= 0.01)[23]. For patients who are rituximab-refractory, bendamustine may be combined with obinutuzumab (BO) based on the outcomes seen in the GADOLIN trial that included patients with indolent B-cell NHL, including FL, grades 1 to 3A[24]. In this study, patients were randomized to receive either BO followed by MO or to bendamustine monotherapy. After a median observation time of 32.6 months (range 0.4 to 65.9) in the obinutuzumab plus bendamustine group and 29.3 months (0 to 65.1) in the bendamustine monotherapy group, progression-free survival was significantly longer with obinutuzumab plus bendamustine (median 25.3 [95% CI 17.4 - 36 months) than with bendamustine monotherapy (14 months [11.3-15.3]; hazard ratio 0.52 [95% CI 0.39-0.69]; p=0.0001). It also showed an OS benefit in the obinutuzumab-arm (Not estimate able versus 53.9 months, p= 0.0061)[24]. Another study recently compared the efficacy of bendamustine in combination with ofatumumab, a second generation anti-CD20 antibody, to bendamustine monotherapy in patients with rituximab-refractory indolent NHL (including FL, grades 1-3A)[25]. Unlike the results seen in the GADOLIN trial, this study showed no benefit to the addition ofatumumab to bendamustine with median IRC-assessed PFS at 16.7 and 13.8 months in the combination and monotherapy arms respectively [hazard ratio (HR) = 0.82; P = 0.1390]. Additionally, the median overall survival (OS) was 58.2 and 51.8 months in the combination and monotherapy arms respectively (HR = 0.89, P = 0.4968). For patients who had already received BR as initial therapy but were not refractory, retreatment with BR may be a reasonable approach at the time of first relapse. Both the StilNHL2 and GADOLIN trials allowed retreatment with bendamustine in the relapse setting if patients were deemed to have been responsive to bendamustine. However, further research is needed to better understand the cumulative long-term effects of re-exposure to bendamustine, and as a result, retreatment is rare.

Given that many patients may have received first-line BR followed by MR, many clinicians choose alternate second-line options such as CHOP, CVP or lenalidomide in combination with rituximab or obinutuzumab (depending on rituximab-refractory status). A small phase II study showed a median time to progression of approximately 47 months for patients with relapsed FL treated with RCHOP[26]. The CALGB 50401 trial comparing lenalidomide with rituximab (LR) to lenalidomide alone (L) showed that LR produced a superior median time to progression (TTP) compared to L alone (2 years versus 1.1 years, respectively)[27]. The same group published results from the AUGMENT trial in which patients with recurrent iNHL (including FL, grades 1 to 3A) were randomized to either the LR arm or the placebo-rituximab arm. The results from this study showed superior PFS in the LR arm compared to the placebo-rituximab arm (**Figure 1**) with a secondary analysis showing favorable OS for FL patients who received LR (hazard ratio 0.45, p= 0.02)[28]. It should be noted that this subgroup analysis was not powered to assess definite OS benefit. Lenalidomide-based combinations have not yet received regulatory approval from Health Canada.

Lenalidomide in combination with obinutuzumab (LO) has also been studied in patients with recurrent FL, grades 1 to 3A. A phase II trial treated recurrent FL patients with LO followed by 1 year of L and 2 years of MO and showed an ORR at the end of induction in the 86 evaluable patients of 79% (95% CI 69–87) with 38% of subjects achieving a CR (95% CI 28–50)[29].

In the event of evident transformed relapsed FL, a CHOP regimen (with R) would be standard for DLBCL[30–32] histology and dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) (with R) may be used if the histology shows high-grade B-cell lymphoma with double or triple hit gene rearrangements[33]. Management of these patients however becomes challenging if the patients experience transformed FL after initial treatment with R-CHOP. In this scenario such patients may be managed with salvage combination agents utilized in the management of DLBCL such as GDP (gemcitabine, dexamethasone,

cisplatin)[34], ICE (ifosfamide, carboplatin, etoposide)[35], DHAP (dexamethasone, high dose cytarabine, cisplatin)[34,35] with or without monoclonal antibodies. In patients who have experienced transformed FL after initial treatment with R-CHOP, salvage therapy followed by ASCT may be considered.

Following systemic second-line chemo-immunotherapy, further consolidative strategies may be pursued such as SCT or maintenance therapy if patients are deemed to be eligible. If patients are candidates for stem cell transplant therapy, then autologous stem cell transplant (ASCT) or allogeneic stem cell transplant (alloSCT) is considered-- especially

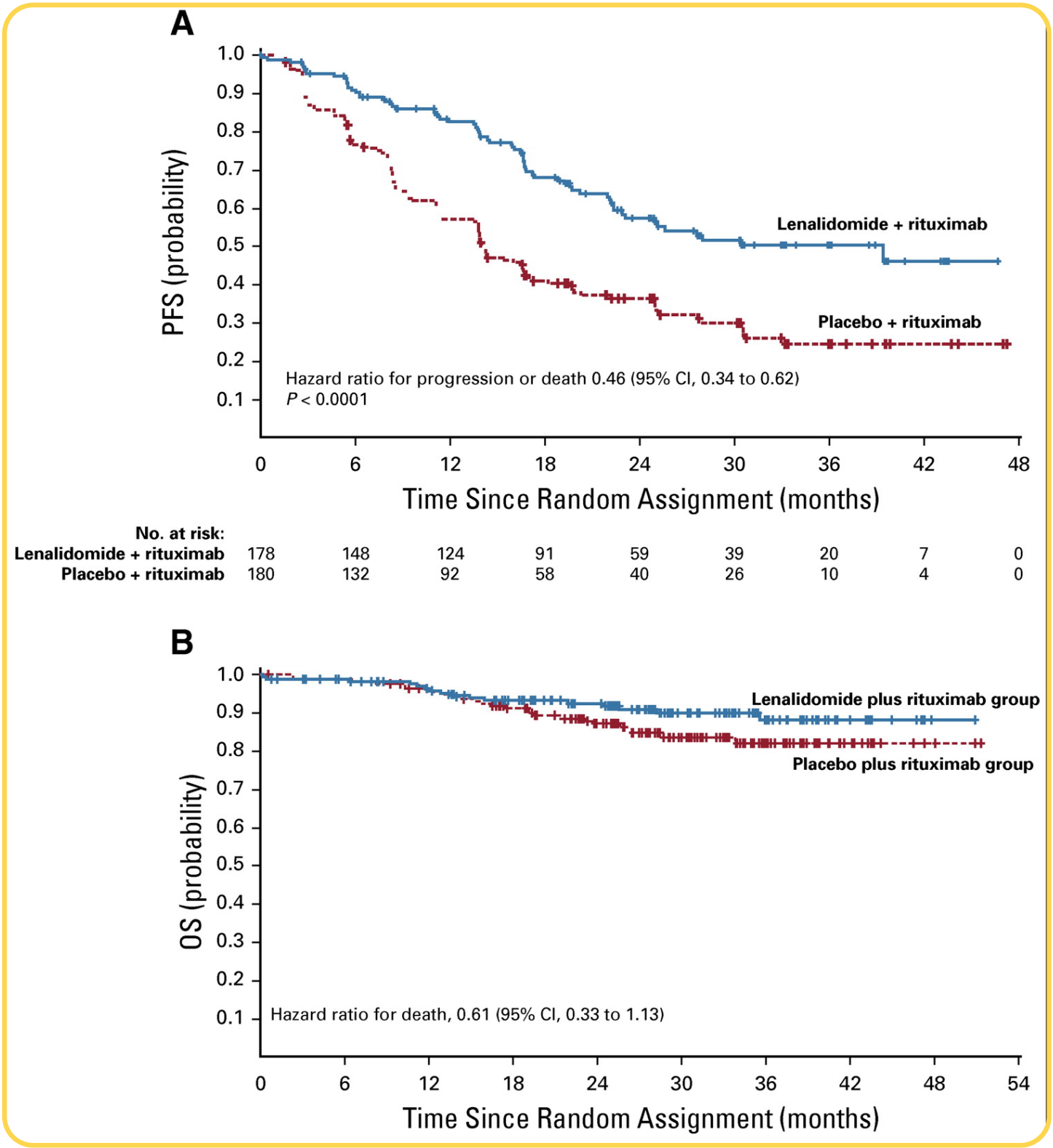



***Figure 1.*** *Survie sans progression (SSP) et survie globale (SG) évaluées par un comité d'examen indépendant auprès de la population en intention de traiter : (A) survie sans progression ; (B) survie globale. Adapté de Leonard, JP et al, 2019*

for those with early relapse. The CUP trial demonstrated significant improvement in PFS and OS for patients who received ASCT compared to chemotherapy alone[36]. The benefit of ASCT was also demonstrated at first relapse in both rituximab-naïve and rituximab re-treated patients[37] in the GELA/GOELAMS FL2000 study which showed a 3-year OS of 92% (95%CI 78-97%) versus 63% (95%CI 51-72%) (P=0.0003), for those that received ASCT versus chemotherapy alone. There is also retrospective evidence demonstrating a survival benefit with early transplant in patients with early treatment failure[38–40]. It should be noted that the benefit of ASCT in the modern era is unclear and there may be varied practices across different centers. In our center we pursue ASCT following second-line therapy at first relapse especially for patients who relapsed within 24 months of first-line treatment. AlloSCT can offer a potential cure due to its graft versus lymphoma (GVL) effect however only few patients would be eligible. Several studies have demonstrated a benefit to alloSCT over ASCT but with higher transplant related mortality (TRM)[41–43]. For patients with early treatment failure both ASCT and matched sibling alloSCT produce a similar 5-year OS (~70%) but with a higher rate of TRM in the alloSCTgroup[44]. The role of alloSCT even in a few select young patients with refractory/relapsed FL, is unclear in the modern era of emerging therapeutic agents. The optimal transplant strategy thus continues to remain unclear.

For patients who are not transplant candidates, maintenance therapy with monoclonal antibodies following salvage chemo-immunotherapy is recommended if maintenance has not been previously given or was administered using a different monoclonal antibody. Maintenance should also be considered post-ASCT if warranted. MR following (R)-salvage therapy in patients with relapsed/refractory FL significantly improved PFS[15]. Even though evidence is lacking on the utility of MR post-ASCT, a recent consensus publication on maintenance therapy after ASCT recommended post-autologous maintenance rituximab for chemo-sensitive, rituximab-naïve patients with FL at relapse[45]. Clinicians may consider the use of MO in patients who received O-chemotherapy followed by ASCT for rituximab refractory FL patients. However, there are no prospective trials to inform this potential therapeutic approach, nor any evidence about potential post-SCT toxicities.

The management at first relapse will continue to evolve as more and more novel therapies are studied in a first relapse setting. Novel chimeric antigen receptor T-cell therapy (CAR-T) is showing promising results in patients with relapsed FL[46]. Additionally, data is also beginning to emerge on the use of bispecific antibodies in FL[47]. In the end, the management of FL at first relapse will undoubtedly consider advances in management strategies as clinicians continue to strive for optimal outcomes for their relapsed FL patients.

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# MYELOPROLIFERATIVE NEOPLASMS IN 2022: A CONCISE REVIEW

**Introduction**

The Philadelphia chromosome(Ph)-negative myeloproliferative neoplasms (MPN) are comprised of a heterogenous group of disorders of myeloid hematopoietic stem cells that include polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (MF). MPN are characterized by constitutional and other disease-related symptoms, an increased risk for thrombotic and hemorrhagic events, and a propensity to transform to acute myeloid leukemia (AML). Progress in our understanding of the molecular pathophysiology of MPN has led to improved prognostic tools, and increasingly personal risk-stratification. In PV, there has been renewed interest in interferon (IFN) for its potential to directly target the malignant clone and exert a disease-modifying effect. In MF, the introduction of Janus Kinase (JAK) inhibitors has significantly altered the therapeutic landscape over the past decade. Ongoing development in the area of JAK inhibitor therapy, as well as several novel pathways, holds promise for improved hematologic responses, lessening of overall burden of illness, increased quality of life, and application to a broader cohort of patients.

**Molecular Pathogenesis**

MPN result from constitutive activation of the JAK/STAT signalling pathway. In the majority of cases, a driver mutation in *JAK2, CALR* or *MPL* can be identified, and those without one of these mutations are classified as "triple negative". The first discovered, in 2005, was a point mutation in exon 14 of the *JAK2* gene which results in a valine to phenylalanine substitution at position 617 (V617F). This mutation results in constitutive activation of the JAK/STAT pathway independent of ligand activation of the erythropoietin (EPO), thrombopoietin (TPO) or granulocyte-colony stimulating factor (G-CSF) and as such, can drive the phenotype of either of the classical World Health Organization (WHO)-defined MPN. In comparison, the second type of driver mutation in JAK2, comprised of a variety of insertions and deletions in exon 12, activates primarily the EPO receptor. JAK2V617F is detected in 95% of PV cases and 50-60% of ET and MF.[1,2] JAK2 exon 12 mutations can be identified in most of the remaining 5%.[3]

Calreticulin (CALR) is a chaperone protein that prevents exportation of misfolded proteins in the endoplasmic reticulum.[4-5] Multiple mutations have been described, but 80% are either type 1, a 52-bp deletion, or type 2, a 5-bp insertion in exon 9. These frameshift mutations result in pathogenic binding of the CALR lectin-binding domain to thrombopoietin receptor (MPL, also known as TPOR) , which activates JAK/STAT signalling. CALR mutations are identified in 20-25% of ET cases and 25-30% of MF cases.[6-7] MPL is the receptor for TPO and gain of function mutations in tryptophan at position 515 (W515) in exon 10 of the *MPL* gene are identified in approximately 3-8% of ET and MF cases.[8]

In addition to driver mutations, the MPN phenotype and evolution over time is modulated by additional mutations such as those in genes involved in epigenetic regulation (e.g., *EZH2, ASXL1*), spliceosome machinery (e.g., *SRSF2, U2AF1*) and the RAS pathway (e.g., *NRAS, KRAS*). Mutations in *ASXL1, EZH2, SRSF2* and *IDH1/29*, denoted "high molecular risk (HMR)", as well as those in *TP53*[10], predict leukemic progression or shortened survival. In addition to these genetic drivers, there is growing evidence that proinflammatory processes play a role in MPN progression, from clonal hematopoiesis to chronic phase MPN to accelerated and blast phase disease.[11,12]

**Goals of Therapy and Risk Stratification**

The goals of treatment in MPN include symptom improvement, prevention of vascular events, control of abnormal blood counts, reduction in splenomegaly and delayed disease progression. Potential consequences of treatment must also be considered and may include side effects, impact on fertility, and the risk of second cancers. The MPN Landmark survey involving 813 patient respondents who had MPNs and 457 hematologist/oncologist respondents who treated patients with these conditions uncovered frequent discordance regarding treatment goals between patients and physicians.[13] Indeed, the goals of treatment may vary depending on the MPN and its clinical and genetic features, as well as an individual's life stage, values, and preferences. Hence, effective patient-physician communication is vital to treatment decision making.

An important component of therapeutic decision making is accurate risk stratification. Traditional stratification in PV and ET is based on age and history of thrombosis, with patients over the age of 60 years or with prior thrombosis considered high risk, and those without either of these factors considered low risk.[14] In ET, the newer International Prognostic Score of Thrombosis for ET (IPSET-thrombosis)[15], which also incorporates the *JAK2V617F* mutation and conventional cardiovascular risk factors as risk factors for thrombosis, is the preferred scoring system.[16,17] In MF, newer risk models incorporating both clinical and genetic information are recommended, particularly to inform decisions around allogeneic stem cell transplantation (SCT). In primary myelofibrosis (PMF), the Mutation-Enhanced International Prognostic Score System (MIPSS-70)[18] or MIPSS-70+ Version 2.0[19] are preferred if both molecular profile and karyotype are available, and the MYelofibrosis SECondary to PV and ET prognostic model (MYSEC-PM)[20] is a validated tool for post-ET or post-PV MF. The Dynamic International Prognostic Scoring System (DIPSS) continues to be the recommended scoring system in clinical practice if genetic information is unavailable.[21]

**Treatment of PV and ET: The Old and the New**

The mainstay of therapy for low-risk PV is low dose acetylsalicylic acid (ASA) and phlebotomy to maintain a hematocrit below 45%.[22] However, in clinical practice, it can be challenging to maintain target hematocrit values with intermittent phlebotomies. Further, phlebotomies do not control progressive thrombocytosis or leukocytosis, and may result in symptomatic iron deficiency.[23] Hydroxyurea is commonly used in this setting for patients who are poorly tolerant or require frequent phlebotomies, however, new strategies are being explored. In the Low-PV study, in which 127 patients with low-risk PV were randomized to receive standard therapy with ASA (100 mg daily) and phlebotomy (300mL for each phlebotomy) with or without ropeginterferon alfa-2b (rIFN) administered subcutaneously every 2 weeks in a fixed dose of 100 µg, more patients treated with rIFN maintained a median hematocrit of 45% or lower without progressive disease during a 12-month period than those receiving standard therapy (84% vs. 60%, p=0.0075). There was no significant difference between grade 3 or higher adverse events, and serum ferritin concentrations progressively increased over time in the rIFN group.[24] Another promising approach to hematocrit control is with the hepcidin mimetic, rusfertide (PTG-300). In a phase 2 study, rusfertide was effective at limiting the number of phlebotomies and maintaining hematocrit below 45%, while the serum ferritin levels increased throughout the treatment period reflecting increase in iron stores.[25]

In high-risk PV, either hydroxyurea or IFN are the currently recommended first-line cytoreductive therapies for patients of any age, however the initial choice is often strongly influenced by cost and drug availability.[15] In Canada, IFN is most often considered in younger patients and in patients who are pregnant and require cytoreduction. In the PROUD-PV study, and its extension phase, CONTINUATION-PV, patients with high-risk PV were randomized to receive rIFN or hydroxyurea. While responses to rIFN occurred later, by 36 months hematologic responses without normalization of spleen size were seen in 71% of patients treated with rIFN vs. 51% of those treated with hydroxyurea (p=0.012).[26] At the 60-month follow-up, 56% of evaluable patients treated with rIFN had a decrease in their JAK2 allele burden to under 10%. Younger age and lower allele burden predicted a better molecular response, suggesting early treatment initiation may result in the greatest long-term benefit.[27]

Most patients with ET likely benefit from ASA for prevention of vascular events. However, in a retrospective review of 433 patients with low-

risk ET, in patients with a CALR mutation, antiplatelet therapy did not affect the risk of thrombosis, but was associated with a higher incidence of bleeding (12.9 vs. 1.8 episodes per 1000 patient-years, p=0.03).[28] Patients with high-risk disease according to the IPSET-thrombosis should receive low dose ASA, while those with low- or intermediate-risk disease should receive ASA if they are 60 years of age or older, have the JAK2V617F mutation, or uncontrolled cardiovascular risk factors.[15] Cytoreduction is recommended for patients aged 60 years and older, those with a history of thrombosis and for a platelet count above 1500 x 109/L. Cytoreduction is recommended for extreme thrombocytosis primarily to reduce the risk of acquired Von Willebrand syndrome and major hemorrhage, as the risk of thrombosis does not appear to be increased.[29] Hydroxyurea is generally favoured as first-line therapy in ET. Anagrelide and IFN are recommended as second line treatments, and the ongoing SURPASS ET study, comparing rIFN to anagrelide in patients with resistance or intolerance to hydroxyurea, may help inform the optimal treatment in this setting (NCT04285086).

**Treatment of MF: JAK Inhibitors and Beyond**

Management of MF starts with risk assessment, as described below (**Figure 1**). In patients with lower risk disease, who have no or minimal disease-related symptoms, active surveillance is recommended. For patients with splenomegaly or MF-related symptoms, ruxolitinib may be beneficial; IFN or hydroxyurea may be indicated if cytoreduction is required,[17,30] and erythropoiesis-stimulating agents may be useful in patients with symptomatic anemia in whom the serum erythropoietin level is under 500 mU/mL.

In patients with higher risk disease, who are eligible for hematopoietic cell transplantation (HCT) and have an available donor, referral for consideration of upfront HCT is recommended.[31,32] For patients with higher risk MF who are ineligible for HCT, do not have a suitable donor, or prefer non-HCT therapy, ruxolitinib has been the mainstay of treatment for nearly a decade. Several studies have demonstrated that ruxolitinib may improve disease-related symptoms, splenomegaly and quality of life.[33,34] Since ruxolitinib's approval, a number of other JAK inhibitors have been developed, most notably fedratinib, which was approved in Canada in September 2020. Momelotinib and pacritinib, which aim to improve the incidence of adverse events such as anemia and thrombocytopenia, respectively, are currently being evaluated in phase 3 trials.

In addition to novel JAK inhibitors, a number of investigational agents are being studied in combination with a JAK inhibitor. The bromodomain and extra-terminal domain inhibitor, pelabresib (CPI-0610), the BCL-2/BCL-XL inhibitor, navitoclax, and the phosphatidylinositol 3-kinase inhibitor, parsaclisib, have all shown clinical benefit in phase 2 studies and are currently in phase 3 trials

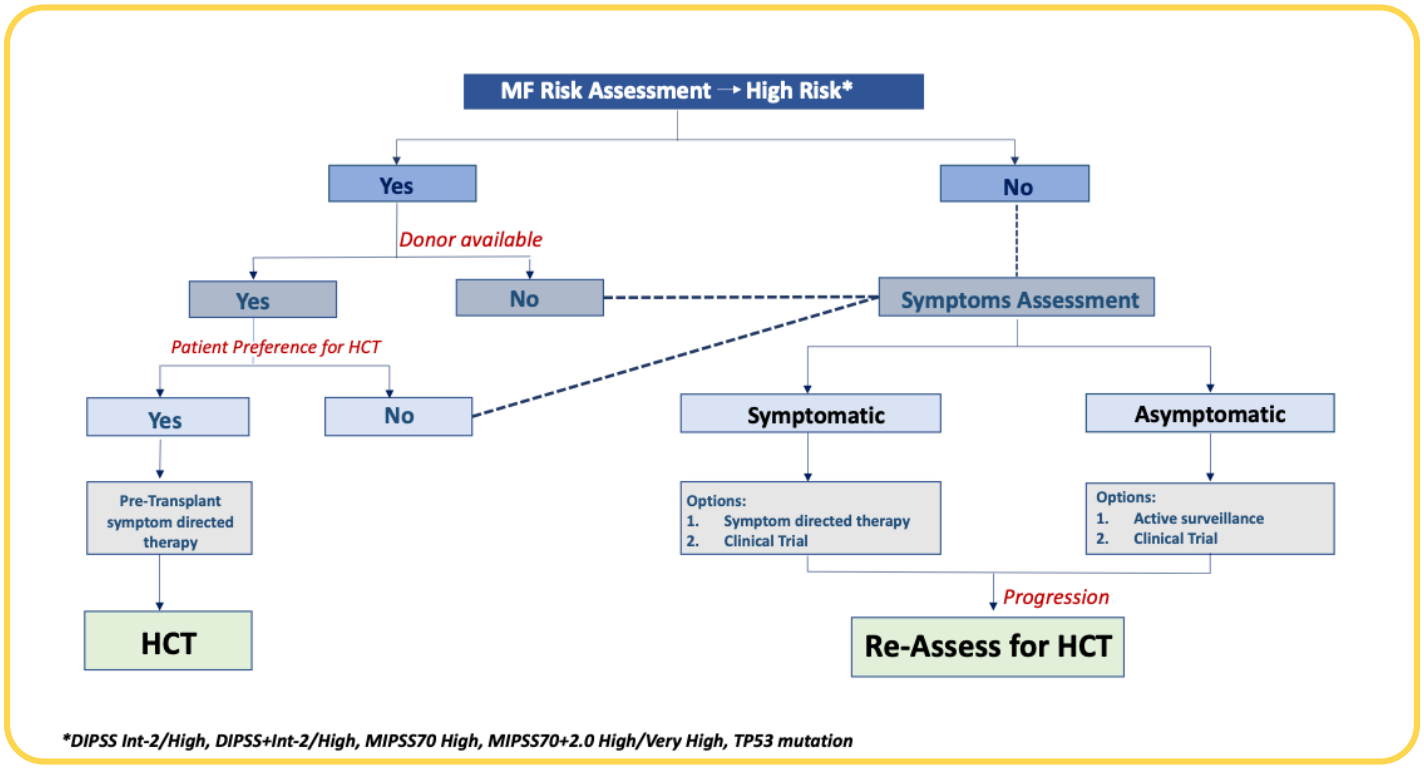



***Figure 1.*** *Management Algorithm for Transplant eligible MF Patients in chronic phase (used with permission from England J, Gupta V. Novel therapies vs hematopoietic cell transplantation in myelofibrosis: who, when, how? Hematology Am Soc Hematol Educ Program 2021; 2021(1): 453-462.)*

(NCT04603495, NCT04472598, NCT04551066). Another agent of interest is luspatercept, which has been demonstrated to improve hemoglobin and reduce transfusion requirements in patients with myelodysplastic syndrome.[35] Symptomatic anemia is an unmet clinical need in MF and in a phase 2 study, luspatercept in combination with ruxolitinib resulted in 27% transfusion-independence for 12 consecutive weeks.[36] This drug is also in phase 3 testing for patients with MF and red cell transfusion dependence in combination with ruxolitinib (NCT04717414).

**Conclusions and Future Directions**

The past decade has seen major shifts in diagnosis, prognostication, and management of MPN. Driver mutations lead to constitutive activation of the JAK-STAT signalling pathway, and the clinical phenotype and disease evolution likely results from a complex interplay of host genomic background, inflammatory pressures, and acquisition of new mutations. There has been renewed interest in IFN for its disease-modifying potential and ongoing trials with long-term follow-up will help inform its place in the MPN therapeutic algorithm. Management of MF begins with risk assessment and a clear understanding of the patient's goals and preferences. For higher risk patients who are ineligible for, or chose not to undergo, HCT, there are several promising new agents and patients should be offered clinical trial participation whenever possible.

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# DIAGNOSIS AND TREATMENT OF AL AMYLOIDOSIS IN 2022

**Introduction**

Light chain (AL) amyloidosis is a rare, progressive and typically fatal disease (when advanced) characterized by organ dysfunction secondary to deposition of misfolded fibrils of immunoglobulin light chains that are produced by clonal plasma cells or B cells.[1] Although less than 10% of AL patients qualify for CRAB criteria of symptomatic myeloma (**C**alcium elevation, **R**enal dysfunction, **A**nemia, and **B**one disease),[2] the majority of these patients have significant impairment of vital organs, such as the heart, kidney and liver. This implies that the common risk factors used for the assessment of multiple myeloma (MM) are not applicable to AL. AL amyloidosis affects 8-12 individuals per million person-years.[3,4] and its clinical presentation is variable depending on the extent and number of vital organs affected. The locations of amyloid deposits can vary among patients, thus contributing to the heterogeneity of the clinical manifestations. The heart and kidney, which are the most affected organs, can lead to renal failure, cardiomyopathy, and pericardial and pleural effusions.[1] Initial symptoms at onset are often non-specific (e.g., weight loss, fatigue). Despite advances in the diagnostic tools and treatment options, early mortality rates remain high; the expected one-year mortality is approximately 30%.[5] Unfortunately, by the time the AL diagnosis is made, and treatment is initiated, the disease has often become advanced.

The diagnosis of AL amyloidosis requires histological demonstration of amyloid deposits in biopsy tissue, followed by amyloid typing to identify the precursor protein associated with the amyloid formation.[6] The tissue source can be the involved organ by amyloid formation. However, a more accessible tissue, such as subcutaneous fat, should initially be pursued when suspicion for amyloidosis is raised.[7] Fat pad aspirate in combination with a bone marrow biopsy will yield the diagnosis in approximately 90% of patients. Congo red is the gold standard staining for recognition of amyloid deposits. Tissue stained by congo red under polarized light demonstrates apple-green birefringence, illustrating the highly organized ultrastructure of the amyloid fibrils. Once the amyloid diagnosis is established, the next step is to type and determine the precursor protein associated to fibril deposition. Several methods of typing are available. The gold standard technique is laser microdissection, followed by mass spectrometry–based proteomic analysis, which has high sensitivity and specificity.[8] Alternative typing methods include antigen-antibody–based analyses, such as immunofluorescence, immunohistochemistry, and immunogold.[9] It should be emphasized that the presence of a monoclonal protein in a patient with amyloidosis does not prove AL type[10] as monoclonal gammopathy of undetermined significance (MGUS) can be found in 30-40% of patients with either wild type or hereditary systemic transthyretin amyloidosis (ATTR).[11] Finally, the distinction between ‘localized’ and ‘systemic’ AL amyloidosis is required. The designation localized applies to AL amyloidosis in which the precursor protein is produced at the site of amyloid deposition and is typically not associated with a detectable circulating monoclonal protein in the serum or urine. The common sites of localized amyloidosis are the

tracheobronchial tree, lungs, urinary tract, skin and soft tissue, oropharynx, gastrointestinal tract, and eyes.[12,13]

Due to the protein clinical manifestations and insidious onset of the disease, indications for diagnostic testing includes a broad range of features including non-diabetic nephrotic range proteinuria, non-dilated cardiomyopathy, increased NT-pro-BNP, unexplained hepatosplenomegaly, carpal tunnel syndrome, edema, purpura, or macroglossia. Biomarkers are also essential in making the diagnosis, as well as in determining the prognosis and evaluating response to therapy. Given the significant prognostic impact of cardiac involvement with early death, several markers of cardiac injury and dysfunction have been reported.[14] Serum levels of NT-pro-BNP and cardiac troponin T (cTnT) were first found to predict survival in several cohorts of patients with AL.[15-17] They were later incorporated into the first widely used staging system for AL amyloidosis (Mayo 2004).[17] The composition and biomarker thresholds were subsequently revised and two modifications of the original score are widely accepted.[18] The European version of the 2004 Mayo system identifies patients with very high NT-pro-BNP levels as having very poor outcomes and splits stage III

into two stages (IIIa and IIIb) based on a cutoff of 8500 ng/L for the values of NT-pro-BNP. More recently, the Boston group reported on the use of BNP and troponin I (TnI) for staging.[19] BNP higher than 81 pg/mL and TnI higher than 0.1 ng/mL were used in this validated staging system.

**Assessment of the monoclonal protein associated to AL amyloidosis**

The screening for a monoclonal protein is done by serum and urine electrophoresis with immunofixation studies as well as serum free light chain (FLC) levels.[20] More recently at the Mayo Clinic, immunofixation has been replaced by the mass spectrometry method (Mass-Fix).[21] The Mass-Fix assay has the ability to detect M-proteins with light chain glycosylation, which has been reported to be a risk factor for progression of AL amyloidosis and other plasma cell disorders.[22] In addition, bone marrow aspiration and biopsy and fluorescence in-situ hybridization (FISH) testing are indicated and can affect treatment decisions during the disease course.

**Immunophenotyping**

Multidimensional flow cytometry (MFC) has emerged as a potential tool highly sensitive for the detection of aberrant plasma cells in the bone marrow. Research has demonstrated that monoclonal plasma cells >2.5% at the time of diagnosis, as detected by MFC was associated with shorter survival.[23] More recently another group developed an automated computerized algorithm to assess clonality and identified three subgroups with different survival outcomes.[24]

**Cytogenetics of the aberrant plasma cells**

FISH abnormalities have been detected in patients with AL amyloidosis. A study conducted in 2009[25] was one of the first to describe the utility of this approach in identifying t(11;14) as an adverse risk factor for AL. Other researchers[26] described the degree of plasma cell burden and their relationship to survival an advanced cardiac disease. Additional research has[27] further stratified patients with t(11;14) who received bortezomib and IMiD-containing regimens showing that this group had an inferior survival compared to those without this translocation. It is important to note that high risk cytogenetics seen in MM (t(4;14), t(14;16) and del17p) are not common in AL. More complex karyotype clones, however, and presence of del17p have an impact on outcomes. Gain of 1q21 has also been described as an independent adverse prognostic factor in a series of 103 AL patients treated with melphalan, dexamethasone, standard chemotherapy, and daratumumab as first-line therapy.[28]

**Treatment of AL amyloidosis**

The aim of treatment of AL amyloidosis is to eradicate the underlying plasma cell clone in order to rapidly reduce the production of misfolded FLC proteins, mitigate further organ damage, and improve overall survival.[29]

**Supportive care**

Supportive measures are key in the management of AL amyloidosis, with the goal of improving quality of life, symptoms and sustaining organ function while the plasma directed therapy takes place.[30] The main pillar of supportive care is the use of diuretics. It should be noted that, in amyloidosis, cardiac function is preload dependent, and thus, avoiding reduction of intravascular volume is fundamental. Angiotensin-converting enzyme inhibitors are usually poorly tolerated due to hypotension. Similarly, calcium channel blockers are contraindicated due to their negative inotropic effects.[31] Patients with severe neurogenic orthostatic hypotension will require therapy with midodrine and/or droxidopa to facilitate diuretic dose titration.

Intracardiac thrombi are another possible complication in AL amyloidosis despite sinus rhythm.[32] Atrial thrombus, mainly located in the right or left atrial appendages, was found by transesophageal echocardiography in 35% of patients with this disease.[33] The incidence of thromboembolism is higher in patients with atrial fibrillation in the presence of cardiac AL amyloidosis than in other more common forms of atrial fibrillation. Therefore, anticoagulation must be considered on an individualized basis counterbalancing the higher hemorrhagic risk of this population due to the potential association of

vascular amyloid deposition, factor X deficiency and liver involvement. As a general recommendation, anticoagulation should be given for any atrial arrhythmia and in patients with sinus rhythm whose echocardiography shows features of left atrial mechanical dysfunction.[34]

Further, organ transplantation should be carefully assessed by a multidisciplinary team since the risk of recurrence of amyloid in the graft and progression of fibril deposition in other organs is often observed. For instance, cardiac transplantation could be considered in young patients with isolated severe cardiac involvement where effective anti-plasma cell therapy is only expected to be delivered if organ replacement occurs. The implantation of left ventricular assist devices is technically feasible for patients with severe heart failure caused by advanced cardiac amyloidosis, but the possible benefit is unclear.[35,36]

**Autologous stem cell transplantation**

In clinical practice, the first question to be asked is whether an AL patient is a candidate for autologous stem cell transplantation (auto-SCT). Among eligible patients, auto-SCT is an excellent option with potential for long-term survival. There are, however, no randomized trial data to support that it is superior to conventional chemotherapy. On the contrary, a phase 3 study concluded that high dose intravenous melphalan followed by auto-SCT rescue was inferior to standard-dose melphalan plus high-dose dexamethasone (MDex) in newly diagnosed patients.[37] On an intention-to-treat (ITT) basis, the median survival for MDex was 57 months vs 22 months for the auto-SCT arm (P=0.04). However, of the 50 patients randomized to receive ASCT, only 37 actually received the planned transplant and 9 of those died within 100 days, indicating an unacceptably high (24%) treatment-related mortality (TRM) rate. In a 6-month landmark analysis, no difference in survival was noted between treatment arms, thus accounting for the survival disadvantage of ASCT to the very high TRM rate. Current clinical trials demonstrate a TRM of less than 5%,[38-40] suggesting inappropriate selection of patients in that study, which in turn limits its conclusions.

**Non transplant therapies**

Historically, therapy for AL amyloidosis was based on targeting the plasma cell clone and treatments that were used in MM were incorporated into the management of AL patients. Treatment should be risk-adapted, considering the severity of organ involvement, characteristic of the clone, and comorbidities and should seek to deliver the most rapid and effective therapy patients can safely tolerate.[30] Early and profound reductions of the amyloid LC are associated with the greatest chance of organ improvement and prolongation of survival outcomes.[41-43] The optimal end point of therapy is still a matter of debate. However, achievement of organ response and profound hematological response should be the long-term goal of therapy. Novel definitions of response and minimal residual disease (MRD) assessment are currently being investigated for AL.[41,44,45]

Most patients with AL amyloidosis are not eligible for auto-SCT. Melphalan with steroids has historically been the first-line approach for the treatment of AL.[46] However, given the efficacy of proteasome inhibition in MM, bortezomib was evaluated in AL amyloidosis. Real-world studies of CyBorD (cyclophsophamide, bortezomib and dexamethasone) demonstrate a need for more effective therapies for AL amyloidosis, with hematologic responses reported in 60-65%, cardiac responses in 17-32%, and renal responses in 15-25% of patients[45,47]. Encouragingly, the phase III ANDROMEDA trial showed that the addition of daratumumab to a CyBorD regimen significantly increased the rates of hematologic complete response (CR) (53% vs 18%, p<0.001), cardiac response (42% vs 22%), and renal response (53% vs 24%), with median time to hematologic CR of 60 days in the daratumumab-CyBorD group compared to 85 days in the CyBorD group (**Figure 2**)[48].

Treatment intensification with high dose melphalan is an option in a subset of patients and it has been suggested as a sequential response-driven approach for patients undergoing CyBorD who don't exhibit a satisfactory response after induction therapy.[49] In addition, a phase 3 study in intermediate risk AL patient demonstrated that bortezomib, melphalan and dexamethasone (BMD) induced a significantly higher HR rate (81% vs 57%, CR, 23% vs 20%; VGPR 42% vs 20%) than MDex, with prolonged overall survival. Cardiac and renal responses were observed in 38% and 44% of cases with BMDex and in 28% and 43% of cases with MDex, respectively.[50]

Approximately 20% of patients have advanced cardiac stage at the time of diagnosis. Treatment of these patients considered high-risk and remains an unmet need. Initially, the European collaborative study reported lower haematological response rates in patients with stage IIIb disease.[51] This is likely a reflection of very advanced cardiac disease. Further, studies have reported on the importance of rapid responses in patients with stage IIIb, demonstrating an improvement in survival for patients treated with CyBorD compared to CTD (cyclophosphamide, thalidomide and dexamethasone); but most importantly described how patients with rapid haematological response in 1-month are associated with improved survival.[52] Based on these reports, recent studies have explored the possible benefit of the substitution of dexamethasone by methylprednisolone with the aim of decreasing toxicity.[53]

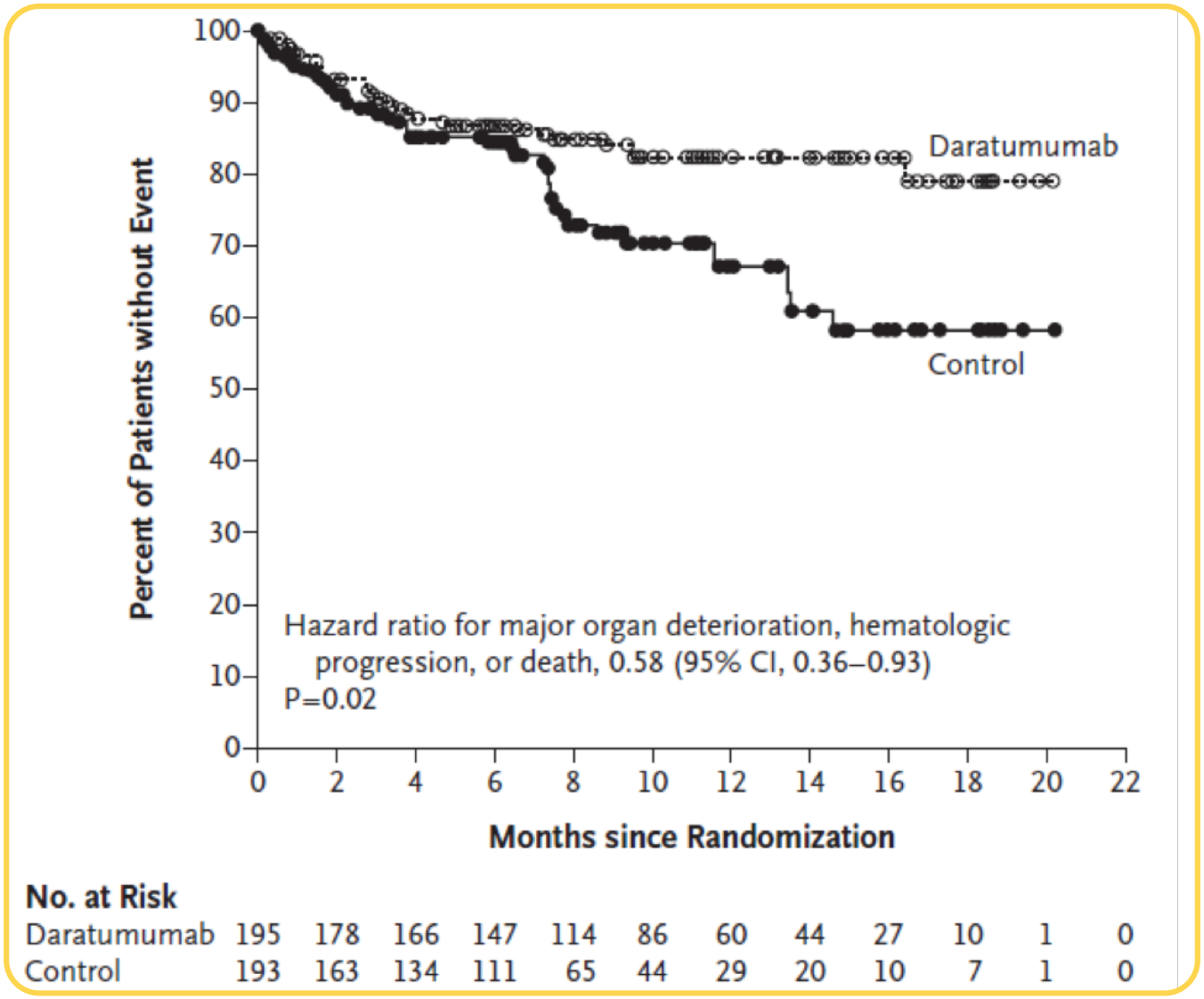



***Figure 2.*** *Kaplan–Meier Estimates of Survival Free from Major Organ Deterioration or Hematologic Progression. Shown are the results of the Kaplan–Meier estimates of survival from major organ deterioration or hematologic progression among patients in the intention- to-treat population. Major organ deterioration was defined as end stage cardiac or renal failure; adapted from Kastritis et al, 2021*

Notably, patients treated with methylprednisolone exhibited faster responses which translated into a better survival rate (2-year OS of 65% versus 43%). Our group presented a preliminary report on the use of CyBorMe (cyclophosphamide, bortezomib and methylprednisolone) for newly diagnosed AL amyloidosis patients treated at a single referral center and compared with a historic group of patients treated with a standard CyBorD regimen used at our institution. Overall response rates (ORR) were similar among the CyBorD and CyBorMe groups (90.6% vs 100%, p=0.7). However, patients in the CyBorMe group had a faster time to first (4 vs 6 weeks) and best response compared to CyBorD (p=0.003 and 0.047, respectively). In addition, a trend towards lower dFLC after one month and higher cardiac response rate was noted (44% and 31% of patients treated with CyBorMe and CyBorD, respectively). Out of 7 evaluable cases for cardiac involvement, 3 patients exhibited cardiac response at a median of 8 weeks.[54]

Venetoclax is also an appealing option for patients with t(11;14), but few data are available to date. Although a change in AL amyloidosis therapy is typically prompted by the occurrence of hematologic or organ progression, there is growing consensus that failure to achieve an optimal response within the first few cycles of treatment should also lead to a change in therapy[55]. Given the poor prognosis of patients with suboptimal response to first-line therapy and the encouraging findings of these studies, further research is warranted to identify the optimal timing of response assessment and to better understand the role for early switch to second-line therapy in AL amyloidosis.

**Anti-fibril directed therapy**

Treatment of AL amyloidosis has been directed at reducing the circulating precursor LC's by targeting the malignant B-cell clone. Recently, two anti-amyloid antibodies have been tested in clinical trials for AL amyloidosis, but despite encouraging preliminary results, further clinical studies were discontinued due to futility or unfavorable toxicity.[56,57] Recently, CAEL-101, a monoclonal antibody that reacts with a conformational epitope present on partially denatured and fibrillar LC's was investigated in phase1a/b study. All patients were exposed to 1 to 10 lines of therapy, and median times from last chemotherapy administration were 2.6 and 7.4 months in the phase 1a and 1b portions of the study. Twenty patients (74%) demonstrated VGPR at the time of first infusion of CAEL-101. Fifteen of 24 patients (63%) had a therapeutic response to CAEL-101 as evidenced by serum biomarkers or objective imaging modalities with a median time of response of 3 weeks.[58] This study provides rationale for the development of a phase 3 clinical trial program for patients with AL amyloidosis with stage IIIa and IIIB that are randomized to CAEL-101 plus CyBorD or CyBorD alone.

**Conclusion**

AL amyloidosis is a rare disease often associated with devastating outcomes due to advanced cardiac disease. As delays in the diagnosis of AL amyloidosis are common, finding biomarkers that could potentially help us diagnose this entity is crucial. Recently, CyBorD plus daratumumab was approved by FDA and Health Canada becoming the first and only treatment approved for patients with newly diagnosed AL amyloidosis. Based on this exciting approval more work is needed to improve awareness and advance research that could potentially lead to early diagnosis and innovative use of novel drug combinations.


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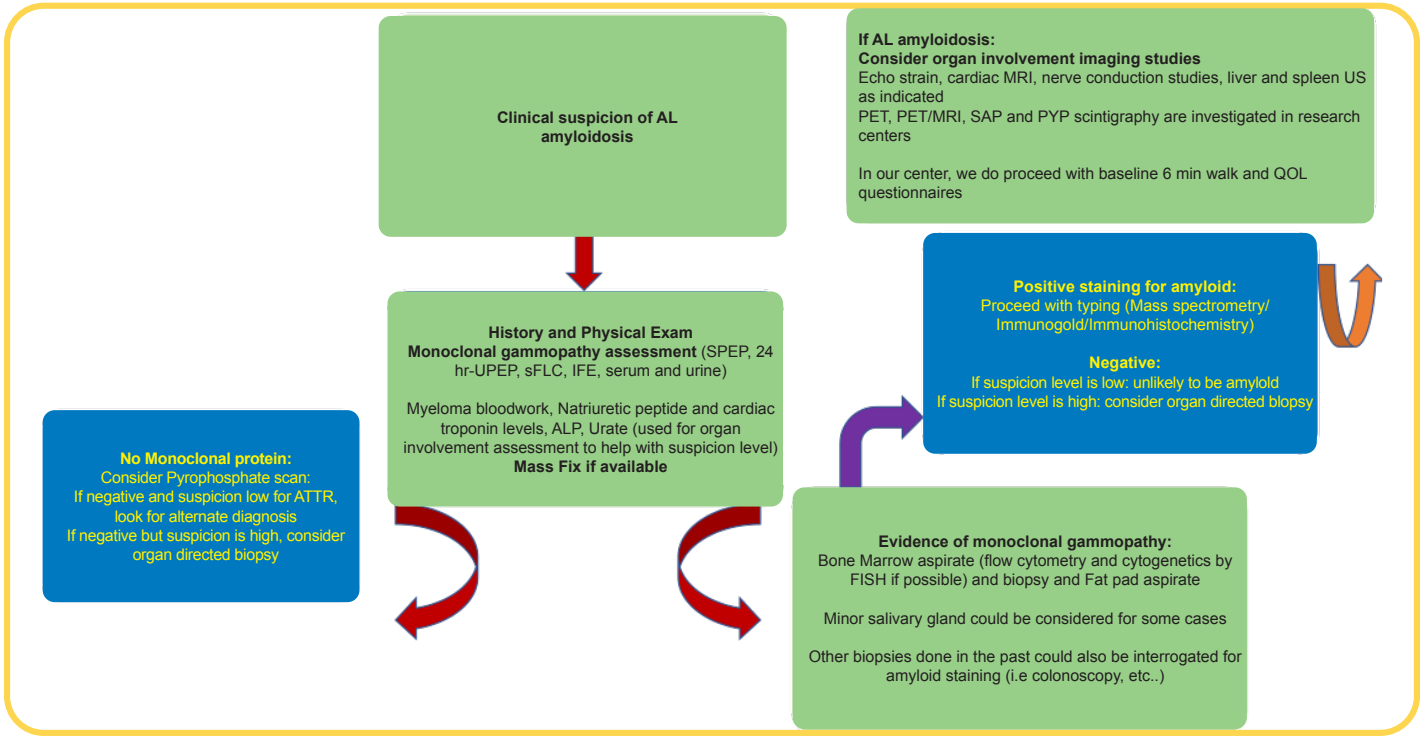




***Figure 1.*** *Diagnostic algorithm for AL Amyloidosis; courtesy of Victor Zepeda, MD*

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Dr Richard LeBlanc is a hematologist and medical oncologist at Maisonneuve-Rosemont hospital. He obtained his MD degree at Laval University in 1995. He was certified in internal medicine in 1998 and in hematology in 2000 at Laval University. Thereafter, he spent two years as a research fellow at the Dana Farber Cancer Institute, Harvard Medical School in Boston, from 2000 to 2002, to develop an expertise in multiple myeloma. In 2004, he completed his training in medical oncology at Montreal University. He is an associate professor of medicine at the department of medicine, Université de Montréal. Since 2012, he is the Myeloma Canada Chair at the Université de Montréal. His practice and interests focus on improvements in care, teaching and research in multiple myeloma.

# TO TRANSPLANT OR NOT TO TRANSPLANT IN MULTIPLE MYELOMA

**Introduction and benefits of autologous stem cell transplantation**

Multiple myeloma (MM) is the second most common hematologic cancer resulting from proliferation and accumulation of abnormal plasma cells (myeloma cells) with a preferential homing in the bone marrow. It causes significant morbidity including lytic bone lesions, renal insufficiency, anemia and infections to name just a few.[1] Although MM remains largely incurable, it is a chemo-sensitive disease. The use of high-dose intravenous melphalan (100-140 mg/m2) in the treatment of MM was first studied almost 4 decades ago.[2] Subsequently, the dose of melphalan was increased and was followed by autologous hematopoietic stem cell to decrease the aplasia-associated toxicity.[3,4] Results from phase 3 studies comparing chemotherapy alone to chemotherapy followed by high-dose melphalan and autologous stem cell transplantation appeared in the mid-90s with the publication of the IFM-90 study[5] demonstrating significant clinical benefits on response rate, event-free survival and even overall survival in a cohort of two hundred previously untreated patients under the age of 65 years. This landmark study was followed by confirmatory studies in the early 2000's.[6-8] Within the last 2 decades, although improvement in the treatment of transplant-eligible patients is mostly the result of better induction regimens[9,10] and due to the addition of maintenance therapies,[11-13] autologous stem cell transplantation remains a cornerstone treatment for MM patients. Indeed, despite novel and more effective treatments for MM, autologous stem cell transplantation continues to demonstrate clinical benefits (**Table 1**).[9,10,14-17] Moreover, tandem autologous transplantation has demonstrated progression-free survival and overall survival benefits for some patients with poor risk cytogenetics.[17]

In 2022, with better knowledge of MM, awareness of potential consequences of high-dose melphalan and with novel and more effective treatment modalities, the role of autologous stem cell transplantation is certainly becoming a question for debate. The purpose of this article is to present the pros and cons of autologous stem cell transplantation in our Canadian reality (**Figure 1**). This article aims to better assess its role as a therapeutic option considering our health system's limited resources in which many novel drugs will not be available/accessible in Canada for several more years to come.

**Risk of high-dose melphalan**

High-dose melphalan is well known for significant risk of adverse effects such as severe bone marrow suppression which can result in infection or bleeding, severe gastrointestinal toxicity such as nausea, vomiting, diarrhea and mucositis with ulceration that further increase the risk of infection via bacterial translocation, among other risks.[18] The risk of early mortality within the first few months after autologous stem cell transplantation is approximately 1-2%[5] and predominantly the result of infectious complications.

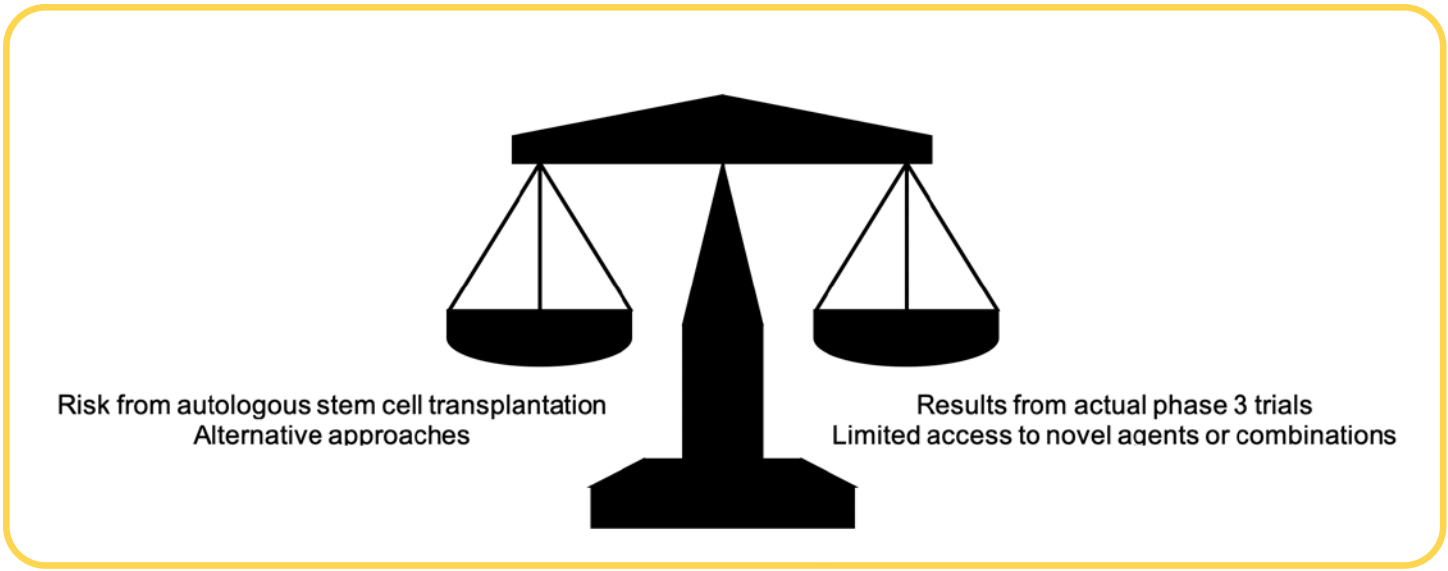



***Figure 1.*** *Weighing the pros and cons of autologous stem cell transplantation; courtesy of Richard LeBlanc, MD*

Beyond the risk of complications and mortality associated with high-dose melphalan is the risk of second primary malignancy. In a retrospective cohort study looking at 841 consecutive MM patients who underwent autologous stem cell transplantation between 1989 and 2009, the overall cumulative incidence of second primary malignancies was found to be 5.3% at 5 years and 11.2% at 10 years when nonmelanoma skin cancers were excluded from the final analysis.[19] In addition, this risk is further increased with the use of lenalidomide in maintenance therapy.[19,20] Melphalan as an alkylating agent that induces DNA damage and high-dose melphalan exposure increases mutational burden detected between diagnosis and relapse by 10-20%.[21] Clinically, melphalan has been shown to increase the relative risk of acute myeloid leukemia by 10-50 fold and the risk of myelodysplastic syndrome by 100 fold in a database analysis of over 9,000 recipients of hematopoietic cell autotransplants between 1995 and 2010 for Hodgkin lymphoma (n = 916), non-Hodgkin lymphoma (n = 3546) and MM (n = 4566), reported to the Center for International Blood and Marrow Transplant Research.[22] This is particularly important since overall survival of myeloma patients is improving. In a recent analysis of 14,532 myeloma patients, the 10-year survival rate favored patients who did not receive transplant.[23] In addition, for long term survivors after autologous stem cell transplantation, the 10-year cumulative incidence of severe and/or life-threatening chronic health conditions is approaching 60%, representing a significant morbidity burden for these patients.[24] As non-transplant regimens become more effective, autologous stem cell transplantation might eventually be regarded as unnecessary and may require a re-examination of its risk-benefit profile.

**Alternative approaches**

The combination of immunomodulatory drugs and proteasome inhibitors in addition to dexamethasone have been shown to have substantial activity against MM.[25,26] These observed benefits from the combination raise questions about the role of autologous stem cell transplantation. The IFM 2009 study compared the bortezomib-lenalidomide-dexamethasone (VRD) combination in induction and consolidation with or without autologous stem cell transplantation, followed by lenalidomide maintenance as a first line treatment for transplant-eligible patients. Although median progression-free survival was significantly longer in the transplant group (50 months vs 36 months; HR 0.65; p<0.001),[9] a long-term follow-up analysis at 95 months, demonstrated median PFS2 to be similar (HR 0.96; p=0.751) between the two groups as well as the rate of overall survival at 60.2% in the VRD arm compared with 62.2% in the transplant arm (HR 1.03; p=0.815).[27] However, 77% of patients randomized in the non-transplant group in first line treatment received autologous stem cell transplantation at time of relapse.[27] Similarly, the FORTE trial compared the carfilzomib-lenalidomide-dexamethasone (KRd) combination in induction and consolidation with or without autologous stem cell transplantation followed by maintenance therapy in first line treatment for transplant-eligible patients with newly-diagnosed MM and who were aged 65 years or younger. Although the overall response rate was similar in both groups, sustained minimal residual disease negativity rate and progression-free survival were in favor of the transplantation group.[10] These trials still suggest a potential role for autologous stem cell transplantation, although, perhaps, not as first-line treatment (**Table 1**).

| Studies References | n | Induction | Intensification | Consolidation | Maintenance | ≥PR | ≥VGPR | ≥CR | MRD | PFS (months) | OS (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RV-MM-PI-209[14] | 273 | Rd x 4 | **ASCT x 2 vs MPR x 6** | None | **Len vs none** | -<br>- | -<br>- | 36%<br>34% | NE<br>NE | m: 43.0<br>m: 22.4 | @4y: 81.6%<br>@4y: 65.3% |
| EMN-441[15] | 389 | Rd x 4 | **ASCT x 2 vs RCd x 6** | None | **Len+pred vs Len alone** | -<br>- | -<br>- | 33-37%<br>23-27% | NE<br>NE | m: 43.3<br>m: 28.6 | @4y: 86%<br>@4y: 73% |
| EMN02[17] | 1197 | VCD x 3-4 | **ASCT x 1-2 vs VMP x 4** | **VRD x 2 vs none** | Len | 95%<br>95% | 84%<br>77% | 44%<br>40% | NA<br>NA | m: 56.7<br>m: 41.9 | @5y: 75.1%<br>@5y: 71.6% |
| IFM2009[9] | 700 | VRD x 3 | **ASCT x 1 vs VRD x 3** | VRD x 2 | Len x 1y | 99%<br>97% | 88%<br>77% | 59%<br>48% | 79%*<br>65%* | m: 50<br>m: 36 | @4y: 81%<br>@4y: 82% |
| FORTE[10] | 474 | **KRd x 4 vs KRd x 4 vs KCd x 4** | **ASCT x 1 KRd x 4 ASCT x 1** | **KRd x 4 KRd x 4 KCd x 4** | **K-Len vs Len alone** | 97%<br>94%<br>91% | 89%<br>87%<br>76% | 54%<br>57%<br>42% | 80%#<br>69%#<br>73%# | m: NR<br>m: 55.3<br>m: 53 | @4y: 86%<br>@4y: 85%<br>@4y: 76% |
| MANHATTAN[40] | 41 | DKRd x 8 | As per SoC | - | As per SoC | 100% | 95% | 95% | 71%∞ | @1y:98% | @1y: 100% |

***Table 1.*** *Studies influencing the decision for autologous stem cell transplantation; courtesy of Richard LeBlanc, MD*

** Performed for patients achieving ≥VGPR after consolidation and maintenance (sensitivity level of $10^{-4}$)*
*# Performed for patients achieving ≥VGPR before maintenance and every 6 months during maintenance (sensitivity level of $10^{-5}$)*
*∞ Performed after DKRd for 8 28-day cycles (sensitivity level of $10^{-5}$)*
***Abbreviation**: ASCT, autologous stem cell transplantation; CR, complete response; DKRd, daratumumab-carfilzomib-lenalidomide-dexamethasone; K, carfilzomib; KCd, carfilzomib-cyclophosphamide-dexamethasone; KRd, carfilzomib-lenalidomide-dexamethasone; Len, lenalidomide; m, median; MPR, melphalan-prednisone-lenalidomide; MRD, minimal residual disease; OS, overall survival; PFS, progression-free survival; PR, partial response; pred, prednisone; RCd, lenalidomide-cyclophosphamide-dexamethasone; Rd, lenalidomide-dexamethasone; SoC, standard of care; VCD, bortezomib-cyclophosphamide-dexamethasone; VGPR, very good partial response; VMP, bortezomib-melphalan-prednisone; VRD, bortezomib-lenalidomide-dexamethasone; y, year; @, at.*
*Characters **in bold** represent part of randomization for these studies*

Aside from immunomodulatory drugs and proteasome inhibitors, monoclonal antibodies against CD38 have emerged as very effective therapeutic options available to clinicians. In randomized phase 3 trials, daratumumab has been shown to significantly improve progression-free survival and overall survival, both in first-line treatment[28-31] and in a relapsed setting.[32-38] Specifically in transplant-eligible patients, the randomized phase 2 GRIFFIN trial comparing lenalidomide, bortezomib, and dexamethasone (RVd) with or without daratumumab (quadruplet) in induction and consolidation treatment in addition to autologous stem cell transplantation and maintenance therapy, demonstrated an impressive 99% overall response rate of daratumumab-based treatment. The daratumumab arm (D-RVd) also achieved a ≥CR of 51.5% compared to the RVd arm at 42.3% , a ≥VGPR of 90.9% compared with 73.2% for the RVd arm and a significantly higher minimal residual disease negativity rate of 51% compared to 20.4% in the RVd arm (P<0.0001).[39] After a median follow-up of 22.1 months, the estimated 24-month progression-free survival was 95.8% (95% CI, 89.2-98.4) in the D-RVd group and 89.8% (95% CI, 77.1-95.7) in the RVd group. Based on these promising results, the phase 2 MANHATTAN nonrandomized clinical trial evaluated the efficacy of the quadruplet treatment daratumumab-KRd in newly-diagnosed transplant-eligible myeloma patients in the absence of high-dose melphalan and autologous stem cell transplantation. Treatment was administered for eight 28-day cycles and resulted in a minimal residual disease negativity rate of 71%, (29 of 41 patients) with a 1-year progression-free survival rate and overall survival rate of 98% and 100%, respectively (**Table 1**).[40]

**Discussion**

Over the last few decades, myeloma patients have achieved longer survival rates as a result of the discovery and approval of novel therapies and combinations.[41] However, in Canada, accessibility to many of these treatments are limited and varies from one province to another. For example, most centers still use cyclophosphamide-bortezomib-dexamethasone (CyBorD) as an induction treatment for transplant-eligible myeloma patients instead of the more effective RVd or KRd combinations.[42] Also, daratumumab-based therapies as first line treatment options, such as those used in the CASSIOPEIA[31], GRIFFIN[39] and MANHATTAN[40] trials, are not available in Canada, nor are the use of quadruplet treatments. In the context of these access limitations and considering the literature showing randomized trials with currently-available agents in Canada still demonstrating clinical benefit with the use of autologous stem cell transplantation, it is prudent to continue the use of transplantation for transplant-eligible myeloma patients as part of the therapeutic armamentarium.

However, impressive results from the MANHATTAN trial with the absence of high-dose melphalan followed by autologous stem cell transplantation are certainly noteworthy. Based on these results, a large randomized, multicenter, 3-arm, phase 2 (ADVANCE) study (NCT04268498) comparing initial treatment with VRd vs KRd vs daratumumab-KRd is presently recruiting. After 8 cycles, patients achieving minimal residual disease negativity will receive maintenance therapy with lenalidomide for up to 2 years. Those with minimal residual disease positivity will have the option to receive an autologous stem cell transplant if available, before initiating the same maintenance therapy. To better evaluate the role of autologous stem cell transplantation, a similar minimal residual disease adapted strategy will be used in the phase 3 MIDAS IFM 2020-02 trial (NCT04934475). After induction treatment with isatuximab-KRd for 6 cycles, patients who achieve minimal residual disease negativity will be randomized to the same treatment as a consolidation for 6 cycles vs high-dose melphalan and autologous stem cell transplantation followed by isatuximab-KRd consolidation for 2 cycles. All patients will receive 3 years of maintenance therapy with lenalidomide. The results of this study may eventually elucidate a sub-population for whom autologous stem cell transplantation can be avoided.

Numerous novel therapies are also emerging with certain immunotherapeutic modalities demonstrating particularly promising results, such as chimeric antigen receptor (CAR)-T and bispecific antibodies. Although still early in their development lifecycle, these therapeutic modalities have shown impressive results in heavily pre-treated relapsed/refractory MM patients.[43-52] Their benefits in earlier use have yet to be demonstrated in clinical trials which will take several more years, but certainly the clinical efficacy of these new agents will have to be compared with that achieved using autologous stem cell transplantation before they are widely adopted.

**Conclusion**

Without doubt, the role of autologous stem cell transplantation will be open for discussion based on the rapid improvement of myeloma therapies. The day may soon arrive when the risks of autologous stem cell transplantation will outweigh its clinical benefits in light of the availability of novel, more effective and safer therapeutic options. Until such time as clinical trials clearly demonstrate that autologous stem cell transplantation can be avoided and alternative therapeutic modalities are fully available for Canadian patients, autologous stem cell transplantation

will remain the standard of care in Canada despite the associated morbidity, mortality and second primary malignancy risks. The eligibility criteria for patients who may be candidates for transplantation are more stringent than those criteria for patients undergoing chemo-immunotherapy alone and, as such, autologous stem cell transplantation as first line treatment should be considered in eligible patients to avoid subsequent ineligibility.

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# EMERGING THERAPEUTIC AGENTS IN THE TREATMENT OF RELAPSED OR REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA

Diffuse large B cell lymphoma (DLBCL) is the most common type of aggressive non-Hodgkin lymphoma (NHL). The median age at diagnosis of DLBCL is 65, and about one-third of patients are older than age 75 at diagnosis.[1,2] The standard of care for frontline treatment is chemoimmunotherapy, consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP). Approximately 60% of patients are cured with standard treatment, but it is inaccessible for up to 25% of patients due to advanced age and underlying comorbidities, including cardiac dysfunction.[2,3] Several biologic factors confer risk of treatment failure, including activated B cell (ABC) cell of origin[4-6] and double expressor phenotype (i.e. and overexpression of c-MYC and BCL2[7,8]).

Patients with DLBCL who relapse or are refractory to RCHOP have a poor prognosis. Currently, the standard approach is platinum-based salvage chemotherapy, such as rituximab, gemcitabine, dexamethasone, and cisplatin (R-GDP), to which approximately half of patients will respond, followed by consolidation with autologous stem cell transplant (ASCT). However this aggressive approach, which yields an overall cure rate of 25 to 35%[2,9], is restricted to younger patients without concurrent medical conditions, leaving a paucity of treatment options for patients ineligible for ASCT. Outcomes for those refractory to frontline or subsequent chemotherapy are particularly poor, with overall response rates (ORR) to salvage treatment of 26% and a median overall survival (OS) of 6 months.[10] Without a curative standard, lower intensity treatment options cited for patients ineligible for ASCT include rituximab, gemcitabine and oxaliplatin (R-GemOx) and bendamustine and rituximab (BR)[1] or lenalidomide[11], but access to these therapeutic agents is not consistent across Canadian treatment centres.

Adoptive cellular therapy using CAR-T cells has recently emerged as a potentially curative option for R/R DLBCL, and it may even supplant ASCT as the preferred second line option for R/R DLBCL. The phase 3 randomized controlled trial (RCT) ZUMA-7 reported superior response rates and event-free survival (EFS) with axicabtagene ciloecel (axi-cel) compared to standard of care (two or three cycles of platinum-based chemoimmunotherapy followed by high-dose chemotherapy with ASCT) in patients who were refractory to or had relapsed no more than 12 months after first-line

chemoimmunotherapy.[12] Both axi-cel and tisagenlecleucel have obtained Health Canada approval for R/R DLBCL after two or more lines of systemic therapy.[13] While eligibility for CAR-T is more inclusive than ASCT, Canadian patients have limited access to CAR-T therapies due to a lack of qualified centres, the limitations of CAR-T product supply and prohibitive cost. Toxicity is also a concern, with a significant incidence of cytokine release syndrome (CRS) and neurologic adverse events (AEs).[14] Therefore R/R DLBCL continues to be an area of unmet therapeutic need with no standard of care, particularly for those patients ineligible for ASCT or CAR-T.

Recently there has been unprecedented development of novel agents for patients with R/R DLBCL who are ineligible for ASCT.[2,14] Select agents are discussed in this article (summarized in **Table 1**), with particular attention to the Canadian regulatory landscape.

**Monoclonal Antibodies:**
Tafasitamab (MOR208) is an Fc-enhanced, humanized monoclonal antibody directed against CD19 approved by Health Canada in 2021.[15] In a phase II open label trial, adults (n=80) with R/R DLBCL who had received no more than three prior lines of therapy and were transplant-ineligible were treated with tafasitamab and lenalidomide (25 mg daily) for up to 12 cycles (28 days each) followed by tafasitamab monotherapy until disease progression.[15] Primary refractory patients were excluded. The initially reported overall response rate (ORR) was impressive at 60% with 43% complete response (CR) (**Figure 1**). The median duration of response (DoR) of 43.9 months was reported in the long term follow up in subjects with at least 35-months of data.[16], and median PFS and OS were 11.6 and 33.5 months respectively. Toxicity was mild with the most common grade 3 or higher AE being neutropenia (48%). After discontinuation of lenalidomide Grade 3 or higher AEs dropped to 29% (from 70%) in the study population.

**Antibody-drug conjugates:**
Polatuzumab vedotin (pola), an antibody-drug conjugate that delivers microtubule inhibitor monomethyl auristatin E (MMAE) directly to mature B cells with its target CD79b was approved by Health Canada

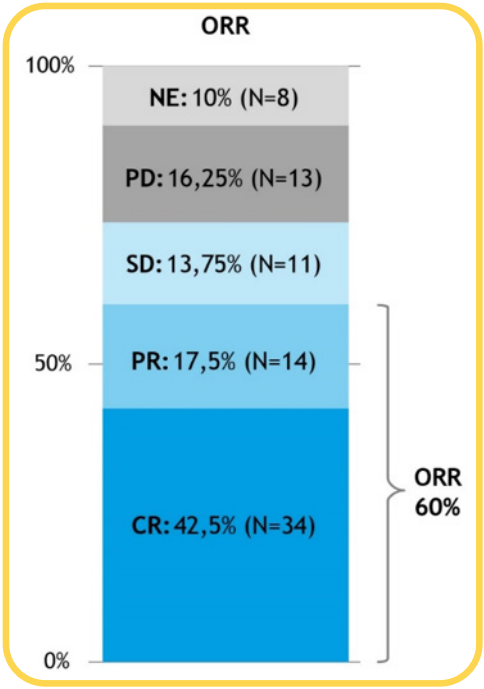



***Figure 1.*** *Response rates with tafasitamab, lenalidomide, and rituximab from L-MIND; Salles et al., 2020.*
*CR, complete response; NE, not evaluable; ORR, overall response rate; PR, partial response; PD, progressive disease; SD, stable disease.*

in 2020.[17] The efficacy of pola was established in a phase II trial that randomized patients with R/R DLBCL who were ASCT-ineligible or had prior ASCT to receive pola in combination with BR versus BR alone[17]. Patients in the pola-BR arm had superior ORR (45% vs. 17.5%) and CR rates (40% vs. 17.5%). The addition of pola to BR also improved both PFS (median 9.5 vs. 3.7 months) and OS (median, 12.4 vs. 4.7 months). There was a higher rate of grade 3-4 AEs in the group that received pola-BR, with the most common being neutropenia (46% vs. 33%) followed by anemia (28.2% v 17.9%), and thrombocytopenia (41% v 23.1%), but a similar rate of grade 3-4 infections (23% vs. 22%). Peripheral neuropathy, a known side effect of MMAE-based drug conjugates, was common (44% pola-BR vs. 8% BR), but all were classified as grade 1-2 and resolved in most patients. The addition of Pola to standard chemoimmunotherapy also enhances response rates and PFS in previously untreated DLBCL.[18] It is also being studied in combination with R-GemOx for R/R.[19]

Another antibody-drug conjugate is loncastuximab tesirine (lonca), a humanized anti-CD19 antibody conjugated to SG3199, a pyrrolobenzodiazepine dimer cytotoxin that causes intrastrand DNA crosslinks.[20] A phase II single-arm open label clinical trial enrolled adults with R/R DLBCL, high grade lymphoma with BCL2+/- BCL6 rearrangements or primary mediastinal BCL; the study population was heavily pretreated with 32% having received more than 3 prior lines of systemic therapy. After receiving lonca as a single agent once every 3 weeks for 1 year or until disease progression, an ORR of 48.3% with a 24.1% CR rate was observed. In this study, patients were allowed to continue their use of lonca beyond the one year timepoint. Median PFS was 4.9 months and median OS was 9.9 months. The most common grade 3 or higher AE was neutropenia (26%) followed by thrombocytopenia (18%), and increased gamma-glutamyltransferase (17%). Eight patients (6%) died while on treatment, but none of the fatalities were deemed to have been drug-related. Lonca tesirine is approved in the U.S. for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy. It is not yet approved by Health Canada.

Despite CD30 not being universally expressed on B-cell lymphomas, brentuximab vedotin (BV), a

monoclonal antibody against CD30 with MMAE drug conjugate, does produce objective responses in R/R DLBCL. In a phase 2 study that enrolled R/R B-cell non-Hodgkin lymphoma patients, 44% of subjects with DLBCL responded and 17% had CR. The median DoR was 5.6 months (16.6 months for complete responders).[21] Neutropenia (37%) was the most common grade 3 and higher AE followed by peripheral neuropathy (28%). BV has also been studied in combination with lenalidomide in a phase 1 dose expansion trial[22], showing an ORR of 57%, a CR rate of 35%, median PFS of 10.2 months and median OS of 14.3 months. Responses were higher in CD30+ DLBCLs (73%) in this study but in the previous study there was no statistical correlation between response and level of CD30 expression.[21] The toxicities observed were consistent with those seen with single agent use, but most patients required G-CSF support. These promising results have led to the current ECHELON3 trial, a phase 3 RCT studying lenalidomide plus rituximab with or without BV.[23]

| Novel Agent | Ab Target or Drug Class | Regimen (comparator) | Phase | ORR % (CRR %) | Median PFS (months) | Median OS (months) |
|---|---|---|---|---|---|---|
| Tafasitamab[15] | CD19 | Tafasitamab + Lenalidomide* | 2 open label | 58 (40) | 11.6 | 33.5 |
| Loncastuximab tesirine[20] | CD19 | Loncastuximab tesirine | 2 | 48 (24) | 4.9 | 9.9 |
| Brentuximab vedotin[21] | CD30 | Brentuximab vedotin | 2 | 44 (17; not reported for PFS and OS) | | |
| Brentuximab vedotin[22] | CD30 | Brentuximab vedotin + Lenalidomide | 1/ dose expansion trial | 57 (35) | 10.2 | 14.3 |
| Polatuzumab vedotin[31] | CD79b | Polatuzumab vedotin + R | 2 (randomized) | 54 (21) | 5.6 | 20.1 |
| Polatuzumab vedotin[17] | CD79b | Polatuzumab vedotin + BR* vs. BR | 2 (randomized) | 45 (40) | 9.5 | 12.4 |
| Blinatumomab[24] | CD19-CD3 | Blinatumomab | 2 | 35 (17) | 3.7 | 5.0 |
| Mosunetuzumab[25] | CD20-CD3 | Mosunetuzumab | 1/1b | 45 (25) | Not reported | Not reported |
| Selinexor[26] | XPO1 | Selinexor | 2b | 28 (12) | 2.6 | 9.1 |
| Venetoclax[29] | BCL2 | Venetoclax | 1 | 18 (12) | 1 | 32 at 12 months |
| Lenalidomide[32] | | Lenalidomide | 2/3 | 27.5 | 13.6 | 31.0 |
| Umbralisib[30] | PI3K | umbra; U2; U2 + benda-mustine | 2 | Umbra 13 (3); U2 32 (11); 43 (17) | Not reported | Not reported |
| Ibrutinib[28] | Bruton's Tyrosine Kinase | Ibrutinib + Lenalidomide + R | 1b/2 | 44 (28) | 5.5 | 9.5 |

***Table 1.*** *Select Agents for Treatment of Relapsed/Refractory DLBCL; courtesy of Anthea Peters, MD*

*BR, bendamustine + rituximab; C, cycle; D, day; NR, not reached; R, rituximab; umbra, umbralisib; U2, umbralisib + ublituximab*
**indicates Health Canada approval*

**Bispecific antibodies:**
Bispecific antibodies, or bispecific T-cell engagers (BiTEs), target B (CD19 or CD20) cells and T (CD3) cells, thereby redirecting T cells to engage with and eliminate malignant B cells. The agent in this class furthest in development is blinatumomab, which targets CD19 and CD3. Blinatumomab has received regulatory approval for adult patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL) and an approval with conditions for pediatric patients with Philadelphia chromosome-negative relapsed or refractory B-cell precursor ALL. In a phase 2 trial of R/R DLBCL, blinatumomab produced a moderate response rate but relatively short survival.[24] There was a high rate of neurologic AEs, including grade 3 encephalopathy and aphasia. Another consideration for the use of this agent is its requirement to be dosed as a continuous intravenous infusion.

Mosenetuzumab is a bispecific antibody with a much longer half-life allowing dosing every three weeks. Durable responses in aggressive and indolent B-cell NHLs in a phase 1 dose escalation study have been recently reported.[25] Despite a heavily pretreated aggressive NHL population in the study, with 34% of subjects having had prior ASCT and 12% having received prior CAR-T, 35% of subjects responded with 20% CRs, and DoR in those with CR was a median of 23 months. Cytokine release syndrome occurred in 27% of all patients but only 1% were classified as grade 3 or higher.

**Other mechanisms:**
Selinexor is an oral selective inhibitor of nuclear export of oncoproteins mediated by XPO1.[26] This agent is already approved in the U.S. for use in multiple myeloma, and was evaluated in patients with R/R DLBCL in the phase 2b SADAL trial in which heavily pretreated patients (n=127) were given selinexor 60 mg taken on days 1 and 3 weekly until disease progression or unacceptable toxicity. Responses with this single oral drug were promising, with an ORR of 28% (36/127) and a CR rate of 12% (15/127). The most common grade 3-4 adverse events were hematologic in nature. The median PFS reported in the SADAL trial was 2.6 months. Selinexor shows promise and is being combined with other agents in further studies, such as a phase 2/3 study in progress using R-GDP with or without selinexor.[27]

Ibrutinib, best-known for its use in chronic lymphocytic leukemia (CLL), does have activity in DLBCL, as shown in a phase 1b/2 trial in combination with lenalidomide and rituximab. The ORR in this study was higher in non-germinal center B-cell-like (non-GCB)DLBCL at 65% vs. 44% in the entire cohort.[28] Another drug typically used for CLL, BCL-2 inhibitor venetoclax, has minimal single agent activity in DLBCL.[29] Umbralisib, a PI3K-inhibitor, has objective activity alone and in combination with ublituximab with or without bendamustine, but results using this agent are preliminary and currently only available in abstract form.[30]

**Conclusions:**
Options for tolerable treatment are needed in R/R DLBCL in patients who are ineligible for ASCT or unable to access CAR-T cell therapy. Herein, several emerging novel agents with promise for further development as single agents or in combination regimens were reviewed. Many of these are monoclonal antibody-based and have a toxicity profile that makes them desirable particularly for the elderly and comorbid patient. The sequencing of agents that target CD19 with regards to CAR-T will be a question of interest.

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