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FLYLEAF

EDITORS FOR THIS ISSUE

Insights into myelodysplastic syndromes from current preclinical models

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Abstract

In recent years, there has been significant progress made in our understanding of the molecular genetics of myelodysplastic syndromes (MDS). Using massively parallel sequencing techniques, recurring mutations are identified in up to 80% of MDS cases, including many with a normal karyotype. The differential role of some of these mutations in the initiation and progression of MDS is starting to be elucidated. Engineering candidate genes in mice to model MDS has contributed to recent insights into this complex disease. In this review, we examine currently available mouse models, with detailed discussion of selected models. Finally, we highlight some advances made in our understanding of MDS biology, and conclude with discussions of questions that remain unanswered.

Key words: Myelodysplastic syndromes; Mouse models; Genetic mutations

Core tip: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic disorders. In recent years, we have witnessed a rapidly expanding

catalog of MDS candidate genes. Mirroring this, there has been an increased number of candidate genes employed to model MDS. Here, we aim to review currently available mouse models of MDS, highlighting models that are robust and well-characterized phenotypically with a particular focus on models that demonstrate close resemblance to the human disease.

INTRODUCTION

Myelodysplastic syndromes (MDS) are neoplastic clonal disorders of ineffective hematopoiesis with an inherent risk of transformation to acute myeloid leukemia (AML)[1]. MDS typically manifests as increased intramedullary apoptosis of maturing clonal cells in a hyperproliferative and pro-inflammatory bone marrow[2-4]. Clinically, this is seen as peripheral blood cytopenia(s) with accompanying dysplasia in a hyper- or normocellular bone marrow.

It should be noted that apoptosis in the bone marrow is more prominent in low risk MDS, driven by an excess of pro-inflammatory cytokines and altered T cell response[5-7]. In advanced MDS, increased expression of BCL2 leads to resistance to apoptosis. Additionally, the acquisition of further molecular defects results in increased proliferation and blocked differentiation in myeloid progenitors, culminating in evolution to AML[8-11].

CLONAL HEMATOPOIESIS FROM A MDS STEM CELL

MDS is thought to arise from mutations in the hematopoietic stem cell/progenitor (HSPC) CD34+ cell[12]. The founder mutation occurs in a MDS stem cell (or MDS initiating cell) that gives rise to clonal hematopoiesis. Support for this model of clonal architecture of MDS has been illustrated in several studies[13,14]. Delhommeau *et al*[13] isolated CD34+ cells from MDS patients and identified *TET2* mutations only in a small fraction of immature CD34+CD38- population with higher proportions detected in the CD34+CD38+ mature progenitors. The findings are in keeping with a model in which a *TET2* mutation arose in an immature progenitor cell and was passed on to its more mature progeny. In another study using whole genome sequencing, Walter *et al*[14] reported that about 85%-90% of unfractionated bone marrow cells were clonally derived from the MDS stage and persisted through to leukaemic transformation.

Of interest, whilst the MDS stem cell can establish clonal hematopoiesis, overt hematological manifestations of disease may be absent. It is likely that additional cooperating genetic and epigenetic events are required to drive disease progression and bring about a clinically apparent phenotype[15]. Indeed, age-related clonal hematopoiesis was first described in a group of healthy women over the age of 65. In approximately 23% of these women, a skewed pattern of X-chromosome inactivation was observed in cells taken from the peripheral blood, with some associated with *TET2* mutations[15,16]. More recently, whole exome sequencing identified the presence of clonal somatic mutations in genes that are recurrently mutated in hematological malignancy in the peripheral blood of ostensibly healthy elderly individuals[17-19]. Jaiswal *et al*[17] reported that the presence of somatic mutations was rare in individuals younger than the age of 40. However, the incidence of clonal mutations increases considerably with successive decades of life thereafter, with the frequency of mutations in individuals 60 years and older exceeding the incidence of hematological malignancy diagnosed in the general population. The most commonly mutated gene was *DNMT3A*, followed by *TET2, ASXL1, TP53, JAK2*, and *SF3B1*. These mutations persisted over time, and were associated with an increased risk, approximately 0.5%-1% per annum, of developing a hematological malignancy. In a second, independent cohort of subjects unselected with respect to hematological phenotypes, Genovese *et al*[18] found that more than 10% subjects aged 65 years or more had evidence of clonal hematopoiesis. In this population the most frequently mutated candidate driver genes were *DNMT3A*, *ASXL1*, *TET2*, *JAK2* and *PPMID* and the presence of a mutant clone was a risk factor for subsequent hematological malignancy or death. Finally, McKerrell *et al*[19] reported that the prevalence of clonal hematopoiesis doubled with each decade of life after the age of 50, rising from 1.5% in those aged 50-59 to nearly 20% in those 90 years and older. The most common mutations were *DNMT3A, JAK2, SRSF2* and *SF3B1.* Notably, spliceosome mutations at *SRSF2* P95, *SF3B1* K666 and K700 were exclusively observed in individuals older than 70 years. The striking degree of overlap between results of these studies with regards to the driver genes identified and the significantly heightened risk of hematological disease in individuals with clonal hematopoiesis serves to underline the generalizability of these findings.

CURRENT MOLECULAR INSIGHTS

MDS is a very heterogeneous disease, underscored by significant genomic instability and a complex genetic landscape. The catalog of MDS candidate genes is rapidly expanding with the application of modern techniques in detecting molecular lesions. However, the pathogenesis of MDS remains elusive. The hierarchical significance and functional interplay of the different mutations in the development and progression of the disease are areas of active investigation. Moreover, there is emerging evidence that MDS is not solely an intrinsic hematopoietic disease, with the niche, *i.e.*, bone marrow

microenvironment, also playing a role[5,6,20,21].

Recently, interrogation of MDS samples by massive parallel sequencing technology has allowed the identification of genetic mutations at single nucleotide resolution. Using this technique, mutations are apparent in up to 80% of cases, including many with a normal karyotype[22]. Recognition that recurrently mutated genes can be grouped according to the function of the proteins that they encode (epigenetic regulators, transcription factors, spliceosome components, *etc*.) has greatly improved our understanding of MDS pathogenesis[14,22,23].

CYTOGENETIC ABNORMALITIES

Cytogenetic analysis of MDS has been instrumental in the diagnosis and prognostication of MDS. Using conventional metaphase cytogenetics, abnormalities are found in up to 50% of patients with MDS[24], with a higher frequency of abnormal and complex cytogenetics (defined as the identification of three or more abnormalities in the karyotype) seen in therapy-related MDS.

As is the case for the recurrent mutations, many cytogenetic lesions seen in MDS are not exclusive to this disorder and also occur in other myeloid malignancies. However, copy number losses or gains are more frequent in MDS than balanced translocations, which tend to predominate in AML. The most common single cytogenetic aberrations include trisomy 8, del(5q), del(20q), and monosomy 7 or del(7q)[24-27]. It is thought that, in most cases, abnormalities detected by conventional cytogenetics are secondary genetic events resulting from genomic instability caused by earlier submicroscopic initiating or founder mutations[28]. Furthermore, cytogenetics only detects large-scale genomic changes, *i.e.*, loss or gain of a whole chromosome or most of a chromosome arm and as such, it is difficult to pinpoint candidate driver genes that are involved in a region of copy number change.

Although the abnormality rate for conventional cytogenetics is limited by the low-resolution inherent in using a microscope-based technology for the detection of genomic lesions, the information it provides remains clinically relevant. Conventional cytogenetics has a key role in identifying clonality. This can be central to MDS diagnosis which otherwise relies on subjective morphologic criteria. Importantly, possibly by virtue of the fact that cytogenetic abnormalities are seldom initiating events, cytogenetics provides powerful prognostic information in additional to diagnostic information, as demonstrated by the revised International Prognostic Scoring System (IPSS-R)[29].

MUTATIONS IN THE SPLICING MACHINERY

Pre-mRNA splicing is catalyzed by the spliceosome, a macromolecule composed of five small nuclear RNAs associated with numerous proteins to form small ribonucleoproteins. It is a highly dynamic structure, conferring accuracy in constitutively spliced exons and at the same time, allowing the flexibility for alternative splicing to generate genetic diversity and complexity.

Recently, whole genome and exome sequencing of human MDS samples has identified frequent somatic mutations in genes that encode components of the RNA splicing machinery, including *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*[22,30-32]. Indeed, RNA splicing is one the most common pathways targeted by mutations in MDS, with up to 50% of patients carrying a mutation in a gene encoding a spliceosome component[22].

Furthermore, mutations in the RNA splicing genes are mutually exclusive and are most often founding events. In fact, the mutant allele burden is typically between 40%-50%, indicating a dominant bone marrow clone that is heterozygous for the mutation[33,34]. Given the essential requirement for RNA splicing in generating protein diversity, biallelic mutations would be predicted to be lethal and evidence from mouse models largely supports this. Mutation hotspots in the three most frequently mutated genes, *SF3B1*, *SRSF2*, and *U2AF1*, have been identified. Almost all described mutations are missense, with no evidence of nonsense or frameshift changes, suggesting that they result in altered function rather than loss of function[30,32,35].

The mutations in individual spliceosome components are associated with different phenotypes and distinct clinical outcomes[22,34]. *SF3B1* mutations are found almost exclusively in patients with refractory anemia with ringed sideroblasts without or with thrombocytosis [refractory anemia with ringed sideroblasts (RARS) and RARS-T respectively], therefore proposing a causal link between mutation and ringed sideroblasts formation. Most patients with *SF3B1* mutations have good risk disease with a protracted clinical course and a low propensity to AML transformation[36]. On the other hand, *SRSF2* mutations are found mainly in patients with multilineage dysplasia and/or excess blasts and predict a high risk of leukemic evolution and poor survival[37-39]. *U2AF1* mutations have been reported in various MDS subtypes and found to predict high risk of progression to AML and hence, shorter survival[30,38].

The observation that spliceosome mutations are mainly founding mutations associated with different clinical outcomes led Papaemmanuil *et al*[22] to hypothesise that they give rise to initiating clones with different genetic predestination. Through specific cooperating genetic lesions, the initial driver mutations likely shape the trajectory of clonal evolution leading to more or less aggressive MDS phenotypes.

Spliceosome mutations are rarely found in childhood myeloid neoplasms. Moreover, they are rarely detected in the blood of young healthy individuals but increase in prevalence in an age-dependent manner in people aged 70 years and over, and confer an increased risk of myeloid malignancy[19]. These findings suggest RNA

splicing mutations are typically acquired with age and support the hypothesis that they occur early in disease pathogenesis[17-19].

MUTATIONS IN GENES INVOLVED IN EPIGENETIC REGULATION

Alterations in epigenetic processes, including DNA methylation, histone modifications and miRNA are now well-described and are pivotal in the pathogenesis of MDS.

Promoter-associated CpG island hypermethylation is seen in about 3%-5% of MDS, may occur early in the course of the disease and is associated with a more rapid progression to AML[40]. Recurrently mutated genes in MDS known to be involved in the regulation of DNA methylation include *TET2*, *DNMT3A* and *IDH1/2*[41-45].

Post-translational modification of histones plays an important part in epigenetic regulation. These proteins can be acetylated, methylated, and ubiquinated by a group of histone-modifying enzymes. Loss-of-function mutations occur in histone modifiers, such as *ASXL1* and *EZH2*, and they are associated with a poor prognosis and reduced survival[46-49].

MUTATIONS IN OTHER PATHWAYS

Mutations in signalling molecules, transcription factors, and *TP53* are often subclonal, driving disease progression and are associated with adverse clinical outcomes[22,50-54]. They tend to occur in advanced disease, with the exception of *TP53*, which may occur at an early stage in del(5q) MDS and therapy-related disease[55]. In the context of del(5q) MDS, mutated *TP53* is associated with lower response rates to lenalidomide treatment and an increased risk of leukaemic transformation[56].

MODELING MDS IN MICE

Animal models are valuable pre-clinical tools to advance our understanding of human diseases, as well as facilitating the development and evaluation of novel therapeutic agents. The laboratory mouse (Mus musculus) is the model of choice to phenocopy and to investigate the biology of human cancer for a variety of reasons including its small size, well-characterized physiology and rapid breeding cycle. Moreover, the frequently used C57BL/6J mouse strain has a fully sequenced genome, with 75% orthology to human[57].

In the case of MDS, mouse models are particularly useful to study the biology of this disease. By expressing MDS candidate genes in mice, the function of the various genes and their role in the pathogenesis and progression of the disease can be evaluated in detail. These models can also serve as a platform to identify and test novel therapeutic candidates. Additionally, they can also be used to evaluate the mechanism of action of therapies currently used in clinic, for example lenalidomide which

is used in 5q- syndrome, and hypomethylating agents such as azacitadine and decitabine.

DIAGNOSING MDS IN HUMANS AND MICE

The diagnosis of MDS in humans is predominantly based on morphology. Based on the 2008 World Health Organisation (WHO) MDS classification, the minimum requirement for diagnosis include the presence of > 10% dysplasia morphologically, significant cytopenia in at least one lineage, and < 20% blasts[1]. The thresholds for significant cytopenias as recommended by the IPSS are hemoglobin < 10 g/dL, absolute neutrophil count < 1.8×10^9/L, and platelet count < 100×10^9/L. The only exception to meeting the minimum diagnostic prerequisites is evidence of clonality, *i.e.*, an abnormality from a pre-defined list of characteristic cytogenetic abnormalities is present. This is sufficient for a diagnosis of MDS, provided cytopenia is present and that AML has been excluded in the basis of the blast count[1].

Using the morphological criteria outlined above, MDS can be sub-classified depending on the number of lineages affected by cytopenias and dysplasia, and the enumeration of blasts in the bone marrow. Groups classified according to WHO criteria have prognostic significance, which can be refined further with cytogenetic information.

The presence of excess blasts, immediately places patients in a high-risk group. There are two groups, stratified according to blast count: Refractory anemia of excess blasts-1 (RAEB-1, 5%-9% marrow blasts) and RAEB-2 (10%-19% marrow blasts). The low-risk MDS comprises subtypes with only single-lineage cytopenia and dysplasia, and includes refractory anemia, and RARS. It should be noted that ringed sideroblasts can also be seen in other subtypes of MDS, and carry no independent prognostic significance. The intermediate-risk MDS, refractory anemia with multi-lineage dysplasia is associated with bi- or tri-lineage dysplasia.

Taking into consideration the diagnostic criteria and other salient characteristics described, the key features of human MDS that are desirable to recapitulate in mouse models include peripheral blood cytopenias, bone marrow dysplasia, ineffective hematopoiesis, a propensity to transform to secondary acute leukemia after a long latency, transplantable disease into secondary mice, and the ability to mimic therapeutic responses to treatments with established efficacy in human MDS.

It should be noted that mouse hematopoiesis differs from human hematopoiesis. In humans hematopoiesis is largely confined to the epiphyses from adulthood and compensatory hematopoiesis can occur in the bone marrow. In steady-state murine hematopoiesis, the bone marrow is 95% occupied leaving the spleen as the major site for compensatory blood cell production, and the spleen in mouse remains an important hematopoietic organ throughout life. As such, compensatory spleno-

	Human	Mouse
Cytopenia	Yes	Yes
Dysplasia	Yes	Yes
Bone marrow cellularity	Usually increased	Usually increased
Cytogenetic abnormality	Yes	No recurrent abnormalities identified to date
Mutations	Yes	Yes
Splenomegaly	Usually absent	Possible

Figure 1 Key features of myelodysplastic syndromes in humans and mouse.

Table 1 Bethesda diagnostic criteria for myeloid dysplasia in mice

(1) At least one of the following
　A: Neutropenia
　B: Thrombocytopenia (without leucocytosis or erythrocytosis)
　C: Anemia (without leucocytosis or thrombocytosis)
(2) Maturation defect in myeloid cells manifest as at least one of
　A: Dysgranulopoiesis, dyserythropoiesis, or dysplastic megakaryocytes with or without increased myeloid immature forms or blasts
　B: At least 20% myeloid immature forms or blasts
(3) Disorder is not AML
Subclassification
Myelodysplastic syndrome if meeting criteria (2)A
Cytopenia with increased blasts if meeting (2)B

Summarised from Kogan *et al*[60]. AML: Acute myeloid leukemia.

megaly in a cytopenic mouse may be considered the equivalent of bone marrow hypercellularity in a human and should not necessarily be regarded as evidence of a myeloproliferative neoplasm[58,59]. Thus, compensated or uncompensated anemia in a mouse with an MDS phenotype may be accompanied by splenomegaly. In addition, AML in mice will often involve both the bone marrow and the spleen, unlike in humans where leukemia tends to be confined to the bone marrow. Figure 1 illustrates the key features of myelodysplasia

expected in both humans and mice, and also points out the differences between them.

DIAGNOSIS OF MDS IN MICE - THE BETHESDA CLASSIFICATION

Recognizing the need for consensus in the classification of murine hematopoietic lesions within the scientific community, the hematopathology subcommittee of the Mouse Models of Human Cancer Consortium (MMHCC) proposed diagnostic criteria for the classification of nonlymphoid hematopoietic neoplasms in mice (Table 1). There is a myeloid dysplasia category and within this, disease can be subclassified as either myelodysplastic syndrome or cytopenia with increased blasts[60]. To qualify as a myeloid dysplasia, acute nonlymphoid leukemia (*i.e.,* AML) must first be excluded. A diagnosis of AML should be applied if there are greater than 20% blasts, disseminated tissue infiltration and biologically aggressive disease that is rapidly fatal[60].

The defining criteria for myeloid dysplasia require the presence of cytopenia. Evidence of myeloproliferation in the form of erythrocytosis, leucocytosis and thrombocytosis must be absent. If there is morphologic evidence of dysplasia in at least one hematopoietic lineage, a myelodysplastic syndrome is said to be present

Table 2 Candidate genes used to model myelodysplastic syndromes in mice and their correlation to human myelodysplastic syndromes

Gene(s) studied	Chromosomal location	Frequency in human MDS	Equivalent human MDS subtype according to WHO 2008 classification	Ref.
Transcription factors				
NUP98-HOXD13	t(2;11)(q31;p15)	Rare	RCMD	[69]
RUNX1	21q22.3	15%-40%	RAEB	[105]
EVI1	3q26.2	Rare	RCMD	[136]
SALL4	20q13.2	15%-40%	RCMD	[139]
NPM1	5q35.1	About 4%	RCMD	[138]
Signalling molecules				
NRAS	1p13.2	About 20%	RAEB	[142]
BCL2	18q21.33	Unknown		
PTEN	10q23.3	Unknown	RCMD	[140]
SHIP	2q37.1	Unknown		
Epigenetic regulators				
TET2	4q24	20%-30%	RCMD/CMML	[115]
ASXL1	20q11.21	15%-20%	RCMD	[122,123]
EZH2	7q36.1	2%-6%	RCMD/MPN	[141]
MLL5	7q22.1	Unknown	No definitive MDS	[145]
RNA spliceosome				
SRSF2	17q25.2	15%-30%	RCMD	[98]
U2AF1	21q22.3	11%	No definitive MDS	[100]
SF3B1	2q33.1	10%-20%	RARS, RARS-T	[101]
Telomere function				
TERT	5p15.33	Unknown	RCMD	[124]
5q-				
RPS14	5q33.1	About 10%	5q-like	[91]
CD74-SMIM3 (NID67)	5q32-q33.1			[92]
SPARC	5q33.1			[97]
MIR145/146A	5q32-34			[143]
APC	5q22.2			[99]
CSNK1A1	5q32			[94]

WHO: World Health Organization; RARS: Refractory anemia with ringed sideroblasts; RARS-T: Refractory anemia with ringed sideroblasts and thrombocytosis; RCMD: Refractory anemia with multilineage dysplasia; RAEB: Refractory anemia with excess blasts; MPN: Myeloproliferative neoplasm; MDS: Myelodysplastic syndromes; CMML: Chronic myelomonocytic leukemia.

(Table 1). Morphological features of dysplasia are subtler in mice than in humans and can be difficult to identify. The MMHCC subcommittee lists features considered speculative evidence of dyspoiesis. In the erythroid lineage, dyserythropoiesis includes megaloblastic maturation, increased mitotic figures, multinucleation, and nuclear irregularity. Ringed sideroblasts are also a feature of dysplasia, but are rare in mice. Dysgranulopoiesis may manifest as hypogranular neutrophils, and lobated neutrophils as opposed to ring-shaped nuclei. For megakaryocytes, micromegakaryocytes, large megakaryocytes with unlobated nuclei or bizarre hypersegmented nuclei, and megakaryocytes with separated nuclei are all regarded as signs of dysplasia. Where AML has been excluded and morphologic dysplasia is lacking but there is cytopenia with more than 20% blasts in the bone marrow or spleen, the diagnosis cytopenia with increased blasts can be made. Provision is also made within the MMHCC criteria for disease in mice that very closely approximates a defined human MDS subtype. In this situation the disease may be labelled "myelodysplastic syndrome with features of a named human MDS"[60].

Little is known about the acquisition of cytogenetic abnormalities in mouse models of MDS. Thus, recurrent cytogenetic abnormalities cannot be used to aid the diagnosis and classification of disease in mice, in the way that they can be used in humans.

AVAILABLE MOUSE MODELS OF MDS

There are more than 20 published mouse models of MDS in the literature, and they are summarised in Tables 2 and 3. Several strategies have been employed to create these models. They include genetic engineering of mouse hematopoietic cells using knock-in or knock-out strategies, and xenotransplantation of human MDS cells, the latter of which has proven to be technically difficult.

Mirroring the rapidly expanding catalog of MDS candidate genes, there have been an increasing number of genetically modified mice being reported as MDS mouse models (Figure 2). These models mostly examine the effects of a single gene modification in the pathogenesis of MDS, and many of them displayed features reminiscent of MDS. Considering the genetic complexity inherent to MDS, it is not surprising that a single unifying model that faithfully mimics the MDS phenotype in its entirety is still lacking. More recently, there have been efforts to incorporate more than one mutation in modeling MDS, which has certainly provided insights into the functional interactions of different genes in the

Table 3 Published mouse models of myelodysplastic syndromes

Gene(s) studied	Model/technique	Tractability to human MDS								Ref.
		Anemia	Multi-lineage cytopenias	Dysplasia	Bone marrow cellularity	HSPC skewing	Secondary leukemia	Latency to leukemia	Survival	
NUP98-HOXD13	Transgenic	Yes	Yes	Yes	Hyper-	Yes	Yes[2]	14 mo	-	[69]
Cd74-Nid67	Large scale chromosomal deletion, RPS14 haploinsufficient	Yes	No	Yes	Hypo-	Yes	No	-	[1]	[92]
SPARC	Knock-out	No	No	Yes	[1]	[1]	No	-	[1]	[97]
MIR-145, MIR146a	Retroviral transduction	No	No	Yes	[1]	[1]	Yes	4-14 mo	-	[143]
APC	Transgenic, haploinsufficient	Yes	No	Yes	[1]	Yes	No	-	3-8 mo	[99]
Csnk1a1	Transgenic, inducible	Yes	Yes	Yes	Yes	Yes	No	-	[1]	[94]
Srsf2	Transgenic, inducible	Yes	Yes	Yes	Normal	Yes	No	-	[1]	[98]
U2af1	Transgenic, inducible	No	No	No	Normal	Yes	No	-	-	[100]
Sf3b1	Transgenic, haploinsufficient	Yes	No	Yes	[1]	[1]	No	-	About 12 mo	[101]
RUNX1-D171N	Retroviral transduction	Yes	Yes	Yes	Normal to hyper-	[1]	Yes	4-13 mo	-	[105]
RUNX1-S291fsX300	Retroviral transduction	Yes	Yes	Yes	Normal to hyper-	[1]	Yes	4-13 mo	-	[105]
RUNX1S291fs/Ezh2	Retroviral transduction	Yes	Yes	Yes	Variable	[1]	No	-	262 d	[125]
Tet2	Transgenic, hypomorphic	Yes	Yes	Yes	Hyper-	Yes	No	-	11 mo	[115]
Tet2/Ezh2	Transgenic, inducible	Yes	Yes	Yes	Hyper-	Yes	No	-	10 mo	[141]
ASXL1	Transgenic, inducible	Yes	Yes	Yes	Hypo-	Yes	No	-	Median 50 wk	[122]
Asxl1	Transgenic, constitutive	Yes	Yes	Yes	Normal to hyper-	Yes[3]	16 mo	8-42 d	-	[123]
TERT	Transgenic, inducible	Yes	Yes	Yes	Hyper	Yes	Yes	[1]	12 mo	[124]
Evi1	Retroviral transduction	Yes	Yes	Yes	Hyper-	[1]	No	-	10-12 mo	[136]
SALL4	Transgenic	Yes	Yes	Yes	Hyper-	Yes	Yes	6 wk to 12 mo	-	[139]
Npm1	Transgenic, haploinsufficient	No	No	Yes	Hyper	No	Yes	24 mo	-	[138]
NRASD12-BCL2	Transgenic, inducible/ constitutive	[1]	No	Yes	[1]	Yes	Yes	3-6 mo	-	[142]
Pten/Ship	Transgenic, Pten +/- Ship -/-	Yes	Yes	[1]	Hypo	Yes	No	-	5 wk	[140]
Dido	Knock-out	Yes	No	Yes	[1]	[1]	No	-	1	[137]
Arid4a	Knock-out	Yes	Yes	Yes	Hyper-	[1]	Yes	5 mo	-	[144]
Mll5	Knock-out	Yes	No	No	[1]	[1]	No	-	-	[145]

[1]Denotes not reported, denotes not applicable; [2]Some mice developed pre-T cell acute lymphoblastic leukemia; [3]Only 1 of 18 mice developed AML. HSPC: Hematopoietic stem and progenitor cells; MDS: Myelodysplastic syndromes; AML: Acute myeloid leukemia.

biology of MDS.

Amongst the published mouse models, anaemia was the most common cytopenia reported with a significant proportion showing multilineage cytopenia including pancytopenia in some models. Accompanying dysplastic changes were often noted in more than lineage. It should be noted that unlike the number of cytopenias which is indicative of MDS severity, the extent of dysplasia carries no prognostic implications. Several of these models also showed alterations in the hematopoietic stem cell (HSC) and a propensity to transform to secondary AML, further improving their tractability to the human disease.

XENOGRAFT MOUSE MODELS OF MDS

The establishment of immunodeficient mice harbouring malignant human xenografts is an attractive approach to model and study malignancy of the hematopoietic system. Establishing human malignant cells in the mouse host has been technically challenging, but has proven feasible in hematological disorders such as AML and acute lymphoblastic leukemia (ALL). In contrast, the propagation of MDS clones has been met with limited success. This has been attributed to a number of factors including host anti-tumor/human immune response, inadequate microenvironment for tumor growth or survival, and toxicity from *ex vivo* manipulation of malignant cells.

Techniques have improved considerably over the last ten years, and the generation of transgenic severe combined immunodeficient (SCID) mice expressing human granulocyte-macrophage colony-stimulating
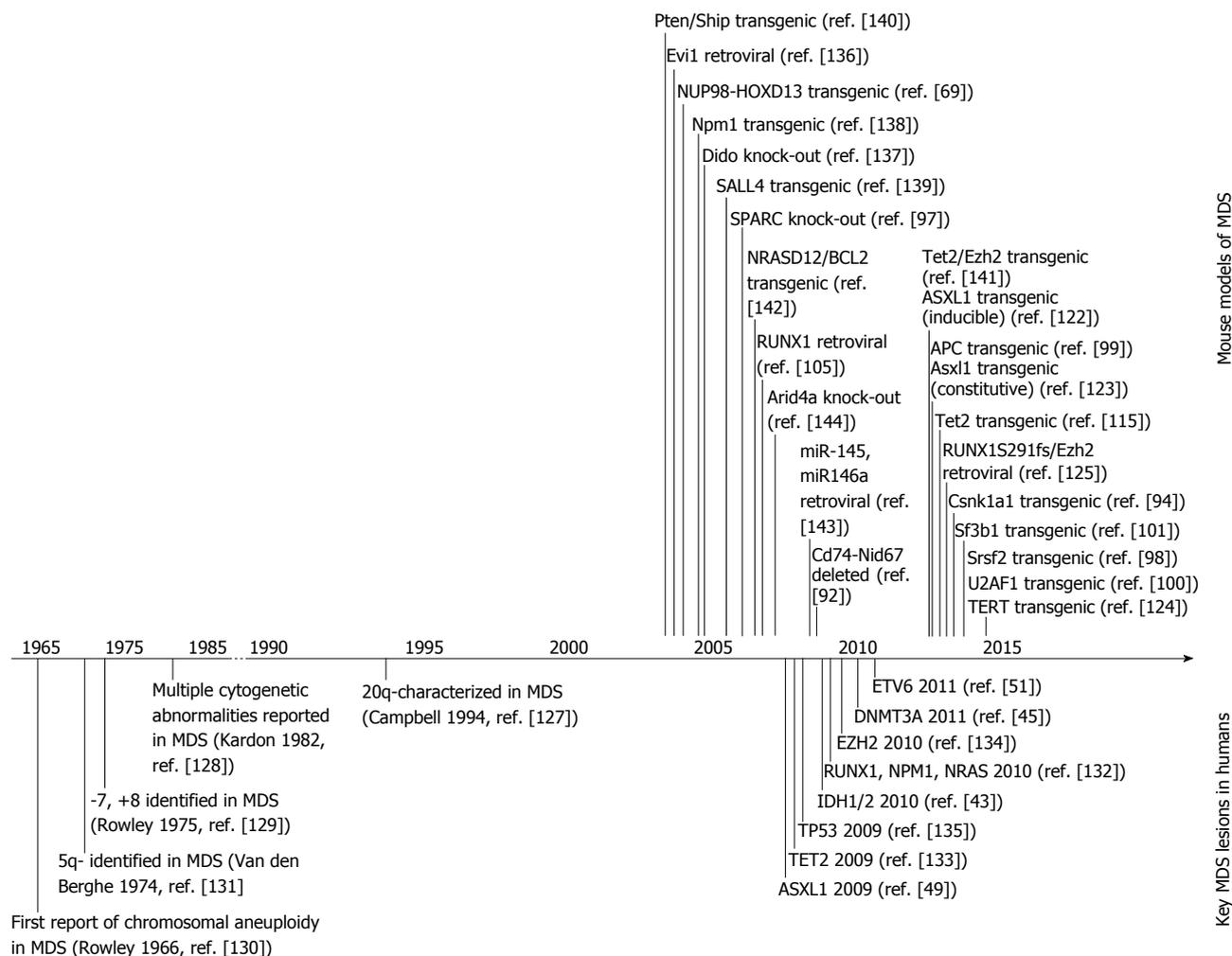
Figure 2 Time-line showing key genetic aberrations identified in myelodysplastic syndromes and published mouse models. MDS: Myelodysplastic syndromes.

factor and interleukin-3 (IL-3) has improved the engraftment of an immortalized cell line derived from a patient with MDS (F-36P). Engraftment was further improved with the pre-administration of IL-2 receptor antibodies, which suppressed natural killer (NK) cell function[61].

This led to the development of non-obese diabetic (NOD)-SCID mice that have reduced natural killer cell function, as well as deficiencies in T- and B-cells. However, injection of progenitor cells from MDS patients with del(5q) and trisomy 8 into these mice showed poor engraftment[62,63]. Although one of seven mice with del(5q) showed low-level of engraftment, no clinical phenotype of MDS was observed. In another study, bone marrow cells from MDS patients and healthy controls were injected into sublethally irradiated NOD-SCID mice, with or without human cytokines[64]. Cells from patients with MDS demonstrated reduced marrow repopulating ability compared to healthy controls. Moreover, previously observed karyotypic abnormalities could not be identified in recipient mice, suggesting that most of the engrafted human cells were derived from normal bone marrow. Taken together, these studies showed

that the NOD-SCID environment could not reliably and reproducibly support the expansion of human MDS cells.

The generation of NOD-SCID beta2-microglobulin-null mice (NOD-SCID-B2m$^{-/-}$) that have suppressed NK cell function, but express human cytokines and steel factor (c-kit ligand) has allowed the repopulation of MDS clones[65]. However, the level of engraftment was less than 1% of nucleated cells, and the mice did not develop clinical disease.

More recently, intravenous co-administration of the human marrow stromal cell line HS27a with CD34$^+$ MDS cells in NOD-SCID gamma (NSG) mice was explored and showed considerable promise with engraftment documented in 44 of 46 (95%) mice[66]. Co-localisation of the stroma and CD34$^+$ cells were seen in the spleen of the recipient mice, and furthermore, these cells also engrafted successfully in secondary NSG recipients. This study suggested that HS27a stromal cells in direct contact with the hematopoietic precursors supported their propagation. In another study, overproduction of niche factors such as CDH2 (N-Cadherin), IGFBP2, VEGFA, and LIF enhanced the expansion of MDS mesenchymal stromal cells, highlighting the complexity of the

disease and that it requires the engagement of both the hematopoietic and stromal elements to propagate[67].

Collectively, these studies demonstrate ongoing progress in the development of xenograft models of MDS. Overall, more robust and more consistent engraftment of MDS cells that can result in clinical disease is needed to improve the utility of this approach. While poor engraftment of MDS cells remains the main drawback, the requirement of an immunocompromised host with this technique makes it unsuitable for the bone marrow niche to be examined.

GENETICALLY ENGINEERED MOUSE MODELS OF MDS

Generally, genetic engineering of hematopoietic cells of mice has been accomplished using two approaches. The first approach involves *in vitro* transduction of bone marrow with viral overexpression/shRNA vectors and subsequent transplantation into a histocompatible, irradiated host. The second approach involves modification of the mouse germline to generate mice with altered expression of a particular gene of interest. These approaches can be further refined with the employment of the Cre-Lox recombination system which allows gene expression to be controlled in a temporal, cell type and spatial manner[68].

Indeed, conditional knock-in mice are currently the most favored technique in generating mouse models of MDS. The gene in question can be manipulated easily and importantly, is expressed at more physiological levels. The host is often immunocompetent, and the bone marrow niche can also be examined. In comparison, retroviral models require transplanting transduced cells into lethally irradiated recipients and hence, results in supraphysiological levels of gene expression. Moreover, the bone marrow niche is altered through the process of irradiation and transplantation. As a result, the observed effects of altered gene expression in this context is not entirely representative.

In the subsequent sections, we will focus our discussions on selected mouse models of MDS (Table 3). We will highlight models that are robust and well-characterized phenotypically, as well as models that illustrated different genetic lesions that are clinically relevant. We have particularly focused on the mouse models that demonstrate synergy to human disease.

MODELLING CYTOGENETIC ABNORMALITIES - CHROMOSOMAL TRANSLOCATIONS

NUP98-HOXD13
Of all the approaches that have been explored to model MDS to date, the *NUP98-HOXD13* mouse model is the best established, and perhaps the only published model that has been able to recapitulate many of the key features of MDS[69].

The *NUP98-HOXD13* involves the fusion of two genes: Nucleoporin protein, *NUP98,* with homeobox D13, *HOXD13*. The *NUP98-HOXD13* fusion gene, which is generated by the chromosomal translocation t(2;11)(q31;p15), was initially identified in a patient with therapy-related MDS (t-MDS)[70]. Although numerous partner genes of *NUP98* have been reported in various hematopoietic malignancies, balanced translocations are rare in MDS[71,72], and there are very few cases of MDS bearing the t(2;11) reported in the literature[73].

The first reported *NUP98-HOXD13* mouse model was established by a retroviral system. Pineault *et al*[74] constructed *NUP98-HOXD13* (*ND13*) cDNA using *ND13* cDNA fragment isolated from a patient with t-MDS, and transplanted transduced murine bone marrow cells into irradiated recipient mice. *ND13* expressing mice showed a preferential increase in myelopoiesis at the expense of B and T-cell lymphopoiesis, and developed overt features of myeloproliferative disease five months post-transplant. The mice did not progress to AML, however accelerated leukemic transformation was observed when the *HOX* cofactor, *Meis1,* was co-transduced with *ND13*.

Subsequently, conditional *NUP98-HOXD13* (*NHD13*) transgenic mice were developed, using a vav promoter to drive *NHD13* expression in hematopoietic tissues[69]. At 4 to 7 mo, these mice developed anemia and neutropenia, with variable degree of macrocytosis and thrombocytopenia. This was accompanied by normal or hypercellular bone marrow with dysplasia observed in multiple lineages. In line with human MDS, about half of *NHD13* mice with MDS developed acute leukemia, typically at 10 to 14 mo of age. Although AML was the most common type of leukemia reported, several mice also developed precursor T-cell lymphoblastic lymphoma/leukemia (T-ALL), which is rarely reported in human MDS. The T-ALL predisposition may be related to increased levels of *Hoxa* cluster genes, such as *Hoxa7* and *Hoxa9,* which have an association with T-ALL[75].

In addition to the key features of MDS described above, it is noteworthy that the *NHD13* mice showed marked reductions in undifferentiated lineage negative (lin^{neg}) hematopoietic precursors *in vitro* and *in vivo,* which are comparable to results from studies performed on MDS patients[76-79]. This was further accompanied by impaired differentiation with the majority of the *NHD13* lin^{neg} cells undergoing apoptosis, which is a salient feature in human MDS. Gene expression profiling of Lineage$^-$, c-kit$^+$, Sca-1$^-$ (LKS$^-$) myeloid progenitor cells from 3-mo-old NHD13 mice that displayed macrocytic anemia showed 3.6-fold reduction in BCL2[79]. Enforced expression of BCL2 inhibited apoptosis at the HSPC level, rescued the macrocytic anemia and interestingly, also abrogated leukaemic transformation[79].

The *NHD13* model has also been used to identify secondary mutations that lead to acute leukemia in the mouse. An increased frequency of *Nras* and *Kras* mutations has been noted in *NHD13* mice that pro-

gressed to leukemia[80]. In contrast, *Npm1, Trp53, Runx1, Kit,* and *Flt3* mutations were not increased, and *Meis1,* which induces leukaemic transformation in the retroviral model, was not altered in the transgenic model[69,80].

The initial transgenic *NHD13* study was performed using FVB/N background mice. The entire study was subsequently repeated with C57Bl/6 mice with similar findings, demonstrating that effects of the transgene were reproducible and not compounded by the genetic background of the mice[69]. In comparison, there are considerable differences between the transgenic and retroviral transduction models, which may be explained by *ex vivo* manipulation of cells, differences in mouse strain, amount of overexpression of NHD13 achieved and/or the differential effects of *ND13* retrovirus on the bone marrow hematopoietic stem/progenitor cells and their subsequent bone marrow reconstitution.

MODELLING CYTOGENETIC ABNORMALITIES - CONTIGUOUS GENE DELETION SYNDROMES

5q-

Deletion of the long arm of chromosome 5, del(5q), is the most common cytogenetic abnormality found in MDS, accounting for approximately 10%-15% of cases[24,81]. The 5q- syndrome is recognized as a distinct clinical entity in the 2008 WHO classification, and is defined by del(5q) being the sole karyotypic abnormality[1]. It has a female preponderance, and a distinct phenotype characterized by refractory anemia with normal or increased platelet count, erythroid hypoplasia, hypolobated megakaryocytes, < 5% blasts, and lenalidomide responsiveness. MDS associated with isolated del(5q) carries a good prognosis with a low risk of transformation to AML[82-84].

There are two distinct commonly deleted regions (CDR) in 5q- syndrome. The more distal CDR is mapped to a 1.5-megabase region between bands 5q31 and 5q33[85,86].

The CDR contains 24 known genes, 16 predicted genes, and four known microRNAs (*MIR584*, *MIR143*, *MIR145* and *MIR378A*)[85]. The more proximal CDR contains 18 genes, and has been associated with more advanced MDS and AML[87,88]. Point mutations in *CSNK1A1* occur in approximately 5% cases of 5q- syndrome[89], however point mutations have not been identified in the remaining coding genes in the distal CDR. The absence of point mutations in the majority of cases suggests that haploinsufficiency of one or more genes, or the epigenetic inactivation of a retained tumor suppressor allele are responsible for the disease phenotype[90]. The study of the haploinsufficient effect of the coding genes in the distal CDR led to the identification of *RPS14*, which encodes a component of the 40S ribosomal subunit. Reduced *RPS14* expression leads to defects in ribosome biogenesis and protein translation, resulting in apoptosis of erythroid cells

and macrocytic anemia[91]. Furthermore, this phenotype was rescued *in vitro* by enforced expression of *RPS14* in CD34+ bone marrow cells derived from 5q- syndrome patients indicating that haploinsufficiency of RPS14 is responsible for the erythroid phenotype in the 5q-syndromes.

Subsequently, Barlow *et al*[92] generated a mouse model using Cre-loxP recombination to delete a large region on chromosome 18 flanked by the *Cd74* gene and small integral membrane protein 3 (Smim3, also known as *Nid67)* in the mouse. The haploinsufficient region in this model is syntenic to a region within the 5q- CDR in humans that contains *RPS14*. The *Lmo2Cre+ Cd74-Nid67* deleted mice developed severe macrocytic anemia, prominent dyserythropoiesis and monolobated megakaryocytes, in keeping with the characteristics of 5q- syndrome. On the other hand, these mice developed thrombocytopenia, which is generally not seen in 5q-syndrome unless in the context of disease progression or leukaemic transformation.

In addition, the *Cd74-Nid67* deleted mice had hypocellular bone marrow with 50%-60% reductions in cell numbers, accompanied by defective production of progenitor cells with proportionally reduced trilineage colony-forming potential *in vitro* compared to controls. Deletion of various segments of mouse chromosomes 11 and 18 syntenic to other regions of the 5q- CDR and exclusive of *Rps14* did not give rise to red cell phenotype. The deleted region of this *Cd74-Nid67* mouse contains five candidate genes (*Synpo, Myoz3, Rbm22, Dctn4* and *Nid67*) in addition to *Rps14*. Nonetheless, the fact that it was only mice in which the region containing *Rps14* was deleted that had a macrocytic anaemia phenotype was consistent with the findings of Ebert *et al*[91] that *RPS14* is the key contributor to the erythroid phenotype seen in 5q- syndrome.

Of interest, *Cd74-Nid67* deleted mice showed increased intracellular Trp53 (p53) in their immature progenitor cells. Although not statistically significant, there was a trend to an increase in annexin-V+ (early apoptotic) cells in the *Cd74-Nid67* deleted marrow compared to control mice, suggesting that cell cycle arrest and apoptosis was probably enhanced by the stabilization of Trp53 in these cells. Following this, Barlow *et al*[92] elegantly showed that homozygous *Trp53* deletion rescued the progenitor deficits and normalized the peripheral blood phenotype observed in *CD74-Nid67* deleted mice. Taken together, these findings suggest that the loss of *RPS14* results in impaired ribosomal biogenesis and consequently TP53 activation, leading to increased apoptosis and erythroid hypoplasia.

More recently, there have been two studies that explored the role of casein kinase 1A1 (*CSNK1A1*) in the pathophysiology and treatment of 5q- syndrome[93,94]. *CSNK1A1* is located in the distal CDR, and encodes a serine/threonine kinase. Gene expression analysis of CD34+ cells from MDS patients with del(5q) demonstrated the haploinsufficiency of *CSNK1A1* with approximately 50% of normal expression[90]. Furthermore, studies in

solid organ malignancies showed that *CSNK1A1* acts as a tumour suppressor gene through regulation of the β-catenin pathway, and also regulates TP53 activity[95,96].

Schneider *et al*[94] generated an *Mx1Cre*-inducible *Csnk1a1* exon 3 knock-out mouse model, and demonstrated that activation of β-catenin activity was proportional to the allelic loss of *Csnk1a1*. Accumulation of β-catenin was noted in both hematopoietic and stromal cells consistent with the expression of *Mx1Cre* in bone marrow stroma[97], with more pronounced expression in homozygous knock-out mice. As such, the function of mesenchymal stem cells in supporting hematopoiesis is significantly impaired in the knock-out mice, with inactivation of β-catenin rescuing the effect.

Homozygous knock-out mice (*Csnk1a1-/- Mx1Cre+*) rapidly developed profound pancytopenia, fulminant bone marrow failure, multi-organ ischemia and death in 5-17 d, demonstrating the critical role of *Csnk1a1* in hematopoiesis[94]. Moreover, there was accumulation of Trp53 and its target, Cdkn1a (p21), leading to induction of early and late apoptosis with a marked decrease in cells in G0 and a significant increase in cells in S/G2/M phases in keeping with stem cell exhaustion.

Transplantation of bone marrow cells from heterozygous knock-out mice (*Csnk1a1-/+*) into lethally irradiated mice showed that transplant recipients developed normal to hypercellular bone marrow, accompanied by increased and mildly dysplastic hypolobated megakaryocytes, as well as thrombocytosis over time[94]. Additionally, non-competitive transplantation of *Csnk1a1-/+* bone marrow showed increased proportions of HSC-enriched Lineage- ckit+Sca-1+ (LKS+) cells in contrast to reduced Lineage- ckit+Sca-1- (LKS-) myeloid progenitor cell populations. This was further demonstrated to be related to exit of *Csnk1a1-/+* HSCs from quiescence, with reduced cells in G0 and a significantly increased proportion of cells in the cycling G1 fraction and S phase, which was due to increases in β-catenin activity and expression of cyclin D1. A competitive advantage was demonstrated in *Csnk1a1* haploinsufficient bone marrow using long-term repopulating assays, where haploinsufficient cells were significantly more abundant than controls at 16 wk following primary and secondary transplant, specifically with increased LKS+ cells and increased myeloid progenitor cells and CD3+ T cells. Collectively, the hypolobated megakaryocytes and self-renewal cells in *Csnk1a1-/+* cells highlight the role of *Csnk1a1* haploinsufficiency and β-catenin in the megakaryocyte phenotype and clonal expansion that occur in 5q-syndrome.

Correlating this clinically, the group performed whole-exome sequencing on MDS samples, and identified a small proportion of patients (3 of 43) with somatic mutations in *CSNK1A1*. All three patients had mutations that resulted in the same amino acid change (E98K or E98V), and all had wildtype *TP53*. Analysis by SNP array showed a high variant allelic frequency of the del(5q) MDS clone, indicating that the deletion of chromosome 5q preceded *CSNK1A1* mutation in remaining allele.

The functional consequence of *CSNK1A1 E98V* mutation was then examined by retroviral transduction of mutant cDNA in *Csnk1a1-/- Mx1Cre+* haematopoietic cells and transplantation into lethally irradiated recipients. Firstly, it was noted that cDNA overexpression of *CSNK1A1 E98V* mutation rescued the HSC ablation in *Csnk1a1-/- Mx1Cre+* cells. Secondly, in comparison to cells transduced with the WT cDNA, *CSNK1A1 E98V* transduced cells have increased nuclear β-catenin activity but do not cause increased p53 activation. Collectively, these findings provided evidence that that *CSNK1A1 E98V* mutation do not cause of loss of function, but conversely confer selective advantage and drives clonal dominance of del(5q) MDS cells.

Finally, the group showed that *Csnk1a1* haplo-insufficiency sensitizes cells to casein kinase 1 inhibition with D4476. Using purified myeloid progenitors, *Csnk1a1* haploinsufficient cells demonstrated reduced viability and increased apoptosis compared to control cells at a range of D4476 drug concentrations.

In another study by the same group, Krönke *et al*[93] showed that reduced *CSNK1A1* levels sensitize hematopoietic cells to lenalidomide. Inhibition of cell growth and proliferation was observed in the presence of lenalidomide using transduced primary human CD34+ hematopoietic stem and progenitor cells with shRNA knockdown of *CSNK1A1*. Overexpression of *CSNK1A1* in bone marrow samples of MDS patients with del(5q) led to reduce *in vitro* sensitivity to lenalidomide in 3 of 5 patients, which correlated with the clinically observed cytogenetic response. In contrast, overexpression of *CSNK1A1* had no effect in normal donors and in MDS with a normal karyotype, highlighting the therapeutic window for selectively targeting MDS cells by lenalidomide in del(5q) MDS.

Lenalidomide induces the ubiquitination of casein kinase 1A1 (CK1a) *via* the E3 ubiquitin ligase CUL4-RBX1-DDB1-CRBN (known as CRL4CRBN) in a species-specific manner. Mice are insensitive to the teratogenic effects of thalidomide. Similarly, *Csnk1a1-/+* murine cells are insensitive to lenalidomide because degradation of CK1a does not occur after binding of lenalidomide to mouse Crbn. This is due to a single amino-acid difference between cereblon in mice and humans. Substitution of isoleucine for the human valine at position 391 of mouse Crbn (CrbnI391V) is sufficient to rescue lenalidomide sensitivity. This data illustrates the importance of taking into account and leveraging differences between mice and humans when using mice to model human diseases.

MODELLING SINGLE GENE MUTATIONS AND OTHER SUBMICROSCOPIC CHANGES

Modelling mutations in the RNA splicing genes

Somatic mutations in components of the 3' pre-mRNA splicing machinery are common, and are frequently early pathogenetic events in MDS. However, the functional

contribution of these mutations in the evolution of MDS remains to be delineated. It is unclear whether mutation in a splicing factor affects the splicing of a single gene or large number of genes, or even whether the downstream impact of these mutations is mRNA splicing-dependent.

SRSF2

SRSF2 is mutated in 20%-30% cases of MDS, and about 50% cases of chronic myelomonocytic leukemia (CMML). Importantly, it is associated with an inferior prognosis[32,35]. SRSF2 is a member of the serine/arginine-rich protein family, and binds to exonic splicing enhancer sequences (ESEs) within pre-mRNA through the RNA recognition motif domain.

To study the functional impact of *SRSF2* mutations on hematopoiesis or splicing, Kim *et al*[98] generated a hematopoietic-specific conditional *Srsf2* knock-in mouse model with the commonly occurring SRSF2[P95H] mutation. Heterozygous transgenic mice were generated and crossed to *Mx1-cre* mice. Bone marrow mononuclear cells from *Srsf2* wildtype (WT), *Srsf2*fl/WT (heterozygous deletion of one copy of *Srsf2*), *Srsf2*fl/fl (homozygous floxed mice for both copies of *SRSF2*), and *Srsf2*P95H/WT were transplanted into lethally irradiated recipients, followed by polyinosine-polycytosine treatment four weeks later.

Mice transplanted with BM cells harbouring the homozygous *Srsf2* deletion or the *Srsf2*P95H/+ mutation developed significant anemia and leucopenia at 18 wk post-transplant[98]. In addition to the observed bicytopenia, *Srsf2*P95H/+ mice also displayed macrocytic erythrocytes, accompanied by normocellular bone marrow with multilineage dysplasia in the erythroid and myeloid lineages, mimicking features of human MDS. In contrast, and consistent with the original published full knock-out model, homozygous *Srsf2* deletion led to profound bone marrow aplasia without evidence of dysplasia morphologically.

Moreover, *Srsf2*P95H/+ mice showed increased LKS^{+} cells, increased early apoptosis and increased proportion of cells in the S-phase of the cell cycle[98]. The increase in HSC and progenitor cells in conjunction with peripheral cytopenia is suggestive of impaired differentiation. Flow cytometry showed that the observed peripheral leucopenia was predominantly due to reduced B-lymphopoiesis. *Srsf2*P95H mice also had reduced early erythroid progenitors with reduced pre-MegE and pre-colony-forming units, erythroid. Of note, none of the non-transplanted *Srsf2* P95H mice developed overt MDS phenotypes or acute leukemia even well past a year of monitoring and the primary phenotypes reported were all present only in the context of transplant studies.

Subsequently, the authors showed that *Srsf2* P95H mutation altered the normal function of SRSF2, instead of resulting in haploinsufficiency or a dominant negative form. Using RNA sequencing, they found that *Srsf2* mutation led to genome-wide alteration, rather than loss, of its normal ESE recognition activity. Wild-type

SRSF2 recognizes the consensus binding sequences CCNG and GGNG with similar affinity[99]. In contrast, *SRSF2* mutation resulted in preferential recognition of cassette exons containing C- *vs* G-rich ESEs. This was further supported by biochemical analysis which demonstrated that SRSF2 mutation was associated with a conformational change in its RNA recognition motif domain, consequently altering the interaction specificity between SRSF2 and pre-mRNA.

At a functional level, the authors proposed that this drives recurrent missplicing of key hematopoietic regulators, including SRSF2 mutation-dependent splicing of *Ezh2*. Missplicing of *Ezh2* leads to nonsense-mediated decay, reduced Ezh2 protein expression and in turn, contributes to impaired hematopoietic differentiation. *SRSF2* and *EZH2* mutations are known to be mutually exclusive in MDS, and findings in this study have provided a potential mechanistic explanation for this observation[22]. Finally, the authors showed that overexpression of normally spliced *Ezh2* cDNA in progenitor cells from *Srsf2* P95H/+ mice partially rescues the hematopoietic defect induced by *SRSF2* mutation in methylcellulose colony forming cell assays.

U2 small nuclear RNA auxiliary factor 1

U2 small nuclear RNA auxiliary factor 1 (*U2AF1*) is one of the most commonly mutated genes in MDS, and can be found in approximately 11% of patients[30,32]. It is typically a founder mutation, and is associated with a less favorable prognosis with a high risk of transformation to AML[14,22]. Previous studies using a retroviral overexpression model of mutant *U2AF1* have demonstrated that transduced murine bone marrow cells have reduced repopulating potential *in vivo*[32].

More recently, Shirai *et al*[100] generated a doxycycline-inducible transgenic mouse model with the most commonly identified *U2AF1* (S34F) mutation. Human cDNA encoding for *U2AF1* (S34F) or *U2AF1* (WT) were inserted into the *Col1a1* locus of KH2 mouse embryonic stem cells, which contain the M2rtTA tetracycline-responsive transactivator protein (rtTA) ubiquitously expressed from the *Rosa26* locus to allow for induction of the transgene. Bone marrow cells from transgenic mice were transplanted into lethally-irradiated wild-type congenic mice, and allowed to engraft prior to the induction of transgene expression with doxycycline treatment for 12 mo.

Peripheral blood leucopenia was observed in the *U2AF1* (S34F) mice after one month of doxycycline treatment, and appears to be related to B-lymphopenia and monocytopenia based on flow cytometry. Leucopenia persisted up to 12 mo, with white cell counts recovering to levels similar to that of controls following withdrawal of doxycycline treatment, suggesting a relationship between expression of mutant *U2AF1* and the phenotype seen.

Strikingly, *U2AF1* (S34F) mice showed increased proportions of HSPC in the bone marrow, particularly in the multipotent progenitors and common myeloid

progenitor (CMP) compartments. Moreover, there was increased cell cycling in the LKS$^+$ population as evidenced by increased Ki67 staining. Overall, the bone marrow cellularity of *U2AF1* (S34F) mice was not significantly different to controls. In mature cell lineage analysis, B-lymphopenia and monocytopenia were also noted in bone marrow. This appeared to be due to neutrophilia and increased in apoptosis of the monocytes.

Interestingly, there was no morphological evidence of dysplasia despite 12 mo of doxycycline treatment. Whilst there were convincing features of perturbed hematopoiesis, and bone marrow characteristics reminiscent of MDS in *U2AF1* (S34F) mice, they do not meet the Bethesda criteria for a myelodysplastic syndrome. They also failed to develop AML. It would be interesting to establish whether other cooperating mutations such as *ASXL1* give rise to MDS and AML in these mice. However, this work remains important as it has shed light on the effects of *U2AF1* mutation on hematopoiesis. In addition, the authors used RNA sequencing data to identify a splice junction sequence-specific pattern of altered splicing induced by *U2AF1* mutation. Exons skipped more frequently and alternative splice sites used more often than canonical splice sites by *U2AF1* (S34F) were enriched for a uracil in the minus 3 position relative to the AG dinucleotide, consistent with published reports of mutant *U2AF1*-associated splicing abnormalities seen in other malignancies[101]. Moreover, integration with human RNA sequencing datasets determined that common mutant *U2AF1*-induced splicing alterations are enriched in RNA processing genes, ribosomal genes, and recurrently mutated MDS and acute myeloid leukemia-associated genes. Taken together, this supports the hypothesis that *U2AF1* mutation alters downstream gene isoform expression, thereby contributing to abnormal hematopoiesis in MDS.

Collectively, studies modelling RNA splicing mutations in mice have provided insights into genetic lesions affecting spliceosome function and mRNA splicing. These findings have already improved our mechanistic understanding of the role of spliceosome mutations in altering the transcriptome, and its effect on normal hematopoiesis and MDS pathogenesis.

MODELLING MUTATIONS IN TRANSCRIPTION FACTORS AND EPIGENETIC MODIFIERS

RUNX1

The *RUNX1* gene, also known as *AML1* or *CBFA,* plays a key role in hematopoiesis, and is frequently mutated in MDS, *de novo* AML and secondary AML[102,103]. The vast majority of *RUNX1* mutations are located in the Runt homology domain (RHD) which mediates binding to DNA and core binding factor beta, although mutations in the C-terminus outside the RHD have also been reported[104].

Watanabe-Okochi *et al*[105] developed a *RUNX1* mouse model using retroviral constructs based on two types of *RUNX1* mutations identified in patients. AML1-D171N (hereafter D171N) has a point mutation in the RHD resulting in the loss of its DNA binding site, while the AML1-S291fsX300 (hereafter S291fs) has a frameshift mutation outside the RHD that results in C-terminal truncation, leading to the loss of transactivation potential but increased DNA-binding ability. Both are dominant negative forms, with the latter being more potent than the former[106,107].

Both D171N and S291fs mice developed macrocytosis, multi-lineage dysplasia, progressive cytopenias in a normal or hypercellular bone marrow, and transformation to leukemia in 4 to 13 mo. However, they displayed quite distinct phenotype and disease kinetics. D171N mice had a more proliferative phenotype with leukocytosis due to increased myelopoiesis, more prominent granulocytic dysplasia, accompanied by marked hepatosplenomegaly, and a higher percentage of blasts. In contrast, S291fs developed pancytopenia with a more marked erythroid dysplasia. This study showed that different mutations within the same gene could induce heterogeneous disease with different biological outcomes. This may be explained in part by the structural and functional differences between the mutants.

Of note, a fraction of the D171N mice had the *Evi1* locus as the retroviral integration site. This reduced the latency of leukaemic transformation to 3 to 5 mo, hence, providing evidence that *Evi1* co-operates with mutant RUNX1 to facilitate disease progression.

Ten-eleven-translocation-2

Ten-eleven-translocation-2 (*TET2*) belongs to a 3-member family of genes (*TET1-TET3*). It encodes a α-ketoglutarate-dependent enzyme that catalyzes the oxidation of 5-methylcytosine (5-mC) to 5-hydroxymethyl cytosine (5-hmC), which is the first step of active demethylation. It is frequently mutated in myeloid malignancies (up to 30% of MDS)[108,109].

Loss of *TET2* leads to a reduction in the amount of 5-hmC, and this has been demonstrated in samples of patients with myeloid malignancies, suggesting that *TET2* acts as a tumour suppressor gene[110]. Indeed, several groups have reported that the loss of *TET2* resulted in deregulated self-renewal of hematopoietic stem cells and the development of CMML-like disease[111-114]. It should also be noted that mice that were hypomorphic or heterozygous for the *TET2* allele showed similar phenotypes, suggesting a haploinsufficiency effect of *TET2* in the development of hematopoietic malignancies.

Recently, Muto *et al*[115] described mice hypomorphic for *TET2* as a mouse model of CMML and MDS. In that study, *TET2* gene trap mice (*TET2*$^{KD/KD}$) were engineered to express approximately 20% of the *TET2* mRNA of WT mice. *TET2*$^{KD/KD}$ mice developed overt features of myeloid malignancy after about 11 mo. Whilst the majority had features of CMML, 3 out of 13 mice developed MDS with pancytopenia, granulocytic dysplasia, and increased erythroid apoptosis.

Comparable to the *NHD13* model, *TET2*$^{KD/KD}$ mice

that developed CMML or MDS showed skewing in their HSPC compartment. These mice had a greater proportion of Lineage⁻, Sca-1⁺, c-kit⁺ (LKS⁺) cells in their bone marrow. Interestingly, mice with CMML had a greater proportion of CMP and granulocyte-macrophage progenitors (GMP) in comparison to mice with MDS who had a greater proportion of megakaryocyte/erythroid progenitors (MEP).

Of note, both mice with CMML and MDS developed splenomegaly, with further analysis showing marked increases in LKS⁺ cells and disruption of the splenic architecture due to extramedullary hematopoiesis. It should be noted that although splenomegaly in association with CMML is well documented clinically, splenomegaly does not tend to feature in MDS patients. This can be explained by the hematopoietically active role of the spleen in the lifespan of the mice, but not in humans. As such, splenomegaly can be seen in MDS mice in the context of a compensatory response to underlying erythroid changes.

This study showed that alterations in TET2 expression resulted in two distinct phenotypes, CMML and MDS, after a considerable latency. Recurrent somatic *TET2* mutations have been identified in normal, elderly individuals with acquired clonal hematopoiesis without overt clinical manifestations[16]. In line with this, findings from this mouse study support a role for *TET2* mutations as early, founder or initiating mutations in myeloid malignancies with later, acquisition of additional, co-operating mutations required to bring about overt disease. The distinct phenotype observed, whether predominantly myelodysplastic, myeloproliferative or myelodysplastic/myeloproliferative may be determined by the nature of the secondary mutations.

Additional sex combs-like 1
Additional sex combs-like 1 (*ASXL1*) plays an important role in regulating *Hox* genes through its interaction with the polycomb group of proteins[116,117]. *ASXL1* mutations are reported in approximately 15%-20% of MDS patients[47,49,118]. They are usually heterozygous with most mutations located in the 5' region of the last exon (exon 12), resulting in the expression of a truncated ASXL1 protein[119]. *ASXL1* mutations are generally subclonal, indicating that they are acquired later in the course of the disease. They have been reported to promote leukaemic transformation. and their presence is an independent predictor of adverse prognosis in MDS[120,121].

There are two mutated *ASXL1* mouse models of MDS in the literature[122,123]. In the first study, Inoue *et al*[122] developed retroviral constructs with a C-terminal-truncating *ASXL1* mutation, FLAG-*ASXL1*-MT1 and FLAG-*ASXL1*-MT2 (collectively termed *ASXL1*-MTs) derived from patients with MDS harbouring mutated genes of 1934dupG;G646WfsX12 and 1900-1922del;E635RfsX15 respectively. GFP-positive cells of *ASXL1*-MTs mice showed preferential myelopoiesis in the marrow at the expense of reduced B-lymphopoiesis at 6 mo post

transplantation. At approximately 12 mo, mutant mice developed features of MDS with display of pancyto-penia, multi-lineage dysplasia and impaired myeloid differentiation. Additionally, some of the secondary transplant recipients progressed to secondary leukemia. Gene expression profiles of hematopoietic cells from mice that developed MDS showed de-repression of homeobox a9 (Hoxa9) through inhibition of polycomb repressive complex 2 - mediated methylation of histone H3K27. Moreover, the *ASXL1* mutation led to upregulation of Mir125a and subsequent suppression of C-type lectin domain family 5, member a (Clec5a), which is involved in myeloid differentiation. Thus, this study identified an ASXL1-MT-Hoxa9-Mir-125a-Clec5a axis that is critical for *ASXL1*-mediated MDS pathogenesis.

In another study, Wang *et al*[123] constitutively deleted *Asxl1* (*Asxl1⁻/⁻*), which resulted in significant embryonic lethality. Surviving *Asxl1⁻/⁻* mice showed profound developmental abnormalities that included dwarfism and anopthalmia. Perinatal mortality was high; 78% of mice died within 24 h of birth and no mice lived longer than 42 d. Hematologically, the surviving mice displayed features of MDS with multilineage cytopenia and dysplastic neutrophils in the peripheral blood. In the bone marrow, *Asxl1⁻/⁻* mice showed normal to increased bone marrow cellularity, with accompanying myeloid hyperplasia and reduced erythroid precursors. Moreover, *Asxl1⁻/⁻* mice had reduced LKS⁺ cells and altered myeloid progenitors with increased GMP and reduced MEP, accompanied by increased apoptosis in the bone marrow.

Subsequently, the generation of heterozygous *Asxl1* mutation (*Asxl1⁺/⁻*) mice showed that haploinsufficiency was sufficient for the development of MDS. These mice generally had a milder MDS phenotype, with some developing a phenotype that was more reminiscent of CMML.

Taken together, these two studies demonstrated that *ASXL1* mutation or deletion gives rise to MDS phenotypes, and suggest a tumour suppressor function for *ASXL1* in hematopoiesis.

TERT
Telomere shortening or dysfunction has been linked to advancing age, however, its direct role in causing MDS is unclear. Colla *et al*[124] recently engineered an inducible telomerase mouse model to study the chronic physio-logical DNA damage in the hematopoietic system. TERT^ER, a telomerase reverse transcriptase-estrogen receptor fusion protein was used, and inter-generational crosses of TERT^ER/ER mice was carried out to elicit progressive telomere erosion. By the fourth and fifth generations (G4/G5), telomere dysfunction with attendant DNA damage signalling and severe tissue degeneration was evident[125].

The G4/G5 TERT^ER/ER mice displayed characteristics of MDS as early as 3 mo. They demonstrated significant cytopenias including anemia, reduced lymphopoiesis with accompanying hypercellular bone marrow, increa-

sed myeloid-to-erythroid ratio, increased apoptosis and multi-lineage dysplasia[124]. Moreover, approximately 5% of the aged G4/G5 *TERT*^ER/ER^ mice progressed to AML.

In the hematopoietic progenitor compartment, G4/G5 *TERT*^ER/ER^ mice showed a significant increase in GMP, accompanied by loss or markedly reduced MEP and CMP that were not attributed to an increase in apoptosis. Further analysis showed a preferential accumulation of γ-H2AX and 53BP1 DNA damage foci in the CMP subpopulation, but not in GMP or MEP. Of note, tamoxifen induction of *TERT* at G4/G5 stage was able to restore telomeres, ceased DNA damage signalling and reversed the degenerative tissue phenotype seen.

Subsequently, long-term HSC isolated from 3-mo-old G0 or G4/G5 mice were transplantable into wild-type congenic recipients[124]. Transplant recipients developed a more severe phenotype, with skewed myeloid diffe-rentiation, trilineage dysplasia and an excess of blasts. Notably, one of the six mice transplanted with G5 HSC progressed to AML. Defective CMP differentiation suggested from *in vivo* studies was confirmed using *in vitro* methylcellulose clonogenic assays, which showed a profound impairment of myeloid differentiation with pre-ferential granulo-monocytic commitment at the expense of the erythroid lineage.

At a molecular level, the defect in CMP differentiation was found to be related to decreased expression of genes involved in the 3'mRNA splicing or processing genes, resulting in abnormal RNA splicing[124]. It was noted that 40% of the aberrant splicing events in *TERT*^ER/ER^ cells resulted in exon skipping, and a higher proportion of in exon retention. Moreover, RNA-sequencing analyses of *TERT*^ER/ER^ CMP cells identified aberrantly spliced genes to be associated with various pathways linked to MDS pathogenesis including DNA repair, chromatin remodeling and histone modification. These gene sets were also enriched in CMP of mice and patients with *SRSF2* mutations.

This report provides the first evidence linking telomere dysfunction to reduced expression of splicing factors, which consequently drives abnormal myeloid differentiation.

CONCLUSION

At present, we have an expanding list of MDS mouse models, most of which displayed a range of typical features of MDS including cytopenias, dysplasia, ineffec-tive hematopoiesis, and some of which also have the ability to transform to leukemia. The bone marrow environment has a distinct role in MDS, as highlighted by the ongoing challenges faced in xenografting MDS cells into the murine system.

Several candidate genes used to model MDS have demonstrated their role in the pathogenesis of MDS, and in some cases, also revealed other collaborating muta-tions leading to leukaemic transformation. Additionally, the impact of specific recurrent mutations within a single gene has also begun to be elucidated. More

recently, the therapeutic potential and the mechanism of action of lenalidomide has also been explored using a del(5q) mouse model, and has certainly enhanced our understanding of this disease with further implications in the clinical context.

In this era of flourishing genetic medicine, there is no doubt that existing and emerging mouse models will continue to be valuable tools in improving our insights into this disease. The availability of the novel CRISPR-Cas9 genomic editing system is likely to hasten the generation of more models, and importantly, the engineering of more complex models that better reflects the disease heterogeneity.

It should be noted that the utility of mouse models in the study of MDS can only be optimized by a careful and systematic approach to their characterization, including the distinction between features that are common to a variety of myeloid malignancies and those that are unique to MDS. Drawing similarities to humans, a diagnosis of MDS is difficult to conclude without the triad of cytopenia, dysplasia and absence of AML (< 20% blasts). Secondarily, evidence of increased apoptosis in the marrow, which is characteristic of MDS, will make the diagnosis more convincing.

To make a diagnosis of MDS, close examination of various haematopoietic samples is essential. The normal ranges of different cellular compositions within the different haematopoietic compartments in a normal mouse should first be appreciated, and this was very well-illustrated and described by Yang *et al*[126]. Ideally, morphological assessments should be carried out by an experienced pathologist.

When mice are monitored for features of MDS, peripheral blood sampling should be examined over various time points. The presence of peripheral blood cytopenia that is persistent over time may point to the development of MDS, particularly if this is accompanied by dysplastic changes morphologically. Anaemia may be normocytic or macrocytic, the latter clearly more convincing of MDS. However, peripheral blood assess-ments alone are insufficient to confirm MDS, and a thorough cytological and histological examination of the bone marrow and spleen is required[59,60]. Additional examination and histological assessment of other tissues may also be required especially if AML is suspected.

Cytological assessments for MDS should include the enumeration of marrow and spleen cellularity, the review of cytopsin preparations for dysplastic features and calculation of myeloid to erythroid ratio. This can be further complemented by immunophenotypic analysis of the various mature and immature cellular populations within bone marrow, including the haematopoietic stem and progenitor compartments. Perls Prussian blue staining should also be carried out on cytospins preparation to assess for the presence of ringed sideroblasts. Ringed sideroblasts are rare in mice, but would be pathognomonic of MDS if present. Histological examination of the bone marrow and spleen is useful to confirm tissue cellularity, and importantly, it provides

better morphological evaluation of megakaryocytes, which can be underrepresented in cytospins preparations.

Finally, the understanding of the MDS initiating cell, and mechanisms responsible for leukemic transformation are some of the major questions that remained to be answered in MDS. It is hopeful that new mouse models created will shed more light on the functional interplay amongst the various genetic mutations present. An ultimate goal of this area of research is to use animal models to facilitate the development of new therapeutics in MDS and improve clinical outcomes.

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MINIREVIEWS
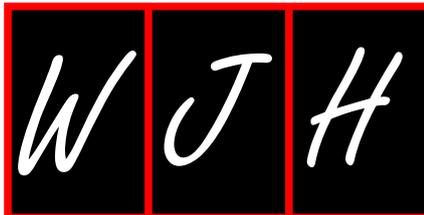
Clinical approach to diarrheal disorders in allogeneic hematopoietic stem cell transplant recipients

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Abstract

Diarrhea is a common complication of allogeneic hematopoietic stem cell transplant (HSCT), with an average incidence of approximately 40%-50%. A wide variety of etiologies can contribute to diarrhea in HSCT patients, including medication-induced mucosal inflammation, infections, graft-*vs*-host disease and cord colitis syndrome in umbilical cord blood transplant. Clinical manifestations can vary from isolated diarrheal episodes, to other organ involvement including pneumonia or myocarditis, and rarely multiorgan failure. The approach for diagnosis of diarrheal disorders in HSCT patients depends on the most likely cause. Given the risk of life-threatening conditions, the development of clinically significant diarrhea requires prompt evaluation, supportive care and specific therapy, as indicated. Serious metabolic and nutritional disturbances can happen in HSCT patients, and may even lead to mortality. In this review, we aim to provide a practical approach to diagnosis and management of diarrhea in the post-transplant period.

Key words: Diarrhea; Medication-induced diarrhea; Allogeneic hematopoietic stem cell transplant; Enteric infection; Graft-*vs*-host disease

Core tip: Diarrhea is a common complication following allogeneic hematopoietic stem cell transplant. However, there is no recent review dedicated to guiding clinicians about the different causes and their management. Our objective is to conduct a thorough review of literature to provide a working schema for the busy clinician on

evaluation and management of diarrhea in this special population.

INTRODUCTION

Hematopoietic stem cell transplant (HSCT) is increasingly used to treat a variety of hematologic disorders. Worldwide, more than 50000 HSCTs are performed every year; approximately 40% of these HSCTs are allogeneic HSCT[1]. Given the increased volume of HSCT procedures, the number of HSCT-associated toxicities is expected to increase. Gastrointestinal complications are common in HSCT recipients and significantly contribute to HSCT-related morbidity and mortality. A wide variety of gastrointestinal complications are reported including nausea, vomiting, oropharyngeal mucositis, dysphagia, diarrhea, gastrointestinal bleeding and graft-*vs*-host disease (GVHD) of the gastrointestinal tract. These manifestations are seen in more than two-third of patients undergoing HSCT[2].

Diarrhea is a common complication of HSCT, with an average incidence of approximately 40%-50%, with a higher occurrence within the first several weeks post-transplant[3,4]. High-dose chemotherapy and radiotherapy used for myeloablation, without any other identifiable etiology, can alone cause diarrhea in up to 50% of patients[2]. Here, we will review a practical approach to diagnosis and management of diarrhea in the post-transplant period.

ETIOPATHOGENESIS

A wide variety of etiologies can contribute to diarrhea in HSCT patients, including mucosal inflammation caused by medications used for myeloablation or immunosuppression, infections, GVHD or other causes seen in non-transplant patients (Tables 1 and 2)[2-7]. Apart from conditioning regimen and immunosuppressant (*e.g.*, mycophenolate mofetil or tacrolimus), antibiotics, proton pump inhibitors, promotility agents (*e.g.*, metoclopramide), and magnesium salts are common causes of medication-induced diarrhea. Infection accounts for 5%-10% of diarrhea in adults HSCT patients[2-7]. Among different infectious etiologies, viruses are the most common pathogens and include cytomegalovirus (CMV), herpes simplex virus (HSV), Ebstein-Barr virus, adenovirus, norovirus and enteric viruses such as rotavirus, coxsackie, and echo virus. Clinical manifestations can vary from isolated diarrheal episodes, to other organ involvement including pneumonia or

myocarditis, and rarely multiorgan failure. Clostridium difficile remains to be an important cause of antibiotic-associated diarrhea[3]. Its occurrence is related to the exposure to multiple antibiotics, antineoplastic agents, prolonged hospitalizations, and extended period of reduced host immunity that can lead to disruption of normal intestinal epithelium. Clostridium difficile may possibly contribute to an increased risk of gastrointestinal GVHD as well by providing the antigenic substrate and adding to the mucosal damage by activated donor T cells that release pro-inflammatory cytokines that drives the immune response towards GVHD[8]. Neutropenic enterocolitis, characterized by cecal involvement and occasionally ascending colon, can result from polymicrobial infection following a combination of mucosal cytotoxic injury, impaired host defense and profound neutropenia. The interaction of pro-inflammatory mediators present in the lumen with innate immune system in the intestinal submucosa leads to the release of pro-inflammatory cytokines resulting in epithelial cell apoptosis and increased mucosal permeability culminating into the syndrome of neutropenic enterocolitis. Microscopically there is a minimal inflammatory exudate along with submucosal edema and hemorrhagic necrosis. Thus, without the presence of any inflammatory response, different bacterial and/or fungal organisms including gram-negative bacilli, gram-positive cocci, anaerobes (*e.g., Clostridium septicum*) and *Candida* species can readily translocate through the bowel wall causing bloodstream infection[9].

Acute GVHD, neutropenic enterocolitis/typhlitis or cytomegalovirus-colitis are among the serious causes of diarrhea and can cause significant morbidity, prolonged hospitalization and increased non-relapse mortality[9-11]. Any moderate to severe diarrhea, regardless of its cause, particularly when associated with vomiting, can contribute to fluid and electrolyte losses, malnutrition, requirement for parenteral nutrition, deconditioning and slow recovery from the effects of conditioning regimen and HSCT.

CLINICAL FEATURES

Medication-induced diarrheal disorders are watery and usually not associated with bloody stool or significant abdominal pain[12]; however, nausea, vomiting, anorexia and mild abdominal cramps are not uncommon in HSCT recipients. Clinical manifestations in viral infections can vary from isolated diarrheal episodes, to other organ involvement including pneumonia or myocarditis, and rarely multiorgan failure[10]. *Clostridium difficile* (*C. difficile*) infection causes multiple episodes of watery diarrhea, associated with abdominal cramp, nausea, low-grade fever and leukocytosis[8]; however, it can also cause severe disease with mortality rate reaching up to 20%, particularly in patients who were previously treated with linezolid[13]. Neutropenic enterocolitis presents with abdominal pain, fever, bloody or watery diarrhea and occasionally nausea and vomiting. The

Table 1 Common causes of diarrhea in hematopoietic stem cell transplant recipients

Etiology	Time period	Percentage of diarrheal episodes	Tests	Management	Comments
Acute GVHD	Early post engraftment	40%-60%, particularly after engraftment	Colonoscopy and biopsy	High-dose prednisone; if no response, other immunomodulators, and extracorporeal photopheresis	Steroid-refractory gut acute GVHD can be fatal
Conditioning regimen, without other etiology	Within 5-7 d after chemotherapy	50%	No-specific tests, other etiologies need to be ruled out	Supportive care	
Medications	During any time, usually within few weeks after initiation	Variable	No-specific tests, other etiologies need to be ruled out	Supportive care, medication withdrawal if possible	Usually diarrhea stops after cessation of the offending medication
Infections	Pre-engraftment for Clostridium difficile infection and typhlitis; early post-engraftment for enterovirus, adenovirus, CMV colitis	5%-10%	Microbiologic, molecular or pathologic tests; CT, CTE, or MRE; colonoscopy with biopsies	Supportive care if viral, antibiotics if bacterial, antifungal therapy if fungal	Neutropenic enterocolitis and CMV colitis can be life threating in severe cases
Cord colitis	Late post-engraftment	10% of cord transplant	Negative cultures and a colon biopsy demonstrating chronic active colitis	Metronidazole	Only occurs in recipients of umbilical cord blood transplant

Also consider other causes unrelated to transplant such as malabsorption syndrome including pancreatic exocrine insufficiency, adrenal insufficiency, lactose intolerance, or other malabsorption syndromes[2-7]. CMV: Cytomegalovirus; CT: Computerized tomography; CTE: Computerized tomography enterography; GVHD: Graft-*vs*-host disease; MRE: Magnetic resonance enterography.

Table 2 Most common medications and infectious etiologies causing diarrhea in hematopoietic stem cell transplant recipients

Categories	Agents
Medications	Conditioning regimen, antibiotics, mycophenolate mofetil, tacrolimus, proton pump inhibitors, promotility agents, magnesium salts
Viral infection	Cytomegalovirus, herpes simplex virus, Ebstein-barr virus, adenovirus, norovirus and other enteroviruses
Bacterial infection	*Campylobacter*, *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Vibrio cholera*, *Clostridium difficile*, other enteric pathogens
Others	Fungal, *e.g.*, candida, parasitic, *e.g.*, Cryptosporidium and *Mycobacterial* infections
Mixed infection	Neutropenic enterocolitis

abdominal pain can be generalized or localized usually in the right lower quadrant. In severe cases, patients may develop acute abdomen with peritonitis, often indicative of complications such as perforation or abscess formation[9].

Acute GVHD is among the most common cause of diarrhea in HSCT patients, accounting for one-third of cases in some studies, especially if diarrhea persists beyond 3 wk of a HSCT[3,4]. Symptoms vary from watery to bloody diarrhea, usually high volume, associated with abdominal pain or cramping. The bleeding can be serious to require blood transfusion. If upper gastrointestinal tract is involved, patients may develop nausea, vomiting and anorexia. Other features of GVHD such as skin rash may also be present[11].

Cord colitis syndrome occurs only in recipients of umbilical cord blood transplant, with an occurrence rate of 10% in such patients. It manifests as watery diarrhea, presenting at about 4 mo after the transplant, and persisting for more than a week. Fever and weight loss are not uncommon, and the majority of patients require hospitalization. Colonoscopy usually reveals erythematous mucosa with or without ulcerations, with occasional granuloma formation[6]. It responds to metro-

nidazole[6] despite negative viral and bacterial cultures, and is felt to be related to infection with bradyrhizobium enteric[14].

EVALUATION AND DIAGNOSIS

The approach for diagnosis of diarrheal disorders in HSCT patients depends on the most likely cause. Given the risk of life-threatening conditions, the development of clinically significant diarrhea requires prompt evaluation, supportive care and specific therapy, as indicated. A thorough history and review of medications may provide information regarding possible medication-induced diarrhea. Stool analysis including viral culture and *C. difficile* toxin assay or polymerase chain reaction (PCR) should be performed. Monitoring of renal function, electrolyte and nutritional status are important. Timing of onset of diarrhea may provide an idea about potential causes.

Pre-engraftment period

Medications including conditioning regimen, calcineurin inhibitors, oral magnesium and prophylactic antimicrobials are frequently used in HSCT recipients, hence

important causes of diarrhea[12]. *C. difficile* is another common cause during this phase and is seen in up to 10%-20% of the patients[8,13,15]. Detection of *C. difficile* toxin or the toxigenic *C. difficile* organism in the stool can be achieved with PCR, enzyme immunoassay for *C. difficile* toxins A/B or *C. difficile* glutamate dehydrogenase[16]. Although non-diagnostic, the classic computed tomography (CT) findings include pancolitis with marked colonic wall thickening (11-15 mm) and wall nodularity, most frequently involving sigmoid colon and the rectum[17,18]. Contrast-enhanced CT scan of the abdomen may show a characteristic but non-specific finding of "target sign" or "double halo sign" due to submucosal edema and mucosal enhancement. Colonic edema may also result in "accordion sign" due to thickening of the haustral folds[19]. Other non-specific imaging features include pericolic fat stranding and ascites. Colonoscopy, performed to rule out other diagnosis, may demonstrate pseudomembrane formation, apoptosis and nonspecific ulceration. However, the absence of pseudomembrane does not rule out the *C. difficile* infection in HSCT patients[20,21].

Neutropenic enterocolitis is a common cause of diarrhea during this phase[9]. Initial workup for suspected typhlitis includes *C. difficile* toxin assays, stool and blood cultures. All patients with suspected neutropenic enterocolitis should have CT scan of the abdomen with intravenous and oral contrast in the absence of any contraindication for contrast use. CT scan of the abdomen has a lower false-negative rate (15%) than ultrasound (23%) or plain radiographs of the abdomen (48%)[22]. CT findings are usually less severe than that seen in pseudomembranous colitis and include bowel wall thickening (7 mm on average, range 4-15 mm), especially in the ileocecal area[23]. Pneumatosis intestinalis, seen in up to 20% of cases[23], is usually not seen in other non-vascular causes of colitis or GVHD[17]. Other non-specific findings include mesenteric stranding (51%), bowel dilatation (38%), and mucosal enhancement (28%)[23]. Plain films of the abdomen are nonspecific but may show fluid-filled, distended cecum with dilated adjacent small bowel loops, thumb-printing or localized pneumatosis intestinalis[24].

Early post-engraftment period

Medications and *C. difficile* infection are common causes of diarrhea during this period as well. Additionally, viral infection (*e.g.,* enteric virus, adenovirus and CMV virus) and acute GVHD of gut are important etiologies. Enteric virus infections (*e.g.,* coxsackie A, rotavirus or norovirus) can cause prolonged gastroenteritis among HSCT recipients. Nevertheless, isolation of virus in a stool specimen, even in cases of diarrhea, may be the result of viral shedding and not be related to intestinal infection[4]. The specific diagnosis of adenovirus infection is also challenging and may require the use of multiple diagnostic tests. Viral culture, direct antigen assays, or PCR of upper nasopharyngeal, throat, urine, stool or rectal samples may detect viral shedding. Results should

be correlated with the clinical picture. Quantitative PCR of blood may be helpful to establish the diagnosis, evaluate the risk of dissemination, monitor response to antiviral therapy and determine prognosis[25-28].

CMV colitis is an important differential of diarrheal disorder in HSCT patients. The diagnosis is challenging and may be complicated by the presence of concomitant gastrointestinal GVHD. The final diagnosis of CMV is based on the presence of any positive microbiological, molecular or pathological tests identifying CMV, and a response to treatment. Diagnostic tests for active CMV disease include CMV phosphoprotein 65 antigenemia assay and quantitative nucleic acid testing, which may be used to establish diagnosis, determine a need to initiate pre-emptive therapy, and monitor response to therapy[29,30]. Viral culture of blood, urine or tissue specimens can be slow and expensive, and is also less sensitive and specific than molecular diagnostic assays. Culture of gastrointestinal tissue for the diagnosis of tissue-invasive disease can be an option when both antigenemia and PCR testing on blood are negative[31]. Sigmoidoscopy with biopsy appears to have equivalent diagnostic yield for CMV colitis, compared to colonoscopy[32]. Typical histopathology finding includes the presence of viral nuclear inclusions but the presence of gastrointestinal gland apoptosis without viral inclusions does not rule out CMV involvement[33]. If a CT scan is done, findings usually include ascending colon wall thickening with an average thickness of 15 mm[34]. The most characteristic feature is mural edema with deep ulcerations, likely due to occlusive vasculitic process, with or without small bowel involvement, surrounding fat stranding and ascites[35].

Acute GVHD of gut is the most frequent cause of diarrhea during the early post-engraftment period, and most commonly occurs within the first few months after HSCT or following a reduction in immunosuppression. The presence of a maculopapular skin rash or otherwise unexplained elevated serum bilirubin or alkaline phosphatase within the first 100 d of HSCT may support the diagnosis. Most but not all cases of acute GVHD of lower gastrointestinal tract can be diagnosed by rectal biopsy[36,37]. Hence, a negative rectal biopsy in patients with a clinical suspicion of acute GVHD requires evaluation with colonoscopy. Additionally, multiple colonic biopsies and pathologic evaluation of tissue are necessary for the diagnosis even when mucosal lining of colon appears normal[37]. Epithelial cell apoptosis, particularly involving cryptic cells, is the characteristic histological feature observed in patients with acute gastrointestinal GVHD[38]. A study has indicated that the presence of 6 or less apoptotic bodies per 10 contiguous crypts may not be predictive of gastrointestinal GVHD[39]. The presence of concurrent GVHD in another organ greatly increases the specificity of isolated crypt apoptosis as a diagnostic feature of gastrointestinal GVHD[21]. Epithelial cell apoptosis can be seen after the use of conditioning regimen but the changes are noted within the first 20 d, whereas acute GVHD is only seen
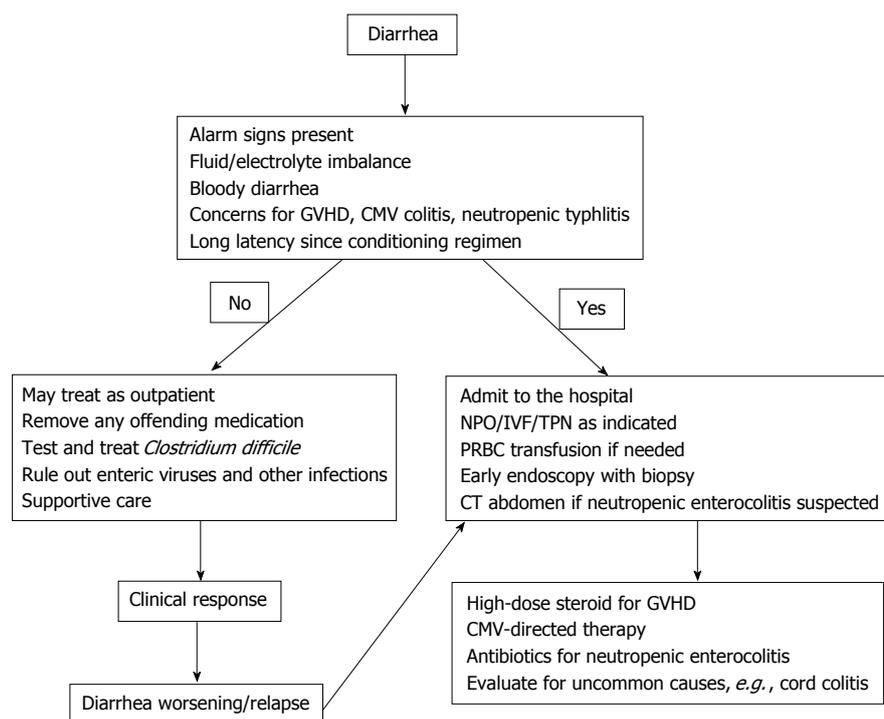
Figure 1 Algorithm for management of diarrhea in transplant recipients. CMV: Cytomegalovirus; CT: Computerized tomography; GVHD: Graft-*vs*-host disease; IVF: Intravenous fluids; NPO: Nil per os; PRBC: Packed red blood cells; TPN: Total parental nutrition.

after engraftment, which occurs after the second or third week following the transplant[40]. Other differentials that can cause gland epithelial apoptosis include drugs such as mycophenolate mofetil and proton pump inhibitors, and viruses such as adenovirus and CMV infection[38]. Radiologic findings are not diagnostic of GVHD. The most consistent CT finding is abnormal mucosal enhancement of the entire gastrointestinal tract, mostly affecting the small bowel. Other frequent features include dilated, fluid-filled bowel loops, submucosal edema with "target sign"[34]. However, a wall thickness of greater than 7 mm can help exclude GVHD[23].

Late post-engraftment period
Diarrhea is more likely to be related to aforementioned infections, but less commonly could be a manifestation of chronic GVHD, particularly if other features of chronic GVHD are also present. The chances of drug-induced diarrhea from mycophenolate, tacrolimus or prophylactic antimicrobial may be relatively less, unless the medications have been changed recently. Other causes of diarrhea, as observed in general population, including pancreatic insufficiency should also be considered. Adrenal insufficiency, possible because of steroid exposure for treatment of GVHD, may also result in gastrointestinal symptoms including diarrhea. If etiologies are not established, a colonoscopy and biopsy may be needed for diagnosis. No histological changes involving the colon are specific for chronic GVHD, but common features may include apoptotic epithelial cells and crypt drop out. These features, however, are also seen in patients with inflammatory bowel disease[38,41]. Cord

colitis syndrome, exclusively seen in cord blood transplant recipients, may mimic chronic GVHD[42]. It is distinguished from other causes of diarrhea by negative viral and bacterial cultures, and a colon biopsy that demonstrates chronic active colitis, frequently with associated granulomas. A response to antibacterial treatment can be both diagnostic and therapeutic[6,14].

MANAGEMENT
Diarrhea in HSCT recipients can induce serious metabolic and nutritional disturbances and may even lead to mortality. Therefore, many HSCT patients with diarrhea may require inpatient admission for volume and electrolytes replacement. However, some patients may be treated as an outpatient with close follow up if they do not have alarming signs such as bloody diarrhea, significant abdominal pain, fever, inability to tolerate oral intake, hypovolemia, tachycardia or hypotension (Figure 1).

Supportive care
If infectious causes of diarrhea are ruled out, loperamide can be used at an initial dose of 4 mg orally, followed by 2 mg with each bowel movement as needed up to a total of 16 mg in 24 h. If diarrhea persists, atropine, diphenoxylate, bismuth subsalisylate, or tincture of opium may be added to the scheduled loperamide. Octreotide may be useful in select cases, particularly when it is started early on in GVHD[43,44]. Other supportive measures that may be used in moderate to severe cases include the use of cholestyramine, bowel rest,

hydration, electrolyte replacement and parenteral nutrition.

GVHD

High-dose steroid remains the preferred frontline therapy for both acute GVHD (1-2 mg/kg per day of prednisone) and chronic GVHD (1 mg/kg per day of prednisone) of gut. High-dose steroid is continued for 1-2 wk or until response, than it is tapered over next several weeks[11,45]. Patients with acute GVHD, who respond to high dose prednisone within the fifth day of therapy, have significantly improved outcomes, compared to non-responders[46]. Non-absorbable steroids such as budesonide can improve response, reduce steroid doses and prevent relapse of gastrointestinal GVHD following the tapering of prednisone[47]. Addition of any other agent to prednisone as a frontline therapy has not been shown to improve outcomes. For steroid-refractory patients, outcomes are poor but several therapy agents have been used including extracorporeal photopheresis, calcineurin inhibitors, sirolimus, etanercept, pentostatin, among others[11,45].

Infections

Identifying the offending organism is crucial in decisions about further treatment of infectious enterocolitis. Culture and susceptibility may guide therapy selection. *C. difficile* infection needs a prompt treatment, and clinicians should treat empirically in suspicious cases because of a high mortality rate. Treatment options include metronidazole, and vancomycin for complicated cases. Fidaxomicin has been recently approved by Food and Drug Administration, and can be considered in refractory cases. Fecal transplant is yet another treatment modality in recurrent/refractory cases[48]. Certain viral diarrhea with enteric viruses may be self-limited but require cautious monitoring; however, where specific agents are available, therapy should be instituted. Ganciclovir or valacyclovir is the therapy of choice for CMV, whereas HSV infection may be treated with acyclovir or valacyclovir for 2-3 wk. Foscarnet is the second-line therapy in both cases[49]. Although data regarding bacterial gastroenteritis are lacking in HSCT patients, empirical antibiotics are not usually indicated in the absence of clinically significant fevers, bloody stools, and evidence of invasive disease based on significant white blood cell or red blood cells in the stool. Trimethoprim/sulfamethoxazole has a good coverage for most of the enteric bacteria and may be used as the first line therapy. Quinolones also provide a good coverage for the enteric bacteria, but the concerns exist regarding an increased resistance to quinolones. Erythromycin is a good therapy option for campylobacter-related diarrhea[50].

Neutropenic enterocolitis

Treatment of neutropenic enterocolitis requires high level of care, as it is potentially life threatening. All patients should be treated as an inpatient with intra-venous fluids, nasogastric tube placement for gastric decompression, and bowel rest. Anti-diarrheal should be avoided as it may aggravate the ileus and worsen the symptoms. Broad-spectrum antibiotics with good coverage against anaerobes and gram-negative rods including pseudomonas are recommended. Suggested empiric regimens include tazobactam-piperacillin, cefepime plus metronidazole, or carbapenem plus metronidazole. The therapy should be adjusted based on the culture results. A lack of response to broad-spectrum antibiotics within 72 h should raise the suspicion for fungal etiology. In such context, addition of antifungal therapy may have to be considered. Intravenous antibiotics can be switched to oral after 48 h of being afebrile and recovery of absolute neutrophil count to above 500/μL. However, antibiotics should be continued for additional 14 d[9,51,52]. Surgical exploration is largely avoided in patients with neutropenic enterocolitis, but may have to be considered in severe cases with suspected perforation or bowel necrosis. Other potential indications for surgical intervention may include clinical deterioration despite the appropriate treatment, or persistent bleeding[9,53].

Cord colitis syndrome

Antibiotic therapy with metronidazole alone or with quinolones for a total of 14 d is the mainstay of the treatment[6].

CONCLUSION

Diarrhea is common in HSCT recipients and accounts for significant morbidity and possibly mortality in these patients. The increasing use of HSCT procedures worldwide indicates that the diarrheal disorder in this patient population is likely to rise. Mediations, infections and GVHD are common causes of diarrhea in post-transplant period[2-7]. Medication history, stool analysis, culture, and *C. difficile* PCR should be routinely performed to establish the underlying cause. CT scan and colonoscopy with biopsies may be performed if the other tests are unrevealing and may help diagnose conditions such as neutropenic enterocolitis and GVHD[23,38,54]. In addition to supportive care such as hydration, monitoring and correction of electrolytes and acid-base balance, timely initiation of appropriate specific treatment is important to improve outcomes. Management often requires a specialized multidisciplinary transplant team with experienced transplant physicians, gastroenterologists, infection disease experts and nursing staff. Many HSCT recipients leave the transplant centers after initial hospitalization and follow-up with community oncologists, hence training of community oncologists and patient education in post-transplant issues are important. Frequent communication between community oncologists, patient/caregivers and transplant physicians can help expedite diagnosis and initiate early management. Certain diagnoses such as neutropenic enterocolitis, steroid-refractory acute GVHD and CMV

colitis are difficult to treat with current therapeutic modalities, hence further research to optimize therapy is needed.

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MINIREVIEWS

Advances and perspectives on cellular therapy in acquired bone marrow failure diseases

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Abstract

Acquired bone marrow failure diseases (ABMFD) are a class of hematopoietic stem cell diseases with a commonality of non-inherited disruption of hemato-poiesis that results in pancytopenia. ABMFDs also are a group of heterogeneous diseases with different etiologies and treatment options. The three most common ABMFDs are aplastic anemia, myelodysplastic syndrome, and paroxysmal nocturnal hemoglobinuria. Stem cell transplantation is the only treatment that can cure these diseases. However, due to high therapy-related mortality, stem cell transplantation has rarely been used as a first line treatment in treating ABMFD. With the advance of personalized medicine and precision medicine, various novel cellular therapy strategies are in trial to increase the efficiency and efficacy of ABMFD treatment. This article aims to review current available stem cell transplantation protocols and promising cellular therapy research in treating ABMFD.

Key words: Bone marrow failure diseases; Aplastic anemia; Cellular therapy; Stem cell transplantation; Paroxysmal nocturnal hemoglobinuria; Myelodysplastic syndrome

Core tip: Stem cell transplantation is the only method can cure acquired bone marrow failure diseases (ABMFD). However, due to the high mortality rate of stem cell transplantation itself, this method is not usually used as the first line treatment for ABMFD. With

the advance of current cellular therapy technology, it is becoming possible to cure ABMFD without significant treatment related complications.

INTRODUCTION

Acquired bone marrow failure diseases (ABMFD) are a group of rare hematologic disorders manifested by insufficient hematopoiesis to produce a sufficient amount of red blood cells, white blood cells, or thrombocytes. ABMFD can occur after exposure to viral infections, toxins, chemicals, or radiation. ABMFD includes aplastic anemia (AA), myelodysplastic syndrome (MDS), and paroxysmal nocturnal hemoglobinuria (PNH). Though the pathogenesis of these diseases is heterogeneous, the high similarity of their clinical manifestation and their bone marrow pathophysiological presentation makes them hard to distinguish from each other. ABMFD can be cured by stem cell transplantation. However, because of the high mortality rate of this therapy, stem cell transplantation has not usually been used as a first line treatment for ABMFD. Currently, there is a lack of literature that offers insight into ABMFD as a class of disorders. This review offers a comprehensive overview of many of the standard and novel treatment options.

CLINICAL PRESENTATION AND CURRENT MANAGEMENT OPTIONS

The common clinical presentations for AA, MDS and PNH are cytopenia in single or multiple hematological lineages, together with signs of impaired quality of life such as fatigue, dizziness, headache, shortness of breath, and other symptoms that are associated with prolonged anemia. The individual clinical presentations of AA, MDS and PNH are illustrated as Figure 1. Due to nearly indistinguishable clinical presentation, peripheral blood smear and bone marrow biopsies are used in the diagnosis of ABMFD.

MDS is the most common form of ABMFD, affecting around 15000 Americans each year[1]. The risk of MDS increases with age[2]: It typically affects people at age 60 years or older. In MDS, myeloid stem cell dysfunction in the bone marrow leads to ineffective hematopoiesis[3]. If left untreated, some of MDS can progress into acute myeloid leukemia. Cancer drugs such as chlorambucil, cyclophosphamide, doxorubicin, ifosfamide, mechlorethanmine, melphalan, procarbazine, and etoposide are associated with onset of treatment related MDS[3].

AA is the second most common form of ABMFD, with an incidence rate of 2.0/million to 7.4/million worldwide[4], and can be triggered by toxins, radiation, chemotherapy, viruses, medicines, autoimmune disorders, or pregnancy[4]. In AA, the bone marrow is injured and the hematopoiesis is interrupted. In most cases, AA is secondary to immune system dysfunction and subsequent premature turnover of hematopoietic cells. AA is commonly seen in young adults.

PNH is a rare hemolytic disease caused by complement system attack on cells with surface membrane glycosylphosphatidylinositol (GPI)-anchor protein deficiency. PNH affects roughly 6000 Americans each year. The clinical presentation of PNH includes hemolytic anemia, thrombosis in large blood vessels, and cytopenia or pancytopenia, depending on severity[5]. PNH appears in suddenly, but recurring episodes can be triggered by stress or physical exertion of the body. Attack on both hematopoietic cells and mature blood cells leads to formation of abnormal blood cells[6]. Abnormally weakened red blood cells will rupture. The ruptured red blood cells will release free hemoglobin that is then excreted through the kidney and stains the urine dark-colored.

TRANSFUSION THERAPY

Transfusion therapy is recommended as a part of supportive therapy for all ABMFDs[7,8]. The current transfusion guidelines suggests transfusion for those patients with platelet counts below 10×10^9/L (or $< 20 \times 10^9$/L in febrile patients), though the ultimate decision for transfusion should be based on the patient's overall clinical condition. Transfusions should be used cautiously because it can induce alloimmunization and autoimmunization that will complicate future treatments, such as hematopoietic cell transplantation (HCT).

Transfusion using blood from family members may induce sensitization against human leukocyte antigens (HLA) of potential HCT donors. The blood units should be carefully screened for common viruses (such as cytomegalovirus, human immunodeficiency virus, human T-lymphotropic retroviruses, hepatitis B and C, and West Nile virus), undergo leukocyte reduction, and be irradiated to avoid graft-*vs*-host disease (GVHD). Platelet transfusion is useful to prevent or stop thrombocytopenic bleeding. Platelet units are useful only for 3-7 d. Platelet shall be stored at room temperature to keep its activity, which, on the otherside, increases the risk of transfusion related infection. White blood cell transfusion is not highly recommended due to efficacy issue.

IMMUNOSUPPRESSIVE THERAPY

For AA and PNH, immunosuppressive therapy (IST) is a front line management to treat immune system dysfunction[9,10]. The complement system is a part of immune system that facilitates leukocytes and antibodies

Table 1 Advantages and disadvantages of hematopoietic stem cell transplantion			
Sources of stem cells for transplantation	Peripheral blood	Bone marrow	Cord blood
Advantages	Abundant supply	Abundant supply	Rapid procedure
	Easy to collect and differentiate	Easy to storage	Less GVHD
	No surgical procedure	Relatively fast engraftment	Tolerance of HLA-mismatching
	Short recovery period	Autologous cells are immune compatible	
	Fastest engraftment		
	Low rates of morbidity and mortality		
Disadvantages	High risk of GVHD	Surgical procedure	Limited number of stem cells
	Requirement of close HLA-matching	Long recovery period	Difficult to grow and differentiate
		High risk of GVHD	Slow engraftment
		Requirement of close HLA-matching	Tissue rejection

GVHD: Graft-*vs*-host disease; HLA: Human leukocyte antigens.

in removing pathogens. However, the over activated complement system attacking GPI-anchor protein deficient stem cells in bone marrow is the mechanism of PNH[11,12]. Different types of immunosuppressive agents, such as antithymocyte globulin (ATG), cyclosporine-A (CSA), or various anti-complement anti-bodies or complement blockers, are used with high degree of response and survival[10,13,14].

ANDROGENS

Androgens (naturally occurring male hormones) have long been used as supportive treatment for many forms of anemia, including ABMFD[10]. Either injection of androgen (testosterone) or giving medications to increase endogenous androgen production are the common approaches to increase serum androgen level. The elevated androgen levels in patient's body may have gender-specific side effects: Men may experience enlargement of breasts or prostate, while women may experience facial hair growth, development of muscles, deepening of voice, or enlargement of the clitoris. Other side effects such as acne, jaundice due to increases in liver enzymes, and liver damage may occur. Due to the wide range of side effects, androgen therapy is limited and is typically used in combination with blood transfusions.

CELLULAR THERAPIES

Hematopoietic stem cell transplantation (HSCT) is the process of treating with a conditioning regimen followed by infusion of a healthy donor's mononuclear cells rich in hematopoietic stem cells and progenitor cells. In general, HSCT can be autologous (obtained from the patient's own cells), syngeneic (obtained from the patient's identical twin), or allogeneic (obtained from another individual); however, autologous HSCT is usually not a choice for ABMFD because the patient's lack of hematopoietic stem cells. The hematopoietic stem cells can be derived from either bone marrow, peripheral blood, or umbilical cord blood. The advantages and disadvantages of each type of hematopoietic stem cell transplantation are shown in

Table 1. GVHD is one of the most common and serious complications. The risk and the severity of GVHD are largely related with the degree of HLA tissue type match between the donor and the recipient. Typically, a sibling has a 25% probability of being a perfect match for the recipient's eight major HLA antigens. The chance of finding an unrelated match ranges from 10% for some minority groups, to around 60%-70% for Caucasians in the United States.

Quite often, ABMFD occurs in patients that receive high doses of radiation therapy and/or chemotherapy. HSCT is often used, following cancer treatments, to facilitate recovery from high doses of radiation therapy and/or chemotherapy by replacing damaged or destroyed stem cells in the bone marrow and restoring hematopoiesis. HSCT for ABMFD has showed promising results[15].

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Peripheral blood stem cell transplantation (PBSCT) involves harvesting stem cells from the peripheral blood cells (peripheral blood is composed of erythrocytes, leukocytes and platelets) of the donor[16]. Before harvesting, donors are usually injected with granulocyte colony-stimulating factor to promote stem cell growth and release into the peripheral blood[17]. Currently, PBSCT is the most commonly performed HSCT due to easy access to peripheral blood stem cells and quick donor peripheral blood cell recovery[16].

BONE MARROW STEM CELL TRANSPLANTATION

Harvesting bone marrow stem cells is particularly complex procedure, compared to harvesting peripheral blood and umbilical cord blood. The donor must be given a general anesthetic and placed in an operation room. During the procedure, an aspiration needle is inserted at multiple points of the iliac crest region to collect approximately one liter (10-15 mL/kg) of bone
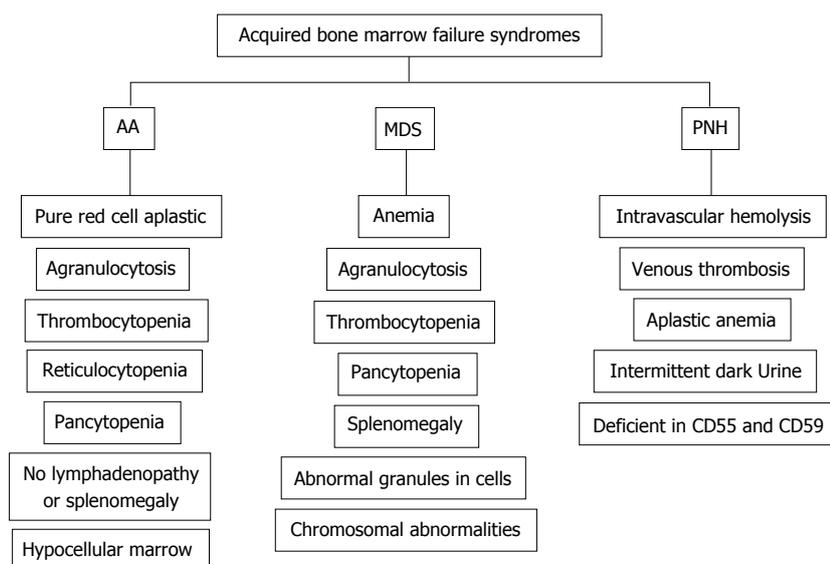
Figure 1 Typical clinical presentation of aplastic anemia, myelodysplastic syndrome and paroxysmal nocturnal hemoglobinuria. AA: Aplastic anemia; MDS: Myelodysplastic syndrome; PNH: Paroxysmal nocturnal hemoglobinuria.

marrow targeting a harvest of 2-4 × 10^8 nucleated cells per kilogram of recipient weight. The marrow is then filtered prior to infusion into the recipient. In the past, bone marrow stem cell transplantation was the only option available for HSCT, but due to the many obstacles in harvesting and health risks to the donor, other HSCT sources are becoming more frequently used. However, bone marrow stem cell transplantation is still a preferred option for ABMFD partially due to fewer amounts of lymphocytes in bone marrow reducing the risk and intensity of GVHD.

UMBILICAL CORD BLOOD TRANSPLANTATION

Umbilical cord blood collections are typically obtained from allogeneic, unrelated donors[18]. Cord blood is harvested from the leftover blood of the placenta and umbilical cord after a birth. The hematopoietic stem cells are filtered from the cord blood and kept frozen in storage. Total cord blood stem cell content is usually less than that obtained from peripheral blood or bone marrow, but the cord blood stem cells have higher hematopoietic potential and are able to produce more blood per cell than their counterparts. Due to the lesser quantity of cord blood stem cells, this type of transplantation is given to children or adults of smaller stature. There does not seem to be a strong association between HLA matching and acquiring GVHD and only one-third of patients can find a HLA-identical donor[19,20]. Thus, cord blood transplantation is beneficial for patients that cannot find an acceptable donor based on their HLA loci[21].

COMPARING IST AND HSCT

As of yet, there have been no clinical trials that have compared IST and HSCT. However, many cohort studies have been completed to analyze overall survival, quality of life and failure-free survival. Survival using HSCT is highly dependent upon the age of patients and donor matching (HLA-identical donor transplants showed the highest proportion of survival). While in general, studies reported that for IST and HSCT overall survival and event-free survival were similar in the two groups, HSCT in patients that received HLA-identical transplants resulted in higher survival than patients receiving IST. Adjusting for quality of life, HSCT patients enjoyed longer periods without symptoms or drug toxicity than IST patients. In the past, most patients received IST due to the inability to find an HLA-identical donor, but with scientific advancement in combatting GVHD and rejection and improved survival in transplants involving unrelated donors, HSCT is being more frequently used.

NOVEL CELLULAR THERAPIES

Clinicians and researchers are working towards developing novel therapies to cure ABMFD. The goal of novel cellular therapies is to increase patient accessibility, improve feasibility, and reduce procedure related complications. The methods range from improvements upon traditional methods, such as haploidentical transplantation, amplified umbilical blood transplantation, and mesenchymal cell transplantation, to novel ideas such as thrombocyte stimulator and chimeric antigen receptor T-cells.

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN COMBINATION THERAPY

Haploidentical HSCT has been used frequently in the past, for patients that are unable to find a HLA identical

donor[22]. Haploidentical HSCT, by itself, leads to the great amount of complications due to unmatched HLA, such as GVHD, graft failure, or infection, resulting in significant morbidity or mortality[23]. Recently, haploidentical HSCT has been used in combination with immunosuppressive techniques to counteract the side effects of unmatched HLA. The overall goal of these combination therapies is to induce acceptance of unmatched donor stem cells in the recipient's bone marrow *via* conditioning.

Full or partial T-cell depletion in combination with non-myeloablative haploidentical HSCT has shown good preliminary results[24,25]. Immunotoxins are used to fully or partially eliminate the T-cells of the HSCT recipient before the transplant. After the transplant, immuno-suppressive agents such as ATG, CSA, or various anti-complement anti-bodies or complement blockers are given on a regular basis to prevent GVHD or rejection of the stem cells. "Megadose" haploidentical HSCT along with full T-cell depletion has also been explored[24,25]. Patients showed success in stem cell engraftment, but they experienced delayed immune reconstitution and higher rate of rejection compared with using partial T-cell depletion with normal HSCT. Variations of these types of therapies are currently being explored; some of them have showed impressive result comparable with HLA matched donor stem cell transplantation. The advantages of haploidentical HSCT combination therapy are the short waiting period in finding a donor and the brevity of the entire HSCT procedure, compared with other methods.

AMPLIFIED UMBILICAL CORD BLOOD STEM CELL TRANSPLANTATION

Umbilical cord blood HSCT offers an option to patients without a HLA matched donor. The recipients of HLA unmatched umbilical cord HSCT have significantly decreased risk of GVHD or graft failure compared to matched unrelated donor HSCT. Typically, cord blood HSCT from one donor is only sufficient to treat children or small adults. Larger adults must receive amplified cord blood from two or more donors.

MESENCHYMAL STEM CELL TRANSPLANTATION

Mesenchymal stem cells (MSC) are found in the bone marrow and fat and are capable of differentiating into hematopoietic cells. MSCs represent a very small proportion of all adult bone marrow cells (< 0.1%), and their exact anatomical location within the bone marrow has yet to be determined[26]. These cells are multipotent and can differentiate into osteoblasts, fat and cartilage, in addition to hematopoietic cells. When transfused into a recipient, MSCs have a tendency to migrate to areas of injury or inflammation and proliferate into resident progenitor cells, but do not induce lymphocyte differentiation, thus immune cells such as T-cells or natural killer cells do not target MSC cells. The MSCs tendency to migrate to injured and inflammatory areas also represents a downside of using this transplantation, leading to poor engraftment. MSC can be used to enhance engraftments after HSCT. Efforts have been made to overcome these difficulties by selecting homogeneous populations of MSCs that exhibit strong osteoblastic potential, through identifying and selecting cells expression of certain surface antigens (such as STRO-1 or STRO-3)[27].

THROMBOCYTE STIMULATOR

For AA and PNH patients, drugs that stimulate throm-bocyte production have been shown to have clinical benefits by improving blood clotting and raising blood cell levels for patients that have failed all standard therapies. This therapy provides a salvage option for AA or PNH patients, who are ineligible for immunosuppression and HSCT[28,29]. The drugs mimic thrombopoietin, which is the principal regulator of thrombocyte production, by binding of the receptor c-MPL on megakaryocytes. Initial clinical trials have shown a median increase in platelet count of 44000 per cubic millimeter for patients receiving the drug. Interestingly, it was observed that 8 of the 11 patients sensitive to the drug kept their response in a median of 10 mo. These drugs have been shown to stimulate erythrocyte and thrombocyte production[28] and are very helpful for patients who are unable to receive stem cell transplantation.

CONCLUSION

ABMFD is a group of rare but serious hematological diseases with a manifestation of insufficient blood cell formation. There are three main forms of ABMFD that share a similar clinical presentation and bone marrow histological appearance. The primary goals in treating ABMFD are to remove the underlining etiologic factors and to rebuild a healthy bone marrow for normal hematopoiesis. Stem cell transplantation is the ideal method to treatment ABFD. However, the high treatment related mortality, long-term complications such as GVHD, and lack of HLA matched donor sources hinder the practical use of this treatment option. With advances in cellular therapy, immunotherapy, and personalized medical therapy, novel gene modification/targeting therapy under precision medicine model opens a new frontier for ABFD therapy.

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ORIGINAL ARTICLE

Retrospective Study

Retrospective study of a cohort of adult patients with hematological malignancies in a tropical area

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Abstract

AIM: To review the characteristics of hematological malignancies in tropical areas, and to focus on the specific difficulties regarding their management.

METHODS: This is a retrospective narrative review of cases of patients with hematological malignancies. All medical files of patients with malignant disease whose treatment was coordinated by the Hemato-Oncology service of the Cayenne Hospital in French Guiana between the 1st of January 2010 and the 31st of December 2012 were reviewed. Clinical data were extracted from the medical files and included: Demographic data, comorbidities, serological status for human immunodeficiency virus, human T-lymphotropic virus 1 (HTLV1), hepatitis B virus and hepatitis C virus

infections, cytology and pathology diagnoses, disease extension, treatment, organization of disease management, and follow-up. The subgroup of patients with hematological malignancies and virus-related malignancies were reviewed. Cases involving patients with Kaposi sarcoma, and information on solid tumor occurrence in virus-infected patients in the whole patient population were included. Since the data were rendered anonymous, no informed consent was obtained from the patients for this retrospective analysis. Data were compiled using EXCEL® software, and the data presentation is descriptive only. The references search was guided by the nature of the data and discussion.

RESULTS: In total, the clinical files of 594 patients (pts) were reviewed. Hematological malignancies were observed in 87 patients, and Kaposi sarcoma in 2 patients. In total, 70 patients had a viral infection, and 34 of these also had hematological malignancies. The hematological diagnoses were: Multiple myeloma in 27 pts, lymphoma (L) in 43 pts, myeloproliferative disorders in 17 pts and Kaposi sarcoma in two patients. The spectrum of non-Hodgkin lymphomas (NHL) was: Burkitt L (1 pt), follicular L (5 pts), chronic lymphocytic leukemia (5 pts), high-grade NHL (9 pts), mucosa-associated lymphoid tissue NHL (4 pts), T-cell lymphoma (4 pts), Adult T-cell lymphoma-leukemia (ATL)/lymphoma/leukemia (12 pts); three patients had Hodgkin disease. The spectrum of myeloproliferative diseases was: Chronic myelogenous leukemia (8 pts), thrombocytemia (5 pts) and acute leukemia (4 pts). There were no polycythemia vera, myelosclerosis, and myelodysplastic diseases. This appears to be due to bias in the recruitment process. The most important observations were: The specificity of HTLV1- related ATL malignancies, and the high incidence of virus infections in patients with hematological malignancies. Further, we noted several limitations regarding the treatment and organization of disease management. These were not related to the health care organization, but were due to a lack of board-certified hemato-oncology specialists, a lack of access to diagnostic tools (*e.g.*, cytogenetic and molecular diagnosis, imaging techniques), the unavailability of radiotherapy, and the physical distance from mainland France. Yet the geography and cultures of the country also contributed to the encountered difficulties. These same limitations are seen in tropical countries with low and intermediate household incomes, but they are amplified by economic, social, and cultural issues. Thus, there is often little access to diagnostic procedures, adequate clinical management, and an unavailability of suitable medical treatments. Programs have been developed to establish centers of excellence, training in pathology diagnosis, and to provide free access to treatment.

CONCLUSION: Management of hematological malignancies in tropical areas requires particular skills regarding specific features of these diseases and in terms of the affected populations, as well as solid public health policies.

Core tip: Management of hematological malignancies is guided by very specialized and up to date guidelines that are based on the biology of the diseases. An important proportion of these diseases are related to viral infections, and this is particularly so in tropical areas. Based on a narrative review of 87 cases of patients managed in French Guiana, we provide an overview of the most important characteristics of these hematological diseases (*e.g.*, human immunodeficiency virus and human T-lymphotropic virus 1 related diseases), the limitations regarding management (*e.g.*, board-certified specialists, pathology labs, imaging techniques, radiotherapy), and possible solutions to improve quality (*e.g.*, centers of excellence, training programs in pathology). These observations may be more broadly relevant in the setting of countries with low and intermediate household incomes.

INTRODUCTION

French Guiana is an Overseas Department (ZIP code: 9700 Guiana) and a Region of France. The political, administrative, and health care organizations are the same as in mainland France. The territory covers 85000 km^2, 95% of which is Amazonian forest. The climate is equatorial. The official population is 229000 inhabitants[1], but there are approximately 40000 illegal immigrants. The population is located primarily along the Atlantic shore. There are three major cities: Cayenne and surrounding area (127000 inhabitants), Saint-Laurent du Maroni (33700 inhabitants) and Kourou (25900 inhabitants). The remainder of the population reside in small villages (2000 to 8000 inhabitants in the general vicinity of the village, and sometimes as little as a few dozen people in the village itself). The majority of the population and associated economic activities are concentrated on the Atlantic Ocean Coast (which is 450 km in length and 30 km wide). The population of French Guiana is expanding[2]. It was 115000 in 1990 and was 229000 at the last census in 2009. In 2040, the population is projected to be 480000 to 650000[3].
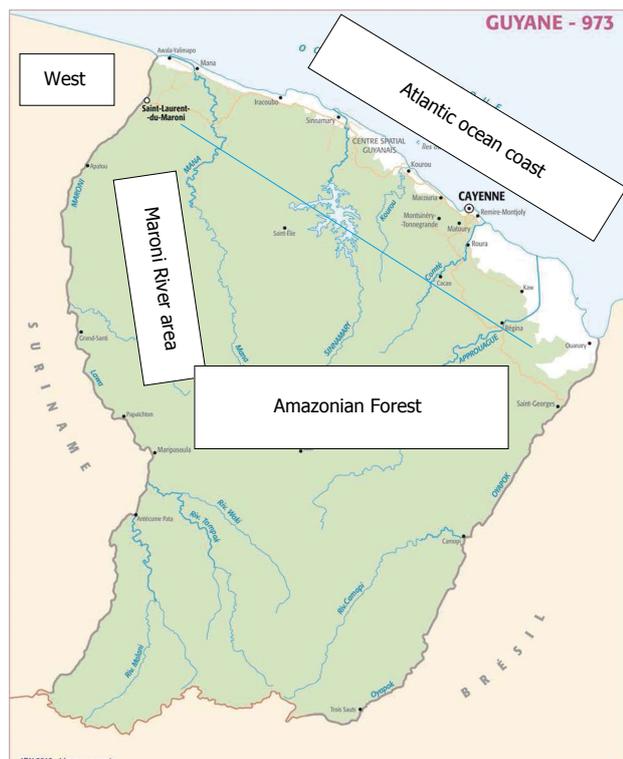
Figure 1 French Guiana map. From: Institut national de l'information géographique et forestièr. IGN: Open licence.

The median age is 28 years. The fertility rate is 3.57 children/woman. The annual population growth rate is 3.9%. The percentage of people 70 years of age or older is 1.5%. The annual birth-rate is 30.4 per 1000 and the annual death-rate is 3.7 per 1000[2]. Economic activity is based on agriculture (0.8%), panning for gold, construction (13.7%), and tertiary activities represented mainly by administrative and military entities (85.5%). The rate of unemployment is high[2]. The population of French Guiana is diverse and comprised of Guianese and French West Indies creoles (40%), metropolitans (10%), Haitians (10%), Brazilians (10%), Surinamese (10%), Chinese, Guyanese, and two specific indigenous populations groups: Native Americans (around 5000-8000 individuals) and Bushinengue ("Noirs marrons" or maroons) (around 15000 individuals). There are major cultural differences between these various ethnic groups.

There are two public hospitals (in Cayenne, and Saint-Laurent du Maroni) and one Red-Cross hospital in Kourou. The Cayenne regional hospital is a university hospital within the framework of the University of the French West Indies - Guiana. The most developed and active medical services are (1) related to obstetrics and pediatrics; and (2) emergency, intensive care unit (ICU), management of trauma and transport of wounded patients. Eighteen health care centers are linked with the Cayenne Hospital, and they are located throughout French Guiana. Figure 1 provides a map of French Guiana.

This study is part of a larger retrospective study of 594 adult patients with cancer who were managed by

the Hematology-Oncology unit of the Cayenne Hospital during 2010 to 2012. The objective was to highlight the major problems regarding management of adult hematological malignancies in a tropical region with a European health organization that was subject to specific limitations due to the tropical setting and the distance from mainland France. These are: An absence of conventional and ICU hematological services, unavailability of board-certified specialists (except monthly visits by specialists from the Centre Léon-Bérard in Lyon, France), a lack of specific labs and radiotherapy on the one hand, as well as the prevalence of tropical infectious diseases and problems due to cultural diversity on the other hand. In this study we have also provided insights regarding virus related malignancies [human immunodeficiency virus (HIV), human T-lymphotropic virus 1 (HTLV1), and a focus on hepatitis B virus/hepatitis C virus (HBV/HCV) lymphoproliferative disorders]. In the Discussion section, we strived to extend the observations made in French Guiana to problems encountered in other tropical countries in the developing world.

MATERIALS AND METHODS

For the purpose of this study, we retrospectively reviewed the clinical charts of all adult patients who were managed by the Hematology-Oncology unit of the Department of Amazonian Health of Cayenne Hospital from the 1st of January 2010 until the 31st of December 2012.

The data collected were: Date of birth, age, gender, home address, place of birth, description and number of comorbidities, cancer type, extension (local, regional, metastatic or specific classification), HIV, HTLV1 and HBV/HCV serological status (no information on EBV infection status), treatment and management in mainland France or the French West Indies. Cultural identity (*e.g.,* Bushinengue or Native American) was annotated if the patient mentioned the fact during the course of the clinical management. Neither the cytology nor pathology examinations were reviewed centrally. The pathology reports were used as provided in the medical files. Examinations were performed at the Cayenne Hospital and in various laboratories in France. Details of biological characteristics of the malignancies (*e.g.,* immunochemistry and immunophenotypes, molecular biology examinations) were rarely available. Available examinations are provided in the Table 1. We restricted the analysis to descriptions; no comparison test was used. The files were rendered anonymous. Complete remission (CR) status, the date of the most recent news and follow-up (FU) duration in years, as well as the clinical status were recorded. Unfortunately, the majority of patients were lost to FU. Clinical files were summarized in an EXCEL® data base. The results were derived using features of the EXCEL® software.

We did not obtain individual informed consent; we used current hospital medical files; all the data presented were rendered anonymous and the chance of

Table 1 Hematological disease distribution and status of accessible and non-accessible examinations		
Diagnosis	**No. of patients**	
		Examinations available in French Guiana
Myeloma	27	Complete blood count with cytology
Lymphoma (43 patients)		Bone-marrow aspiration and biopsy
Burkitt	1	Common limited immunophenotypes
Follicular	5	Standard blood chemistry
CLL	5	LDH and β2-microglobulin
High-grade B-NHL	9	Standard immunological studies
MALT NHL	4	Immunoelectrophoresis (blood and urine)
T-NHL	4	Serological testing for HIV, HTLV1, HBV, HCV, EBV, as well as PCR/RT-PCR for these viruses, HHV8, HPV and the majority of infectious diseases (opportunistic or not)
		Standard bone X-rays
		CT scan
		MRI
		Examinations not available in French Guiana
ATL/lymphoma	10	PET scan
ATL/leukemia	2	Complete immunophenotypes
Hodgkin	3	Genetic testing
Myeloproliferative diseases (17 patients)		
CML	8	
Thrombocytemia	5	
Acute leukemia	4	
Specific HIV-related entity (2 patients)		
Kaposi	2	

CLL: Chronic lymphocytic leukemia; NHL: Non-Hodgkin lymphoma; ATL: Adult T-cell lymphoma-leukemia; CML: Chronic myeloid leukemia; PET: Positrons emission tomography; MRI: Magnetic resonance imaging; MALT: Mucosa-associated lymphoid tissue; HIV: Human immunodeficiency virus; HTLV1: Human T-lymphotropic virus 1; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CT: Computed tomography; LDH: Lactico-DeHydrogenase; PCR: Polymerase chain reaction; RT-PCR: Real-time polymerase chain reaction; EBV: Epstein-barr virus; HHV8: Human herpes virus type 8; HPV: Human papilloma virus.

patient identification was extremely low.

A reference search was conducted using PUBMED with the key words: "French Guiana; cancer; neoplasm; Kaposi; lymphoma; leukemia, multiple myeloma, myeloproliferative diseases". However, we also reviewed the literature relating to recommendations published for hematological malignancies (National Cancer Centers Network - NCCN[4-7], French Hematological Recommendations[8]). Epidemiological data were derived from Globocan 2012[9,10] and from the French National Cancer Institute report of 2014[11]. The authors declare no conflict of interest.

In total, we reviewed 594 medical files of patients with malignancies, of whom 87 had hematological malignancies. We also included two cases of HIV-related Kaposi sarcoma. For the purpose of having a review of systemic virus infections in these patients, we specifically analyzed the group of patients with an HIV, HTLV1, HBV or HCV positive serological status. The entire 594 patient study was the subject of a thesis[12] for an MD degree (Bianco L), and it was reviewed and approved by the University of French Guiana and West Indies Medical School Institutional Review Board.

This article provides a narrative review of general information for the entire 89 patient cohort, and specific information on the various groups of diseases.

RESULTS

There were 50 men and 49 women; the median age was 46 years (ranging from 18-85 years of age). The majority of patients (61) lived in the Cayenne area; 15 patients lived in the Western part of French Guiana (*i.e.,* Saint-Laurent du Maroni and Mana areas) and 6 patients lived in the Maroni River area (refer to Figure 1). In 6 cases, the home address was unknown. The place of birth was the Cayenne area for 19 patients, the Western part of French Guiana for 10 patients, the Maroni River area for 6 patients, unknown for 25 patients, and countries other than French Guiana for 29 patients (Surinam 9, the French West Indies 5, Haiti 4, Brazil 3, mainland France 3, and other foreign countries for 5 patients). Comorbidities were frequent in this population of young patients: 28 had one comorbidity, 11 had 2, 8 had 3, and one had 4, while 40 had no comorbidities (for one patient, comorbidities remained unknown). The most frequent comorbidities were hypertension (25 patients), diabetes (16 patients), congestive heart failure (5 patients), lung diseases (asthma and chronic obstructive bronchopathy in 2 and 3 patients, respectively), stroke in 4 patients, and dementia and psychosis in one patient for each of these mental conditions. Two patients had chronic renal insufficiency (creatinine clearance < 30 mL/min). Tuberculosis, malaria, and leprosy were present in one patient for each of these conditions. One patient had an albinism, one had cirrhosis related to chronic HBV hepatitis, and three patients suffered of drepanocytosis.

The HIV, HTLV1, and HBV/HBC serological status were unknown in 31, 40, and 34 patients, respectively.

No.	Gender	Age	Ig	Stage	Regimen 1	Regimen 2	Regimen 3	FU (yr)
					Table 2 Characteristics, treatment and evolution of 22 patients with active multiple			
1	M	65	IgAλ	ⅢB	Bortezomib + DEXA	Melphalan DEXA	0	6
2	F	60	IgGκ	NA	Bortezomib + DEXA	0	0	3
3	M	66	IgAκ	NA	Bortezomib + DEXA	0	0	< 1
4	F	77	IgAκ	ⅢB	Thalidomide + Melphalan	Melphalan + DEXA	0	< 1
5	F	80	NA	ⅢA	Thalidomide	0	0	< 1
6	M	56	IgAκ	ⅢB	Thalidomide + DEXA	Thalidomide + Melphalan	0	2
7	F	71	IgGκ	NA	Bortezomib + DEXA	Thalidomide	0	4
8	F	85	IgGκ	Ⅰ A	Melphalan + DEXA	0	0	6
9	F	62	IgAλ	NA	Melphalan + DEXA	Bortezomib + DEXA	0	7+
10	M	83	NA	NA	NA	0	0	< 1
11	F	65	IgGλ	ⅢA	Bortezomib + DEXA Thalidomide	VAD	0	11
12	F	81	NA	ⅢB	Thalidomide + Melphalan + DEXA	0	0	< 1
13	M	57	IgGκ	NA	Bortezomib	0	0	6
14	F	69	IgGκ	ⅢA	Melphalan + Thalidomide,	Lenalidomide	0	4
15	F	59	IgGκ	ⅢA	Bortezomib + DEXA + Thalidomide	HDCT	0	3+
16	M	44	IgAκ	ⅢB	Bortezomib + DEXA	HDCT	0	3
17	F	60	IgGκ	ⅢB	DEXA+ Melphalan + Thalidomide	HDCT	Lenalidomide	6
18	M	52	IgGλ	ⅢA	Bortezomib + DEXA	HDCT	0	2
19	F	61	IgGκ	ⅢB	Bortezomib + DEXA + Thalidomide	HDCT	0	2
20	M	62	NA	NA	Bortezomib + DEXA	HDCT	Lenalidomide	2
21	M	53	IgAκ	ⅢA	VAD	HDCT	0	12
22	F	55	IgGκ	ⅢA	Bortezomib + DEXA + Thalidomide	HDCT	0	< 1

M: Male; F: Female; age in years; stage according to Durie-Salmon; Ig: Immunoglobulin; FU: Follow-up (+: died); DEXA: Dexamethasone; HDCT: High-dose chemotherapy; VAD: Vincristine, adriamycine, dexamethasone; NA: Not available.

Nine out of 58 patients had an HIV positive serological status and viremia, four of whom had been treated and had achieved infection control. Fifteen out of 49 patients had HTLV1 positive serological status. Ten out of 55 patients had a positive hepatitis virus status: 8 were HBV[+]/HCV[-] and two were HBV[-]/HCV[+]. One patient had a positive serological status for both HIV and HTLV1. One patient had previously had prostate cancer.

The hematological malignancy diagnoses are detailed in the Table 1.

Multiple myeloma

Twenty-seven patients were managed during this period: 10 men and 17 women. MGUS was not included in this series. Median age was 62 years (ranging from 44 to 85 years of age). The number of comorbidities was 0, 1, 2 and 3 in 10, 10, 4 and 3 patients, respectively. There were 10 cases of hypertensions, 5 involving diabetes, one with drepanocytosis and one with chronic renal insufficiency. One patient had a solitary plasmocytoma, IgGκ, in the sphenoid bone of the skull base, 4 patients had smoldering myeloma and 22 patients had active myeloma, 8 of whom were eligible for high-dose chemotherapy (HDCT). The monoclonal component was IgAλ in 3, IgAκ in 5, IgGλ in 2 and IgGκ in 10 patients, while this information was not available for 6 patients. The Durie-Salmon stage[13] was IA in 4 patients, ⅢA in 7, ⅢB in 7 and not available for 8 patients. One patient was HIV[+], but had achieved disease control following treatment, while one patient had an HBV[+] serological status. The patient with a plasmocytoma had a partial resection of the tumor and radiotherapy followed by adjuvant Bortezomib treatment for 6 mo. Patients with smoldering myeloma were followed without being given a specific treatment, and one of them died two years later. Treatment and evolution of the 22 patients with active myeloma are shown in the Table 2. All these patients received biphosphonates treatment and supportive care.

Eleven patients were treated in mainland France. These were the eight patients who had high-dose chemotherapy (one cycle of high-dose Melphalan) and autologous bone-marrow support (ABMS), the patient with plasmocytoma, and two other patients.

Lymphoid malignancies

Forty-three patients had lymphomas, including adult T-cell lymphoma-leukemia (ATL)/lymphoma/leukemia and Hodgkin disease. There were 28 men and 15 women; median age was 50 years (ranging from 18 to 84 years of age).

Clinical entities are summarized in the Table 2.

Chronic lymphocytic leukemia

Five patients had chronic lymphocytic leukemia (CLL). They were four men and one woman, aged 46, 50, 60, 62 and 70 years. The Binet stage[14] was A, B, C in 2, 1 and 2 patients, respectively. One patient had auto-immune hemolytic anemia (a Bushinengue patient with stage C disease). Patients had standard immuno-phenotyping (Matutes score)[15], but none of these patients underwent fluorescence *in situ* hybridization (FISH) and molecular analysis. One patient had an HTLV1 positive serological status. Two patients were only monitored (less than 1 and 6 years FU, respectively), one received Chlorambucil (less than 1 year FU). Two patients received Fludarabine and Rituximab (3 years FU), one of whom eventually received R-CHOP (5 years FU).

Follicular lymphoma

Five patients had follicular lymphoma. They were 2 men and 3 women; median age 55 years (ranging from 40 to 66 years of age). Two patients had HBV positive serological status, one of whom had post hepatitis cirrhosis. One patient was HIV⁺, although he had received treatment and achieved disease control. The Ann Arbor stage[16] was: ⅡAE (1 patient, E: Breast), ⅢA (2 patients), Ⅳ (2 patients). All of these patients were treated in mainland France. Four patients received R-CHOP and one Rituximab only.

Mucosa-associated lymphoid tissue lymphoma

Four patients (3 men and one woman; aged 42, 68, 69, 84 years) had mucosa-associated lymphoid tissue (MALT) lymphomas. Two patients had gastric MALT, Ann Arbor stages ⅡE and Ⅳ (one patient had a positive HCV and HTLV1 serological status) and two patients had skin MALT, both stage Ⅳ (one of whom had a positive serological HTLV1 status). All patients received Rituximab, on its own for one patient, and combined with Fludarabine for two patients, with CHOP either as first line or second line, depending on the patient. Three patients reached a CR status and one attained stable disease. One patient died after 2 years of disease progression, another patient died after 10 years of cardiac heart failure, and two patients underwent 4 and 9 years FU.

Burkitt lymphoma

A 46-year-old male immigrant from Senegal had a high-risk Burkitt lymphoma. He had a prior record of malaria and a controlled HIV infection. He was transferred to Paris for treatment where he received R-COPADEM-CYVE regimen chemotherapy[17]. This individual went into complete remission (at 1 year of FU).

High-grade B-cell lymphomas

High-grade B-cell lymphomas were observed in 9 patients. For 3 patients the data was incomplete, as full pathology and immunophenotyping were not available[18,19].

DLBCL was observed in 9 patients. Five patients were treated in the West French Indies or mainland France. Two patients were of Bushinengue descent, and one was a Native American. Disease extension was not available in 3 patients. The Ann Arbor stage was ⅡA and ⅢB in one patient each, Ⅳ in 4 patients. Treatment modalities were available for 7 patients: 6 patients received R-CHOP[20] and one R-ACVBP[21]. Two patients had HDCT and ABMS (2 CR) and two patients required DHAP[22] salvage chemotherapy regimen (one CR). In total there were 4 cases of CR (5, 10, 10, and less than 1 year FU), 4 other patients had less than 1 year FU, and one patient died after 2 years. The case of this latter patient is notable. He was a 53-year-old male of Bushinengue descent who lived along the Maroni River and who had an albinism. He had multiple exereses of basal-cell carcinoma of the skin. Serological status for HIV, HTLV1, HBV and HCV were all negative. He had a stage ⅢB DLBCL and received R-CHOP that was complicated by neutropenic fever. He received R-DHAP after disease progression, and died of disease progression after 2 years of this treatment.

Hodgkin disease

Two women and one man, aged 18, 44 and 51 years, had Hodgkin disease. The histological type was nodular sclerosis in all 3 patients. The Ann Arbor stages were ⅡA (2 patients) and ⅢA. The 51-year-old male had been treated for HIV infection, which was under control, and a renal insufficiency. All three patients received ABVD regimen[23] of chemotherapy. The patient with stage ⅡA disease was referred to mainland France for radiotherapy. All of them entered into CR status, but were then soon lost to FU.

T-cell lymphoma

Four patients has T-cell lymphomas, all of them had very uncommon clinical history with unfortunately a lot of missing information.

Two men, aged 40 and 75 years, had T-cell lymphoma. Both received CHOP regimen chemotherapy. The 75 year old patient had a response to treatment, but he was lost to FU after 5 years. Unfortunately, disease extension was not available. The other patient was a 40-year-old of Bushinengue descent and residing in the Maroni River area. He had an active untreated and uncontrolled HIV infection. HTLV1 and HBV/HCV serological status were negative. The Ann Arbor stage was Ⅳ with osteolytic bone involvement and hypercalcemia. The patient received CHOP chemotherapy regimen. He died, however, of progressive disease after 4 mo.

One patient had a very complex disease, for which many of the data were not available. This individual (a 49-year-old male) had respiratory insufficiency and hyper eosinophilia. Serological status was negative, and he had no parasitic disease. The lung biopsy demonstrated a bronchocentric granulomatosis. The blood cytology and in the medullary aspirate showed a monoclonal T-cell proliferation. Nonetheless the precise phenotype is not available. Various treatment sequences were administered. He was lost to FU after 10 years.

The last patient was a 21-year-old male from the Maroni River area and he was of Bushinengue descent, with an Ann Arbor stage Ⅳ lymphoma which was classified as precursor B-cell lymphoblastic lymphoma. He received R-CHOP and entered into CR, and relapsed and died one year later. Nevertheless the retrospective study of the pathological report (liver biopsy) shows a profile of large-cell anaplastic T-cell lymphoma (ALK-).

ATL/lymphoma/leukemia

Nine patients had ATL/lymphoma and two patients ATL/leukemia. There were 6 women and 5 men; their median age was 46 years. Nine patients were of Bushinengue descent, eight of whom lived in the

Table 3 Characteristics, treatment and evolution of adult T-cell lymphoma-leukemia/lymphoma/leukemia

No.	Gender	Age	Stage	Tumor sites	Regimen 1	Regimen 2	Regimen 3	FU
ATL/lymphoma								
1	M	28	NA		L/Z + P-IFN + oral ETO	CHOP	0	2
2	M	34	IV		L/Z + P-IFN	CHOP	DHAP	3
3	M	38	NA		L/Z + P-IFN	CHOP	DHAP	1
4	F	46	III	Abdomen, Ca^{2+}		CHOP	VBl	1
5	M	49	IV			CHOP	DHAP	< 1
6	M	49	IV	Colon, CNS	L/Z + P-IFN	CHOP	IT-MTX	< 1
7	F	51	I E	Nasal, sinus	0	CHOP	0	< 1
8	F	58	II A		L/Z + P-IFN	CHOP	0	3
9	F	62	NA		L/Z + P-IFN + ETO oral	CHOP	0	5
10	M	42	IV	Ca^{2+}	R-CHOP	0	0	3
ATL/leukemia								
10	M	41	-	Liver, cavum	L/Z + P-IFN	CHOP	0	< 1
11	F	47	-		L/Z + P-IFN + oral ETO	CHOP	0	4

Hydroxydaunorubicine, oncovin®, prednisone; M: Male; F: Female; age in years; stage according to Ann Arbor staging system; ATL: Adult T-cell lymphoma-leukemia; FU: Follow-up in years; Ca^{2+}: Hypercalcemia; CNS: Central nervous system; L/Z: Lamivudin + Zidovudin; P-IFN: Peg-interferon α-2a; ETO: Etoposide; CHOP: Cyclophosphamide, R-CHOP: Rituximab-cyclophosphamide; DHAP: Dexamethasone, aracytine, cisplatin; VBl: Vinblastine; IT-MTX: Intra-thecal methotrexate; NA: Not available.

Western part or in the Maroni River area. Four patients had hypertension; two had diabetes, one suffered from drepanocytosis. One patient had a serologically positive HBV^+HCV^- infection. One patient had a treated and controlled HIV infection. All patients had serologically positive HTLV1 infection. It is noteworthy that four patients had aggressive Strongyloides stercoralis GI infections. Three patients were treated in Paris and one in the Netherlands. The patient treatments and evolution are shown in the Table 3. To date, no patient has died, while two patients are in CR.

One patient had a singular history. He was a 42-year-old man from the Maroni River area and of Bushinengue descent with a stage IV lymphoma (lymph nodes and bone osteolytic lesions). He had hypercalcemia. This patient had serologically positive untreated HIV and HTLV1 infections. He had also a retinitis and CNS toxoplasmosis. The disease was initially diagnosed as diffuse large B-cell lymphoma (DLBCL). The patient received two cycles of R-CHOP, but contact with this patient was lost soon thereafter, thereby precluding FU. But the diagnosis was reviewed and changed to an ATL/lymphoma.

Myeloproliferative syndromes
Chronic myelogenous leukemia: Eight patients had chronic myelogenous leukemia (CML). They were 5 men and 3 women, with a median age of 51 years (ranging from 37 to 82 years of age). Aside from the older patients, none of them had significant comorbidities. The diagnosis was established by cytology, and the presence of Ph1 by FISH. Three patients underwent bcr-abl transcript analysis, one of whom lacked Ph1 [he was bcr-abl(-), but JAK^{2+}], and two after developing resistance to Imatinib. The possibility of bcr-abl point mutations was, however, not addressed. The older patient, who was bcr-abl(-), received hydroxyurea. Three patients received first line hydroxyurea, followed

by Imatinib and then Dasatinib. Three patients received first-line Imatinib, and then Dasatinib, while one patient received Dasatinib first-line. None of the patients were transferred to mainland France, and none died of the disease after a median FU of 3 years. None of them was considered for allogenic bone marrow transplantation.

Essential thrombocytemia: Three women and two men, aged 34, 41, 44, 49, 54 years, had essential thrombocytemia. One patient had hypertension and another had hypertension, arteritis and stroke, while the younger patient had a portal thrombosis. Three patients had a $JAK6^{V617F}$ mutation. This mutation did not occur in two patients, one of whom was bcr-abl(-). These two patients had no evaluation of MPL mutation. One patient received Anagrelide, and all of these patients were treated with hydroxyurea. They were alive after 3-4 years of FU.

Acute leukemia: Three men, one woman, aged 22, 39, 46, 54 years were diagnosed with acute myeloid leukemia. All of them were transferred to Paris for treatment. According to the FAB classification[24] there were one LAM5 and three LAM3: One of them was promyelocytic RARα[+], another had a Flt3 duplication with t(15;17). Patients were treated according to standard regimens[8]: The patient with LAM5 had an allogenic bone-marrow transplantation; patients with LAM3 received Idarubicin, all-trans-retinoic acid, and arsenic trioxide. Patients entered into CR, and they were free of disease at less than 1, 1, 2 and 5 years of FU.

Malignancies related to viral infections
The distribution of tumor types in patients infected with viruses is shown in the Table 4.

HIV infection: Nine patients had an HIV infection in this series, 4 with treated and controlled disease. Five

Table 4 Distribution of tumor types in patients infected by viruses			
Virus	**Hematological malignancies**	**Solid tumors**	**Total**
HIV	9	19	28
HTLV1	15	4	19
HBV	8	10	18
HCV	2	3	5
Total	34	36	70

HIV: Human immunodeficiency virus; HTLV1: Human T cell leukemia/lymphoma virus 1; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

patients had lymphomas (one follicular lymphoma, two DLBCL, one ATL/lymphoma, one Burkitt lymphoma). The other patients either had myeloma or Hodgkin disease.

Two patients had the HIV-related malignancy Kaposi sarcoma. They were two men who were aged 38 and 54 years. One had an uncontrolled HIV infection. He had serous involvement by the disease, and although he received liposomal Doxorubicin, the disease progressed and this patient was soon lost to FU. The other patient, an immigrant from Haiti, had a treated and controlled HIV infection, and he had involvement of the colon, lymph nodes and particularly the skin of both legs. He received liposomal Doxorubicin and experienced stable disease. He was alive with the disease after 3 years of FU.

Of the total of 28 patients who were HIV$^+$ in the series of 594 patients, 9 were described above. The tumor types in the 19 other patients were: Prostate, gastric (3 each), cervix, breast, lung, head and neck (2 each), penile, kidney, pancreas, esophageal and colon cancers (one each).

HTLV1 infection: Nineteen of the 594 patients had a positive serological status. Fifteen patients had hematological malignancies, of which 12 were ATL/lymphoma/leukemia; two MALT non-Hodgkin lymphoma (NHL), one CLL. The four other patients either had colon, liver, prostate or kidney cancer.

HBV infection: Of the entire 594 patient cohort, 18 patients had a positive serological status: 8 had hematological malignancies while the others had various tumor types comprised of breast (3), liver (2), gastric (2), cervix, prostate and head and neck cancer (one each).

HCV infection: Of the entire 594 patient cohort, 5 patients had a positive serological status: 2 had hematological malignancies and the others had either liver (2), or lung cancer (1).

DISCUSSION

This study reports on 87 patients with hematological malignancies (and two with Kaposi sarcomas) who were managed during a three-year period at the main hospital in French Guiana. However the study scale is small, but it provides several interesting insights that are of considerable relevance in terms of the provision of medical care. First of all, there is the effect of bias. There are biases of recruitment of patients in the series: Patients may have been managed in the two other hospitals of the territory. This may not have occurred so much in Kourou (which is only 50 km from Cayenne), but rather in Saint-Laurent du Maroni, which is 250 km far from Cayenne. It is noteworthy that Saint-Laurent is the most substantial city (around 50-70000 inhabitants) of the Western part of French Guiana, of the Bushinengue area and also of both banks of the Maroni River (French and Surinamese), as Paramaribo, Surinam's capital is 150 km west of Saint-Laurent. Moreover, there are many illegal immigrants, but since the border control is 100 km down the road running from Saint-Laurent to Cayenne, many patients are managed at the local hospital. Other biases are the fact that myelodysplastic syndromes are either managed by other hospital wards, or they are misdiagnosed; an unknown proportion of patients are directly referred to mainland France; and lastly, an unknown proportion of patients from the Western and Maroni River areas are certainly not diagnosed due to the absence of robust medical network.

Other interesting insights are the gap between the needs for hematological disease management and the facilities that are available. This aspect can be equated with countries in tropical areas that have low and intermediate household incomes. Lastly, this series shows the importance of infections in the occurrence of some hematological malignancies to be reviewed.

Distribution of diseases

In this series, out of 594 patients with malignancy, hematological malignancies occurred in 87 patients (*i.e.*, 14.5%).

The incidence of hematological malignancies could, however, not be measured. We are cognizant of the fact that it is scientifically inexact as it is an approximate assessment of the prevalence of hematological diseases which were accounted for in the period from 2010 to 2012. In 2008, in mainland France, the incidence of hematological malignancies relative to solid tumors was found to be 6.5%[25]. The comparative morbidity figures for solid tumors in mainland France (2005) *vs* French Guiana (2003-2009) was determined to be 1.30 and 1.31 for men and women, respectively[26]. The incidence of cancers therefore seems lower in French Guiana than in mainland France. GLOBOCAN 2012 incidence data[9,10] show that French Guiana has a global incidence of cancer in keeping with other northern and North-Western parts of South America (137.5/100000), but lower than in Brazil, Argentina, Chile and specially Uruguay (where the incidence is the highest, and at a level that is similar to Western countries). The incidence of hematological malignancies in French Guiana is similar to that of

mainland France, but higher than in the majority of South American countries, at 6.5/100000 for leukemia (as in Ecuador), 2.4/100000 for multiple myeloma (as in Surinam) and 7.0/100000 for NHL (as in Colombia and Uruguay).

Multiple myeloma

Patients with multiple myeloma had the same median age as in mainland France, while there was a slight predominance among women. The precise staging procedures at the initial diagnosis were not always fully available in the medical files. Nevertheless, with the exception of the cytogenetics of plasma cells[5], the most important criteria were available. The most important genetic abnormalities should be in regard to chromosomal 13q deletion, and detection by FISH of t(4;14), t(14;16), and del17p. These are poor prognostic factors. The international staging system is nonetheless used in clinical practice[27]. The majority of patients benefited from proteasome inhibitors, and Thalidomide. Eight out of 22 patients had high-dose Melphalan with ABMS. The conditions to indicate HDCT and ABMS are based on response to first-line treatment[28] and practical opportunity to perform HDCT. Treating multiple myeloma is hence not a problem in the setting of this organization. In patients not eligible to HDCT, new regimens including targeted drugs are more active than Melphalan plus prednisone regimen[29], and a Bortezomib-DEXA regimen seems to have the same activity as the three drug regimens (with Thalidomide or Melphalan)[30].

Lymphomas

The pathology diagnosis of lymphoma was established by different pathologists in French Guiana, the French West Indies or mainland France. The standard procedure first comprised morphological analysis, followed by a standard immunophenotyping panel with at least CD20, CD79a for B-cell malignancies and the same negative readout with CD3, CD5 for T-cell malignancies. Depending on the first screening and the capabilities of the laboratory, other antigens were also tested. Precise information regarding the pathology procedures and classification was not generally available. Since 2010, the biopsy samples have been referred to one of the pathology reference centers affiliated with the French National Cancer Institute (LYMPHOPATH network). Clinical staging of lymphomas (both NHL and Hodgkin Disease) used the Ann Arbor classification, as originally reported[16], because the lack of access to TEP-scanning did not allow use of the Lugano staging system[31].

Patients with CLL exhibited classical features of the disease. There were diagnosed using an immunophenotyping panel that allowed application of the Matutes score[15].

Staging of the patients was established according to the Binet classification[15]. None of the patients had FISH to screen for a del(11q) or del(17p), which plays an important role in the identification of specific treatments, nor Ig mutational profile and expression of protein

ZAP70, which are highly relevant for the prognosis[8]. Patients therefore received treatment according to their clinical status. Thus, the patient with auto-immune hemolytic anemia received R-CHOP regimen. The treatment eventually conformed to recognized standards.

Patients with follicular lymphomas received a standard work-up, including a bone-marrow aspirate and biopsy. They were staged according to the Ann Arbor classification. It is noteworthy that these patients were all referred to mainland France for treatment. There is no specific explanation for this, except that two of them were from a metropolitan area. All of them nonetheless benefited from extensive work-up, and they received Rituximab with or without a CHOP regimen of chemotherapy for extensive or progressive disease. This is standard disease management. Two patients had an HBV infection, which has not been reported to be linked with the occurrence of follicular lymphoma[32]. HIV infection also does not seem to be related to this NHL type.

Two patients had gastric MALT lymphoma. Information regarding infection by *Helicobacter pylori* was not available, and FISH t(11;18) was not performed. One of these patients had positive HCV and HTLV1 serological status (stage ⅡE). Unfortunately, no information on splenic involvement was available, as it has been described in splenic marginal zone lymphoma related to HCV infection[33]. Two patients had skin MALT lymphoma. Information on infection by Borrelia burgdorferi was not available. One of these patients had a positive serological HTLV1 status. The staging procedure was standard. With gastric MALT lymphoma, no information on *Helicobacter pylori* treatment was known, but all of them received induction immunotherapy (Rituximab). In three cases, associated chemotherapy was given because of stage Ⅳ disease.

The patient with Burkitt lymphoma had standard current characteristics: Malaria[34], African descent, and HIV infection[35-37]. He was treated in keeping with the best treatment standards in Paris[17].

High-grade lymphomas were diagnosed according to morphological aspects and standard minimal immunophenotyping. The Ann Arbor stage was used, but the International Index was not available[38]. Five patients were transferred to mainland France and therefore had a complete diagnostic and clinical work-up. When the information was available, these patients nonetheless received standard chemotherapy regimens[39] with Rituximab. Two patients with stage Ⅳ disease, who responded to conventional chemotherapy, received HDCT and ABMS. The two Bushinengue patients with specific disease were not transferred to mainland France. This may have been due to the absence of coverage for their health expenses. The native American patient was transferred to Paris for treatment. These individuals are usually French citizens, and they hence have the appropriate level of social security coverage. DLBCL belongs to the spectrum of hematological malignancies related to HIV[40], but that are unrelated to HTLV1 and HBV. The precise diagnosis of albinism was not done,

but it is likely to have been a type 2 oculocutaneous albinism. This disease does not seem to increase the risk of lymphoma (although this patient did experience repeated basal cell carcinomas).

Two patients had large cell T-cells lymphoma. Unfortunately, information regarding the expression of ALK was not available[6]. The response to CHOP was in keeping with the diagnosis of anaplastic large cell lymphoma. It is very rare to come across a T-cell lymphoma in a patient with an untreated uncontrolled HIV infection. A misdiagnosis must hence be suspected.

The three patients with Hodgkin disease did not have exceptional characteristics, and they were treated in keeping with the standard treatment[4]. One patient had a controlled HIV infection, and this might have been involved in the mechanism of the disease, although it unexpectedly exhibited a nodular sclerosis pattern, which is less frequent than mixed cellularity in this setting[41].

Eleven patients had ATL/lymphoma/leukemia, as defined by the Shimoyama classification[42]. The serological status was established by the ELISA method and confirmed by Western blot. When results from these assays were inconclusive, HTLV1 PCR was performed (at the Pasteur Institute). All patients had been infected by HTLV1. It is noteworthy that 9 of them were Bushinengue. This is a very well-recognized observation in French Guiana[43]. The Bushinengue population has a high prevalence of this infection[44], which is seen extensively in children[45] as the virus is transmitted from the mother by breast feeding[46,47]. Clinical work-up was in agreement with recommendations[6]. Four patients had Strongyloides GI involvement, which is often observed with this disease[48]. Patients received Ivermectin or Abendazole treatments. Patients also received standard treatment focused on retrovirus control, as a combination of Lamivudin + Zidovudin and Peg-interferon α-2a[49]. It also included standard chemotherapy regimens, such as CHOP[50] as first-line and DHAP as second-line treatments[6]. Five patients were treated in mainland France. This may present a difficult socio-cultural problem for the patients who live in the Maroni River area, and is an issue which will be discussed below. Due to cultural reasons, the majority of patients were lost to FU.

Myeloproliferative syndromes
The eight patients with CML were managed in a very conventional manner. Unfortunately, they were under-diagnosed and potentially undertreated. Apart from the cytological diagnosis and Ph1 FISH, the initial work-up ought to have included analysis of bcr-abl transcripts and screening for JAK2, CALR and MPL mutations, as well as a complete karyotyping[7]. No patient underwent mutational analysis for TKI second-line resistance so as to evaluate further indication of Nilotinib, Bosutinib, Ponatinib and Omacetaxine. Criteria for cytogenetic[51] and molecular[52] responses were not performed. Therefore, patients received Hydroxyurea and/or first-line Imatinib, and Dasatinib when they progressed. The profile of the patients did not fit with conditions for treatment by allogenic bone-marrow transplantation. Furthermore, organizing a prolonged stay in mainland France to select the indication and to perform the procedure is fraught with difficulties[53].

Surprisingly, we observed five cases of thrombocytemia. The same criticism can be made in terms of the lack of a complete work-up to at least eliminate secondary thrombocytopenia[8]. These diseases are however not specific to this tropical region.

The failure to encounter cases of polycythemia vera, myelofibrosis and myelodysplastic syndromes is certainly due to a bias in the recruitment process; such as the patients not being referred to the unit, not been diagnosed or, in some cases being directly referred to mainland France by their treating physician.

Lastly, four patients had acute myeloid leukemia. All of them were transferred within two or three days after diagnosis to a hematology unit in Paris by medical transport using the daily flight between Cayenne and Paris. This may present a substantial bias since these four patients were adults who had achieved CR status, while some patients may have been transferred only to return directly home with progressive disease, or they may even have died while in Paris. Further, some patients may have been children who were not managed in the hematology-oncology unit, while some patients may have died prior to diagnosis due to the distance from their residence to the hospital (for example, a patient who lives in the Maroni River area, with no doctor nearby, could face a two days trip by canoe to Saint-Laurent du Maroni, and then at least another day for administrative processing and a day for transfer to Cayenne…).

Viruses and hematological malignancies
The importance of viral infections in this patient population from this tropical region has been reported in various epidemiological studies[32]. Half of the virally-infected patients presented with hematological malignancies, while only 14% of the entire patient population of the series exhibited hematological malignancies. HIV is the most common viral infection. Hematological B-cell malignancies correspond with being afflicted with AIDS[40]. Two patients had very aggressive Kaposi sarcomas, which are typically encountered with HIV infected patients[54]. In this series, HBV serum positive status is often encountered, but there is no evidence in the literature that this virus could be involved in the occurrence of hematological malignancies. HCV has however been implicated in the occurrence of marginal B-cell lymphomas[33]. The small number of positive patients does, however, not allow such a conclusion to be drawn from this series.

Conversely HTLV1 infection is quite common. Moreover, screening for HTLV1 infection is part of the diagnosis of ATL/lymphoma/leukemia. Although serological positivity in French Guiana is quite high, this does not necessarily mean that affected individuals are

going to develop hematological malignancies. Indeed, the majority of patients are highly unlikely to suffer any adverse consequences from this infection.

Practical management

The Table 1 aims to give an indication of what is available for the diagnosis of patients with hematological malignancies. Thus, basic examinations are reasonably available, while some of the more in depth examinations may be obtained at a metropolitan laboratory. What is most relevant, however, is to have one or two board-certified (hemato-oncology) specialists available to manage the patient, specify the work-up, monitor patient progression, and get feedback from colleagues in mainland France regarding the patient cases. Lastly, they can recommend the transfer of patients for specialized management. This is not currently the case. At present, this is done by monthly 3 d visits of specialists from Centre Léon-Bérard in Lyon, and weekly contact with the tumor board by video conferencing. The current daily management is performed by two generalist practitioners. The issues encountered require a medico-scientific view of the situation. A very strong relationship between the Hemato-Oncology unit in Cayenne and a center of excellence in mainland France is paramount in providing these patients access to optimal treatment. Two useful logistical entities can be developed on site: Extension of the laboratory of pathology, and the implementation of TEP-scanning. Conversely, implementation of radiotherapy is not feasible to date.

Thus, in this regard French Guiana is, in fact, fully linked with mainland France, and can be seen as being independent of its neighboring countries Surinam and Brazil. Indeed, there are no transportation means to readily reach urban centers in Brazil. Although there actually is a bridge across the Oyapock River (at a distance of 200 km from Cayenne), there is no paved road to Belem (600 km). Moreover, the health care system in Brazil is mainly private. Only illegal Brazilian immigrants could benefit from having their disease managed in Brazil. Cooperation with Surinam could be easier to organize, as a Surinamese company operates flights between Cayenne and Paramaribo. It is also feasible to cover this 400 km distance by motor vehicle. Such an arrangement would however also require a genuine willingness to cooperate at a political level, and this has not been scheduled to date.

Socio-cultural problems

Social aspects are very important considerations. The majority of patients, even illegal immigrants, are covered by one or other of the various national health insurance systems. In French Guiana 60%, 28%, 12% of the population is covered by the national health insurance, the universal social insurance and the emergency medical help, respectively. This means that the administrative management for these patients is extremely time consuming and needs multiple players, including the police, social security, social services, *etc*.

Cultural aspects are also very important. The recommendations produced by the French National Cancer Institute for cancer patient management have been implemented, and they match international criteria. This nonetheless only applies to a small proportion of patients, being mainly the residents of metropolitan areas and a small proportion of Guianese and French Caribbean creoles. Even in this population the word "cancer" is taboo. This applies even more to Bushinengue and native Americans patients. In general, knowledge of the illness is expressed clearly (using the word "cancer"). The problem lies more with the significance of such a diagnosis, particularly when it comes to interpretation of the cause of the disease, which is often assigned to evil spirits or somebody who has cast a spell[55]. A great proportion of patients therefore seek advice from a wizard or medicine man. This does not preclude the possibility of conventional treatment, although it does generally delay it. These aspects are inadequately scrutinized and overcome by cancer patients. More progress has been made for patients with HIV infections[56]. An important aspect is that it is often thought that Bushinengue patients who become ill should return to their own village to die. This view hence tends to fully preclude taking the risk that the patient might die elsewhere, such as in a hospital, possible far away, such as in mainland France. The view is often that, should this occur, it would cast a pronounced spell on the deceased's lineage. It is therefore important that transcultural mediation is developed, as has been done for HIV patients[56].

Translation to management of patients with hematological malignancies in tropical countries with low and intermediate household incomes

This series summarizes some of the problems that are encountered in other tropical countries that have low and intermediate household[57]. Unlike French Guiana, these countries do not have the option, however, of providing disease management in mainland France. In these countries the most severe shortcomings are the lack of a sufficient number of board-certified specialists, the equipment and skill of pathology laboratories, the lack of access to new drugs, and particularly targeted drugs (mainly due to cost), as well as the lack of imaging and radiotherapy equipment. Various initiatives have been instigated to implement centers of excellence[58] and programs to provide access to new drugs, as done for Imatinib in CML and GIST[59,60]. The implementation of radiotherapy is also an important initiative, as it makes one of the most efficient therapeutic means more accessible[61]. Another important initiative is the implementation of diagnostic pathology learning programs. These are promoted by the International Network Cancer treatment and Research[62]. Initiative have been promoted for surgery training[63]. Training and research programs are priorities of international organizations[64,65].

In conclusion the specificity of tropical hematology (and oncology) is important: Firstly, in terms of gaining knowledge and understanding of disease mechanisms,

and, secondly for decision making and organization of the management of these malignancies in the tropical areas. The latter requires particular skills relating to the specificity of these diseases and of the affected populations, as well as solid public health policies. Finally the study reflects the problems the hematologists face in the daily practice in this area.

ACKNOWLEDGMENTS

COMMENTS

Background

This study is part of a larger retrospective study of 594 adult patients with cancer who were managed by the Hematology-Oncology unit of the Cayenne Hospital during 2010 to 2012.

Research frontiers

The objective was to highlight the major problems regarding management of adult hematological malignancies in a tropical region with a European health organization that was subject to specific limitations due to the tropical setting and the distance from mainland France.

Innovations and breakthroughs

This aspect can be equated with countries in tropical areas that have low and intermediate household incomes. Lastly, this series shows the importance of infections in the occurrence of some hematological malignancies to be reviewed.

Applications

In conclusion the specificity of tropical hematology (and oncology) is important: Firstly, in terms of gaining knowledge and understanding of disease mechanisms, and, secondly for decision making and organization of the management of these malignancies in the tropical areas. The latter requires particular skills relating to the specificity of these diseases and of the affected populations, as well as solid public health policies.

Peer-review

It is an interesting retrospective study regarding hematological malignancies in french guiana. The study also reflects the problems the hematologists face in the daily practice.

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