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Review

Blasts in context: the impact of the immune environment on acute myeloid leukemia prognosis and treatment

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ABSTRACT

Acute myeloid leukemia (AML) is a cancer that originates from the bone marrow (BM). Under physiological conditions, the bone marrow supports the homeostasis of immune cells and hosts memory lymphoid cells. In this review, we summarize our present understanding of the role of the immune microenvironment on healthy bone marrow and on the development of AML, with a focus on T cells and other lymphoid cells. The types and function of different immune cells involved in the AML microenvironment as well as their putative role in the onset of disease and response to treatment are presented. We also describe how the immune context predicts the response to immunotherapy in AML and how these therapies modulate the immune status of the bone marrow. Finally, we focus on allogeneic stem cell transplantation and summarize the current understanding of the immune environment in the post-transplant bone marrow, the factors associated with immune escape and relevant strategies to prevent and treat relapse.

1. Introduction

The bone marrow is both a hematopoietic and immune organ, as well as the site of disease of the overwhelming majority of acute myelogenous leukemias (AML). Neoplastic microenvironments are central to cancer evolution and resistance or sensitivity to treatments [1]. Among the various cell types that interact with malignant cells schematized in Fig. 1, immune cells are of special interest given the increasing importance of immunotherapeutic approaches being investigated and used to treat hematopoietic cancers, including AML [2,3]. This review focuses on the lymphoid cells that populate the bone marrow in AML and their relevance to prognosis and therapy. Several other cell types contribute to the medullary AML environment, such as myeloid cells (macrophage and myeloid-derived suppressor cells – MDSC), dendritic cells, or mesenchymal stem cells that can support the leukemogenic process and influence positively or negatively the immune response against AML [2,4–9]. While these cells will not be examined in detail in this review, their relevance to lymphoid cell function in AML will be discussed when appropriate.

2. Bone marrow as a lymphoid organ

The bone marrow (BM) acts primarily as a hematopoietic organ. However, the bone marrow hosts a variety of immune and non-hematopoietic cells that have an active role in immunity.

Among these, lymphoid cells including conventional and regulatory T cells, natural killer (NK) cells, gamma-delta (γδ) T cells, B and plasma cells, are relevant to both normal bone marrow physiology and pathological states [10].

Post thymic naïve T cells circulate in peripheral blood or reside in secondary lymphoid organs where they encounter antigen, and further, differentiate into effector and memory T cells. A significant fraction of the memory T cell pool transits and resides in the bone marrow [11–13]. Specifically, 8-20% of BM mononuclear cells are lymphocytes showing T

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cell:B cell ratio of approximately 5:1 [10]. Although the majority of T cells present in the BM have already encountered an antigen, studies in mice demonstrated that primary T cell responses to antigens also occur in the medullary environment. This implies that circulating naïve T cells can home to and be primed to respond to antigen in the BM [14]. The BM stroma is a significant source of homeostatic cytokines such as IL-7 and IL-15 that support proliferation of T cells in the absence of antigen, making the marrow microenvironment an "immune memory reservoir" [11,12,15]. High numbers of antigen-specific CD8 T cells have been shown to persist in the BM for several months after antigen encounter and display anti-infectious or anti-tumor activity. Indeed, adoptive transfer of BM from lymphochoriomeningitis virus-immunized mice to immunodeficient recipients protected them from chronic infection [10,13,16]. While capable of inducing potent anti-leukemic effects following allogeneic stem cell transplantation (HSCT), central memory CD8 T cells decrease the risk of alloantigen recognition and graft versus host disease (GVHD) [17,18].

T cells benefit from the BM environment, and reciprocally provide a supportive environment for hematopoiesis through the secretion of cytokines and the expression of chemokine receptors [19]. T cells contribute to normal hematopoiesis as demonstrated by various *in vivo* observations in both mice and humans. Athymic mice show peripheral granulocytopenia associated with an accumulation of immature hematopoietic precursors in the BM [20] and T cells are required for successful engraftment of allogeneic stem cells in transplant recipients [21,22]. T-cell deficiency associated with severe combined immune deficiency (SCID) can be associated with variable degrees of cytopenia, although functional hematopoiesis in SCID patients suggests that diverse mechanisms can palliate for T cell deficiency [19,23]. The frequency of regulatory T cells (Tregs) is higher in the BM than in the blood or other lymphoid organs and BM Tregs contribute to normal hematopoiesis and engraftment capacity of allogeneic stem cells [10,24]. Finally, adaptive immunity participates in bone remodeling through regulation of the OPG-RANKL axis through BM B and T cells [19].

This review describes how these interactions in the BM may affect acute myeloid leukemia development and treatment.

3. The bone marrow lymphoid environment in AML

Acute myeloid leukemia (AML) results from the malignant transformation of hematopoietic myeloid precursors. Despite advances in first-line regimens and consolidation therapies, most AML patients relapse and subsequently die from the disease. AML is characterized by molecular and cytogenetic inter-individual heterogeneity, as illustrated by the 2017 European Leukemia-Net (ELN) classification [25], but also within individual patients due to clonal evolution and variable differentiation states [26]. BM sampling performed at diagnosis and after treatment focuses on blasts almost exclusively and overlooks the immune cells [27,28]. However, accumulating evidence suggest that heterogeneity in the immune environment and its interaction with AML blasts contribute to the variable outcomes and response to therapy. Hence, immune-related features should also be considered in AML classification, response evaluation and prognosis.

3.1. Characterization of immune cells in AML

3.1.1. Conventional T cells

The characteristics of the blood and BM lymphoid compartments of AML patients is a matter of debate, with salient findings from several studies described in Table 1 [2,29–31]. Several groups found that peripheral blood (PB) T-cell counts (especially CD8) were higher in AML patients relative to healthy patients, but returned to normal after induction chemotherapy and achievement of a complete response [29,32]. Using multiplexed immunohistochemistry, Brück *et al.* studied the AML BM immunologic niche at diagnosis and found that compared to control BM and to other leukemias, AML BM contained decreased lymphocyte

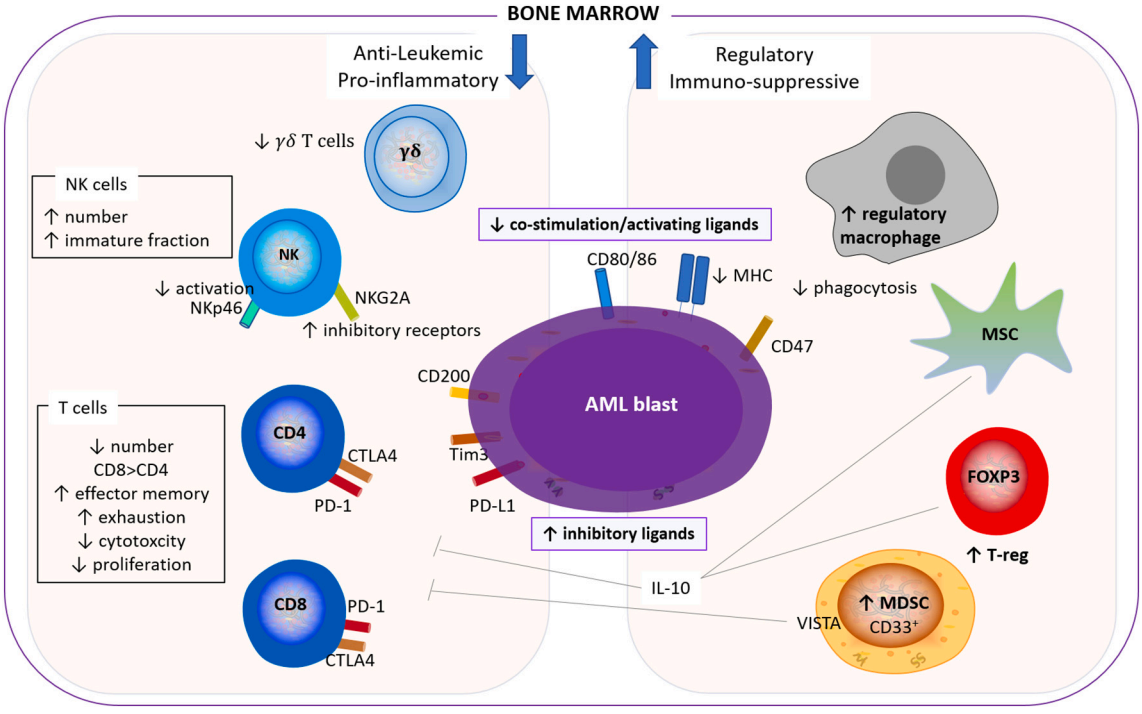

Fig. 1. Immunosuppressive bone marrow immune environment of acute myeloid leukemia at diagnosis.
The main cells and molecules are represented with their mutual interactions. Cytotoxic lymphocytes with anti-leukemic activity (left panel) are decreased in number and display exhaustion markers while suppressive cells (right panel) are highly functional resulting in a global immunosuppressive milieu. AML: acute myeloid leukemia, NK: natural killer, T-reg: regulatory T cells, MDSC: myeloid-derived suppressive cell, MSC: mesenchymal stem cell, MHC: major histocompatibility complex, IL: interleukin. Illustrations made with ScienceSlide® Suite 2010 edition. The full lines represent a production or secretion of a cytokine, and the full line with a bar indicates a suppressive effect.

Table 1
Lymphocyte populations.

Main findings AML as compared to healthy controls	Source	sample type	timing	Sample size	Method	reference
↑absolute number of T cells (↓ relative numbers) ↓CD4/CD8 ratio ↑CD3 + CD56+ cells with cytotoxic and activated phenotype (CD57+ CD28- CD25 + CD69+)	PB	PBMC	Diagnosis	36 (17 controls)	Flow	[29]
↑lymphocyte count, restored after chemotherapy ↑ CD8 T cell at diagnosis ↔ NK and *γδ* T cells at diagnosis	PB	PBMC	Diagnosis and during treatment	29 (15 controls)	Flow	[32]
↓T cells and CTLs	BM	aspirates	Diagnosis and during treatment	16 (5 controls)	Single cell RNA sequencing	[33]
↓T cells and CTLs, ↓CTL:T cell ratio ↑T-regs	BM	Trephine	Diagnosis	15 (15 controls)	IHC	[33]
↔T cell infiltration on biopsies in AML versus controls	BM	Trephine	Diagnosis	13 (14 controls)	IHC	[34]
↔absolute T cell numbers	PB	Whole blood	Diagnosis	13 (8 controls)	Flow	[30]

Overview of the main studies quantifying T cells in AML patients.
Abbreviations: PB: peripheral blood; BM: bone marrow; PBMC: peripheral blood mononuclear cells; IHC: immunohistochemistry; Flow: flowcytometry; CTLs: cytotoxic lymphocytes. Arrows refer to increase (↑), decrease (↓) or unmodified (↔) in AML as compared to healthy controls or otherwise specified comparison.

populations, including T cells, B cells, NK cells and NK T cells. In the same study, BM aspirates and paired peripheral blood (PB) AML samples at diagnosis were characterized by flow cytometry in 8 patients. The study was limited to NK and CD8 T cells and showed a more differentiated "effector memory cell" status in the BM as compared to PB. Interestingly these differences were more pronounced in AML samples, than in healthy controls, suggesting that AML enhances the effector differentiation of BM T cells [27]. Another study reported a decreased T cell infiltrate in AML BM [33], but another showed no difference in T cell BM frequency after adjusting for cellularity [34]. Such discrepancies might be related to the varied approaches (single cell RNA sequencing, flow cytometry, immunohistochemistry) and samples (aspirates vs core biopsies) used by the different investigators to assess T-cell infiltration.

AML cells and the host immune system are interacting at multiple levels (Fig. 1) [35]. AML cells are associated with an immunosuppressive microenvironment with reductions in population and/or function of T and NK cells and accumulation of T-regs, and immunoregulatory myeloid cells which may protect leukemic stem cells and predispose to relapse. The AML blasts themselves display immune evasion mechanisms including reduced expression of major histocompatibility complex (MHC) molecules, enhanced expression of inhibitory ligands such as Tim-3, Gal9 and PD-L1, and reduced expression of NKG2L and DNAM-1 on the blast surface leading to impaired NK cell activation [2,3]. These suppressive effects of AML cells on lymphocytes can be recapitulated *in vitro* by co-incubation of AML blasts with healthy NK or T cells, which results in impaired cytotoxicity and antigen-driven proliferation [36]. In addition, T cells from AML patients display aberrant activation profiles as well as phenotypic and transcriptional features of exhaustion (co-expression of multiple immune checkpoint molecules) terminal effector differentiation and/or senescence, further compromising an effective endogenous immune response against AML [37–41]. However, these findings do not appear to be specific for AML but consistent across hematological malignancies [42]. At diagnosis CD4 T cells of AML patients were shown to produce less interferon(IFN)γ upon stimulation as compared to healthy controls [43]. Circulating AML T cells also showed decreased expression of genes involved in immune synapses [29] and single cell RNA sequencing revealed T cell suppression signatures [33]. By immunohistochemistry, BM T cells of AML patients displayed less cytolytic and co-stimulation markers. In terms of immune checkpoint receptors, PD1 expression in T cells of AML patients exceeded expression in control subjects, while the contrary was observed for LAG3 and TIM3 [27]. However, another study identified co-expressing PD-1 and TIM-3 on CD8+ T cells in AML patient suggesting a high degree of T-cell exhaustion [44,45]. Increased inhibitory checkpoint molecule or exhaustion-associated transcription factor expression is consistently found in AML BM T cells. Studies suggest this expression pattern at diagnosis is attenuated upon response to chemotherapy, as shown for PD1 and Tim-3 [41,44] but more pronounced in progressive or relapsed patients. For example, PD1 and CD244 T cell expression was increased at relapse post HSCT in one study [46]. However, it is unclear whether this reflects T-cell exhaustion or a population shift to differentiated effector T cells [2,46–48]. Nevertheless, as presented in Table 2, most studies point to lymphocyte dysfunction (encompassing states such as exhaustion, terminal differentiation and senescence [47,49]) as a feature of the AML immune environment with potential prognostic and therapeutic consequences that will be developed in Sections 2.2 and 3 [2,50].

3.1.2. *Regulatory T cells*

Regulatory T cells (Treg) prevent excessive immune responses through various mechanims. Elevated T-regs are consistently documented across studies in the PB and for some of these reports in the BM of AML patients [2,33,34,40]. In a large study of 182 patients, Shenghui et al. describe an elevated T-reg frequency in the PB and even more pronounced in the BM of newly diagnosed AML patients as compared with healthy volunteers [51]. Moreover, suppression assay using *ex vivo* isolated Treg showed higher suppressive activity in AML patients. Interestingly, Treg frequency decreased upon achievement of complete remission and increased at relapse [2,51]. However, the clinical relevance of these findings and the exact role of Treg in the mechanism of AML development and relapse remains to be elucidated.

3.1.3. *Gamma-delta T cells*

Gamma-delta (γδ) T cells are unconventional T cells that are activated through both MHC-independent and non-classical class-I MHC molecules-dependent interactions. Among these, γδ T cells subsets recognize lipids by the CD1 family of MHC class I-like proteins or MHC-related protein 1 (MR1) [52,53]. These T cells are present in the BM at variable frequencies and as such, are a component of the AML immune environment [32,54–56]. In a Canadian study of 33 AML patients compared with healthy volunteers, circulating γδ T cells tended to be lower at diagnosis in patients with high leukemic burden but nearly normalized after achievement of complete response. By contrast, a sharp increase of γδ T cells was noted at early relapse in another study, but the mechanisms underlying this phenomenon are unclear [56]. These findings underline the potential role of γδ T cells in leukemic immune surveillance [54]. *In vitro* evidence shows that γδ T cells can recognize and kill leukemia blasts, but their network of interactions with the tumor environment *in vivo* remains poorly understood [54,56]. Given the

Table 2
Function, differentiation, exhaustion and senescence.

Main findings AML as compared to controls or as indicated	Source	Sample type	Timing	Sample size	Method	Reference
↑ relative numbers of $CD3^+$ cells - ↑ Tregs ↑ PD1- and OX40-positive T cells - ↑ ICOS-positive CD4 T cells	BM	Aspirate	Diagnosis	107 (8 controls)	Flow	[34]
↑PD1, CD160, 2B4, Tim3, KLRG1, and CD57 ↑ terminally differentiated CD8 T cells at diagnosis Expression of inhibitory receptors decreased when achieving CR and increased in non-responders ↑TNF***α*** and IFN***γ***, ↓IL-2 expression ↑senescence and exhaustion ↓adhesion and migration (partially restored in responders)	PB/ BM	Aspirate	Diagnosis Post induction	72 (70 controls)	Flow Functional GEP	[41]
↓T, B, NK, NK T cells ↓ GrB and CD57, CD27, LAG3 and TIM3 ↑PD1 ↔CTLA4 Higher Treg % associated with poor prognosis $CTLA4^-LAG3^-$ T-helper cells associated with longer survival	BM	Trephine	diagnosis	69 (12 controls)	IHC/image analysis	[27]
Impaired T cell proliferation and cytokine production Proliferation rescued by adding CPI to the assay	PB/ BM	Aspirate	diagnosis	49 (8 controls)	Flow, ICS, proliferation assay, CyTOF	[37]
↑terminally differentiated CD8 T cells ↓CD127 and TCF-1 and ↑ TOX ↑$PD\text{-}1^+TIGIT^+CD73^-CD8^+$ T cells ↑$CD39^+$ $TIGIT^+CD73^-CD8^+$ T cells (normalized in remission)	PB/ BM	PBMC/aspirate	Diagnosis, relapse or CR	43 (12 PB controls)	Flow	[48]
Relapse associated with higher LAG3 gene expression ↓ Th1 cells and exhaustion markers associated to relapse	BM	aspirate	Diagnosis	15 41	Single cell RNA sequencing Flow	[74]
↔CD244, PD-1, CD160, and TIM-3 ↑PD1 at relapse after allogeneic SCT ↔proliferation and cytokine production (exception: ↓CD4 T cell IFNγ production at diagnosis)	PB	PBMC	Diagnosis Relapse and post HSCT relapse	23 (30 controls) 14	Flow/ICS/Proliferation	[46]
↑CD244 T cell expression in AML as compared to controls ↑ CD8 T cell CD244 expression at relapse post HSCT ↑ T cell PD1 and Tim-3 expression at relapse post HSCT ↑terminal effector memory differentiation at all time points	BM	aspirate	Diagnosis Relapse and post HSCT relapse	31 (5 controls) 13	Flow	[46]
↓relative numbers of $CD3^+$ cells - Absence of Tregs ↑ LAG3 and PD1 ↓ IFN***γ*** and TNF***α***, IL-2, IL-5 and IL-6 production	PB	PBMC	Relapse post-transplant	24 (9 controls)	Flow/ICS	[43]
Aberrant TCR signaling and T cell activation patterns Differential regulation of genes associated with cytoskeleton	PB	Sorted CD4 and CD8 T cells	diagnosis	10 (10 controls)	GEP	[29]
↓ immunologic synapses of T cells with blasts	PB	T cells autologous blats	diagnosis	10 (10 controls)	cell conjugation assay	[29]
↑$PD1^+$ $Tim\text{-}3^+$ $CD3^+$ T cells in non-responding patients compared with CR	PB/ BM	PBMC/aspirate	diagnosis	9	Flow	[44]

Overview of the main studies reporting T-cell dysfunction in AML patients.
Abbreviations: PB: peripheral blood; BM: bone marrow; PBMC: peripheral blood mononuclear cells; CR: complete remission; HSCT: hematopoietic stem cell transplantation; ICS: intracytoplasmic staining; GEP: gene expression profile; Flow: flow cytometry. CPI: checkpoint inhibitor. Arrows refer to increase (↑), decrease (↓) or unmodified (↔) in AML as compared to healthy controls or otherwise specified comparison.

sensitivity of leukemic blasts to γδ T cells and the propensity of these cells to recognize non canonical antigens, γδ T cells are candidates for cellular immunotherapy, especially in allogeneic transplantation where they are associated with a potent GVL effect without increase in GVHD [57,58]. Accordingly, a higher frequency of γδ T cells in peripheral blood at day 56 post-transplant has been associated with better HSCT outcomes [58]. The exact role of different γδ T-cell subtypes in AML has yet to be established.

3.1.4. NK cells

NK cells are innate cytotoxic lymphocytes exerting direct and indirect cytotoxic effects on cells displaying distress signals and constitute 2 to 4% of the BM lymphocytes. A historical study of PB and BM lymphocyte subsets in AML suggested that relative and absolute numbers of BM NK cells were increased in AML as compared to healthy controls and enriched in the CD16-negative, more immature, NK cell fraction [59]. By contrast, a more recent study showed the accumulation of CD56-negative CD16-positive NK cells in the PB of AML patients [60]. NK cells exert cytotoxic activity against leukemic cells but display dysfunctional features in AML that appear to be directly induced by interactions with AML blasts [2,61]. These features include downregulation of activating receptors such as NKp46, upregulation of inhibitory receptors such as NKG2A, impaired cytokine production and degranulation. Interestingly, in a study of 29 patients, downregulation of the activating receptors NKp30 and NKp36 observed at diagnosis compared to healthy controls, was restored 6 weeks after induction chemotherapy [32], suggesting some of the dysfunctional features of AML NK cells are reversible.

AML cells further evade NK cell suppression through synergistic mechanisms leading to impaired recognition by NK cells and direct suppression of NK cell functions [2,62–64]. These mechanisms include overexpression of CD137L and CD200, whose interaction with their respective receptors on the surface on NK cells leads to suppression of NK cell cytotoxicity and IFNγ production [65,66].

The close interactions between NK cells and blasts in the AML microenvironment can be modulated and as detailed below, NK cell-based immunotherapies are of considerable interest to treat this disease.

3.2. T cell infiltration and status correlate with outcome and response to treatment

3.2.1. Quantification of the AML immune environment

The magnitude of T cell infiltration as measured by flow cytometry in the BM at AML diagnosis has been associated with increased overall survival [40,67,68]. Also, multiplex immunohistochemistry at diagnosis identified AML-specific immune profiles that segregated with age and correlated with disease-free survival [27].

The immune contexture of cancers, as defined by the nature, the density, the functional orientation and the location of immune cells, is an established predictor of clinical outcome in several malignancies [69–72]. The Tumor Inflammation Signature (TIS) score results from the expression level of 18 immune-related genes reflecting the degree of adaptive immune response inside the tumor. The TIS score was mostly described in solid tumors with the aim of predicting response to immunotherapy. However, when applied to 163 AML marrow samples using a pan cancer genome atlas, it revealed that AML present an average low score, implying an overall corresponding limited sensitivity to immune interventions [73]. However, recent data point out important heterogeneity in the AML immune environment as developed in the next sub-sections.

3.2.2. Immune contexture of AML contributes to prognostic classification

AML risk classification is largely based on cytogenetic abnormalities [25]. In the case of AML with normal karyotype, ELN criteria are insufficient to explain the heterogeneity of outcomes. Among these AML cases, relapsing patients had lower CD4 Th1 cell infiltration in the BM and their T cells expressed an exhausted signature as compared with patients with long term remission. This suggests that the proportion of Th1-differentiated CD4 T cells at presentation is associated with unfavorable outcomes in AML patients treated with standard chemotherapy [74]. In another report, Vadakekolathu and colleagues identified two distinct immune profiles in AML using targeted immune gene expression profile of 290 BM samples [75]. Around 50% of the samples were identified as “immune-infiltrated” whereas the other half was “immune-depleted” [2,75–77]. AML cases with immune-infiltrated profiles had higher transcript levels of IFNγ-stimulated genes, T cell recruiting factors (STAT1, CXCL10, and IRF1), T cell markers and cytolytic effector molecules (CD8A, CD8B, GZMB, and PRF1), counter-regulatory immune checkpoints and immunotherapy drug targets (IDO1, CTLA4, PD-L1, and BTLA), as well as molecules involved in antigen processing and presentation (TAP1, TAP2, HLA-A, HLA-B, and HLA-C) [75]. Expression of STAT1, a key component of the IFNγ signaling pathway, was more strongly correlated with the presence of T cell inhibitory receptors such as Tim-3 and LAG-3 and with IFNγ-stimulated genes in the immune-infiltrated relative to the immune-depleted subtype [75]. Together these gene expression data are indirect signs of an IFNγ-driven adaptive immune response taking place in the inflamed BM immune environment of the immune-infiltrated subtype. In the same study, authors could identify T-cell rich regions with CD3-positive cells co-localizing with cells expressing proteins involved in antigen presentation and processing in immune-infiltrated samples. In immune-depleted samples, CD3-positive cells expressed memory (CD45RO) and exhaustion (PD-1) markers [75]. Further analysis of the same dataset identified PD-L1, FOXP3, PTEN and BCL2 as gene markers of the immune-infiltrated BM samples and correlated with TP53 mutation status and with adverse ELN cytogenetic features. Interestingly, in this cohort of 290 patients, the presence of an immune infiltrate was associated with a better prognosis for patients with favorable risk, whereas it correlated with worse prognosis for patients with adverse ELN cytogenetic risk [75]. Altogether, these data identify BM immune gene signature in AML as an important prognostic factor with distinct effects depending on genetic classification [75]. In a separate study, immunogenomics were also used to build a “cytolytic score” of hematological malignancies based on the expression of genes directly involved in T/NK-cell mediated cytotoxicity such as *GZMA, GZMM, GZMH, PRF1* and *GNLY*. This score was generally low for AML as compared to other diseases such as lymphoma but among AML samples, the cytolytic score correlated with *TP53* mutation, myelodysplasia-related changes and associated cytogenetic abnormalities. In contrast, the common AML driver mutations *FLT3* and *NPM1* preferentially occurred in samples with low cytolytic activity [78]. Altogether these studies suggest that together with genetic markers, immune contexture heterogeneity contributes to AML prognostic classification.

3.2.3. T cell dysfunction markers correlate with AML outcome

T cell dysfunction markers described in Section 2.1.1 can have a prognostic significance. Sallmann et al. showed an impact of immune checkpoint dysregulation on overall survival in AML that was more marked in *TP53*-mutated patients. For *TP53*-mutated AML, the BM environment was possibly immunosuppressive with a high proportion of $ICOS^{high}PD1^{low}$ T-regs and reduced expression of co-stimulatory molecules such as OX40, a marker of T cell activation, by conventional T cells [79]. Other groups showed an impact of the degree of immune suppression in the immune environment on AML outcome and response to treatment. In a murine model, the co-expression of the exhaustion markers PD1 and Tim-3 on CD8 T cells was associated with defective T cell effector function and AML progression [45]. In a large AML cohort from MD Anderson, PD1 and OX40 BM expression was higher in AML patients as compared to healthy donors. PD1- and TIM3- and to a lesser extent PD1- and LAG3- double-positive T cells showed a bimodal distribution in BM samples from different patients. These exhausted T cells were found in a higher proportion in newly diagnosed AML patients than in age-matched healthy volunteers and were even higher at relapse (Table 2 and [34]). Altogether these results suggest that heterogeneity of T cell functional status may contribute to heterogenous outcome in AML.

In conclusion, evidence presented in Section 2 and summarized in Fig. 2, suggest that heterogeneity in the BM immune infiltration contributes to the variability of AML clinical outcome and could predict response to treatment. In Section 3 we will review how the immune status of the BM environment can help identify patients who could benefit from immune-based therapies.

4. Impact of bone marrow environment on lymphocyte-mediated immunotherapy

The GVL effect has long been established as potentially curative in AML [80]. However, no non-transplant T cell-mediated immunotherapeutic strategies has been incorporated in clinical practice [81]. The barriers to the development of immunotherapy in AML include the heterogeneous interactions between blasts and AML cells characterized by variable degree of immune suppressive environment and immune evasion by AML blasts [2,36,40]. Addressing this issue has become critical and, as such, was the topic of several recent reviews [2,3,36,81–83]. The wider availability of genome-wide sequencing technologies, is allowing for a more comprehensive assessment of the genetic determinants of the immune environment such as the *TP53* mutation status, and could help predict the likelihood of response to immunotherapy through accurate AML immune subtyping [75,76,84].

4.1. Immune Checkpoint inhibition

Immune checkpoint inhibition (CPI) is now extensively used in oncology as a mean to reinvigorate exhausted antigen-experienced T cells [85,86]. As highlighted above, several immune checkpoints (and their ligands) are expressed in the context of AML, providing a strong rationale for CPI in this disease [87]. The different CPI that were tested for AML treatment include antibodies against PD-1, PDL-1, CTLA4 and more recently novel agents targeting Tim-3, the leukocyte immunoglobulin-like receptor B4 (LILRB4), CD47, CD70 or CD200 [84,88]. The targeting of NK cell inhibitory receptors such as NKG2A is

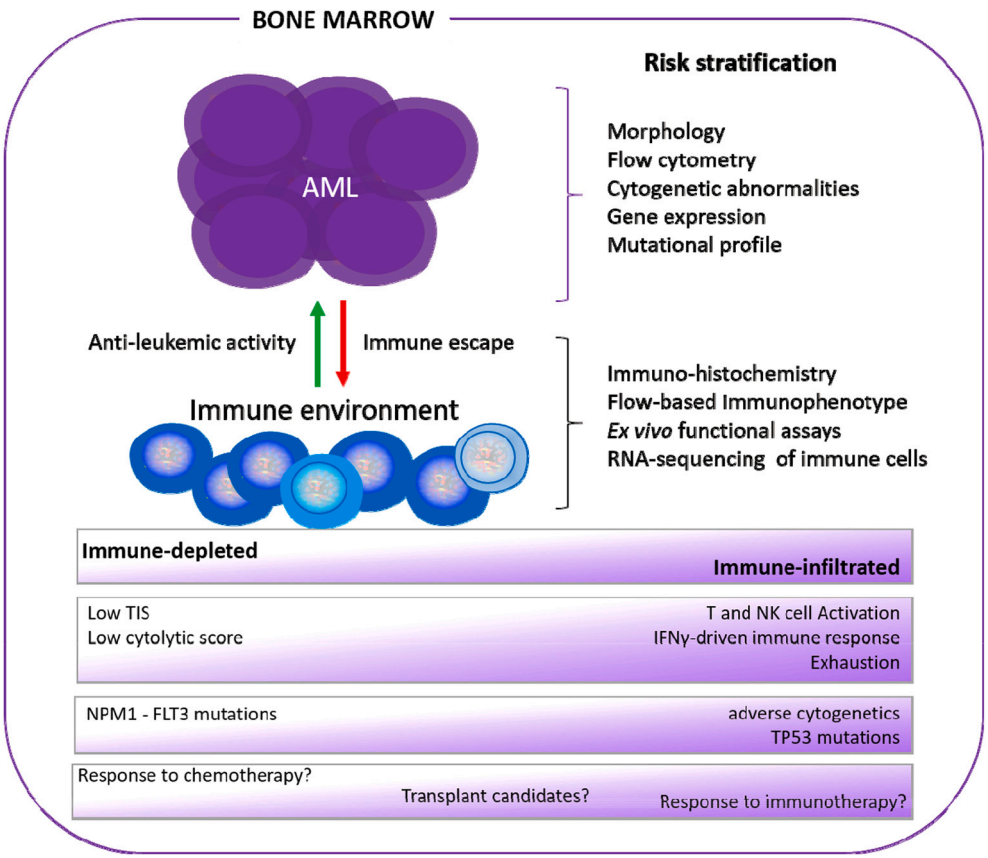

Fig. 2. Immune stratification of acute myeloid leukemia.
Next to conventional validated molecular and cytogenetic features of the AML blasts (upper panel), the characteristics of the immune environment (middle panel) may have a prognostic role, potentially impacting the choice of immunotherapeutic strategies. Most AML patients have an immune-depleted bone marrow with low cytolytic activity. However, subgroups of patients display an immune-infiltrated bone marrow with IFNγ-driven inflammation and T cell exhaustion. Emerging data suggest these features correlate with the presence of adverse cytogenetic and molecular features and could be better candidates for therapies aiming at reverting the immune suppressive environment. The color gradients reflect the heterogeneity in indicated parameters. Illustrations made with ScienceSlide® Suite 2010 edition.

also a potential strategy for AML treatment [89,90]. Unfortunately, many single arm phase I/II trials have failed to show any clinically relevant efficacy and when they have, real world practice has been disappointing [2,82,91,92]. One of the only randomized trials in the field compared the combination of azacitidine (AZA) with the anti-PD-L1 antibody durvalumab with azacitidine monotherapy in 129 AML patients and didn't show clinically meaningful benefits for the combination [93]. From a mechanistic point of view, the combinations with hypomethylating agents (HMA) could be more effective than CPI alone because HMA are known to increase PD-L1 expression on myeloid cells and are believed to increase the immunogenicity of AML blasts through antigenic expression [94,95]. However, 5-azacitidine may impair effector T-cell differentiation and function [96]. In the above-mentioned randomized study, azacitidine induced overexpression of PD-L1 in healthy myeloid cells but not in leukemic blasts. Because the expression of checkpoints is highly variable in time and between patients, CPI may work differentially in subgroups of AML patients [84]. In an attempt to identify these subgroups, recent studies report biomarkers predictive of response to CPI in AML. These markers include the frequency and phenotype of BM CD8 T cells [97–99], higher polyfunctionality and Th1-polarized BM CD4 T cells, as elegantly shown using single cell functional proteomic profiling [100]. TP53 mutation has been associated with a slight increase in PDL1 expression in precursor cells, which could possibly lead to higher sensitivity to CPI according to a study on 30 TP53-mutated AML compared with 73 controls [79], whereas loss of chromosome 7/7q was associated with resistance to CPI in a smaller cohort of 8 patients [98]. The negative results obtained with CPI in immunologically uncharacterized AML does not rule out that subgroups of AML patients, selected based on pre-treatment immune markers, could benefit from CPI-based combinations [101].

4.2. *T cell recruiting antibodies*

Bispecific antibodies are recombinant proteins that recruit T cells in a TCR-independent manner, through direct CD3 engagement after binding of a surface antigen expressed by tumor cells [102]. Following the approval of the CD19-directed blinatumomab for the treatment of B-acute lymphoblastic leukemia, a large variety of antibody constructs and molecular forms are currently being studied in different hematological malignancies [102,103]. In the case of AML, the issue is to identify suitable targets [49,102]. Myeloid lineage-restricted surface antigens such as CD33 and CD123 are expressed by leukemic blasts and healthy hematopoietic cells, and leukemia-associated antigens such as WT1 are overexpressed by AML cells but also detected on healthy tissues, causing potential on-target off- tumor toxicities [49,102]. Bispecific antibodies against CD33, CD123, FLT3, WT1 and CLL1 are currently being studied in phase I/II clinical trials [102,104,105].

The status of T cell BM environment is highly relevant for the design of bispecific antibody-based therapies for AML, as “immune-infiltrated” and “immune-depleted” subtypes show differential response [75]. Flotetuzumab (MGD006), an investigational bispecific antibody-based molecule to CD3 and CD123 was tested in a phase I/II clinical trial on 88 relapse/refractory AML patients [103,106]. Although the overall response rate in the total cohort was modest (13,6%), the authors identified a subgroup with better response. This subgroup included primary induction failure and early relapse patients that preferentially had an immune-infiltrated marrow environment. Dissecting immune-related gene expression data, the investigators showed that the complete response rate was up to 60% in the patients with the highest immune infiltration. The ranking of gene expression data allowed the identification of a 10 gene signature predictive of response. The signature included IFNγ-driven inflammation, components of the TIS score and other T cell-associated pathways. Strikingly this signature could statistically better predict response to flotetuzumab than the ELN risk

category [106]. In a comparison of post-cycle 1 BM samples to baseline samples, exposure to flotetuzumab resulted in increased immune cell infiltrate and immune activation scores. This suggests that treatment with bispecific antibodies could not only eradicate blasts but also shift the microenvironment to a more inflamed type [107]. In line with this finding, AMG330, an anti-CD33 bispecific antibody, could elicit robust cytotoxic responses against AML cells even when used on exhausted BM T cells [108] and the number of endogenous T cells correlates with drug-induced cytotoxicity *in vitro* [109]. The efficacy and mechanism of action of these drugs requires further study but these preliminary observations highlight once again the need to include immune environment markers in interventional AML immunotherapy clinical trials.

4.3. Adoptive T cell therapy

Different forms of adoptive T cell therapy have been and are currently being tested for the treatment of AML as recently reviewed [83,89,102,110,111]. By adoptive T cell therapy, we mean the use of autologous or allogeneic T cells, transferred to patients, that target leukemic cells and which can induce their apoptosis through direct and indirect cytotoxic mechanisms. As mentioned for T-cell recruiting antibodies, the major difference with CD19-expressing malignancies is the lack of an obvious adequate antigenic target in AML. To address this unmet need, the Sadelain group integrated protein and RNA expression profiles of AML cells with an algorithm designed to detect ideal CAR-targets. This method identified promising combinatorial pairings such as CD33 + ADGRE2, that was present in more than 97% of cells in AML samples with non-overlapping expression in normal tissues [112]. The other limitation in the development of effective T cell therapy for the treatment of AML is the presence, in a significant proportion of patients, of an immunosuppressive microenvironment that could dampen the antitumor activity of adoptive T cells [113].

In the context of allogeneic stem cell transplantation, the use of *in vitro* generated leukemia-specific donor T cells could enhance the GVL effect without triggering GVHD [110,114]. With the same objective, Chapuis et al. studied the prophylactic infusion of T cells expressing a high affinity WT1-specific TCR with encouraging results in a 12 patient-cohort [115]. Another promising approach, again following HSCT is the use of cytokine-induced killer (CIK) cells, which are activated T-cells that acquire NK-cell-like cytotoxicity after culture with cytokines [83,110].

Chimeric antigen receptor (CAR)-T cells are genetically engineered to express a variable heavy and light immunoglobulin chain coupled with intracellular stimulation and co-stimulation machinery. The CAR binds malignant cell surface antigens with high specificity independently of MHC. Based on encouraging preclinical data [116], CD33- and CD123-directed CAR-T cells have been clinically tested, so far with disappointing results. Further, both CD33 and CD123 are expressed on normal hematopoietic cells potentially resulting in unacceptable myelosuppression. An elegant strategy to avoid these shortcomings and applicable to CD33 targeting is to engineer CD33-deficient allogeneic stem cells and infuse them along anti-CD33 CAR T-cells that will recognize and eliminate CD33-positive blasts but not the CD33-negative donor stem cells [117]. CAR T cells targeting FLT3, CD117 and LeY as well as the recently identified Siglec 6 [118] could be more promising as these markers are unique to or more prominent on AML cells. Finally, multi-target and dual anti-CD33/CD123 CAR T cells are under clinical investigation [83,110]. A recent report from the Zeiser group illustrates the interaction of cellular therapy with the immune microenvironment [119]. In this xenograft AML model, the authors infused an anti-CD123 CAR-T construct with potent anti-leukemic effect *in vitro* and *in vivo* without excessive myelotoxicity. Furthermore, the combination of this anti-CD123 CAR T cell with azacitidine resulted in increased expression of the target CD123 on leukemic blasts and increased anti-CD123 CAR T cell function. Interestingly, the CAR population was enriched in CTLA4-negative CD4 CAR T cells which exhibited stronger anti-leukemic and memory properties than their CTLA4-positive counterparts. Interestingly, 28 days post infusion, this CTLA4-negative T-cell population was enriched in the BM and not in the PB in one of the murine models. Higher numbers of CTLA4-negative CD4 and CD8 T cells were also found in the PB and BM of mice treated with azacitidine and anti-CD123 CAR T cells as compared with the CAR-T cell monotherapy group. These observations suggest a modulatory effect of the combination AZA/CAR on the immune environment of AML [119]. Other ways to overcome immune dysfunction and enhance adoptive T cell therapy were recently reviewed by our group and others [47,113,120,121]. Clinical translation of this approach is eagerly awaited and should include investigations on immune environment markers.

4.4. NK cell-based immunotherapy

The interactions between NK cell receptors (activating and inhibitory) and corresponding ligands on target cells determine whether the NK cells will effectively kill neoplastic or infected cells. In this context, AML blasts may be more susceptible to NK cell mediated killing than other cancers since they express ligands that are more recognized by activating, rather than inhibitory receptors on NK cells [122]. This property is the basis of the well-known NK-mediated GVL effect that contributes to the curative effect of HSCT, best demonstrated in HLA-mismatched transplants where inhibitory killer immunoglobulin-like receptors (KIR) cannot interact with self MHC [123]. However, AML cells display mechanisms to evade NK cell recognition, such as down-regulation of ligands for the activating receptor NKG2D [122]. In a phase I study on 13 patients, post-haploidentical stem cell transplantation *ex vivo* expanded donor NK cell infusion improved NK cell function and was associated with a low relapse rate and incidence of viral infections without severe GVHD, and without significant impact on T cell immune reconstitution [124]. In another small trial on 10 patients with advanced myeloid malignancies, post-transplant CD56+ enriched donor cell infusion was associated with an early and rapid rise of mature NK cells as well as CD4 T cells and T-regs [125]. NK cells can also be infused pre-transplant with the objective of increasing GVL effects [126].

The cytotoxic activity of autologous NK cells is dampened by KIR/self MHCI interactions. Therefore allogeneic KIR-mismatched NK cells are preferred for most applications, even in non-transplantation settings [89,127,128]. Nevertheless, autologous NK cells can be recruited and engaged through antibody constructs similar to T cell-engaging bispecific antibodies. As an example of development in this very dynamic field, the University of Minnesota recently reported encouraging preclinical data on the use of a trispecific killer engager (TriKE) combining an anti-CD16 antibody, an IL-15 molecule to support the CD16-positive NK cell and an anti-CLEC12A to target the leukemic cells [129]. The anti-tumor activity and persistence of adoptively transferred NK cells can be optimized through *ex vivo* expansion in specific cytokine conditions, that mostly include IL-15 or genetic modification to enable autocrine IL-15 secretion [130]. Combination strategies with epigenetic modulators, checkpoint inhibition or even donor lymphocyte infusion in the post-transplant setting are other investigated options [127,131,132]. The validity of adoptive NK cell transfer for the treatment of AML has been demonstrated [133] and many trials are ongoing in this rapidly evolving field [83,89,110,122,127].

Finally, CAR-NK cells have also been developed for targeting AML antigens such as CD33, CD123, CD7 and CD4. CAR-NK cells combine intrinsic and engineered anti-tumor features that could overcome some of the obstacles to AML immunotherapy related to antigen escape and heterogeneous gene expression as demonstrated *in vitro* and *in vivo* by the Rezvani group and others [122,131]. CAR-NK cells can be generated from allogeneic donors or from cell lines, with the advantage of off-the-shelf availability [89]. Unsurprisingly, the AML immune environment also poses obstacles to successful CAR-NK cell therapy [131]. The obstacles include the presence of immunosuppressive soluble factors such

as TGF-β which can adversely affect NK cell function [122,134]. Circumventing such factor through genetic engineering of CAR-NK cells is a focus of research for several groups [122]. One proposed approach has been to use the CRISPR-Cas9 gene-editing technology to delete the TGF-β receptor 2 gene (*TGFBR2*) in NK cells, which renders them resistant to this immunosuppressive cytokine [122].

In conclusion, immune-based therapies could be a valid option for selected AML patients. However, challenges such as the cost, availability and toxicity of these therapies currently limit their use and are the object of intensive research efforts. Among recent advances that may lead to better targeted and safer therapies are the refinements in CAR T-cell design [112], introduction of fate-regulating transgenes to rapidly curtail toxicity [135] and transgenic TCR technologies [115,136]. Such therapies will nevertheless, have to compose with the BM immune environment features that have an impact on lymphocyte-based cellular therapy for AML and conversely, cellular therapy may modulate the immune status of AML patients. Therefore, one could suggest that the assessment of the immune environment should be an integral part of cell therapy clinical trials.

The place of immune-based therapies in the AML therapeutic arsenal also remains to be defined. Because refractory disease and post-HSCT relapse are the biggest unmet medical needs, and given their specific immune contexture, we argue that these settings should be explored in priority. However, low burden disease states such as the immediate post HSCT period or MRD after chemotherapy might be optimal settings to test immune based interventions given the relative small number of leukemia blast to target and yet to be defined changes that may occur in the AML microenvironment following therapy. In Section 4 we will review how AML immune environment impacts allogeneic stem cell transplant strategies.

5. Impact of immune microenvironment on transplant outcomes

Allogeneic hematopoietic stem cell transplant (HSCT) is established as a curative therapy for intermediate and high-risk AML [25,137,138]. This strategy is based on the efficacy of a GVL effect that is primarily mediated by donor-derived T and NK cells [80,123]. AML is the leading indication for HSCT worldwide [139], yet disease relapse remains a major cause of treatment failure and progress is needed in terms of patient and donor selection, conditioning regimen and post-transplant maintenance therapy [137].

The AML immune environment is relevant for the optimization of pre-transplant management, for adequate immune reconstitution and in the relapse setting.

5.1. Optimization of pre-transplant risk stratification and pre-transplant management

The 2017 ELN classification stratifies AML in three risk categories based on cytogenetic and molecular characteristics [25]. Since its publication, this classification has been validated and refined with the use of next generation sequencing (NGS) technology [140,141]. Measurable residual disease (MRD) is also recognized as a major predictive factor of relapse in AML, and post HSCT [142,143].

Currently, pre-transplant immune environment features are not included in the routine pre-transplant workup and decisional process. Knaus et al. demonstrated that response to induction chemotherapy correlated with the restoration of the altered T cell function present at AML diagnosis. In contrast, non-responders displayed an increased frequency of senescent T cells and upregulated exhaustion markers such as PD-1 and Tim3 in PB and BM [41]. It is plausible that similar observations can be made when comparing T cell status of AML patients who achieve negative MRD with patients for whom MRD remains detectable which offers a rationale to use immunotherapy to achieve a deeper negative MRD status and improve subsequent transplant outcomes [142].

Finally, the T cell composition of the graft impacts outcome. In a multivariate analysis of 147 haploidentical transplantations, an optimal CD4:CD8 ratio close to 1 was associated with the best transplant outcome [144]. The absolute number of T cells in the infused product is a simple but surprisingly underused parameter and could be developed further for more personalized transplant strategies [145]. Successful engraftment and effective GVL require a minimal number of donor T cells. However, donor T cell depletion is required to avoid severe GVHD and can be achieved through *in vitro* graft engineering, or by the *in vivo* depletion of T cell replete grafts using pre-transplant anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTcy). Accordingly, the optimal dose and timing of ATG administered as GVHD prophylaxis depends on body weight, but also on lymphocyte count; excessive or insufficient exposure to ATG negatively impacts transplant outcomes [146].

5.2. Immune reconstitution as a major determinant of transplant outcome

Immune reconstitution is a major determinant of transplant outcomes, but has mostly been studied in the peripheral blood [147]. The GVL effect enables donor immune cells to eliminate host leukemic cells by engaging a multicellular response including T cells, NK cells and antigen-presenting cells to overcome the multiple immunosuppressive mechanisms and clonal heterogeneity observed in AML [2]. Timely and appropriate immune reconstitution ensures persistent GVL activity and prevents infectious complications, while mitigating GVHD risk [147,148].

5.2.1. Conventional T cells

In the early post-transplant period, expansion of donor T cells results from antigen priming of alloreactive T cells and from cytokine-driven proliferation in the lymphodepleted host. This phase is followed by thymic production of naïve T cells after differentiation of donor stem cells. CD4 T cells recover slower than CD8 T cells and rely more on *de novo* thymic differentiation from lymphoid precursors generated in the BM. Reaching normal CD4 T cell counts can take up to 2 years [148,149]. A robust and timely CD4 and CD8 T cell recovery is an important predictive marker of post-transplant survival across HSCT platforms [150–152]. Accordingly, timely achievement of full donor T cell chimerism is predictive of post-transplant survival [153]. In addition, the re-acquisition of a broad polyclonal T cell repertoire is associated with better transplant outcomes [154,155]. Finally, intrinsic properties of donor T cells such as polymorphisms in the *CTLA4* gene could modulate GVHD and overall survival, as suggested by a recent meta-analysis [156].

Thymic recovery is associated with robust immune reconstitution and lower opportunistic infections [157]. Age-related impaired thymus activity contributes to the well-known inferior outcomes of HSCT in the elderly [158]. Ageing also impacts post HSCT BM microenvironment. On one hand, hematopoietic stem cells from older donors are intrinsically altered through several mechanisms leading to reduced lymphoid progenitors generation [149,158,159], while on the other hand, changes in the BM stromal environment of the elderly contributes to defective lymphopoiesis. Strikingly, transplantation of old stem cells in a young microenvironment is sufficient to partially reverse these age-related defects [149,158,160]. Conversely, the transfer of expanded young (cord blood) progenitors with high lymphoid potential may improve thymic output [157]. Normal ageing is also associated with mutation-driven acquired clonal hematopoiesis (CH) [161]. Interestingly, the presence of donor CH impacts transplant outcomes through mechanisms that include immune modulation [162]. The presence of DNMT3A mutation was associated with improved progression free survival, reduced relapse and increased chronic GVHD, possibly due to a positive effect on Th1 polarization and IFNγ production by CD4 T cells [163,164].

Although massively disrupted by the conditioning regimen and

concomitant procedures [165], BM has a major role in post HSCT immune reconstitution. Post HSCT BM hosts donor memory T cells and supports their homeostatic expansion. The BM contains residual hosts antigen presenting cells (APCs) which present allo-antigens to donor T cells. The BM further ensures efficient donor hematopoiesis and differentiation of donor lymphoid progenitors [166]. An inflammatory IFNγ-rich BM plasma has been associated with primary graft failure, suggesting an impact of BM environment on early engraftment and hematopoiesis [167]. In the context of GVHD, IFNγ produced by donor T cells inhibits stem cell proliferation and induces lymphocyte apoptosis [168]. The BM is also affected by post-transplant immune insults such as GVHD and subsequent inflammation, infections and immunosuppressive therapy. Moreover, BM stromal niche, just as other organs, is a target of alloreactivity and "marrow GVHD" leads to impaired hematopoiesis and delayed immune reconstitution [169].

Evidence of a strong impact of T cell composition of post-transplant BM was recently published in the setting of autologous stem cell transplant (ASCT) for multiple myeloma. The authors identified a group with a specificpattern of post ASCT BM immune reconstitution, characterized by higher levels of naive and terminally differentiated T cells, some of which were expressing markers of T-cell exhaustion. This group had a significant inferior overall survival and time to myeloma progression as compared with the patients that did not display these markers[170]. To our knowledge, similar information is lacking in the context of HSCT for AML but we review existing data in Section 4.3.

5.2.2. NK cells

NK cells reconstitute early post-HSCT and their recovery is associated with clinical outcomes and protection against relapse [144,171–173]. Furthermore, the dose of NK cells in the infused graft product correlates with relapse-free survival [171]. Accordingly, T-depleted haploidentical SCT historically lead to better survival in case of KIR mismatch in the graft versus host direction [123] though this concept is currently challenged with the broad use of post-transplant cyclophosphamide as GVHD prophylaxis [175]. However, even in the context of T cell-replete transplant platforms, NK cell alloreactivity can be relevant. For example, a study on cord blood transplant for AML showed an association between a specific KIR-HLA combination and lower relapse indicating NK-mediated modulation of alloreactivity [176]. Altogether these data indicate that NK cells contribute to the GVL effect. This is illustrated by the success of allogeneic NK cell therapy and harnessing NK cell alloreactivity is an important tool for preventing and treating post-transplant relapse [89].

5.2.3. Other immune cells

An immunomodulatory and prognostic role in the setting of HSCT has also been attributed to $\gamma\delta$-T cells [57,58], invariant natural killer T cells [177], myeloid-derived suppressor cells [177], Treg [148,178–183] and neutrophils [184]. Post-transplant BM B cell quantification has been associated with frequency and severity of GVHD; patients with GVHD having decreased lymphopoiesis [148,185]. In addition, elevated numbers of immature B cells were found in PB of patients with severe infections and active chronic GVHD [148,186]. Such delayed B cell recovery leads to increased infectious complications and impaired response to vaccines post HSCT [187].

Although mainly based on analyses performed on PB, the associations between immune reconstitution and transplant outcome highlight the importance of the complex post-transplant immune environment. In Sections 4.3 and 4.4 we will review the interplay between immune environment and AML relapse.

5.3. Immune escape and environment at relapse

Recurrence of the initial disease remains the main cause of HSCT failure. AML blasts evade the immune system through different mechanisms [188]. These include abrogation of leukemia cell recognition due to loss of HLA genes, T cell exhaustion, production of anti-inflammatory factors, loss of proinflammatory cytokine production, and acquisition of novel driver mutations that promote leukemia outgrowth [188,189]. Exome sequencing of 15 paired BM samples at diagnosis of AML and at relapse post-HSCT showed no new AML-specific mutations or structural variations in immune-related genes [190]. However, dysregulation of pathways that may influence immune function, including downregulation of MHC class II genes, which are involved in antigen presentation have been observed. Downregulation of MHCII expression at the blast surface was confirmed by flow cytometry [190]. As compared to blasts at diagnosis, relapsed leukemic cells had a diminished capacity to stimulate a third-party CD4 T cell [190]. Of note, *in vitro* exposure to IFNγ rapidly reversed this phenotype, hinting at transcriptional or epigenetic mechanisms underlying these defects. [190]. In line with these findings, another group reported impaired CD8 T cell production of IFNγ and TNFα in AML patients relapsing post HSCT [43].

The immune features in PB and BM at relapse remain poorly understood but recent studies have uncovered some important characteristics of the immune environment of relapsed AML (Fig. 3). Noviello *et al.*, compared post-transplant BM samples of patients at relapse and in sustained complete remission (CR) [191]. The frequency of BM- but not PB Tregs was significantly higher in relapsed patients compared with CR patients. A higher proportion of early-differentiated memory stem (TSCM) and central memory BM-T cells expressed multiple inhibitory receptors such as PD-1, CTLA-4 and TIM-3 in relapsing patients than in CR patients. At relapse, T cells displayed a restricted TCR repertoire and impaired effector functions compatible with an exhausted phenotype. In addition, the early detection of severely exhausted (PD-1^{+}Eomes^{+}T-bet^{-}) BM-TSCM was predictive of relapse in a retrospective analysis. Accordingly, leukemia-specific T cells in patients prone to relapse displayed exhaustion markers, absent in patients maintaining long-term CR [191]. Similar findings were reported by another group on peripheral blood [192]. Toffalori *et al.*, analyzed the PB transcriptome in AML patients relapsing post HSCT. The relapse signatures were highly enriched in immune-related processes, including T cell co-stimulation and antigen presentation. The authors further documented deregulation of multiple co-stimulatory ligands on AML blasts and concomitant exhaustion markers on T cells leading to defective T cell-mediated allorecognition and elimination of leukemic cells [193]. From a mechanistic point of view, it was recently demonstrated that post HSCT, relapsed AML cells reduce the glycolytic activity of T cells through pH modification, which leads to changes in their transcriptional profile and subsequent dysfunction. Interestingly, this effect could be counteracted by sodium bicarbonate suggesting a pharmacological application of this finding [194]. More recently, McCurdy et al. used multimodal machine learning to identify signatures of relapse specifically after HSCT when PTcy was used as GVHD prophylaxis [174]. They found that loss of NK and CD8 T cell inflammatory signaling predominated at relapse. In addition relapse was characterized by a loss of inflammatory gene signatures in NK cells and a transcriptional exhaustion phenotype in CD8 T cells [174].

Hence, relapse immune environment results from the combination of defective post-transplant immune reconstitution on one hand and immune escape from AML blasts on the other hand (Fig. 3). A systematic study of post-transplant immune readouts could help identify the best options to prevent and treat relapse.

5.4. Immune strategies to treat and prevent relapse

Many established and experimental options exist to prevent and/or treat post-transplant AML [188,195]. These options include molecules targeting specific mutated genes, hypomethylating agents, immune- and cell-therapy and share the common goal of enhancing an exhausted alloimmunity or avoid AML immune escape. Induction or stimulation of a pro-inflammatory environment can enhance GVL. Tyrosine-kinase inhibitors (TKI) with activity against mutated Fms related receptor

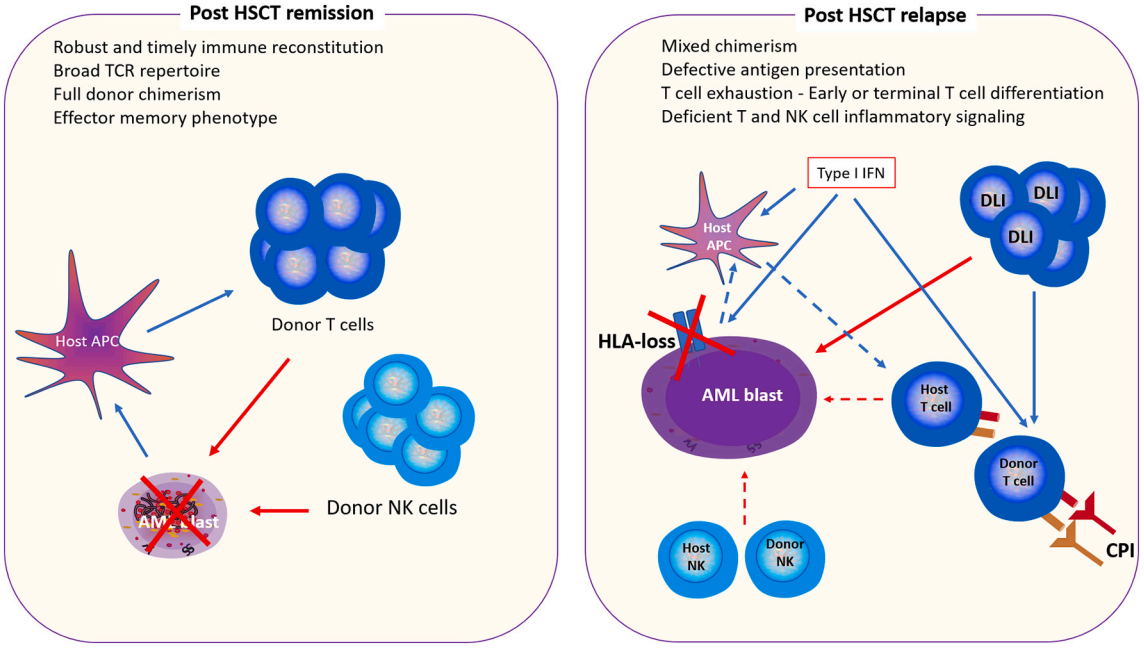

Fig. 3. Post-transplant immune environment, factors associated with relapse and corresponding therapeutic targets.
Schematic representation summarizing key findings and likely interactions in post HSCT remission or relapse. Post-transplant remission relies on a robust graft versus leukemia effect associated with on a broad donor-derived T cell repertoire with effector memory T cell differentiation, and on the presence of potent natural killer (NK) cells. Immune escape mechanisms leading to relapse include loss of HLA expression by leukemic blasts which impairs both priming by antigen presenting cells (APC) and T cell mediated killing. Relapse is associated with incomplete donor chimerism and defective NK cell function. Immunotherapeutic interventions include donor lymphocyte infusions (DLI) which both directly target AML blasts and indirectly activates pre-DLI donor T cells. Type I interferons (IFN) can stimulate APCs, increase HLA molecules expression by AML blasts and activate T cells. Checkpoint inhibitors (CPI) can counteract T cell exhaustion by targeting markers such as CTLA4 and PD1 (represented by orange and red bars on the surface of T cells).

tyrosine kinase 3 (FLT3) are used in the post-transplant maintenance setting for FLT3-mutated AML [196]. In addition to the TKI effect targeting the leukemic blasts, these agents may interact with the immune system to accelerate the development of a GVL effect. For example, mitochondrial activity of human CD8 T cells was enhanced upon sorafenib exposure in responders, but not in non-responders, to this therapy [137,197]. Type I interferons are also used in prevention or treatment of post-HSCT AML relapse [198,199]. In this setting, the use of type I IFN was associated with persistence of cross-presenting dendritic cells and circulating leukemia antigen-specific T cells [199]. In the next paragraphs we describe treatment modalities specifically directed to relapsed AML microenvironment and the particular case of extramedullary relapse.

5.4.1. *Donor lymphocyte infusions*

Donor lymphocyte infusions (DLI) can be used prophylactically in high risk disease or after T cell-depleted transplantation, pre-emptively when MRD rises or as treatment for overt relapse, alone or in combination with other modalities [189,200–204]. Such infusion of polyclonal intact T cells impacts the immune environment status by hastening immune reconstitution as indicated by increased T cell recovery rate and improved donor chimerism. Post DLI analysis of PB T cells shows oligoclonal leukemia-specific expansion [205,206]. Bachireddy et al. integrated BM-derived single T cell gene expression, chromatin accessibility and TCR sequencing at relapse, pre- and post DLI in chronic myeloid leukemia (CML). They showed that responding patients' BM was enriched in late differentiated T cells before DLI, which was associated with rapid and durable expansion of early differentiated T cells after adoptive transfer. In contrast, BM T cells from patients resistant to DLI displayed dysfunction features. Strikingly, the early differentiated T cells identified in responders originated mainly from the pre-DLI leukemic microenvironment rather than from the infused product [207]. A recent study done on PB similarly showed that DLI favored the expansion of the pre-DLI repertoire in responding AML patients [208].

5.4.2. *Epigenetic modulation*

Epigenetic modulators have multiple immunomodulatory effects relevant to post-HSCT relapse, including reversal of HLA expression loss, increased Treg frequency, increased expression of PD-L1 and PD-L2 on AML cells, upregulation of tumor antigens capable to induce a CD8 T cell response, and global promotion of inflammation [2,40,209,210]. Post-transplant maintenance therapy with azacitidine alone did not improve outcome [137,211]. However, panobinostat, another epigenetic modulator acting through deacetylase inhibition showed encouraging effects on relapse, GVHD and survival [212]. Most promising results were obtained by combining hypomethylating agents and immune- or cell-therapy [2,213,214].

5.4.3. *Extra-medullary relapse*

Extra-medullary (EM) AML is a rare entity referred to as myeloid sarcoma in the 2016 WHO classification [215]. EM AML can occur with or without concomitant BM involvement and involve any organ. Correlative evidence suggests that BM environment could provide a stronger GVL effect than other tissues that can act as immune sanctuaries. Extramedullary localizations are over-represented in post-HSCT relapsed AML, 41% of all post-HSCT relapse in a Korean cohort [216]. Moreover, retrospective data identified the presence of chronic GVHD and late relapses with an increased risk of EM as compared to BM-only relapses. Finally, patients with EM relapse appeared to respond to cytotoxic therapy but not to DLI which further suggest that the BM may better support an effective GVL effect [217].

Similarly, mechanisms of relapse after adoptive transfer of leukemia-specific donor T cells included extra-medullary infiltration of known immune sanctuaries in a recent study [218]. On the other hand, some tissues such as skin could offer an environment favorable to CPI

therapeutic efficacy, as patients with post-HSCT EM AML seemed to better respond to CTLA4 blockade in a phase Ib study [219]. In that study, responses were associated to in situ infiltration of cytotoxic CD8 T cells, decreased activation of Treg, and expansion of subpopulations of effector T cells in the PB [220]. These studies and others suggest CPI could be useful in selected cases of post-HSCT relapsed AML, particularly in EM relapse [221].

6. Conclusion and future directions

In this review, we recapitulated the roles of the BM as a hematopoietic but also lymphoid organ. We have detailed what is known of the AML immune microenvironment at diagnosis and across therapies with a focus on T cell counts, phenotypes and functions. Despite important heterogeneity, the immune status of AML BM is emerging as a critical biomarker that predicts outcome following standard treatments and immune-based interventions. Based on the available evidence, and with the increasingly available sequencing methods, we argue for the use of immune characterization along with the other well-known criteria to stratify AML in terms of prognosis and choice of therapy. Such a stratification could help identify the patients most likely to benefit from immune-based therapies. Similarly, current evidence indicates that robust immune reconstitution and persistence of an active non-exhausted immune environment are key to counteract AML immune escape, prevent relapse and ensure successful HSCT. Emerging data suggest early post-transplant immune markers could predict relapse and identify appropriate preventive and therapeutic strategies. Despite major advances in the recent years, cure of high-risk and relapsed AML remains an unmet medical need. We postulate that large prospective studies systematically analyzing immune environment readouts at diagnosis, across therapy and at relapse could address the intra- and interindividual heterogeneity of the disease and eventually allow the development of efficient immunotherapy for selected AML patients.

Practice changing bullets

- There is an immunological basis for AML heterogeneity
- Immune subtypes of AML partially correlate with established cytogenetic and molecular prognostic categories
- The quantity and functional features of lymphoid cells in AML microenvironment contribute to the prognosis of the disease and possibly predict response to immunotherapy
- Carefully selected immune-based interventions potentially revert the suppressive AML immune environment

Research agenda

- Prospective and comprehensive analysis of the immune microenvironment, at diagnosis, after achievement of complete remission or in states of refractoriness and at relapse. Such prospective studies should include large number of patients to address the important inter- and intra-individual variability and age-related changes.
- Correlate immune features with response to immune-based therapies to identify the best targets and the best candidates for immunotherapy.
- Use of immune markers to predict response to immune therapy in the groups with the greatest medical needs (refractory AML, MRD-positive and post-HSCT relapse.

Full red lines represent a pro-cytolytic activity, full blue lines indicate a positive interaction and dotted lines indicate an impaired mechanism. Illustrations made with ScienceSlide® Suite 2010 edition.

Author contributions

YS wrote the original draft of the manuscript, YS and JSD wrote and edited the manuscript, YS, JH, LB, FM, CER, SA, SL and JSD reviewed and edited the manuscript.

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Declaration of Competing Interest

The authors have no conflict of interest to disclose relevant for this review.

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