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REVIEW

Effective Immunosurveillance After Allogeneic Hematopoietic Stem Cell Transplantation in Acute Myeloid Leukemia

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Abstract: The number of patients receiving allogeneic hematopoietic stem cell transplantation (alloHCT) has increased constantly over the last years due to advances in transplant technology development, supportive care, transplant safety, and donor availability. Currently, acute myeloid leukemia (AML) is the most frequent indication for alloHCT. However, disease relapse remains the main cause of therapy failure. Therefore, concepts of maintaining and, if necessary, reinforcing a strong graft-versus-leukemia (GvL) effect is crucial for the prognosis and long-term survival of the patients. Over the last decades, it has become evident that effective immunosurveillance after alloHCT is an entangled complex of donor-specific characteristics, leukemia-associated geno- and phenotypes, and acquired resistance mechanisms. Furthermore, adoption of effector cells such as natural killer (NK) cells, alloreactive and regulatory T-cells with their accompanying receptor repertoire, and cell–cell interactions driven by messenger molecules within the stem cell and the bone marrow niche have important impact. In this review of pre- and posttransplant elements and mechanisms of immunosurveillance, we highlight the most important mechanisms after alloHCT.

Keywords: acute myeloid leukemia, AML, allogeneic stem cell transplantation, alloHCT, graft-versus-leukemia, GvL, relapse, immunosurveillance

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHCT) is currently the leading consolidation treatment for high-risk malignant hematologic diseases like acute myeloid leukemia (AML) with a potentially curative approach and increasing numbers of transplants performed each year worldwide.[1–5] In fact, AML is the most common disorder for which alloHCT is used.[4] For AML patients having intermediate- or adverse-risk genetic alterations, as well as relapsed or refractory disease, alloHCT achieves the highest rates of long-term survival as post remission or combined salvage treatment.[6–8] Still, disease relapse remains the main cause of treatment failure.[9,10] In case of relapse, the prognosis is generally very poor despite comprehensive salvage therapies.[11–16] This stresses the vital role of a strong anti-leukemic effect in the alloHCT setting.

The key mechanism for durable remissions is preserving a graft-versus-leukemia (GvL) effect targeting residual disease after intense conditioning therapy and sustained engraftment.[17] Donor-derived alloreactive cytotoxic CD8+ T-cells play an important part in mediating GvL disease control by recognizing leukemia-specific antigens, eg, major and minor histocompatibility antigens, thereby conferring anti-leukemia

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alloreactivity. The concept of GvL was established after several observations showed an increased risk of relapse after matched-sibling or T-cell depleted transplantation while lowering the risk of acute graft-versus-host disease (GvHD).[17] Vice versa, decreasing the risk of relapse by tolerating acute and chronic GvHD, respectively, and retrieving disease control by donor lymphocyte infusions (DLI) in case of imminent relapse has unveiled the delicate interconnection between GvL and GvHD.[17–19]

With the awareness of an immunologic GvL effect, the use of less intensive conditioning regimens became more feasible in the transplant setting. Therefore, it has broadened the spectrum of patients with AML being eligible for an intensive treatment like allogeneic HCT as a curative therapy worldwide.[20,21] Currently, alloHCT can now be offered to many fit elderly patients with acceptable toxicity.[22]

However, achieving and enhancing a strong GvL effect while minimizing GvHD reactions is still a challenging endeavor in clinical practice and a major goal of translational research. Although GvHD and GvL appear to be concomitant phenomena in clinical practice, subsequent reports have highlighted the differences in between these two posttransplant events, yielding clinical potential to offer proper GvHD prophylaxis while the GvL effect remains unaffected for disease control.[23–26] Preserving a sufficient GvL effect is a complex interplay of different cellular components and characteristics, involving the interaction of the immune environment and immune system components, efficacy of allogeneic T-cell reactivity, leukemia-specific features, potential and side effects of conditioning intensity, applied GvHD prophylaxis, and anti-leukemic potential of specific maintenance therapy (Figure 1). The complexity of immunosurveillance after alloHCT is still not fully understood yet. Providing sufficient immunosurveillance will optimize outcome after alloHCT noticeably and improve the prognosis of AML patients (Table 1). Whether further

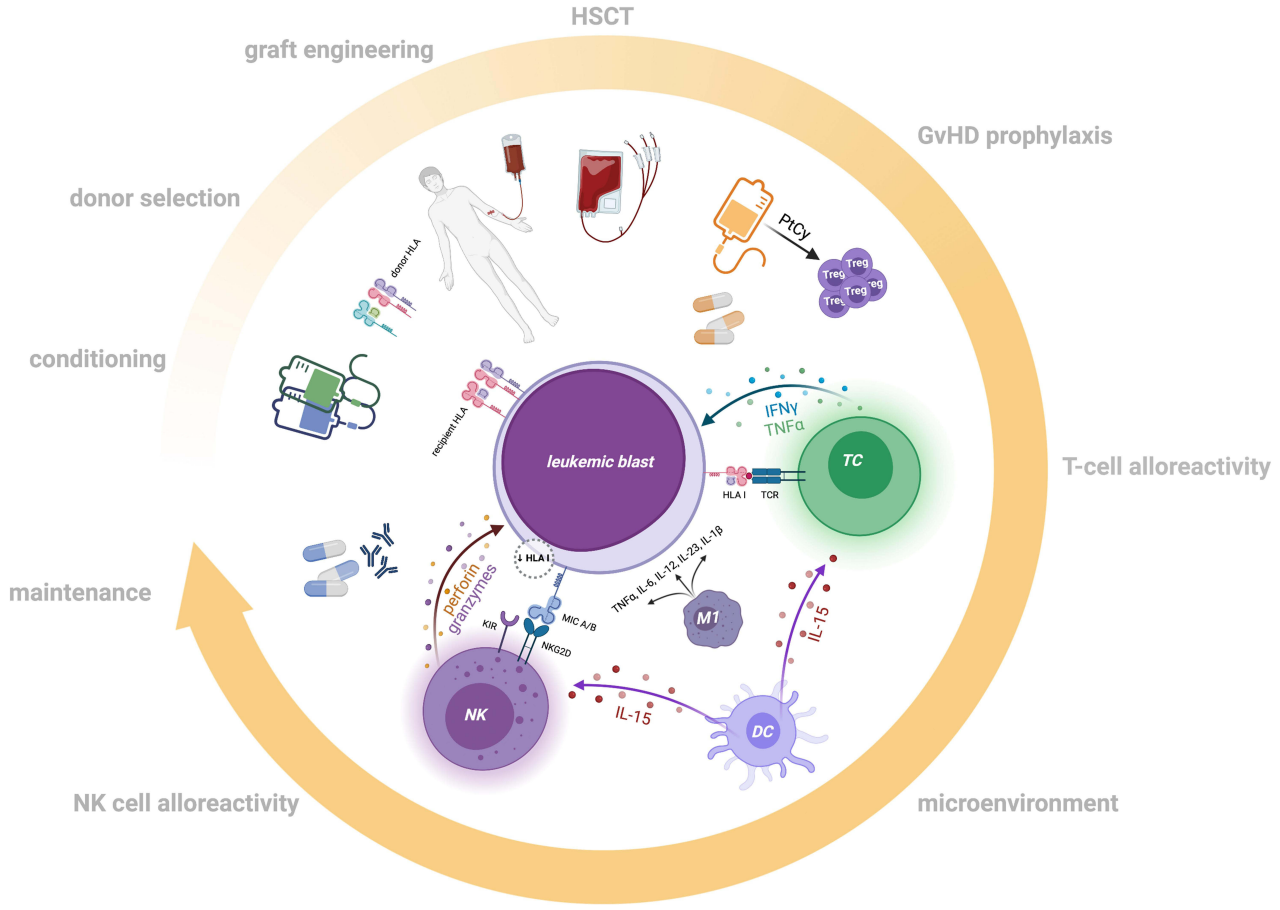

Figure 1 Components associated with Graft versus Leukemia activity. This cartoon depicts the main components associated with Graft versus Leukemia (GvL) and the interdependency of anti-leukemic alloreactivity. During the course of alloHCT several factors are crucial to support and provide a strong GvL effect. These elements include: intensity of conditioning, donor-specific characteristics (eg, HLA profile [red/violet shared alleles, blue/green mismatched alleles], sex, age, and kinship), stem cell source and graft engineering, cellular composition of the graft, effects of GvHD prophylaxis, effector T-cells and NK cells, microenvironmental features and targeted maintenance therapies (eg, HMAs, TKIs, and antibodies). Figure created with BioRender.com.

Table 1 Clinical Trials Addressing Augmentation of Graft versus Leukemia

Approach	Trial	Population Characteristics	N	Main Outcomes
DLI	Schmid C et al 2007[13]	(i)r/r AML ≧ 16 years after alloHCT (ii)RIC or MAC (iii)HLA identical donor or other	339	Remission vs no remission at time of DLI administration significant prognostic factor for OS (RR for OS 5.8 [95% CI 2.5–13.7], p < 0.0001)
	Schmid C et al 2019[100]	(i)AML or ALL ≧ 18 years (ii)RIC or MAC (iii)MSD or MUD	178	Improved outcome in high-risk AML (OS: prophylactic DLIs 69.8% vs 40.2% in controls, p = 0.027)
NK	Ciurea S et al 2017[120]	(i)High risk AML, MDS or CML 18–60 years after alloHCT (ii)Flu/Mel with 1 dose 200 cGy TBI (iii)haploHCT	13	Significantly improved NK-cell number and function, lower viral infections, and low relapse rate posttransplant
	Ruggeri L et al 2021[113]	(i)AML or ALL (ii)RIC or MAC (iii)haploHCT	138	NK cell alloreactivity with no impact on GRFS in unmanipulated grafts (HR 1.66 [95% CI 0.9–3.1], p = 0.1), but beneficial impact on GRFS in T-cell-depleted transplants (HR 0.6 [95% CI 0.3–1.2], interaction p < 0.001)
CIK	Laport G et al 2011[173]	(i)Relapsed AML, APL, ALL, CLL, NHL, MM, MDS or HL ≧ 18 years after alloHCT (ii)RIC or MAC (iii)MSD	18	Median EFS 4 months, median time to relapse 6 months (range 2–37 months), median OS 28 months
ICi	Davids M et al 2016[150]	(i)Refractory/progressive AML, AML with extramedullary disease, ALL, MM, NHL, HL, MDS or MPN ≧ 18 years after alloHCT (ii)RIC or MAC (iii)Related or unrelated donor	28	Ipilimumab dose of 10 mg/kg: 23% complete response, 9% partial response, 27% decreased tumor burden, complete responses in patients with extramedullary AML; 4 patients with durable response for more than 1 year; Immune-related adverse events 21%
	Daver N et al 2019[153]	(i)r/r AML ≧ 22 years (ii)additional azacitidine treatment	70	ORR 33%: 22% CR/CRi, 1 partial response, 10% with hematologic improvement, 9% stable disease; ORR 58% HMA–naïve vs 22% HMA-pretreated; Grade 3 to 4 immune-related adverse events 11%
TKI	Mathew N et al 2018[118]	(i)In vitro murine and human models (ii)Relapsed *FLT3*-ITD^{mut} AML	/	Sorafenib increases IL-15 production by *FLT3*-ITD^{mut} leukemic cells and CD8+CD107a+IFN-γ+ allogeneic T-cells
	Burchert A et al 2020[127]	(i)*FLT3*-ITD^{mut} AML ≧ 18 years (ii)Hematologic CR after alloHCT	83	Sorafenib maintenance lowers risk of relapse/death (HR 0.39 [95% CI 0.18–0.85], p = 0.013) and improves RFS (HR 0.256 [95% CI 0.10–0.65], p = 0.002) after alloHCT for *FLT3*-ITD^{mut} AML

Abbreviations: ALL, acute lymphoblastic leukemia; alloHCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; cGy, centigray; CI, confidence interval; CIK, cytokine-induced killer cells; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CR, complete remission; CRi, complete remission with insufficient recovery of counts; DLI, donor lymphocyte infusion; EFS, event free survival; Flu, fludarabine; *FLT3*-ITD^{mut}, mutated Fms-related tyrosine kinase 3 internal tandem duplication; GRFS, GvHD/relapse-free survival; haploHCT, haploidentical hematopoietic stem cell transplantation; HL, Hodgkin's lymphoma; HLA, human leukocyte antigen; HMA, hypomethylating agent; HR, hazard ratio; ICi, immune checkpoint inhibitors; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; Mel, melphalan; MM, multiple myeloma; MPN, myeloproliferative neoplasm; MSD, matched sibling donor; MUD, matched unrelated donor; NHL, non-Hodgkin lymphoma; NK, natural killer cells; ORR, overall response rate; OS, overall survival; RFS, relapse free survival; RIC, reduced intensity conditioning; RR, relative risk; r/r, relapsed/refractory; TBI, total body irradiation; TKI, tyrosine kinase inhibitor.

cell-based therapies such as chimeric antigen-receptor (CAR) T-cell therapies in combination with alloHCT will add further anti-leukemic pressure is still a matter of debate and will be investigated in the near future.

Donor-Specific Characteristics

Human Leukocyte Antigen (HLA) Profile

Disparities in HLA class I (HLA-A, -B, -C) and class II (HLA-DRB1, -DQB1, -DPB1) have important

implications for both GvHD and GvL. In fact, the HLA region is the most polymorphic system with 17.191 class I and 6.716 class II alleles described to date.[27] With the beginning of HLA typing, first as a serologic approach spanning HLA-A, -B, -C, and -DRB1, and later as a high-throughput next-generation sequencing-based technique broadening our knowledge of the HLA system, donor availability, and allowing the usage of unrelated donors, paralleled by dramatically rising numbers of donor volunteers (approximately 38 million in 2021; World Marrow Donor Association [WMDA], https://statistics.wmda.info), alloHCT has become a safe and widely used treatment option.

Despite the huge number of volunteers, approximately 10% of patients in need of an allograft lack an HLA-matching related or unrelated donor. A higher degree of HLA disparity is associated with a higher transplant-related mortality (TRM) and lower overall survival (OS) compared to well-matched donors.[28,29] Notably, comparison of mismatched settings, eg, partially matched-unrelated donors (MMUD) and related haploidentical donors (MMRD) suggests comparable outcomes, as non-relapse mortality (NRM), relapse rate, and occurrence of GvHD did not differ significantly in retrospective studies.[30] By contrast, a higher number of HLA mismatches were associated with increased event-free survival (EFS).[31] One could easily argue that greater HLA disparity leads to an enhanced GvL effect because of higher numbers of displayed unshared HLA epitopes with supporting data showing decreased relapse rates, though no survival benefit was achieved because of increased NRM.[32] Nevertheless, prospective studies comparing MMUD with MMRD in times of refined GvHD prophylaxis are still ongoing and are expected to shed light on one of the most open questions in the transplant community to evaluate OS in MMUD versus MMRD (NCT03275636) and MUD versus MMRD (NCT04232241).

Considering functional HLA class II mismatches, donors harboring an HLA-DPB1 graft-versus-host (GvH) disparity have been associated with a reduced risk of relapse as mismatched HLA-DPB1 is a target structure for alloreactive T-cells.[33,34] Further studies have found that HLA-DRB1 mismatches in the GvH direction are also associated with an improved survival because of reduced NRM.[32] Even smallest sequence dimorphisms like the concept of HLA-B M- and T-leader peptides, describing single nucleotide variants in exon one of the HLA-B allele, strongly affect T-cell and natural killer (NK) cell alloresponses.[35] These data underline the importance of deciphering pro-GvL and pro-GvHD-associated HLA alleles and antigens as a main goal for future studies to improve donor selection and achieve maximum therapeutic success.

Donor Age

In addition to donor/recipient HLA profile concordance, sex, parity, cytomegalovirus (CMV) serostatus, and blood group ABO match, donor age has become one of the major determinants for survival after unrelated alloHCT, with a reported 5.5% increase for overall mortality for every 10-year increment in donor age.[36] The advantage of younger donor age could also be associated with a significantly lower rate of acute and chronic GvHD.[37] Interestingly, the benefit of younger donor age is more important in older recipients (>40 years) than in younger patients with AML in a haploidentical setting.[38] Likewise, younger donor age (≤40 years) was determined as an independent predictor for better OS in older AML patients (≥55 years) receiving haploHCT.[39] Noteworthy, donor age appears to have no adverse effects on functional fitness of hematopoietic cells after alloHCT.[40] A recently published retrospective multicenter analysis of a large cohort of haploHCTs using posttransplant cyclophosphamide (PtCy) as GvHD prophylaxis confirmed a higher incidence of acute GvHD and a trend for higher NRM with increasing donor age, though a significant reduced risk of disease relapse and improved progression-free survival (PFS) was associated with older donors. Donor/recipient kinship, especially maternal donors, predicted worse PFS and OS.[41]

Donor Gender

Female donor T-cells recognizing minor histocompatibility antigens (mHAgs) encoded on the male Y chromosome (H-Y) have been hypothesized to induce GvHD and therefore cause increased NRM in a male recipient/female donor setting. As H-Y antigens can also be expressed on leukemic blasts, the primary negative circumstance could be highly beneficial for GvL and therefore lower the risk of relapse. This effect plays an important part especially in HLA-matched transplantations, where mHAgs are the only target structures for alloreactive T-cells.[42,43] By contrast, considering that haploHCT male donors should be preferred for male recipients, as female donors for male recipients are associated

with inferior survival.[31] In addition, compared to nulliparous female and male donors, transplantation from parous female donors is associated with a higher NRM because of GvHD.[36]

Stem Cell Source

Filgrastim-mobilized peripheral blood stem cells (PBSCs) have become the main source of donor stem cells for related and unrelated alloHCT.[44] They have been associated with beneficial events such as early engraftment and lower incidence of graft failure compared with bone marrow stem cells (BMSCs), but also induce significantly higher rates of acute and chronic GvHD related to T-cell repleted PBSC allografts.[45] Still, these T-cell-rich grafts offer a significant GvL potential to confer increased leukemia-free survival (LFS) and OS despite elevated risk of TRM.[46] Especially, in the setting of reduced intensity conditioning (RIC), PBSCs mediate anti-leukemic effects, thus resulting in superior survival and significantly decreased risk of relapse with no difference in NRM compared with BMSCs.[47] However, the increased risk of acute and/or chronic GvHD after peripheral blood grafts needs to be clinically and scientifically addressed in further investigations improving GvHD prophylaxis without nullifying GvL to reduce late morbidity and mortality and achieve long-term LFS and cure, respectively. Recently, interesting data have revealed a modified allograft composition when additionally mobilized with plerixafor (AMD3100). An increase in the total number of nucleated cells, including CD4+ and CD8+ T-cells, as well as regulatory T-cells (Tregs), CD19+ B-cells, plasmacytoid dendritic cells (DCs) and primitive immature CD34+CD38-CD133+ progenitor cells harboring high self-renewal potential were detected in grafts mobilized with G-CSF and plerixafor.[48,49] Studies have also demonstrated the beneficial effects of Tregs in the alloHCT setting concerning improved OS, lower rates of acute GvHD, and GvL preservation.[49–51] Nonetheless, further immunologic pathways apart from cellular components might be initiated, as plerixafor preferentially mobilizes a plasmacytoid DC precursor that produces high interferon-α (IFN-α) levels and a favorable balance of immune effectors that lowers the risk of GvHD.[49,52]

Disease Status Prior to Transplant

To achieve long-term survival in AML patients after alloHCT, maximum reduction of leukemic burden prior to transplant is essential for outcome. Patients with an uncontrolled and active disease status as well as patients in morphologic complete remission (CR), but who still show measurable residual disease (MRD), are at increased risk for relapse and are likely to have a short OS.[53–56] To determine MRD, cytogenetic testing is established in everyday routine, whereas the value of MRD detection through next-generation sequencing has yet to be evaluated and flow-cytometry-based MRD needs to be harmonized.[57,58] A way to counteract the disadvantage of persistent disease or MRD, respectively, and to minimize pretransplant tumor burden is the application of sequential or intensified conditioning such as melphalan, intensified busulfan, high-dose cyclophosphamide, or TBI-based regimens.[59–64] However, trying to achieve MRD negativity by additional therapies or salvage treatments before alloHCT also contributes to increased toxicity and selection or development of highly resistant leukemic clones and altogether postpones application of alloreactive anti-leukemic CD8+ T-cells and therefore delays the inevitable effect of GvL for high-risk AMLs. The question of the ideal point of time when to transplant is an objective of ongoing trials (NCT02461537).[65]

Conditioning Regime

As early as the 1990s, intensive myeloablative conditioning (MAC) regimens have been associated with reduced risk of relapse.[66] Especially, in the matched-sibling setting, retrospective data showed a superior benefit concerning relapse in MAC conditioning compared with RIC.[67,68] However, the use of myeloablative alloHCT is limited because of organ toxicities. The advent of RIC regimens has both broadened the spectrum of patients being eligible for transplant and decreased the toxicity and TRM of myeloablative alloHCT.[69] Moreover, emerging evidence presents that in case of good response after induction therapy, RIC and MAC protocols are equally effective in terms of OS, with a tendential benefit for RIC regarding reduced TRM, though RIC presents with a significantly higher rate of relapse than MAC in prospective trials ($p < 0.01$).[20,70,71] Consequently, MAC should be considered whenever possible, and conditioning dose intensification should be applied if physically feasible. Furthermore, sequential application of potentially toxic conditioning components (eg, melphalan and fludarabine/TBI) is thought to generate moderated toxicity. Recently, conditioning regimens are increasingly personalized to optimize transplant outcomes. For patients requiring RIC, an underlying key tool for acceptable relapse rates and long-term

survival is attributed to a potent GvL effect, which made the usage of less intense conditioning and achieving of satisfying results possible. Notably, especially, in the setting of RIC alloHCT, robust GvL effect and chronic GvHD are strongly intrinsically connected processes, challenging treating physicians in the posttransplant follow-up.[72]

GvHD and GvHD Prophylaxis

Withdrawal of immunosuppression has been one of the simplest and well-studied interventions to prevent imminent relapse and give way to stronger GvL over the last years.[73] Still, development of GvHD often hampers rapid tapering of immunosuppression. GvHD is one of the most dreaded complications after alloHCT.[74] Recognition of major and minor histocompatibility antigens expressed in patients' non-hematologic tissues by stimulated donor-derived T-cells can lead to acute and/or chronic GvHD, a relevant number of patients even have to face a lethal course. The greater the disparity between donor and recipient's HLA matching, the higher the risk of GvHD development. Tissue damage caused by conditioning, especially MAC regimens, can further trigger GvHD.[75] The yet unresolved crux of this therapeutic challenge is the interdependency of GvHD and GvL, as they are mediated by the same CD8+ T-cells.[76] GvHD has a protective potential regarding disease reoccurrence. Still, evidence reveals that these two phenomena are characterized by individual and differential pathophysiologic pathways, which could potentially give way to new therapeutic strategies, shifting posttransplant T-cell reconstitution from GvHD to an intensified GvL activity, therefore refining GvHD prophylaxis while leaving GvL unaffected.[25]

The introduction of PtCy launched a whole new era of T-cell-replete alloHCT differing an entire HLA haplotype between donor and recipient with satisfying rates of engraftment, OS, EFS, NRM, and acute and chronic GvHD, thus expanding donor availability for a considerable number of patients lacking a related or unrelated matched donor who would otherwise have no option for curative treatment.[77–81] Preclinical studies have provided evidence of CD4+FOXP3+ Tregs as the key essential effectors of alloimmune regulation after PtCy application.[82,83] Tregs mitigate GvHD while preserving GvL. Therefore, it is an attractive alternative and revolutionary GvHD prophylaxis option in contrast to conventional pharmacologic immunosuppression (eg, anti-thymocyte globulin, calcineurin inhibitors, and methotrexate) yielding singularly on the depletion of donor alloreactive T-cells and therefore potentially minimizing GvL. Another approach beyond pharmacological immunosuppression to support GvL strengthening is the infusion of adoptive FOXP3+ Tregs or regulatory Type 1 T-cells posttransplant or graft manipulation such as selective depletion of alloreactive T-cells.[84,85]

Recently, adoptive T-cell therapy applications in the context of GvHD prophylaxes while augmenting GvL were brought into focus. Several active Phase 1 and/or Phase 2 GvHD prophylaxis studies (eg, NCT04678401, NCT01660607, and NCT03977103) aim to determine the safety, feasibility, and efficacy of immunosuppression-free strategies by infusion of T-cell repleted grafts, followed by T-cell add back of donor Tregs and conventional T-cells (Tcons) in a matched or haploidentical setting, respectively. Additionally, by achieving effective prevention of severe GvHD and long-term tolerance, subsequent and rapid tapering and discontinuation of immunosuppressive medication will strongly give rise to CD8+ donor-derived T-cells, prompting necessary anti-leukemic potential and important anti-infectious functions.[86,87]

T-Cell Alloreactivity and T-Cell Subpopulations

A generally accepted consensus in the transplantation area is that alloreactive CD4+ and CD8+ T-cells are the central and primary mediators of a powerful GvL and that cell recognition of recipients' mHAgs is mainly the effector mechanism of T-cell response in many hematologic entities. A dreaded parallel event is the manifestation of GvHD mediated by the same effector cells, especially αβ T-cells, while innate-like γδ T-cells are supposed to exert an anti-leukemic effect without inducing GvHD.[88] Hence, several approaches of (selective) αβ T-cell depletion have been pursued, unfortunately resulting in increased risk of graft failure and relapse.[89,90] To partially compensate this downside of total T-cell depletion, T-cell add back by infusing either Tcons, Tregs, or donor-specific T-cells equipped with a suicide gene has been investigated to shift the alloreactive power back to GvL.[91,92]

Although associated with higher rates of NRM, posttransplant CMV replication and infection have been repeatedly reported to correlate with a lower risk of leukemic relapse.[93,94] Matching a CMV-positive donor to a CMV-positive recipient has shown to improve outcomes after MAC.[95] This beneficial aspect might be due to the expansion of donor-derived $V\delta2^{neg\gamma\delta}$ T-cells, though their target antigens are still not completely identified.[94] Another possible

immunologic explanation of this observation is an increased expression of HLA-C on CD56-CD16+ NK cells and a higher number of killer immunoglobulin-like receptors (KIRs), CD56, CD57, and NKG2C coexpressing CD8+ T-cells in CMV-positive alloHCT recipients, leading to the regulation of KIR expression and interaction with HLA-C by proliferating CMV-specific CD8+ T-cells, which eventually might contribute to GvL effects posttransplant.[96] Worth mentioning are conflicting results demonstrating that donor CMV serostatus after T-cell replete haploHCT has no effect on NRM or OS, making it difficult to determine CMV-dependent donor preferences in this setting.[97]

Studies investigating the T-cell receptor (TCR) repertoire are expected to add further knowledge for clinical applications. Interestingly, existing data indicate a correlation between advanced diversity in the TCR repertoire early after alloHCT and certain T-cell subclones with a lower risk of relapse and less GvHD.[98] Noticeably, at the time of AML relapse, upregulation of inhibitory TCRs mirror alterations of leukemic blasts expressing the respective ligands. Furthermore, exhausted T-cells display a restricted TCR repertoire and are detectable months before relapse.[99] Therefore, the expression of T-cell exhaustion markers could be used as predictive indicators for guidance of preemptive therapies.

DLIs

Continuous posttransplant monitoring is crucial for rapidly detecting imminent relapse and, by utilizing specific relapse treatments, improving OS. Following routine MRD diagnostics, measurement of donor-specific chimerism (or, if available, CD34+ subset chimerism) is a standard practice for disease monitoring and evaluation of donor engraftment after alloHCT. In case of imminent relapse or low leukemic burden, administration of DLIs is a simple and feasible means to reinforce GvL yielding a polyclonal T-cell response and to improve survival, especially in high-risk AML.[13,100,101] Unfortunately, DLIs are ineffective in overt morphologic leukemia relapse, allocating a tight time frame for clinical use.[13,100] Still, one of the major pitfalls is triggering GvHD. The incorporation of suicide genes and transfusion of T-cell subsets without alloreactivity have tried to circumvent this potentially fatal complication.[102–104] After haploHCT with PtCy, administration of escalating doses of haplo DLIs showed a satisfying rate of CR with acceptable rates of acute GvHD without cases of severe or chronic GvHD.[105] The combination of DLIs with hypomethylating agents (HMAs) has been associated with increased susceptibility of leukemic clones to the applied treatment and demonstrated effectiveness particularly in AML with low disease activity at the time of relapse.[106]

Future studies will reveal whether other T-cell based antigen-targeted therapies such as CAR-T-cells can improve therapeutic response to salvage therapy and improve survival in relapsed/refractory AML (r/r AML).

NK Cells and KIR Receptors

A growing body of evidence illuminates further mediators in the process of alloreactivity. NK cells and KIR-mediated alloreactive effects via epitopes presented by HLA class I molecules gain an increasing role in new therapeutic approaches reinforcing the GvL effect in preemptive strategies or once relapse has occurred. The balance between inhibitory and activating signals is crucial for the physiological function of NK cells. Interactions with HLA class I molecules trigger inhibitory signals, protecting cells from NK cell lysis. Inhibitory KIR receptors play a major role for NK cell activity. Similar to HLA, KIR genes are highly polymorphic sites with a variable number of KIR genes, coding for either activating or inhibitory cell-surface receptor activity. NK cells with a KIR-ligand mismatch, especially the KIR B haplotype (B/x), demonstrate a strong alloreactive potential without inducing GvHD.[107,108] Again, in the haploidentical setting, donor KIR B haplotype could be associated with a significantly reduced risk of relapse and NRM.[109,110] Later, the concept of "missing self" was introduced, providing a more veritable model of KIR interaction with recipients' KIR ligands.[111] Especially ligand incompatibility between donor and recipient in KIR3DL1 recognizing the HLA-Bw4 group, KIR2DL2 and KIR2DL3 recognizing the HLA-C1 group, and KIR2DL1 recognizing the HLA-C2 group in haploHCT could be associated with increased GvL effects and significantly lower rates of relapse in AML.[112] Recently, the beneficial effect of haplo NK cells has been shown particularly in T-cell repleted grafts.[113] Therefore, in addition to comprehensive HLA typing, KIR sequencing and KIR genotypes should be further investigated regarding their impact in alloHCT and whether they can be implemented in the future by selecting donors with favorable KIR genotypes in KIR-mismatched transplantation for improving outcome for AML patients undergoing alloHCT.

Missing inhibitory ligands activate NK cytotoxicity. NKG2D that binds to major histocompatibility complex class I chain-related protein A and B (MIC A and MIC B) and UL16 binding proteins (ULBPs) is an important activating receptor expressed by NK cells and cytotoxic T-cells alike.[114] The fact that leukemic stem cells often lack NKG2D activating ligands is a perfect example of leukemic immune evasion from NK and, after alloHCT, alloreactive surveillance.[115] A similar mechanism has been attributed to decreased expression of cytotoxic receptor DNAM-1 on NK cells in AML patients.[116] NK cell dysfunction investigated in AML patients has been detected in almost all patients at first diagnosis.[117] Among the underlying mechanisms are decrease or loss of activating natural cytotoxicity receptor (NCR) expression via direct cell–cell contact between leukemic blasts and NK cells or via cytokine-dependent NCR downregulation or NK stimulation.[117,118] CMV-mediated expansion of donor-derived NKG2C+ NK cells recognizing HLA-E of residual leukemic blasts and/or the lack of HLA molecules according to the missing self-theory have been hypothesized to be beneficial for reducing rate of disease relapse.[119]

Data suggest that GvL mediated by NK cells may be initiated immediately once the donor graft is transfused.[120] In a previous study, relapse rates were decreased in a subcohort that was characterized by higher numbers of NK cells within the graft than the median cohort NK-cell number when compared with another subgroup with NK cell numbers below the median value (5% vs 43%).[121] Consistently, high numbers of NK cells early after alloHCT provide potential GvL effect via NK cell alloreactivity demonstrated by lower relapse rates and increased survival.[122] The NK cell GvL effect may be a powerful tool for enhancing the efficacy and safety of allogeneic hematopoietic transplantation without the risk of GvHD. However, further knowledge about NK cell education and the T-cell interplay is needed to provide safe clinical use.

Microenvironment

As primarily described in solid tumors, alterations in the microenvironmental composition induced by malignant cells gain increasing importance in the field of hematologic neoplasms and add an additional level of complexity in the understanding of relapse biology. Several investigations have demonstrated the potential of leukemic blasts to optimize immune tolerance by influencing physiological mechanisms of the bone marrow niche favoring their own survival benefit.[123] This immune evasion is achieved by several modifications of both host and donor components. One of the best described pathways of tumor extrinsic immune modulation is a modification of the leukemic microenvironment by a deregulated release of cytokines by residual leukemic blasts. This process includes a limited production of interferon-γ (IFN-γ), interleukin 15 (IL-15), or G-CSF during leukemic transformation or an accelerated release of IL-10 and transforming growth factor beta (TGF-β), shifting the surrounding niche from a proinflammatory and therefore immunogenic environment into an immunosuppressive milieu and finally evading effector T- and NK cell mediated GvL. IL-15 occupies a special role in cytokine composition of the bone marrow niche, which is physiologically produced by DCs.[124] IL-15 has an activating function concerning T-cell and NK cell function and expansion and further leads to the creation of memory T-cells.[125] Consequently, low levels of IL-15 appear to be a predictive marker of relapse posttransplant, and maintenance therapies aiming for IL-15 induction have been established in the clinical routine for certain AML subgroups.[126,127] Further metabolites such as indoleamine 2,3-dioxygenase-1 (IDO1) or amino acid arginase (Arg) expressed by leukemic blasts or immunoregulatory DC, respectively, significantly interfere with immunomodulatory pathways within the niche. This comprises inhibition of T-cell activity and function while inducing Treg expansion (IDO1), causing further effector T-cell anergy, or stimulating phenotypic changes of macrophage populations from proinflammatory M1 to immunosuppressive M2 phenotype (Arg).[128,129] In addition, extracellular adenosine generated by ectonucleotidases CD39 and CD73 expressed on leukemic blasts bind to adenosine receptor A_{2A} (A_{2A}AR) and thereby promote suppression of anti-leukemic T- and NK cell function.[130–132] Recently, murine models explored the interdependency between leukemia-derived lactic acid and hampered T-cell glycolysis and IFN-γ production; surprisingly the detrimental effect of lactic acid was reversed by sodium bicarbonate, reestablishing GvL in human and murine T-cells and restoring IFN-γ production.[133]

Leukemia-Specific Characteristics and Immune Evasion

Although the majority of AML relapse after alloHCT occurs within the first 12 months, approximately 15% of patients present with a late relapse.[134] Favoring escape

from GvL as an evolutionary process, gain of adverse-risk mutations, development of immune resistance mechanisms, expression of inhibitory ligands, or downregulation of target molecules are found frequently during leukemic relapse. Interestingly, a considerable number of AML patients acquire immune evasion mechanisms during leukemic evolution, which further highlights the importance of GvL for tumor control.

HLA Loss

The abrogation of T-cell mediated anti-leukemic interactions by alterations in the expression and functionality of HLA classes I and II in hematologic diseases after alloHCT has revolutionized our knowledge of relapse characteristics. In 2009, Vago et al demonstrated an acquired uniparental disomy (aUPD) of chromosome 6p by copy-neutral loss of heterozygosity in the HLA locus (HLA loss) after haploHCT in one-third of relapsed cases.[135] For the MMUD setting, HLA loss is observed in about 5–25% of cases; after MUD transplantation, HLA loss has been detected in approximately 15% of relapsed cases, and reports have demonstrated cases of HLA loss after MSD transplantation.[134,136,137] By contrast, at the time of AML diagnosis, this phenomenon is very uncommon.[138,139] Compared with "classic" AML relapses, HLA loss relapse occurs significantly later and is associated with active disease at transplant, which stresses the importance of low or negative MRD before alloHCT.[140] Moreover, HLA loss appears to occur less often in T-cell depleted haploidentical grafts, and larger studies need to investigate this aspect. Further analysis should also focus on the question whether certain HLA loci are more prone to HLA loss than others.

Through fundamental genomic loss of the mismatched patient-specific HLA haplotype and its replacement with the homologous copy, leukemic blasts lose their immunodominant and crucial GvL target, escaping immunologic pressure and becoming "invisible" for alloreactive T-cells. As a "proof of principle", the application of DLIs becomes ineffective while preserving their risk of GvHD. From a translational view, the inability of some NK cells to recognize HLA loss despite the frequent loss of HLA alleles that also represent inhibitory KIR ligands, is still undergoing intensive research.[141] Second allogeneic transplantation with a donor favorably expressing the complementary haplotype and therefore possibly intensifying GvL, adoptive and designed NK-based treatments or non-HLA restricted immunotherapies such as CAR-T-cells or bispecific antibodies redirecting T-cell and/or NK cell response (eg, CD3 for T-cells and CD16 for NK cells targeting leukemic CD33) are possible salvage options in case of HLA loss relapse.[142–144]

Another HLA alteration by epigenetic downregulation of HLA class II molecules through a downregulated major histocompatibility class II transactivator (CIITA) has been identified, translating into a lack of recognition of affected leukemic blasts by CD4+ T-cells and therefore compromising GvL. Likewise, higher counts of T-cells infused with the graft were associated with a higher chance of HLA II downregulation, though the frequencies of HLA II downregulation were similar in matched and unmatched donors, suggesting that alloreactivity is directed mostly against other antigens (eg, mHAgs).[145,146] High levels of IFN-γ induced by T-cell cross-recognition restituted expression of downregulated HLA class II molecules and eradicated relapse by reestablishing GvL, therefore making IFN-γ an attractive object of future studies and therapeutic approaches and implying a beneficial aspect of a proinflammatory environment as seen in GvHD.[145] Still, the side effects of proinflammatory molecules secreted in the leukemic microenvironment, such as IFN-γ, which is also a potential driver for upregulation of inhibitory ligands like programmed death-1 ligand (PD-L1) and thus favoring immune escape, need to be considered.

Immune Checkpoint Alterations

In addition to HLA-dependent immune evasion mechanisms, multiple inhibitor molecules impairing T-cell responses after alloHCT have been revealed over the last years. Recently, inhibitory ligands have been found to be increasingly expressed in up to 40% of relapse cases.[145] The interaction of T-cell inhibitory ligands such as PD-L1 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) with the respective TCR effectively abrogates the cytotoxic function and proliferation of alloreactive T-cells, thus mitigating or nullifying GvL and finally generating T-cell exhaustion.[147] New immunotherapeutic methods are needed to conquer these highly aggressive immune escape mechanisms. For example, the use of monoclonal PD-L1 antibodies such as nivolumab for leukemic blasts overexpressing PD-L1 or monoclonal antibodies like ipilimumab targeting the CTLA-4/B7 axis partially restored anti-leukemic T-cell functions.[148–150] However, inhibitory ligand expression is characterized by a high inter-patient variability, and shared regulatory pathways should be

identified and therapeutically addressed. Also, the development of acute GvHD during immune checkpoint inhibitor therapy should be taken seriously and monitored precisely.[151] Furthermore, combinatory therapies including HMAs and immune checkpoint blockade deliver promising results.[152,153] Ongoing studies (eg, NCT02890329, NCT02845297) are investigating the combination of immune checkpoint inhibitors and HMAs in the posttransplant setting. These observations might translate into clinical practice to either prevent imminent relapse or to offer potent relapse strategies, as uncontrolled r/r AML is associated with a very poor prognosis and a short survival.

Tumor-Specific Antigens (TSAs)

So far, AML patients have not benefited from recent achievements in immunotherapeutic approaches compared with patients having solid cancer. This is mostly due to the lack of targetable leukemic-specific immunogenic ligands. To date, the frequency of newly acquired inhibitory molecules is also difficult to reveal because of the complex expression patterns and the generally low expression at baseline.[154] Lately, encouraging studies investigating the proteogenomic features of HLA class I molecules of AML patients have shown that leukemic blasts and stem cells express TSAs. These antigens represent strong targets for GvL, as they demonstrated elicitation of CD8+ T-cells in vivo and in vitro and improved survival. Astonishingly, TSA are mostly seen in intronic and therefore non-coding genomic regions and may be intrinsic predictors for sufficient GvL. Intron retention and epigenetic modulations are supposed to be essential for TSA biology, as epigenetic modifiers such as *DNMT3A* mutations correlated with TSA RNA expression.[155] These data stimulate further detailed analysis of immunopeptidomes in AML patients and may offer chances to break new grounds in AML immunotherapy.

Maintenance Therapy for AML After alloHCT

Disease relapse occurs in approximately 40% of patients with AML after transplantation and is the main cause of death.[11] The success of salvage treatments is aggravated by the fact that AML is a biologically heterogenous and aggressive disease and presents with various relapse mechanisms.[156] Considering the very poor prognosis in relapse cases, preventive strategies are highly desirable to improve outcome. Maintenance therapies have therefore become a popular means to maintain disease control or prolong DFS. Targeted therapies with regard to the clonal landscape of leukemic stem cells have been a clinically favored option for patients with AML harboring molecular targets. Tyrosine kinase receptor inhibitors are probably the most established form of targeted therapy in AML and other malignant entities.[157,158] One important representative of alterations in tyrosine kinase receptor genes are Fms-related tyrosine kinase 3 (*FLT3*) mutations, which are frequently mutated in AML and simultaneously define worse prognosis because of frequent relapse.[159] Therefore, multiple inhibitory molecules for *FLT3*-internal tandem duplication (ITD) and *FLT3*-tyrosine kinase domain (TKD) have been developed and successfully implemented in daily practice.[160–162] Sorafenib plays a pivotal role in the maintenance of *FLT3*-ITD-mutated AML after alloHCT, demonstrating improved outcomes after alloHCT based on a significantly reduced risk of relapse.[127] Additional immune-mediated efficacy of sorafenib, as well as tyrosine kinase inhibitors tandutinib, midostaurin, crenolanib, and quizartinib has been attributed to enhanced IL-15 transcription in *FLT3*-ITD-positive leukemic clones, leading to increased anti-leukemic CD8+CD107a+IFN-γ+ T-cell counts and consequently intensified GvL effect.[118] NK cells are another cellular profiteer of elevated IL-15 levels, increasing their anti-leukemic activity and equipping sorafenib a broader spectrum of action than just solely targeting *FLT3*-ITD mutations. Further results of studies investigating the potential of gilteritinib as *FLT3*-ITD inhibitor maintenance after alloHCT are eagerly awaited and could add further therapeutic players for GvL (NCT02997202).

Regarding other molecular AML entities (eg, *IDH1, IDH2, RUNX1*, and *EZH2*) and, in times of high-throughput panel sequencing, other emerging targetable structures (eg, *CDK9*, and *BTK*), respectively, innovative therapeutic approaches are gaining increasing clinical interest and scientific focus, either as a single agent or as combinational therapy. However, most of them are investigated in preclinical models and have not been applied in clinical posttransplant settings yet.[163–167]

Pharmacological strategies modifying GvL after transplant have been introduced by implementation of HMAs like azacitidine or decitabine. In addition to the inhibition of DNA methyltransferases, these agents upregulate leukemia-associated antigens (eg, PRAME and MAGE-A), mHAgs, HLA class I and II molecules, costimulatory molecules such as ULBPs and MIC A and an intensified

CD8+ T-cell response, hereby augmenting GvL.[143,168–170] Furthermore, azacitidine stimulates the conversion of CD4+CD25- T-cells into Tregs by inducing FOXP3 expression both in vitro and in vivo and expand the total number of Tregs.[170,171] By contrast, HMA-associated upregulation of inhibitory molecules on the leukemic surface and therefore inhibition of GvL are raising the question of combined approaches in the case of relapse.[172]

Conclusions

Grand strides in performing alloHCT have enabled physicians worldwide to offer alloHCT for nearly all eligible AML patients. However, enhancing an enduring GvL effect to efficiently control AML relapse is still an immunomodulatory and therapeutic challenge in the context of therapy-related toxicity and GvHD. Based on the compiled knowledge obtained in numerous trials in the last decades, MAC regimens should be applied for high-risk AMLs and MRD positivity whenever possible. If RIC is required, PBSC grafts are recommended over BMSC grafts because of longer OS and lower risk of relapse.

Donor age becomes an increasingly significant characteristic in the process of donor selection. HLA disparity is still a key selection criterion, but new knowledge about the effect of small nucleotide variants, HLA subgroups, and NK cells/KIRs will broaden our perspective and influence our decision for choosing "the donor". T-cells and their respective sub-entities play the main part for therapy success or failure. New therapeutic approaches, such as NK cell infusion during transplant, or immunomodulatory molecules have to be defined and evaluated in prospective trials. The role of PtCy beyond the haploidentical donor setting and its combination with RIC protocols will be one of the most attractive foci of future clinical trials. The underlying mechanisms differentiating alloreactive processes and processes inducing tolerance are still insufficiently understood and need to be intensively investigated. Deciphering AML relapse mechanisms provides an opportunity to develop and utilize targeted therapies either in the induction or maintenance therapy and thus to either achieve MRD negativity before transplant or to augment GvL by lowering the leukemic burden. Regardless which component will be the most effective to induce and maintain GvL, GvL is the key tool to achieve long-term survival and cure for patients with AML.

Disclosure

The authors report no conflicts of interest for this work.

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