Donor lymphocyte infusion after allogeneic haematopoietic cell transplantation for haematological malignancies: basic considerations and best practice recommendations from the EBMT

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Lancet Haematol 2024; 11: e448–58

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Since the early description of three patients with relapsed leukaemia after allogeneic haematopoietic cell transplantation (HCT) who obtained complete remission after donor lymphocyte infusions (DLIs), the added value of this procedure to induce or maintain graft-versus-leukaemia immunity has been undisputed. For more than 30 years, DLIs have become common practice as prophylactic, pre-emptive, or therapeutic immunotherapy. However, as with many aspects of allogeneic HCT, centres have developed their own routines and practices, and many questions related to the optimal applications and toxicity, or to the immunobiology of DLI induced tumour-immunity, remain. As a part of the Practice Harmonization and Guidelines Committee and the Cellular Therapy and Immunobiology Working Party of the European Society for Blood and Marrow Transplantation effort, a panel of experts with clinical and translational knowledge in transplantation immunology and cellular therapy met during a 2-day workshop in September, 2023, in Lille, France, and developed a set of consensus-based recommendations for the application of unmanipulated DLI after allogeneic HCT for haematological malignancies. Given the absence of prospective data in the majority of publications, these recommendations are mostly based on retrospective studies and expert consensus.

Introduction

Allogeneic haematopoietic cell transplantation (HCT), the most paradigmatic form of cellular immunotherapy, is currently the only curative treatment for many haematological disorders.[1] The graft-versus-malignancy effect is the alloreactive response ensuring disease control via the recognition of tumour-associated antigens by donor-derived T-cell effectors, and guarantees the success of allogeneic HCT procedures.[2] However, the discrimination of allogeneic HCT from graft-versus-host disease (GVHD)—mainly related to a deleterious attack of the recipient's healthy tissues by the donor's adaptive immune system—remains elusive, and transplantation procedures are still hampered by notable rates of morbidity and mortality associated with GVHD and disease recurrence.[2,3] The infusion of lymphocytes and other immune effector cells from the original donor, after establishing hematopoietic donor chimerism, has been implemented as immunomodulatory strategy capable of restoring or boosting the therapeutic index of allogeneic HCT, improving graft-versus-malignancy effect and immune surveillance (ie, the control that the immune system enacts on residual malignant cells), although with a theoretical increase of GVHD risk.

Since the early description of the added value of donor lymphocyte infusions (DLIs) after allogeneic HCT in 1990,[4] many revised summary papers have been published by different generations of physicians;[5,6] we identified approximately 20 reviews per year since 1999. However, despite the routine use of DLIs at allogeneic HCT centres worldwide, no true consensus has been defined concerning indications, prerequisites, and application details. Recently, the European Society for Blood and Marrow Transplantation (EBMT) has attempted to provide guidance, in the 2024 version of the *EBMT Handbook*, on topics such as fresh versus frozen DLI, DLI generated during the stem cell harvesting process or during a separate procedure, and dosage and timing of DLIs.[7] However, a recent retrospective study on the use of DLI after haploidentical allogeneic HCT still indicates broad variation in centre practices.[8] This heterogeneity raises questions about the effect of the vast number of consensus papers on daily clinical practice. Therefore, in addition to revising the existing handbook's advice on DLI management in 2024, and conducting an extensive literature review,[6] we also engaged in a consensus-driven discussion with the experts attending the workshop to deliberate on and establish best practice recommendations on behalf of the EBMT.[9] During these debates, we delved deeply into the biological principles of, and open clinical questions about, DLI use. Hence, in this Review, we provide a structured overview of centre clinical practices, capture real-world data, and encourage a more data-driven approach to decision-making processes regarding the administration of unmanipulated DLI after allogeneic HCT for haematological malignancies.

Methods

These consensus recommendations were developed by an international panel of experts during a 2-day

DLI Harmonisation Workshop held in September, 2023, in Lille, France. This Review was generated dring the workshop according to the method published by the EBMT Practice Harmonization and Guidelines Committee.[9] Workshop members were a diverse group of experts invited by the Cellular Therapy and Immunobiology Working Party of the EBMT, with clinical and translational expertise in tumour immunology, transplantation immunology, and cellular therapy, which allowed a broad reflection of the biological basics necessary for making clinical decisions on DLI use. A comprehensive literature review was carried out by the workshop participants, serving as the basis for the subsequent discussions.

Because this literature search yielded, among prospective studies, mostly trials investigating immune subset-modified cell therapies, rather than unmanipulated DLIs, guidance was primarily based on retrospective analyses, collections of common practice data, and expert opinions of the committee members.[6,7] Given the scarcity of high-quality evidence from randomised trials in the area of unmanipulated DLIs, recommendations were not graded.

Current state of the art

Composition of DLIs

Since its first use,[4] the infusion of lymphocytes derived from a stem-cell donor was conceived as an immunotherapeutic strategy able to reinforce post-transplantation graft-versus-leukaemia effects. A less frequently used application of DLI is to restore anti-infectious control by providing a pool of immune effectors ready to operate different types of immune responses.[6] The standard of care for this post-HCT adoptive immune strategy is the infusion of unmanipulated donor-derived lymphocytes. The DLI product typically consists of T cells (80–90% of the entire product), B cells (~5%), and natural killer (NK) cells (5–20%). Among T cells, the αβ T-cell effector subset is the most represented, accounting for 90% of the T-cell repertoire, followed by γδ T cells (5–10%), αβ T regulatory cells (5%) and natural killer T cells (NKT cells; <1%).[6] The variation in proportions depends on the donor's immune status, the use of G-CSF before cell collection, and the conservation status of the DLI product.

Graft-versus-malignancy immunity

Over the years it has become clear that any of the lymphocyte subsets present in allograft and DLI products can contribute to graft-versus-leukaemia immunity or graft-versus-lymphoma immunity (figure 1). In patients with relapsed chronic myeloid leukaemia, DLI-induced complete remission occurred at the same time leukaemia-directed antibodies appeared in the serum.[10] In patients with high-risk acute myeloid leukaemia or relapsed myelodysplastic syndrome, durable humoral graft-versus-leukaemia responses were observed, directed against membrane-expressed tumour-specific antigens.[11,12] Major targets of T-cell alloreactivity are considered to be the minor histocompatibility antigens, either restricted to haematopoietic stem cells (theoretically targets of graft-versus-leukaemia) or ubiquitously expressed (possibly enhancing graft-versus-host disease); mismatched HLA; neoantigens derived from mutated proteins; butyrophilins and butyrophilin-like proteins, participating in the interactions with γδ T cells; and antigens derived from wild-type proteins with abundant expression in malignant cells (eg, WT1).[2,6] Specificity and diversity of T-cell receptor repertoires, particularly in the αβ subset, are supposed to be major molecular features of alloreactivity, driving both graft-versus-leukaemia and GVHD. Specifically, recent evidence highlights that patients responding to DLI harbour higher T-cell heterogeneity compared with non-responders, reinforcing the importance of having diversified phenotypes to induce an efficient antileukaemic action.[4] Patients who have graft-versus-leukaemia without GVHD have shown lower molecular diversity of their CD8 T-cell receptor repertoires than patients who develop GVHD.[13,14] These findings underline the importance of having competent, diverse, and specific T-cell repertoires to

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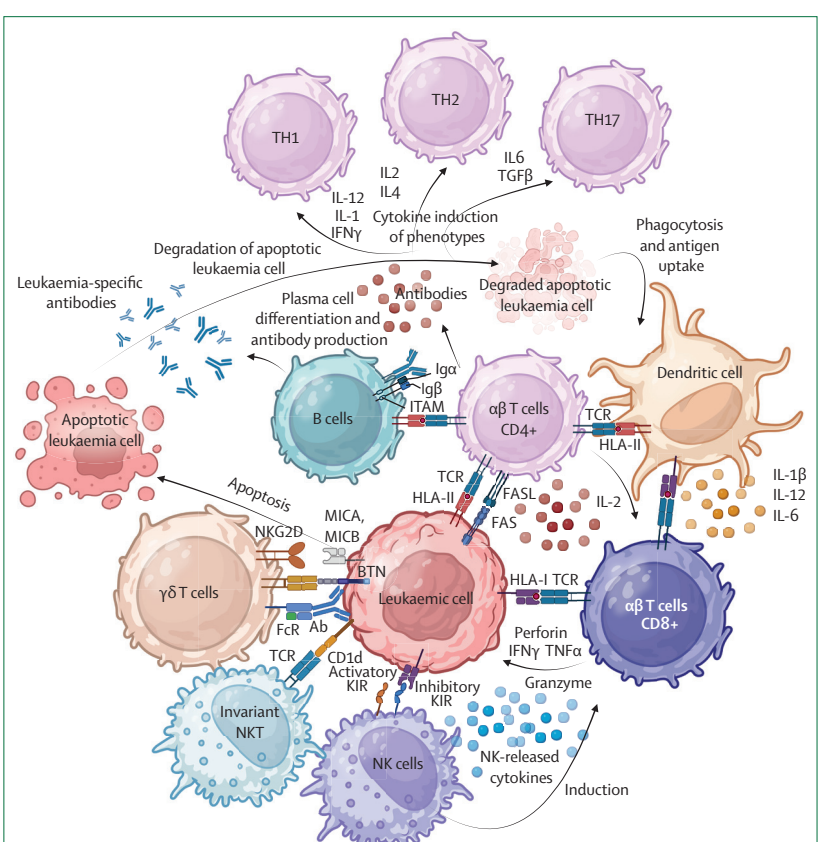

Figure 1: **Graft-versus-tumour immunity**
Immunobiology of graft-versus-tumour or leukaemia effect, describing the known immune effectors and molecular patterns able to induce an alloreactive response against tumour cells or leukaemic blasts. NK cells=natural killer cells. NKT=natural killer T cells. TCR=T-cell receptor. TGF=tumour growth factor. TH=T helper cell.

enhance the efficacy of alloreactive T-cell-mediated responses. Among other cell types, CD8+ terminally differentiated effector memory cells have been shown to develop clonal expansion and tumour-specific activation in patients with acute myeloid leukaemia responding to DLI, supporting antigen specificity in graft-versus-leukaemia effect.[15]

Analysis of DLI cell products reveals a large heterogeneity in the proportion of naive and antigen-experienced T cells, which might have an effect on disease control. DLI cell products administered for acute myeloid leukaemia relapse, and containing low levels of effector memory cells and high amounts of naive T cells, were associated with long-term remission.[16]

γδ T cells show anti-cancer activity in both solid and haematological malignancies.[17,18] These cells interact with stress-induced molecules expressed by cancer cells or with mismatched HLA molecules.[19] As γδ T cells act independently of HLA haplotypes, they are considered to drive graft-versus-leukaemia responses without causing GVHD.[20] Similarly, invariant NKT cells express an invariant T-cell receptor (Vα24-Jα18 and Vβ11) and recognise glycolipidic antigens (such as α-galactosylceramide) via non-polymorphic class I-like HLA molecules (eg, CD1d).[21] High invariant NKT-cell numbers in stem-cell grafts have been associated with a reduced incidence of GVHD, and improved GVHD-free and progression-free survival.[22,23] Preclinical studies have shown the capacity of these effectors to kill acute myeloid leukaemia blasts.[24,25] NK cells identify self-peptides derived from classical and non-classical HLA, adhesion molecules, and other structures, via activating or inhibitory killer-cell immunoglobulin-like receptors (KIRs).[26] Specifically, this subset of NK cells recognises specific molecular configurations characterising transformed cells, such as the downmodulation of HLA class I molecules, thereby sparing healthy tissues.[27] NK cell function is particularly important in the context of haploidentical settings, whereby KIR ligand mismatches between donor and recipient might trigger beneficial alloreactive responses. The presence of some donor inhibitory KIRs that are known to interact with HLA molecules can provide protection against leukaemia relapse following HLA-matched unrelated donor HCT for acute myeloid leukaemia. This observation suggests that the selection of donors with certain KIR genes (such as *2DL5A*, *2DS1*, and *3DS1*) can be associated with a reduced rate of leukaemia relapse, emphasising the potential of using NK-cell alloreactivity as a therapeutic strategy in the context of allogeneic HCT.[27,28]

NK cells, NKT cells, and γδ T cells show cytotoxic activity via granzyme and perforin release. The production of cytokines (such as IFN-γ, TNF-α, and IL-4) upon stimulation enables these cells to interact with other immune effectors (T cells, B cells, NK cells, monocytes, and granulocytes) at tumour sites.[17,29]

Mechanisms of immune escape

As a reflex of the unique biology of post-transplantation relapses, graft-versus-leukaemia and DLI efficacy can be impaired by many immune evasion mechanisms, ranging from the molecular dysfunction of the antigen presentation machinery, to variegated patterns of tumour-induced T-cell exhaustion, or decrease in tumour-specific antibodies, enabling the development of immune-resistant disease phenotypes (figure 2).[30,31] In particular, the loss of expression of HLA molecules, either through genomic or epigenetic mechanisms, has been acknowledged as a frequent aberration in leukaemic relapses. Loss of HLA heterozygosity, loss of HLA haplotypes, and mutations or small deletions in specific class I and class II HLA alleles, have all been identified as mechanisms of tumour escape, occurring in both matched and mismatched settings.[32–34] Transcriptional downmodulation, possibly because of epigenetic reprogramming, has decreased the intensity of class II-related presentation (and in particular of DRB1 alleles), highlighting the importance of CD4+-mediated immune pressure.[34,35] Although baseline HLA expression is determined by molecular disease subtype (eg, NPM1 mutant leukaemias have low HLA-DR expression),[36–38] changes in HLA expression in post-transplantation acute myeloid leukaemia relapses have been observed across disease subtypes.[34,35] In a recent study, epigenetic silencing of class II HLA was shown to be regulated by polycomb repressive complex 2, the selective inhibition of which was able to restore HLA class II expression and antigen presentation to alloreactive CD4 T cells.[39] The transcription of other non-HLA immune-related genes (ie, IFN-γ response pathway and other genes intervening in the antigen presentation machinery) has been shown to be impaired in cases of post-transplantation relapse of acute myeloid leukaemia.[34] Finally, acute myeloid leukaemia cells have the capacity to rapidly develop an array of inhibitory signals, such as membrane expressed inhibitory ligands and checkpoint inhibitors or soluble factors (eg, aryl hydrocarbon receptor agonists), leading to escape from NK or CD8+ T-cell-mediated tumour control.[40–43]

Manipulation of DLIs to selectively enhance graft-versus-leukaemia effects

Early after introduction of DLI as means to cure relapsed leukaemia, reports on the manipulation of DLIs to selectively enhance graft-versus-leukaemia immunity without inducing GHVD appeared. It became clear, for example, that the enrichment in CD4+ T cells in the product could mitigate the risk of GVHD,[44] and relapsed chronic myeloid leukaemia could potentially be cured by DLIs that were CD8+ T-cell depleted.[45] More recent efforts in the last two decades have focused on the enrichment or the depletion of other cell subsets in DLI products.[46] For example, invariant NKT-cell expansion from cryopreserved DLIs has been proposed

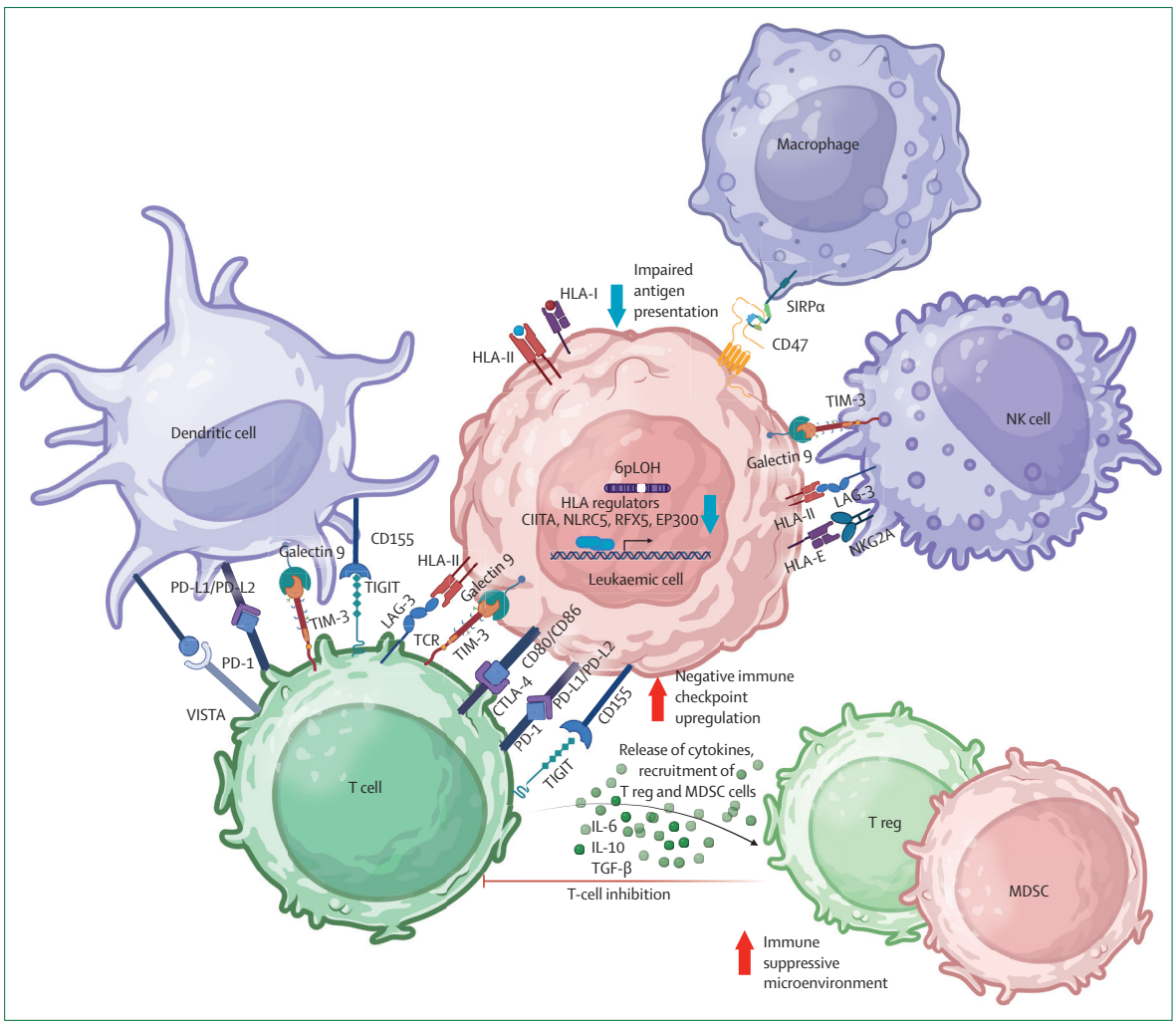

Figure 2: **Principles of immune escape**
The possible known mechanisms of immune evasion of leukaemic blasts from the graft-versus-leukaemia effect are shown. These mechanisms have been mostly acknowledged in acute myeloid leukaemia; however, their recurrence in other disease settings at the moment of post-transplantation relapse is not excluded.[47] 6pLOH=loss of heterozigosity on chromosome 6p. MDSC=myeloid-derived suppressor cells. NK cell=natural killer cell. T reg=T regulatory cells.

as a strategy to enhance antileukaemic control, overcoming GVHD risk.[24] However, simple DLI dosing, on the basis of the number of CD3 T cells in the product is, so far, the only strategy that has been broadly established across transplantation centres.

Workshop recommendations

Indications for DLI

At present, there exist three distinguished indications for DLI (panel 1). Prophylactic DLIs are used as maintenance therapy for prevention of post-allogeneic HCT relapse, and are applied to patients without evidence of the underlying disease, who are estimated to bear a high risk of disease recurrence, based on one of three factors: high-risk disease characteristics (eg, unfavourable genetics or secondary disease);[48] transplantation in refractory or advanced disease; and use of ex-vivo lymphocyte depletion as GVHD prophylaxis. Prophylactic DLIs can be considered in the case of non-myeloablative conditioning or in the absence of druggable molecular targets, irrespective of the conditioning regimen.

The term pre-emptive DLI is used in patients in haematological disease remission, but with incomplete or decreasing donor chimerism, or with detectable disease at a very low level, either as measurable residual disease or when the first signs of subclinical relapse are observed (eg, molecular or cytogenetic relapse), or detection of recurrent disease by flow cytometry.

Therapeutic DLIs are given as part of the management of overt haematological relapse or graft failure, preferably after disease control has been obtained by systemic therapies (ie, chemotherapy, hypomethylating agents, or targeted therapies).

Currently, we are conducting a survey, on behalf of EBMT, to track European practices of DLI management. Preliminary analyses show that more than

Panel 1: **Indications for donor lymphocyte infusion (DLI)**

Prophylactic DLI[49,50]

Common practice

- High-risk disease (as defined by the European Leukaemia Net 2022 criteria)
- Transplantation in advanced or refractory disease
- Ex-vivo lymphodepleted allogeneic haematopoietic cell transplantation

Can be considered

- Non-myeloablative conditioning*
- Absence of druggable targets (ie, FLT3)*

Pre-emptive DLI[51,52]

Common practice

- Mixed chimerism
- Persistent minimal residual disease
- Molecular or cytogenetic relapse

Can be considered

- Infections

Therapeutic DLI[4,44,46,53]

Common practice

- Haematological relapse
- Extramedullary relapse

Can be considered

- Post-transplantation lymphoproliferative disease†

*Data to substantiate recommendations are currently scarce. †Given the shortage of supporting studies for the use of the DLI in post-transplantation lymphoproliferative disease, and the current development of other forms of antiviral cellular therapies, we seek to acknowledge the possibility for selected cases of Epstein–Barr virus-related lymphoproliferative disorders to use third party allogeneic anti-EBV cytotoxic T lymphocytes.

114 (97%) of 117 EBMT centres that responded are using DLI as part of their cellular therapy programmes, with prophylactic, pre-emptive and therapeutic approaches in 47% (n=53), 84% (n=96), and 77% (n=88) of these cases, respectively (unpublished).

As a means to improve immunological reconstitution and diversity of immune effector responses, DLIs can also be given to prevent recurrent infections in patients with delayed immune recovery, and to treat post-transplantation lymphoproliferative disorders. However, there is a paucity of data on indication, dosing, timing, and efficacy, making it impossible to define clear recommendations for provision of DLIs in this setting. In these situations, given the development of other forms of cellular therapies, third party antiviral lymphocytes can be considered a possible treatment option.

Due to the variability of applied techniques with different sensitivities for measurement of chimerism and for monitoring of residual disease or incipient relapse, published data on prophylactic and pre-emptive DLIs bear considerable uncertainty in regard to their efficacy.

Practical aspects of unmanipulated DLIs

Due to the variability of types of DLI, practical recommendations can only relate to the most frequently used application (ie, the transfusion of unmanipulated donor lymphocytes). Even among studies reporting on this kind of therapy, a certain heterogenicity exists. DLIs can be obtained from the original transplant, where cells have been exposed to G-CSF and cryopreserved at the time of stem-cell harvest. Alternatively, donor lymphocytes can be obtained by a second apheresis procedure, which involves gathering peripheral blood mononuclear cells without any stimulation. This process is typically carried out as needed, shortly after the initial transplantation, and does not use granulocyte G-CSF to mobilise the cells. Usually, the first DLI dose is administered as a fresh dose immediately after apheresis, whereas the majority of collected cells are cryopreserved in different portions of either equal or escalating doses. Available data do not suggest substantial differences in outcomes between either of these two approaches.[53,54]

For all indications, the balance between efficacy of DLI against the underlying disease and the risk of side effects, especially GVHD, requires particular attention. The risk of post-DLI GVHD is mainly influenced by donor type and HLA matching, cell dosage, timing and frequency of DLIs, previous history of GVHD, and whether immunosuppressive medication is still required at the time of DLI. These factors are acknowledged in the generally accepted prerequisites for conducting DLIs, and in the guidelines regarding the appropriate cell doses, the timing between transplantation and first DLI adminstration, and the interval between subsequent DLI adminstrations. Different regimens are used depending on the different strength of anti-leukaemic efficacy required in the prophylactic, pre-emptive, or therapeutic indications (table 1).

On the basis of the most frequently used strategies, we provide recommendations for DLI prerequisites (panel 2).

In clinical practice, these recommendations might be considered on an individual basis, considering individual risk, type of donor, graft source, GVHD prophylaxis, and conditioning regimens.

After DLI, close clinical and laboratory monitoring in the transplantation outpatient clinic is mandatory, considering all possible manifestations of GVHD and other side-effects. In the absence of prospective data, we recommend weekly or fortnightly monitoring (depending on the patient's awareness of signs and symptoms of toxicity) of complete blood counts and biochemistry, including liver function tests. In the case of

sequential DLIs, the aforementioned prerequisites should be carefully re-evaluated, and DLI administration promptly postponed or cancelled if any alteration is observed. Furthermore, in patients who develop GVHD, periodic monitoring (ideally every 3 months or 6 months) of lung function tests to exclude lung GVHD should be performed, even in asymptomatic patients.

As about 30% of acute myeloid leukaemia relapses are associated with loss of HLA expression,[55] we recommend performing molecular characterisation of HLA on relapsed acute myeloid leukaemia, at least in the context of haploidentical and mismatched unrelated allogeneic HCT, whenever possible. This recommendation acknowledges the absence of standardised immunogenetic procedures, with many laboratories using in-house developed bioanalytical pipelines. The presence of such molecular features should discourage the administration of pre-emptive or therapeutic DLI, given their ineffectiveness in HLA-negative relapses, and the potential and unnecessary risk of GVHD.

Disease-specific considerations, and possible combination of DLI with medical treatments

In general, DLIs can be applied either in combination or in sequence with medical treatment to increase their efficacy against the underlying malignancy (table 2). However, identifying the place of DLI in the management of different diseases is not a uniform process. Particular attention must be paid to the sensitivity of the underlying disease to the allogeneic graft-versus-malignancy effect.[6,56] The 2010 workshop on relapse after allogeneic HCT, organised by the National Cancer Institute, put forth a comprehensive evaluation of the sensitivity of various diseases to DLI.[57] The findings show that sensitivity levels vary: chronic myeloid leukaemia, myelofibrosis, and low-grade non-Hodgkin lymphoma and multiple myeloma show notably high sensitivity to DLI; chronic lymphocytic leukaemia, acute myeloid leukaemia, myelodysplastic syndrome, and Hodgkin lymphoma are considered to have intermediate sensitivity; and acute lymphoblastic leukaemia and diffuse large B-cell lymphoma, are considered to have a lower sensitivity to this therapeutic approach. This difference in sensitivity, underlying the success of allogeneic HCT and DLI outcomes, is probably equally related to tumour immunogenicity, tumour growth (with higher burden in rapidly proliferative disorders), and the propensity to develop immune escape features. Awareness of differences in sensitivity could help tailor treatment strategies to the specific needs and characteristics of each disease, avoiding the toxicity of the treatment itself.

In patients with overt haematological relapse, DLIs are usually applied after initial control of the malignancy by chemotherapy, disease-specific drugs, or immunotherapy (eg, bispecific antibody therapy, chimeric antigen receptor [CAR] T cells). This order of application is particularly important in rapidly progressive diseases, such as acute leukaemia. In patients receiving prophylactic or therapeutic DLI, the combination with disease-specific drugs has been studied. However, it must be stressed that almost none of the drugs discussed here are approved for use in combination with DLI. In particular, the immune-modulating drug lenalidomide[58] and checkpoint inhibitors (eg, nivolumab, pembrolizumab, and ipilimumab) have been associated with the development of severe GVHD, and should, therefore, be avoided in combination with DLI.[59,60] When given in combination with other drugs, the indication, dosage, and timing of DLIs might need to be adjusted when an increased risk of GVHD is expected.

Beyond the direct cytotoxic effects on the malignancy, some of the disease-specific drugs available have additional properties that increase the therapeutic potential of DLI. The FLT3-inhibitor sorafenib has shown synergistic effects with DLI, by restoring IL-15 production that is downregulated in acute myeloid leukaemia blasts.[61] Similarly, treatment with the hypomethylating agent 5-azacitidine is able to upregulate tumour-associated antigens.[62] Clinically, in patients with acute myeloid leukaemia, response to 5-azacitidine was associated with with the expansion of specific $CD8^+$ cytotoxic T cell clones against leukaemia-associated antigens.[62] However, other studies have reported upregulation of inhibitory immune checkpoints under hypomethylating agent treatment, which has been associated with inferior outcomes in both

	Recommended DLI dose for matched related donor	Recommended DLI dose for matched unrelated donor	Recommended DLI dose for mismatched unrelated donor or haploidentical donor	Number of DLIs
Prophylactic[48–50]				
3 months (ex-vivo TCD: any risk; no ex-vivo TCD: high risk or refractory disease)	1×10^5 cells/kg	1×10^5 cells/kg	1×10^5 cells/kg*	1–3†
6 months‡	1×10^6 cells/kg	1×10^6 cells/kg	5×10^5 cells/kg	1–3†
Pre-emptive[51,52]				
3 months	$1–5\times10^5$ cells/kg	1×10^5 cells/kg	1×10^5 cells/kg	1–4§
6 months‡	$1–3\times10^6$ cells/kg	1×10^6 cells/kg	5×10^5 cells/kg	1–4§
Therapeutic[4,44,46,53]				
After systemic therapy¶	1×10^7 cells/kg	1×10^7 cells/kg	1×10^6 cells/kg	1–4‖

The interval between two consecutive DLIs in prophylactic settings should be at least 6 weeks, whereas in pre-emptive or therapeutic settings the delay can be shorter (usually 4 weeks is accepted). DLI=donor lymphocyte infusion. GVHD=graft-versus-host disease. HCT=haematopoietic cell transplantation. TCD=T-cell depletion. *In clinical practice in a mismatched donor scenario, prophylactic DLI infusion at 3 months remains challenging for the risk of GVHD and the need for longer immunosuppression requirement; this practice needs, therefore, to be considered on the basis of patient disease risk profile and clinical conditions. †For second and third dose, increase each DLI dose by 0·5–1 log; both further DLI administration and dose increment should be clinically guided and restricted in patients developing signs of GVHD. ‡Starting at 4 months after allogeneic HCT, using DLI doses in the range used at 3 months or 6 months can be discussed. §The number of DLIs should be guided by minimal residual disease or chimerism, consider continuation until negative minimal residual disease and full chimerism are reached; in the absence of a minimal residual disease marker (including in prophylactic settings), DLI should be guided by GVHD with dose escalation possible in the absence of any GVHD sign. ¶In cases of acute leukaemia, when complete haematological remission has been reached. ‖For the second to fourth doses, increase each DLI dose by 1 log, to a maximum dose of 1×10^8/kg.

Table 1: **Practical aspects of DLI by timing of first dose since allogeneic HCT**

Panel 2: **Recommended pre-requisites for donor lymphocyte infusion (DLI)**

- At least day 90 after transplantation (might be amended on the basis of type of donor, graft source, graft-versus-host disease (GVHD) prophylaxis, and conditioning regimens).
- Absence of infection.
- No requirement of systemic immunosuppressive medications (eg, calcineurine inhibitors or mycophenolate) for 3–6 weeks (this delay can be longer in cases of mismatched donor transplantations). Under certain conditions (eg, unfavourable risk disease profiles, mismatched settings, or centre experience) DLI can be considered earlier than 3–6 weeks after cessation, or even in patients still requiring immunosuppression. An increased risk of GVHD has to be taken into account as a result of shorter delays. On the basis of the immune regulatory effects of G-CSF, mobilised peripheral blood mononuclear cells followed by short-term immunosuppression have been applied by several groups in this situation.[6] However, after deep discussion during our workshop, our conclusion was not to suggest DLI administration under ongoing immunosuppression to avoid counteracting DLI effects.
- Considerations for previous or ongoing GVHD in cases of:
  - Prophylactic DLI: active acute or chronic GVHD is considered an absolute contraindication. A history of cortico-sensitive acute GVHD (grade 2–4), or moderate or severe chronic GVHD, are defined as relative contraindications, depending on the timing of immune suppression withdrawal, the timing of GVHD, and the risk of the underlying malignancy, and should always be discussed on a case-by-case basis.
  - Pre-emptive or therapeutic DLI: although a history of acute GVHD (grade 2–4), or moderate or severe chronic GVHD, do not serve as a contraindication for DLI, it is crucial to carefully assess the benefit–risk balance with the patient. DLI administration must be approached with utmost caution under these circumstances, depending also on the timing of GVHD episodes in relation to relapse and DLI.
- A dose escalating regimen should be used if more than one DLI is planned, as this has been shown to reduce the risk of DLI-induced GVHD (table 1).

transplantation[63] and non-transplantation settings.[64] Hence, the crosstalk between medical and cellular therapy is not well understood, and drugs might have contradictory effects on immune cells with unclear consequences for both efficacy and toxicity of DLIs.

A specific point of debate is whether some of the newer drugs or immunotherapies can replace DLI, particularly in regard to B-cell malignancies and myeloma, where CAR T cells and bispecific antibodies have shown potent results in remission induction post transplantation. It remains to be established whether these therapies should be preferred to DLI or should be considered a bridge to DLI as consolidation therapy. A summary of drugs that have been studied in combination with or as alternative to DLI is provided in table 2, and further details can be found in a recent comprehensive review by Schmid and colleagues.[6]

Early and late toxicity post DLI

The induction of GVHD is the most relevant toxicity of DLI. The risk of post-DLI GVHD is mainly influenced by donor type, cell dosage, timing and frequency of DLIs, previous history of GVHD, and whether immunosuppressive medication is still required at the time of DLI. In a retrospective registry study by the EBMT Acute Leukaemia Working Party, on pre-emptive and prophylactic DLIs in the HLA-matched setting, cumulative incidences of grade 2–4 acute or chronic GVHD were 11·9% (95% CI 8·2–16·3%) for pre-emptive DLIs and 30·7% (24·9–36·6%) for prophylactic DLIs. In this series, 6% of patients died from DLI-induced GVHD. An age of 60 years or older, advanced stage at transplantation, shorter interval from allogeneic HCT, and previous acute GVHD of grade 2 or higher were risk factors for DLI-induced GVHD.[49] In another study, high-intensity DLI (defined by higher T-cell dose or earlier application than recommended by international guidelines) was a strong risk factor for acute GVHD.[65] In the haploidentical setting, data from the EBMT registry revealed cumulative incidences (CIs) for grade 2–4 acute GVHD and chronic GVHD of 17% (95% CI 7–27%) and 53% (40–67%) for the prophylactic DLI group, 20% (2–38%) and 21% (3–39%) for the pre-emptive DLI group, and 17% (9–24%) and 24% (15–33%) for the therapeutic DLI group, respectively.[8] However, despite the considerable risk of GVHD caused by DLI, various studies—primarily assessing the use of prophylactic DLIs—have shown the clinical value of this approach while demonstrating acceptable toxicity and reduced relapse rates compared with matched control groups.[50,66,67]

Haematological toxicity related to DLI is more debated. It could be difficult to disentangle from other complications (eg, viral infections, drug induced cytopenias, disease recurrence, or clonal evolution), especially in the therapeutic setting, and in particular when DLIs are combined with other systemic therapy (eg, hypomethylating agents or tyrosine kinase inhibitors). This type of toxicity is poorly codified in retrospective studies, possibly due to the difficulties in tracking these events in post-transplantation contexts, making it impossible to define the incidence and characteristics of these events. That said, we suggest all types of cytopenia appearing from 1 day to 30 days after DLI are considered DLI related, but only after excluding all other possible causes.

	General sensitivity to DLI*	Drugs that are used for remission induction or maintenance	Remarks
Myeloid malignancies			
Acute myeloid leukaemia	Intermediate	Cytarabine, sorafenib, midostaurin, gilteritinib, and other TKIs, hypomethylating agents, and venetoclax	Relapse: in cases of loss of HLA-I, DLI is not advised
Myeloproliferative neoplasms	High	Ruxolitinib and IFN-α	High risk of GVHD; consider lower DLI dose
Chronic myeloid leukaemia	High	TKIs and IFN-α	..
Lymphoid malignancies			
B-cell acute lymphoblastic leukaemia	Lower	TKIs, blinatumomab, and CAR T cells	DLI as remission consolidation
T-cell acute lymphoblastic leukaemia	Lower	Decitabine, nelarabine, and CD38 antibodies	DLI as remission-consolidation
Follicular lymphoma	High	CD20 antibodies, CAR T cells, and bispecifics	CAR T-cell therapy; bispecifics probably also effective but data are scarce
Mantle cell lymphoma	High	Ibrutinib and CAR T cells	..
Diffuse large B-cell lymphoma	Lower	Ibrutinib (ABC type), CAR T cells, and bispecifics	DLI as remission consolidation
Hodgkin lymphoma	Intermediate	Brentuximab vedotin	Checkpoint inhibitors should be considered as alternative to DLI in post-allogneic HCT setting due to the risk of alloreactive complications
Chronic lymphocytic leukaemia	Intermediate	Bruton's tyrosine kinase inhibitors and venetoclax	..
T-cell non-Hodgkin lymphoma	Unknown	Brentuximab vedotin (if CD30$^+$)	Mogamulizumab might be effective in cutaneous non-Hodgkin lymphoma, but data are scarce
Multiple myeloma	High	Proteosome inhibitors, CAR T cells, CD38 antibodies	High risk of GVHD in combination with lenalidomide

CAR=chimeric antigen receptor. DLI=donor lymphocyte infusion. GVHD=graft-versus-host disease. HCT=haematopoietic cell transplantation. TKI=tyrosine kinase inhibitor. *Sensitivity to DLI according to the National Cancer Institute consensus definitions[57] and other sources.[68,69]

Table 2: **Disease-specific considerations**

Although not well characterised, from an immunobiological point of view, it has been postulated that this kind of cytopenic syndrome might be a direct effect of donor-derived immune effectors against haematopoietic stem cells or more mature cells.[70] The pathophysiology can arise from a T-cell-mediated reaction against haematopoietic stem cells and progenitors, sometimes associated with T-cell oligoclonality or large granular lymphocytes occurrence, that might mimic bone marrow failure.[71] Alternatively, this kind of cytopenic syndrome can be associated with donor-derived B-cell recirculation and antibody production. This last condition, in more serious cases, can be associated with haemolytic anaemia, immune-mediated thrombocytopenia, and autoimmune neutropenia, either isolated or variously combined.[72,73] An oriented and exhaustive diagnostic investigation (including the search for pathogenic agents, vitamin deficiency, peripheral antibodies, and bone marrow failure causes) should always be conducted to correctly manage the underlying condition. When post-DLI grade 3–4 haematological toxicity is identified, it is not advised to protract DLI administration, and depending on the situation, the initiation or intensification of growth factors, steroids, or immune suppressive treatments, along with anti-infectious prophylaxis, could be necessary.

Unanswered questions and further research

Despite DLIs being routinely used at all transplantation centres for many decades, and over 4400 publications on the subject, many questions remain unanswered—partly attributable to the fact that DLIs are not classified as a drug, and to a lack of financial support for prospective studies in a field with a high diversity in transplantation platforms and protocols across transplantation centres. We must acknowledge the need to better document current DLI practices. To initiate this documentation, a questionnaire was implemented during the DLI Harmonisation Workshop to capture the diversity in DLI applications. This initiative aims to establish a framework for evaluating which data should be systematically collected in the EBMT registry, and enabling matched-pair analysis to study transplantation outcomes, with or without prophylactic DLI. Furthermore, variations in practical routines of transplantation centres might provide valuable insights into how to determine the appropriate DLI dosage for different indications, including prophylactic, pre-emptive, and therapeutic use, and for assessing the added value of DLIs in preventing or treating infections and post-transplantation lymphoproliferative disorders. Another crucial aspect under consideration is the role of measurable residual disease and chimerism in guiding

Search strategy and selection criteria

Pubmed, ClinicalTrials.gov, and abstract books from the most recent haematological and transplantation meetings, were used as research support. The search strategy included specific keywords and MeSH terms such as "donor lymphocyte infusion", "graft-versus-leukemia effect", and "relapse after transplantation". Inclusion criteria concerned peer-reviewed articles in English reporting clinical trials, observational studies, case series, and reviews, which provided insights into the efficacy, dosage regimens, immune response, and adverse effects of donor lymphocyte infusions. The first studies included were reported in 1990, no date restriction was considered.

pre-emptive DLI therapy, which should take into consideration the high variability, poor standardisation, and different sensitivities of the molecular methods used to measure these biological parameters.[74] There is also an urgent need to establish a standardised definition for a mixed chimerism threshold that requires intervention, including specifying the relevant compartment for assessment. Finally, the added value of HLA loss and HLA mutation-driven decision making in patients with relapse should be tested prospectively. Recognising that comprehensive data collection can pose a substantial burden for transplantation centres, the initial step forward could involve prioritising data collection for a limited DLI dataset before expanding to more comprehensive records. Performing DLIs according to internationally accepted guidelines will help bring uniformity, and will lay the foundation for future studies to resolve remaining questions.

Contributors

IY-A and AR proposed the idea of the workshop; IY-A, RG, and FO were coordinators of all the workshops of the Practice Harmonisation Committee held in Lille, in September, 2023. AR, MDH, and JK conceived the project outline and the issues to be discussed. SP, CS, JK, MDH, FS, and NS participated in the in-person panel discussion in Lille. IS-O provided organisational and logistical support for the workshop. SP, CS, JK, and MDH wrote the first draft and conceived the figures. SP, AR, JK, CS, MDH, FS, NS, TG, GB, IS-O, and IY-A edited the document. All authors approved the final version of the manuscript.

Declaration of interests

SP has received travel expenses or honoraria for participation in advisory boards, symposia, or other scientific events from Alexion, Novartis, Jazz Pharmaceutical, Therakos, OneLamda, and Sobi; and received research funding from Janssen Horizon. CS has received travel expenses or honoraria for participation in advisory boards, symposia, or other scientific events from Novartis, Jazz Pharmaceutical, Neovii, Janssen, and Kite Pharma. JK has received grants from Novartis, Miltenyi Biotech, and Gadeta; is a Gadeta shareholder; and holds patent licenses with Gadeta and Miltenyi Biotech. All other authors declare no competing interests.

Acknowledgments

AR is a member of the Cellular Therapy and Immunobiology Working Party of the EBMT. The EBMT is a non-profit medical and scientific organisation, which performed administrative and legal management, and funding acquisition in relation to the organisation of the workshops of the Practice Harmonization Committee. No pharmaceutical company and no other funding source were involved in any way, nor had a role in the study design, data collection, data analysis, data interpretation, or writing of the report.

References

1 Passweg JR, Baldomero H, Chabannon C, et al. Hematopoietic cell transplantation and cellular therapy survey of the EBMT: monitoring of activities and trends over 30 years. *Bone Marrow Transplant* 2021; **56:** 1651–64.
2 Negrin RS. Graft-versus-host disease versus graft-versus-leukaemia. *Hematology Am Soc Hematol Educ Program* 2015; **2015:** 225–30.
3 Pagliuca S, Prata PH, Xhaard A, et al. Long-term outcomes and risk factor analysis of steroid-refractory graft versus host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2021; **56:** 38–49.
4 Kolb HJ, Mittermüller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; **76:** 2462–65.
5 Biederstädt A, Rezvani K. How I treat high-risk acute myeloid leukemia using preemptive adoptive cellular immunotherapy. *Blood* 2023; **141:** 22–38.
6 Schmid C, Kuball J, Bug G. Defining the role of donor lymphocyte infusion in high-risk haematologic malignancies. *J Clin Oncol* 2021; **39:** 397–418.
7 Frederik Falkenburg JH, Schmid C, Kolb HJ, et al. Delayed transfer of immune cells or the art of donor lymphocyte infusion. In: Carreras E, Dufour C, Mohty M, Kröger N, eds. The EBMT Handbook. Cham: Springer International Publishing, 2019: 443–48.
8 Santoro N, Mooyaart JE, Devillier R, et al. Donor lymphocyte infusions after haploidentical stem cell transplantation with PTCY: a study on behalf of the EBMT cellular therapy and immunobiology working party. *Bone Marrow Transplant* 2023; **58:** 54–60.
9 Yakoub-Agha I, Greco R, Onida F, et al. Practice harmonization workshops of EBMT: an expert-based approach to generate practical and contemporary guidelines within the arena of hematopoietic cell transplantation and cellular therapy. *Bone Marrow Transplant* 2023; **58:** 696–700.
10 Wu CJ, Yang XF, McLaughlin S, et al. Detection of a potent humoral response associated with immune-induced remission of chronic myelogenous leukemia. *J Clin Invest* 2000; **106:** 705–14.
11 Gillissen MA, Kedde M, Jong G, et al. AML-specific cytotoxic antibodies in patients with durable graft-versus-leukemia responses. *Blood* 2018; **131:** 131–43.
12 Gillissen MA, de Jong G, Kedde M, et al. Patient-derived antibody recognizes a unique CD43 epitope expressed on all AML and has antileukemia activity in mice. *Blood Adv* 2017; **1:** 1551–64.
13 van Bergen CAM, van Luxemburg-Heijs SA, de Wreede LC, et al. Selective graft-versus-leukemia depends on magnitude and diversity of the alloreactive T cell response. *J Clin Invest* 2017; **127:** 517–29.
14 Bachireddy P, Azizi E, Burdziak C, et al. Mapping the evolution of T-cell states during response and resistance to adoptive cellular therapy. *Cell Rep* 2021; **37:** 109992.
15 Maurer A, Park CY, Mani S, et al. Coordinated immune cell networks in the bone marrow microenvironment define the graft versus leukemia response with adoptive cellular therapy. *Blood* 2023; **142** (suppl 1)**:** 363 (abstr).
16 Odak I, Sikora R, Riemann L, et al. Spectral flow cytometry cluster analysis of therapeutic donor lymphocyte infusions identifies T cell subsets associated with outcome in patients with AML relapse. *Front Immunol* 2022; **13:** 999163.
17 Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat Rev Immunol* 2013; **13:** 88–100.
18 Janssen A, van Diest E, Vyborova A, et al. The role of γδ t cells as a line of defence in viral infections after allogeneic stem cell transplantation: opportunities and challenges. *Viruses* 2022; **14:** 117.
19 Melandri D, Zlatareva I, Chaleil RAG, et al. The γδTCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. *Nat Immunol* 2018; **19:** 1352–65.
20 Yazdanifar M, Barbarito G, Bertaina A, Airoldi I. γδ T cells: the ideal tool for cancer immunotherapy. *Cells* 2020; **9:** 1305.
21 Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007; **25:** 297–336.

22 Chaidos A, Patterson S, Szydlo R, et al. Graft invariant natural killer T-cell dose predicts risk of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. *Blood* 2012; **119:** 5030–36.

23 Malard F, Labopin M, Chevallier P, et al. Larger number of invariant natural killer T cells in PBSC allografts correlates with improved GVHD-free and progression-free survival. *Blood* 2016; **127:** 1828–35.

24 Jahnke S, Schmid H, Secker KA, et al. Invariant NKT cells from donor lymphocyte infusions (DLI-iNKTs) promote ex vivo lysis of leukemic blasts in a CD1d-dependent manner. *Front Immunol* 2019; **10:** 1542.

25 Kent A, Crump LS, Davila E. Beyond αβ T cells: NK, iNKT, and γδT cell biology in leukemic patients and potential for off-the-shelf adoptive cell therapies for AML. *Front Immunol* 2023; **14:** 1202950.

26 Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol* 2016; **17:** 1025–36.

27 Joncker NT, Fernandez NC, Treiner E, Vivier E, Raulet DH. NK cell responsiveness is tuned commensurate with the number of inhibitory receptors for self-MHC class I: the rheostat model. *J Immunol* 2009; **182:** 4572–80.

28 Ruggeri L, Vago L, Eikema DJ, et al. Natural killer cell alloreactivity in HLA-haploidentical hematopoietic transplantation: a study on behalf of the CTIWP of the EBMT. *Bone Marrow Transplant* 2021; **56:** 1900–07.

29 Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human gammadelta T cells. *Science* 2005; **309:** 264–68.

30 Vago L, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. *J Clin Invest* 2020; **130:** 1552–64.

31 Gillissen MA, de Jong G, Levie SE, et al. AML relapse after rituximab treatment for GvHD: crucial role for B cells in GvL responses. *Bone Marrow Transplant* 2016; **51:** 1245–48.

32 Vago L, Perna SK, Zanussi M, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med* 2009; **361:** 478–88.

33 Toffalori C, Zito L, Gambacorta V, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nat Med* 2019; **25:** 603–11.

34 Pagliuca S, Gurnari C, Hercus C, et al. Leukemia relapse via genetic immune escape after allogeneic hematopoietic cell transplantation. *Nat Commun* 2023; **14:** 3153.

35 Christopher MJ, Petti AA, Rettig MP, et al. Immune escape of relapsed AML cells after allogeneic transplantation. *N Engl J Med* 2018; **379:** 2330–41.

36 Dufva O, Pölönen P, Brück O, et al. Immunogenomic landscape of hematological malignancies. *Cancer Cell* 2020; **38:** 380–399.e13.

37 Pagliuca S, Gurnari C, Zhang K, et al. Comprehensive transcriptomic analysis of VISTA in acute myeloid leukemia: insights into its prognostic value. *Int J Mol Sci* 2022; **23:** 14885.

38 Ferraro F, Miller CA, Christensen KA, et al. Immunosuppression and outcomes in adult patients with de novo acute myeloid leukemia with normal karyotypes. *Proc Natl Acad Sci USA* 2021; **118:** e2116427118.

39 Gambacorta V, Beretta S, Ciccimarra M, et al. Integrated multiomic profiling identifies the epigenetic regulator PRC2 as a therapeutic target to counteract leukemia immune escape and relapse. *Cancer Discov* 2022; **12:** 1449–61.

40 Boudreau JE, Liu XR, Zhao Z, et al. Cell-extrinsic MHC class I molecule engagement augments human NK cell education programmed by cell-intrinsic MHC class I. *Immunity* 2016; **45:** 280–91.

41 Cantoni C, Wurzer H, Thomas C, Vitale M. Escape of tumor cells from the NK cell cytotoxic activity. *J Leukoc Biol* 2020; **108:** 1339–60.

42 Shahid S, Ceglia N, Le Luduec JB, et al. Immune profiling after allogeneic hematopoietic cell transplantation in pediatric acute myeloid leukemia. *Blood Adv* 2023; **7:** 5069–81.

43 Gournay V, Vallet N, Peux V, et al. Immune landscape after allo-HSCT: TIGIT- and CD161-expressing CD4 T cells are associated with subsequent leukemia relapse. *Blood* 2022; **140:** 1305–21.

44 Alyea EP, Soiffer RJ, Canning C, et al. Toxicity and efficacy of defined doses of CD4(+) donor lymphocytes for treatment of relapse after allogeneic bone marrow transplant. *Blood* 1998; **91:** 3671–80.

45 Giralt S, Hester J, Huh Y, et al. CD8-depleted donor lymphocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation. *Blood* 1995; **86:** 4337–43.

46 Ye Y, Yang L, Yuan X, Huang H, Luo Y. Optimization of donor lymphocyte infusion for AML relapse after allo-HCT in the era of new drugs and cell engineering. *Front Oncol* 2022; **11:** 790299.

47 Sauerer T, Velázquez GF, Schmid C. Relapse of acute myeloid leukemia after allogeneic stem cell transplantation: immune escape mechanisms and current implications for therapy. *Mol Cancer* 2023; **22:** 180.

48 Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 2022; **140:** 1345–77.

49 Schmid C, Labopin M, Schaap N, et al. Long-term results and GvHD after prophylactic and preemptive donor lymphocyte infusion after allogeneic stem cell transplantation for acute leukemia. *Bone Marrow Transplant* 2022; **57:** 215–23.

50 Legrand F, Le Floch AC, Granata A, et al. Prophylactic donor lymphocyte infusion after allogeneic stem cell transplantation for high-risk AML. *Bone Marrow Transplant* 2017; **52:** 620–21.

51 Rettinger E, Willasch AM, Kreyenberg H, et al. Pre-emptive immunotherapy in childhood acute myeloid leukemia for patients showing evidence of mixed chimerism after allogeneic stem cell transplantation. *Blood* 2011; **118:** 5681–88.

52 Rujkijyanont P, Morris C, Kang G, et al. Risk-adapted donor lymphocyte infusion based on chimerism and donor source in pediatric leukemia. *Blood Cancer J* 2013; **3:** e137–137.

53 Lamure S, Paul F, Gagez AL, et al. A retrospective comparison of DLI and gDLI for post-transplant treatment. *J Clin Med* 2020; **9:** 2204.

54 Abbi KKS, Zhu J, Ehmann WC, et al. G-CSF mobilized vs conventional donor lymphocytes for therapy of relapse or incomplete engraftment after allogeneic hematopoietic transplantation. *Bone Marrow Transplant* 2013; **48:** 357–62.

55 Vago L, Toffalori C, Ciceri F, Fleischhauer K. Genomic loss of mismatched human leukocyte antigen and leukemia immune escape from haploidentical graft-versus-leukemia. *Semin Oncol* 2012; **39:** 707–15.

56 Kröger N, Bacher U, Bader P, et al. NCI First International Workshop on the biology, prevention, and treatment of relapse after allogeneic hematopoietic stem cell transplantation: report from the committee on disease-specific methods and strategies for monitoring relapse following allogeneic stem cell transplantation. Part I: methods, acute leukemias, and myelodysplastic syndromes. *Biol Blood Marrow Transplant* 2010; **16:** 1187–211.

57 Alyea EP, DeAngelo DJ, Moldrem J, et al. NCI First International Workshop on the biology, prevention and treatment of relapse after allogeneic hematopoietic cell transplantation: report from the committee on prevention of relapse following allogeneic cell transplantation for haematologic malignancies. *Biol Blood Marrow Transplant* 2010; **16:** 1037–69.

58 Kneppers E, van der Holt B, Kersten MJ, et al. Lenalidomide maintenance after non-myeloablative allogeneic stem cell transplantation in multiple myeloma is not feasible: results of the HOVON 76 trial. *Blood* 2011; **118:** 2413–19.

59 Davids MS, Kim HT, Costello C, et al. A multicenter phase 1 study of nivolumab for relapsed hematologic malignancies after allogeneic transplantation. *Blood* 2020; **135:** 2182–91.

60 Holderried TAW, Fraccaroli A, Schumacher M, et al. The role of checkpoint blockade after allogeneic stem cell transplantation in diseases other than Hodgkin's Lymphoma. *Bone Marrow Transplant* 2019; **54:** 1662–67.

61 Mathew NR, Baumgartner F, Braun L, et al. Sorafenib promotes graft-versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells. *Nat Med* 2018; **24:** 282–91.

62 Goodyear O, Agathanggelou A, Novitzky-Basso I, et al. Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. *Blood* 2010; **116:** 1908–18.

63 Feng X, Velázquez GF, Bolscheid S, Schmid C, Schmetzer H. The 49th Annual Meeting of the European Society for Blood and Marrow Transplantation: physicians—poster session (P001–P708). P022 Potential modification of dysregulated expressions of inhibitory checkpoint (ICM) and their ligands on T cells and blasts in AML relapses after stem cell transplantation. *Bone Marrow Transplant* 2023; **58:** 153–627.

64 Yang H, Bueso-Ramos C, DiNardo C, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia* 2014; **28:** 1280–88.

65 Velázquez GF, Weller JF, Rubeck A, et al. The 49th Annual Meeting of the European Society for Blood and Marrow Transplantation: physicians—poster session (P001–P708). P121 Multistate model of prophylactic/pre-emptive donor lymphocyte infusion for high-risk acute myeloid leukemia and myelodysplastic syndrome in complete hematologic remission after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2023; **58:** 153–627.

66 Jedlickova Z, Schmid C, Koenecke C, et al. Long-term results of adjuvant donor lymphocyte transfusion in AML after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2016; **51:** 663–67.

67 Schmid C, Schleuning M, Schwerdtfeger R, et al. Long-term survival in refractory acute myeloid leukemia after sequential treatment with chemotherapy and reduced-intensity conditioning for allogeneic stem cell transplantation. *Blood* 2006; **108:** 1092–99.

68 Gagelmann N, Wolschke C, Badbaran A, et al. Donor lymphocyte infusion and molecular monitoring for relapsed myelofibrosis after hematopoietic cell transplantation. *HemaSphere* 2023; **7:** e921.

69 Rampotas A, Sockel K, Panitsas F, et al. Adoptive immunotherapy via donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation for myelofibrosis: a real-world, retrospective multicenter study. *Transplant Cell Ther* 2023; **29:** 687.

70 Nicolini FE, Wattel E, Michallet AS, et al. Long-term persistent lymphopenia in hematopoietic stem cell donors after donation for donor lymphocyte infusion. *Exp Hematol* 2004; **32:** 1033–39.

71 Awada H, Mahfouz RZ, Durrani J, et al. Large granular lymphocytic leukaemia after solid organ and haematopoietic stem cell transplantation. *Br J Haematol* 2020; **189:** 318–22.

72 Moulinet T, Moussu A, Pierson L, Pagliuca S. The many facets of immune-mediated thrombocytopenia: principles of immunobiology and immunotherapy. *Blood Rev* 2024; **63:** 101141.

73 Migdady Y, Pang Y, Kalsi SS, Childs R, Arai S. Post-hematopoietic stem cell transplantation immune-mediated anemia: a literature review and novel therapeutics. *Blood Adv* 2022; **6:** 2707–21.

74 Stadler M, Venturini L, Bünting I, et al. Navigating pre-emptive and therapeutic donor lymphocyte infusions in advanced myeloid malignancies by high-sensitivity chimerism analysis. *Front Oncol* 2022; **12:** 867356.