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Dissecting the biology of allogeneic HSCT to enhance the GvT effect whilst minimizing GvHD

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Abstract

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) was the first successful therapy for patients with haematological malignancies, predominantly owing to graft-versus-tumour (GvT) effects. Dramatic methodological changes, designed to expand eligibility for allo-HSCT to older patients and/or those with co-morbidities, have led to the use of reduced-intensity conditioning regimens, in parallel with more aggressive immunosuppression to better control graft-versus-host disease (GvHD). Consequently, disease relapse has become the major cause of death following allo-HSCT. Hence, the prevention and treatment of relapse has come to the forefront and remains an unmet medical need. Despite >60 years of preclinical and clinical studies, the immunological requirements necessary to achieve GvT effects without promoting GvHD have not been fully established. Herein, we review learnings from preclinical modelling and clinical studies relating to the GvT effect, focusing on mechanisms of relapse and on immunomodulatory strategies that are being developed to overcome disease recurrence after both allo-HSCT and autologous HSCT. Emphasis is placed on discussing current knowledge and approaches predicated on the use of cell therapies, cytokines to augment immune responses and dual-purpose antibody therapies or other pharmacological agents that can control GvHD whilst simultaneously targeting cancer cells.

ToC Blurb

Haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for several haematological malignancies. Improvements in HSCT methodologies have considerably reduced treatment-related morbidity and mortality, thus broadening eligibility and placing increased emphasis on the prevention of disease relapse. In this Review, the authors discuss approaches to

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Author contributions

The authors contributed equally to all aspects of the conception and preparation of this manuscript.

Competing interests

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dissecting the biology of HSCT and exploiting the biological insights to enhance the graft-versus-tumour response, in particular with adoptive cell therapies and other immune-directed therapies, whilst minimizing graft-versus-host disease.

Introduction

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) and autologous HSCT (auto-HSCT) have been a cornerstone of cancer therapy, primarily for haematological malignancies, for three decades. Allo-HSCT and, to a much lesser extent, auto-HSCT are predicated on exploiting the graft-versus-tumour (GvT) effect, defined as an immune-mediated reaction by engrafted donor cells against tumour cells. The annual number of such transplantations continues to rise, with >8,000 allo-HSCT and >14,000 auto-HSCT procedures performed in the USA in 2018[1]. In adults, the majority of allo-HSCTs are performed for the treatment of acute leukaemias, approximately two-thirds in patients with acute myeloid leukaemia (AML)[1]. Other major indications include myelodysplastic syndrome (MDS) or lymphoma (predominantly non-Hodgkin), and to a lesser extent, multiple myeloma (MM), chronic myeloid leukaemia (CML) and chronic lymphocytic leukaemia (CLL)[1]. With regard to auto-HSCT, two-thirds of recipients have MM; the remaining third mostly have non-Hodgkin or Hodgkin lymphoma[1]. Allo-HSCT for non-haematological cancers has also been evaluated, although with less robust responses observed[2]. HSCT is increasingly being offered to older patients, including a growing number aged >70 years[1]. This trend largely reflects improvements in supportive care, especially infection prophylaxis and treatment, as well as the development of reduced-intensity and/or non-myeloablative conditioning and improved immunosuppressive therapy[3]. Indeed, over the past three decades, non-relapse mortality (NRM) has been the greatest cause of mortality in the first 200 days after HSCT, and reductions in NRM are the predominant factor responsible for the improvements in outcome[1,3]. The risk of disease relapse has also declined, albeit less dramatically than NRM, over the past three decades; beyond 200 days after HSCT, relapse is now responsible for the majority (>50%) of deaths after allo-HSCT and >70% of deaths after auto-HSCT[1,3]. Thus, the dramatic changes in methodologies and indications for HSCT have resulted in the prevention and treatment of disease relapse becoming the major unmet needs in the field, focusing efforts to enhance the GvT effect without increasing the risk of graft-versus-host disease (GvHD).

The source of donor stem cells for allo-HSCT has also changed dramatically over the past 5 years, with haploidentical — that is, 50% human leukocyte antigen (HLA)-matched — family donors increasingly favoured over other alternative sources (for example, umbilical cord blood or HLA-mismatched unrelated donors), owing to the ease of access to such donors and the excellent outcomes achievable in the era of post-transplant cyclophosphamide (PT-Cy)-based immunosuppression[4]. Indeed, an acceptable donor stem cell source can be found for almost all patients. Age and co-morbidities no longer present strict limitations on eligibility for most patients. Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) are used for most allo-HSCT procedures in adults (>85%)[1]. In paediatric patients, however, transplantation for nonmalignant conditions, typically immunodeficiencies, haemoglobinopathies, anaemias

and congenital errors of metabolism, are proportionately higher[5], with bone marrow as the preferred source of donor cells. This preference reflects the fact that PBSCs are associated with a higher rate of chronic GvHD (cGvHD, occurring primarily beyond 100 days of transplantation)[6], which presents an unnecessary risk in most of these patients. Regardless of the source of donor cells, immunosuppressive therapies for both acute GvHD (aGVHD, occurring primarily within 100 days after transplantation) and cGvHD, probably impair the GvT effects of HSCT, a major issue complicating efforts to delay or prevent disease relapse.

In this Review, we provide an overview of the findings of preclinical and clinical studies that have informed our current understanding of the GvT effect. Emphasis is placed on current knowledge of the mechanisms of disease relapse after HSCT, allogeneic and autologous, and on immunomodulatory therapeutic strategies targeting these mechanisms, which include cell therapies, immunostimulatory cytokines and dual-purpose antibodies or other pharmacological agents that can enhance efficacy without compromising safety.

Antitumour immune effects of auto-HSCT

Auto-HSCT has traditionally been thought of as a means of enabling the use of high-dose (that is, supra-myeloablative) chemoradiotherapy, which can result in complete or near-complete eradication of the cancer cells and necessitates haematopoietic stem cell rescue. However, the effects of auto-HSCT likely extend beyond cytoreduction and lymphodepletion, owing to restoration of a balanced immune system. In MM, disruption of the bone marrow microenvironment after auto-HSCT is likely to have tumour-promoting immunological effects, supporting the exploitation of applying tumour-specific immunotherapies that may invoke T cell-mediated and MM-specific disease control by re-establishing a state of immune equilibrium[7]. T cell exhaustion and immune escape are implicated in disease relapse after auto-HSCT in preclinical models and patients with MM[8,9] and can be countered through immune-checkpoint inhibition (ICI), especially co-targeting of the inhibitory T cell immunoreceptor with Ig and ITIM domains (TIGIT) and programmed cell death 1 (PD-1) pathways[10]. Understanding and harnessing the immunological properties of auto-HSCT is thus an area of increasing research interest. In addition, the period of profound immunodeficiency following auto-HSCT and states of low disease burden might also provide a window for application of adoptive cell immunotherapy (for example, with T cells engineered to express chimeric antigen receptors (CARs) that bind cell-surface antigens expressed on malignant cells; NCT03455972).

Antitumour immunity after allo-HSCT

Preclinical modelling of GvT effects.

This Review will not be a compendium of strategies that augment the GvT effect because the GvT response, in its truest sense of the tumour-specific effects of donor cells, has received far less attention than GvHD or the efficacy of direct antitumour therapies as the primary readout of preclinical models of allo-HSCT. Herein, we have focused on graft-versus-leukaemia (GvL) effects, which are the most extensively studied GvT responses. Moreover, allo-HSCT is most commonly used to treat patients with leukaemia — although, cell therapy approaches to the treatment of solid cancers are gaining considerable interest, in

particular, with the advent of genetically engineered third-party or ‘off-the-shelf’ T cell products expressing CARs and T cell receptor (TCRs) targeting antigens associated with solid tumours.

The predominant preclinical models of allo-HSCT use inbred laboratory mice owing, in part, to low costs, short times to end points and broad accessibility to reagents and strains, as well as the potential for high levels of reproducibility between different laboratories[11,12]. Preclinical studies that exploit mouse models to examine the GvT effect usually involve engraftment of long-term propagated transplantable tumour cell lines followed by allo-HSCT consisting of both donor HSCs and additional T cells to induce GvHD. Reflecting the focus of most of these studies, however, investigations of the GvL effect are often performed only to ensure that the antitumour activity is not compromised by therapeutic interventions to minimize GvHD. Notably, however, fewer T cells are needed to mount specific GvT responses than result in GvHD; as such, preservation of the GvT effect might reflect the fact that a low threshold number of T cells might be sufficient for antitumour responses, rather than true selectivity of interventions to suppress GvHD (reviewed in[13]). Thus, preclinical models have been optimized for GvHD analysis, whereas GvL modelling is less developed, thus hindering clinical extrapolation of findings relating to the GvL effect.

In contrast to the clinical scenario, preclinical studies involve well-characterized tumour cell lines transplanted into inbred mice that are healthy, young, lean and are housed within specific-pathogen-free (SPF) facilities, all of which are likely to result in antitumour immune responses that do not accurately mirror those associated with the antigen-experienced immune system of patients with cancer (TABLE 1). Simply altering one preclinical variable, such as age or obesity status, has been demonstrated to result in markedly different outcomes and toxicities, including cytokine dysregulation[14]. Furthermore, incorporating human-modifying variables such as obesity can sometimes result in parodoxical but clinically validated outcomes, highlighting the importance of considering such variables in preclinical modelling[15].

Full major histocompatibility complex (MHC) and multiple minor histocompatibility antigen disparate mouse strain combinations continue to be a mainstay of preclinical GvL models, owing to the fact that GvHD is readily titratable by varying T cell doses and that strains deficient in cytokine, co-stimulatory molecules and other relevant proteins are commonly available, enabling demonstration of true cause-and-effect relationships. Beyond the considerable species differences between the mouse and the human immune system, mouse models of GvHD commonly rely on a limited number of strain combinations associated with induction of a particular GvHD type or pathology, **w**ith only aGvHD or cGvHD (but less commonly both), which is not reflective of the frequently observed clinical presentation. Mouse allo-HSCT usually involves conditioning with total body lethal irradiation alone (that is, without chemotherapy), the use of bone marrow as a source of HSCs and typically requires the use of donor T cells (usually from a splenocyte source) to induce GvHD (TABLE 1). The limited range of transplantable mouse tumour lines available are highly aggressive and proliferate rapidly, such that only a minimal tumour burden is treatable. Other GvHD and GvT models consist of human T cells (and typically allogeneic human tumours from either established cell lines or primary tumour cells from patients)

grafted into immunodeficient mice, which cause xenogeneic GvHD[12]. While advantageous in assessing interactions between human cells and the effects of experimental therapies in vivo, these models are limited by species-specific factors (for example, human TCRs cannot recognize mouse MHCs and many human cytokines to do not bind the relevant mouse cytokine receptors) and the unclear mechanisms underlying the pathogenesis of xenogeneic GvHD. Large-animal models, primarily in canines and non-human primates, are also used for studies of allo-HSCT, but predominantly for investigations of GvHD owing to high costs, the outbred nature of the animals that often limits the experimental control of MHC disparities, limited validated immune reagent availability and **i**mmune monitoring capabilities, and the lack of established GvT models in non-human primates (TABLE 1). These challenges further are coupled with difficulties in applying unproven or high-risk experimental therapies to client-owned canines with cancers and limited ‘in-patient’ facility capabilities or support (TABLE 1).

Thus, while SPF, inbred mice are the most informative means of initially assessing therapeutics for enhancing GvT activity, clinical GvT responses clearly need to be better reflected in these models, considering that patient outcomes are markedly affected by age, health status, obesity, previous therapies, immune system status and prior exposure to opportunistic pathogens, including latent cytomegalovirus (CMV) and Epstein–Barr virus (EBV), as well as cancer burden (TABLE 1). The clinical allo-HSCT paradigm involves various combinatorial radiotherapy, chemotherapy and immunosuppressive regimens chosen, in part, on the basis of the degree of HLA compatibility, stem cell source, patient age and co-morbidities, and cancer type. In contrast to cancer models involving relatively homogeneous tumour cell lines transplanted to healthy treatment-naive mice, human cancers arise over longer time periods and are heterogeneous with respect to tumour burden, utilization of immune suppressive or evasion pathways and treatment exposure prior to allo-HSCT. Prophylactic GvHD immunosuppressive agents profoundly affect immune cell and haematopoietic cell reconstitution in patients but are rarely applied in mouse models, although they are used routinely in large-animal models[11,12]. Donor *HLA* compatibility is crucial in clinical allo-HSCT, with preferred use of related donors, whereas many mouse models have full MHC mismatching on a background of allelic disparities for multiple different genes. These models are immunologically problematic for GvT activity, in which donor T cell responses against the tumour cells might be promoted by some degree of MHC compatibility with the recipient’s cells. Changes in the demographics of the population of patients undergoing allo-HSCT (that is, increasing numbers of older recipients, patients with obesity and those from minority populations with HLA disparate donors[1]) are likely to affect GvHD and GvT responses. Additionally, the microbiota has been demonstrated to markedly affect the outcomes of allo-HSCT and anticancer immunotherapy, thus warranting re-examination of appropriateness of the existing SPF mouse models, which also do not incorporate systemic antibiotics that alter the microbiota[16]. In addition, some clinically used immunomodulatory and/or immunosuppressive drugs present difficulties related to species-specific effects and might not be active in the absence of transgenic modification (for example, immunomodulatory imide drugs (IMiDs))[17]. Finally, some transplant-relevant malignancies (for example, EBV-driven lymphomas) and target antigens (such as the minor histocompatibility antigen HA-1 or NY-ESO) are uniquely human and very difficult to

model in preclinical systems, thus necessitating studies in patients from the outset (TABLE 1).

GvL effects after allo-HSCT.

In the context of allo-HSCT, the observation of direct cytostatic or cytotoxic effects on malignant cells does not prove that the graft — rather that the allo-HSCT procedure itself — has anticancer activity, although immune-mediated GvT responses constitute the principal curative pathway associated with this therapeutic approach. Target antigens on leukaemia cells that are recognized by donor T cells are typically alloantigens, and are less frequently haematopoietic or leukaemia-specific antigens, and thus GvL activity is often associated with deleterious GvHD responses. Separating GvL from GvHD responses, while a principal therapeutic goal, has been difficult in clinical practice. With this in mind, the selective generation of GvL responses to recipient haematopoietic alloantigens is a highly attractive approach to avoiding off-target effects on other recipient tissues (that is, GvHD). A number of haematopoietic cell-restricted minor histocompatibility antigens have been identified (such as HA-1 and HA-2) and corresponding adoptive T cell therapies are under development[18–20] (BOX 1). While targeting leukaemia and haematopoiesis-specific antigens reduces the risk of GvHD, preclinical modelling is needed to better understand the mechanisms initiating and maintaining GvL responses that are potentially distinct from GvHD and thereby develop strategies to promote immunity in bone marrow rather than parenchymal tissue.

MHC class I (MHC I)-dependent, $CD8^+$ T cell-mediated GvHD is initiated by recipient haematopoietic antigen-presenting cells (APC)[21], which activate and induce the differentiation of naive T cells from the donor[22,23]; no single APC subset (for example, dendritic cell (DCs), macrophages or B cells) is fundamentally required, indicating substantial functional redundancy[24,25]. Both MHC I-dependent and MHC II-dependent GvL responses require recipient haematopoietic APCs and cognate interactions with alloantigens on leukaemia cells[26] (FIG. 1), while donor APCs are minimally required[27,28]. No convincing evidence suggests that any one specific subset of APCs is essential to initiate MHC I-dependent GvL responses. Whereas GvHD is dominantly caused by naive T cells, both naive and memory T cells, once primed by recipient APCs, can mediate GvL effects[29]. Recipient DCs can limit both GvHD and GvL in rodent models by promoting activation-induced cell death of alloantigen-specific donor T cells[25,30]. Non-haematopoietic APCs (for example, epithelial or stromal cells) seem to have a limited role in initiating MHC I-dependent GvL responses in mice, although they are associated with T cell exhaustion and thus impairment of GvL activity[27,31]. By contrast, non-haematopoietic APC are involved in the initiation of MHC II-dependent GvHD responses[25,32] and, in addition to donor granulocyte–macrophage colony-stimulating factor (GM-CSF)-dependent DCs in the gastrointestinal tract, are an essential factor maintaining and amplifying GvHD[33,34]. Whether these tissue-resident APCs are important determinants of GvL effects requires further testing, particularly given the increasingly recognized importance of loss of MHC II expression on leukaemia cells at disease relapse[35,36]. Optimal antitumour immune responses require $CD4^+$ T cell activation by MHC II-presented neoantigens in the tumour microenvironment, even in tumours without inherent MHC II expression on the malignant

cells[37] (FIG. 1). Myeloid malignancies in bone marrow usually have low expression of MHC II, and thus the role of MHC II-dependent antigen presentation within the bone marrow on GvL activity deserves increased attention in future research.

Antigen presentation by GvHD-initiating APCs is highly regulated by the intestinal microbiota, is absent in germ-free mice and is augmented in dysbiotic mice[32,33], correlating with historical clinical data demonstrating that gut decontamination with broad spectrum antibiotics and protective isolation of patients has beneficial effects on GvHD outcomes in some settings[38–40]. Nevertheless, microbiota components, especially butyrate-producing anaerobic bacteria, can be protective against GvHD[41]. Associations between microbiota composition and disease relapse are now emerging[42], warranting cause-and-effect testing and investigation of the immunological mechanisms in preclinical models.

Mechanisms of relapse after Allo-HSCT.

Disease relapse owing to GvL failure is mediated by tumour intrinsic and extrinsic mechanisms. Immune escape mechanisms (as opposed to leukaemic clonal evolution with acquisition of driver mutations) include loss of MHC expression, increased expression of immune-checkpoint ligands (such as programmed cell death 1 ligand 1 (PD-L1), CD155 or B7 molecules), secretion of inhibitory cytokines (for example, IL-10) and upregulation of immunosuppressive enzymes (including indoleamine 2,3-dioxygenase (IDO) 1 and/or 2, CD73 or CD79), all of which facilitate the malignant cells in evading GvL responses (reviewed in[43]) (FIG. 2). Concurrent GvHD might contribute to ineffective GvL responses because chronic exposure of T cells to alloantigens increases their expression of cognate immune-checkpoint molecules, such as PD-1 and/or TIGIT on the T cell[31]. Moreover, while secretion of IFNγ early after allo-HSCT enhances antigen presentation by leukaemia cells[44], persistent cytokine secretion by alloresponsive donor T cells invokes immunosuppressive pathways, such as secretion of IDO enzymes and/or stromal and endothelial expression of PD-L1, thereby exacerbating T cell dysfunction[31].

The presence of minimal (or measurable) residual disease (MRD) prior to allo-HSCT correlates with and predicts a higher rate of disease recurrence[45]. Whether high relapse rates reflect the residual disease burden or and inherently aggressive malignancy, residual leukaemia cells are likely to directly subvert GvL responses via the immunosuppressive pathways detailed above. Hence, optimal GvL activity would be initiated in an MRD-negative state, within the bone marrow, against haematopoietic-restricted antigens and in the absence of GvHD. Indeed, given that the induction of severe aGvHD or cGVHD is unacceptable in composite end points of allo-HSCT studies, approaches to enhancing GvL activity warrant rethinking with a focus on the mechanisms unique to the antitumour immune responses.

Modulating anticancer immunity post HSCT

In patients with haematological cancer, most cases of disease relapse after allo-HSCT or auto-HSCT occur within a year[3]; therefore, approaches to enhance GvL responses probably need to be initiated within the first 3 months of transplantation, for prevention of disease relapse, or at first detection of MRD. Alloreactivity is key to the GvL effect, and thus

immunotherapy approaches are most tractable when alloreactivity is well controlled without substantial ongoing pharmacological immunosuppression. With regard to auto-HSCT, tumour immune escape seems to be driven largely by T cell exhaustion in response to the malignancy itself[10,46]. With allo-HSCT, approaches to modulating GvL activity are dependent upon augmenting donor T cell alloreactivity and are hindered by T cell-depleting agents[47]. Accordingly, deliberate use of lower than standard doses of cyclosporine, early or rapid cyclosporine withdrawal, or donor lymphocyte infusions can induce remission in patients with relapse of myeloid malignancies receiving immunosuppression, albeit with an increased risk of GvHD[48,49].

ICI for the treatment of relapsed disease after allo-HSCT is problematic, given the propensity for induction of GvHD[50], but might be tolerated when used prior to bone marrow transplantation with the use of post-transplant cyclophosphamide based immune suppression[51]. Cytokines such as type I interferons can enhance GvL activity (BOX 1), but do so at the risk of invoking GvHD[52]. Similar studies with type II interferon (IFNγ) are logical, owing to the roles of this cytokine in promoting antigen presentation by malignant cells, and their results are highly anticipated[35,44]. Alternatively, cytokines important in promoting GvHD whilst also providing survival signals to malignant cells, such as GM-CSF[34] and macrophage colony-stimulating factor 1 (CSF-1)[53] in AML and IL-6 in acute lymphocytic leukaemia (ALL) and MM[54], are attractive as targets for neutralization after allo-HSCT.

After auto-HSCT, ICI focused on TIGIT and/or PD-1 might prevent immune escape without increasing the risk of GvHD[10] (BOX 1). Moreover, combining T cell-directed ICI and inhibition of cytokine-signalling pathways by targeting, for example, CSF-1[10,53] or IL-18[55] that support immunosuppressive cell populations in the bone marrow, including tumour-associated macrophages and myeloid-derived suppressor cells (MDSCs) (FIG. 2), presents a potentially synergistic strategy to prevent immune escape after auto-HSCT. Rigorous testing of this approach in preclinical models is required before its evaluation as a preventative strategy (as opposed to a treatment for relapsed disease) in clinical studies.

Adoptive cell therapy with T cells genetically modified to express CAR targeting specific tumour-associated antigens, such as CD19[56] and BCMA[57] on malignant B cells and plasma cells, respectively, is increasingly being deployed for the treatment of relapsed disease[58,59]. Notably, lymphodepleted and MRD-negative states occurring after auto-HSCT present an advantageous context for CD19-directed or BCMA-directed CAR T cell therapy for B cell lymphomas or MM, respectively (BOX 1). CAR T cells might also be useful prior to allo-HSCT, in order to generate MRD-negative states; however, transplantation might be counterproductive in patients with CAR T cell persistence, owing to CAR T cell depletion by allogeneic donor cells. Preclinical data from mouse models suggest that allogeneic donor-derived CD19-directed CAR T cells, at least those harbouring CD28 co-stimulatory domains, can be utilized after allo-HSCT without exacerbating GvHD, with the CAR T cells expressing alloreactive TCRs having enhanced stimulation relative to those without alloreactive TCRs, leading to clonal deletion of the alloreactive cells[60]. By contrast, allogeneic T cells expressing 4–1BB-co-stimulated CARs invoked GvHD[60], likely

necessitating safety measures to eliminate these CAR T cells, such as inducible, dimerization agent-induced caspase-9 suicide switches[61].

Similarly, donor T cells genetically modified to express TCRs specific for leukaemia-associated or haematopoietic antigens, such as Wilms tumour protein (WT1)[62] or HA-1[18], respectively, are being studied for the treatment of disease relapse after allo-HSCT, with promising initial findings — including in patients using WT1-targeted T cells. Antibody-based approaches to targeting WT1, and bi-specific T-cell engager (BiTE) antibodies (BOX 1) that link tumor associated proteins such as CD19 on malignant B cells to CD3 on T cells[63], are also showing promise. Alternatively, a vaccine-based approach targeting the PR1 peptide derived from proteinase 3 has shown promise in patients with myeloid malignancies, some of whom had previously undergone allo-HSCT[64]. Notably, adoptive cell transfer of allogeneic EBV-specific T cells to treat EBV-driven malignancies, particularly post-transplant lymphoproliferative disorders and type II latency lymphomas occurring after allo-HSCT, is highly effective, without invoking GvHD, and is now an established standard of care[65,66] (BOX 1).

A particularly appealing strategy to treat haematological disease relapse is predicated on the use of small-molecule inhibitors of intracellular signalling pathways or monoclonal antibodies (mAbs) targeting cell-surface antigens with the potential to simultaneously eliminate malignant cells and immune cell populations crucial for deleterious inflammation during GvHD (for example, JAK inhibitors could potentially be used to treat both myeloproliferative diseases and GVHD[67]) (BOX 1). Other examples include the anti-CD20 mAb rituximab, which is approved for the treatment of CLL and non-Hodgkin lymphoma and has also been used to prevent or treat cGvHD in the clinical setting[68,69]. Disrupting the Notch pathway using mAbs or γ-secretase inhibitors offers the possibility of both direct anti-leukaemia effects and aGvHD or cGvHD prevention and therapy[70,71]. Small-molecule inhibitors targeting proteins upregulated or mutated in malignancies, such as BCR–ABL1 in Philadelphia chromosome-positive CML or ALL, FLT3 in AML, BCL-2 in CLL, ALL or AML, and JAK2 in myelofibrosis, are attractive maintenance therapies that can sensitize malignant cells to GvT effects by deprivation of crucial growth signals and/or disruption of pathways involving in resistance to immunological killing. For example, the multi-protein kinase inhibitor sorafenib, which can target the FLT3 internal tandem duplication (FLT3-ITD) associated with a subtype of AML, induces GvL responses possibly via stimulation of IL-15 production by leukaemia cells[72] (BOX 1).

Certain kinase or proteasome inhibitors used for cancer therapy have been explored specifically for GvHD prevention and therapy. Ibrutinib targets Bruton tyrosine kinase (BTK) and inducible T cell kinase (ITK) expressed in B cells and T cells, respectively, has direct anticancer effects in patients with CLL and some lymphomas (including small lymphocytic, marginal zone and mantle cell lymphomas) and has also been granted FDA approval for cGvHD therapy[73,74]. Splenic tyrosine kinase (SYK) inhibitors, which also suppress B cell receptor signalling and have thus been proposed as treatments for CLL and lymphoma, and proteasome inhibitors such as bortezomib, which are approved for the treatment of MM, increase the susceptibility of tumour cells to immune elimination and are in clinical use or testing for cGVHD therapy[75–78]. Ruxolitinib, a JAK1 and JAK2 inhibitor,

is approved for the treatment of myeloproliferative disease (that is, myelofibrosis) and for aGVHD therapy[67], and PI3Kδ inhibitors that are approved for patients with CLL or certain lymphomas also have activity in experimental models of GvHD[79]. These agents offer opportunities to prevent or treat disease relapse after allo-HSCT by directly targeting key survival pathways and indirectly by diminishing the immunosuppression resulting from agents used for GvHD prophylaxis or therapy. However, use of these pharmacological inhibitors, especially prior to a clear GvL response, might complicate interpretation of whether elimination of the disease reflects the contributions of these agents to a true GvL effect or, alternatively, their direct antitumour activity.

T_{reg} cells in GvHD and GvL effects

Thymus-derived $CD4^+$ regulatory T (T_{reg}) cells, which co-express CD4, CD25 and the master transcriptional regulator FOXP3, are essential in maintaining immune homeostasis and inhibiting alloresponses[80–83]. Infusion of ex vivo expanded T_{reg} cells in high numbers at the time of allo-HSCT, or freshly isolated donor T_{reg} cells in lower numbers several days before donor T cells are transferred, permits in vivo T_{reg} cell expansion in a lymphopenic environment, heightening T_{reg} cell-mediated suppression of GvHD in mice[81–83] and patients[84,85]. Early preclinical studies revealed that T_{reg} cell administration does not overtly dampen GvL responses or lymphoma-cell cytotoxicity by the engrafted mature allogeneic T cells[86,87], with only a modest reduction in cytotoxicity against P815 mastocytoma cells reported[71]. Third-party human T_{reg} cells also have demonstrated efficacy in suppressing GvHD in xenogeneic HSC recipients[88] and allogeneic T_{reg} cells reduced the incidence and severity of GvHD in patients undergoing allo-HSCT[84]. In immunodeficient mouse models, the combined use of clinical-grade ex vivo-expanded T_{reg} cells together with conventional T (T_{conv}) cells suppressed GvHD while retaining GvL activity against human AML, Philadelphia chromosome-positive ALL and Burkitt-like cell lines[88]. Interestingly, human T_{reg} cells have been shown to have direct cytotoxicity against glioblastoma cells via the granzyme–perforin pathway in vitro, which can be augmented when combined with bi-specific T cell-engaging antibodies[89]. Intriguingly, T_{reg} cells can also use cytolytic pathways, such as perforin and granzyme A, to kill autologous immune cells[90], which could potentially be exploited for not only direct tumour-cell lysis but also control of immune responses. Thus, infusion of antigen-specific or CAR-expressing T_{reg} cell together with allo-HSCT might not only reduce GvHD and any accompanying immune suppression, but also amplify GvL effects by focusing cytolytic T_{reg} cells responses towards relevant target antigens. Nevertheless, the typically high sensitivity of leukaemia cell lines, especially those derived from mice, to T cell-mediated cytotoxicity and thus GvL effects is a caveat in extrapolating these data to the clinical setting. Furthermore, the finding that GvL effects can be observed in patients without preceding or concurrent GvHD has led to the plausible hypothesis that fewer functional T cells are required for eliminating mouse leukemia cell lines than are needed to cause GvHD of sufficient magnitude to manifest clinically (reviewed in[13]).

Despite the theoretical concern that the immunosuppressive activities of adoptively transferred T_{reg} cells might lead to increase rates of disease relapse, the results of a study in patients with high-risk ALL ($n = 33$) or AML ($n = 10$) indicate significantly lower relapse

rates with this approach compared to 114 historical controls (5% versus 21%; $P = 0.03$), probably owing to superior lymphoid reconstitution and reduced GvHD-induced and/or GvHD treatment-induced immune suppression[91]. These findings contrast with reports from studies of AML that T_{reg} cell-depletion improves anti-leukaemia cytotoxic T cell responses in mice with established AML[92] and that the abundance of T_{reg} cells is inversely correlated with the responsiveness of AML to induction chemotherapy in patients with active disease[93], and were observed despite the fact that bone marrow, a predominant site of leukaemia, serves as a T_{reg} cell reservoir[94]. Differences between the predominant sites of T_{reg} cell trafficking (for example, bone marrow, peripheral blood or lymphoid organs) and metastasis (for example, central nervous system, skin, lung, liver or reproductive organs) might account, in part, for a reported low relapse rate in the aforementioned patients with high risk ALL or AML[95].

A second major type of T_{reg} cells comprise induced T_{reg} (iT_{regs}) cells, which are generated from T_{conv} cell in vitro or can be induced in vivo in the context of low-dose or tolerigenic antigen exposure. iT_{reg} cells generated in mice in vivo after allo-HSCT contribute to ameliorating GvHD[96,97], with several reports indicating higher frequencies of $CD8^+$ versus $CD4^+$ iT_{reg} cells[96,98]. Polyclonal or antigen-specific $CD4^+$ iT_{reg} cells can suppress allogeneic[99,100] and human xenogeneic GvHD[101] in mouse models. By comparison, $CD8^+$ iT_{reg} cells seem to have a modestly reduced capacity to protect against GvHD, but provided superior GvL effects[102]. Indeed, $CD8^+$ iT_{reg} cells can overcome $CD4^+$ iT_{reg} cell-mediated abrogation of GvL activity, and the highest degree of GvHD prevention and GvL preservation was observed when both of these cell populations were administered together[102].

In lineage-tracing studies in mice, natural thymus-derived T_{reg} (tT_{reg}) cells were seen to lose stability — reflected in loss of FoxP3 expression — in an inflammatory environment, at frequencies ranging from 4% to 15%[103,104], with consequent loss of immunosuppressive capacity. These ‘ex-T_{reg}’ cells acquire a pro-inflammatory phenotype and function and can, therefore, contribute to autoimmunity[103] and perhaps also to a GvT effect. In contrast to tT_{reg} cells, iT_{reg} cells lack a locked-in gene-expression signature of five transcription factors (*Eos*, *Irf4*, *Gata1*, *Lef1* and *Satb1*) needed for stabilization of FoxP3 activity[105]. Additionally, methylation of an evolutionary conserved CpG-rich element in the first intron or the conserved non-coding sequence 2 (CNS2) region of *FoxP3* renders iT_{reg} cells susceptible to becoming ex-T_{reg} cells. Inflammatory factors can destabilize tT_{reg} cells, iTreg and $FoxP3^-$ T regulatory type 1 (T_R1) cells in the context of mouse GvHD[106], with IL-1 and IL-6 having similar effects on human tT_{reg} cells in the context of xenogeneic GvHD[107], which could potentially promote GvT responses. In another mouse model, deletion of the one or both alleles of *Card11* in a fraction of tumour-infiltrating T_{reg} cells, thereby disrupting the CARD11–BCL10–MALT1 signalosome complex, was sufficient to increase IFNγ production by these cells, activate macrophages and upregulate MHC I and PD-L1 expression on tumour cells, while avoiding systemic autoimmunity[108]; when this approach was combined with PD-1-targeted ICI, the mice were able to reject tumours[108]. By contrast, phenotypic and functional stabilization of iT_{reg} cells has been demonstrated with retinoic acid[109–111] and vitamin C[112–115], and the stability of these cells is dependent on the chromatin-modifying enzyme EZH2 (potentially providing opportunities to enhance GvT

effects using EZH2 inhibitors)[116,117]. Notably, stabilization of $CD8^+$ iT_{reg} cells does not necessarily lead to impaired GvT responses: GvL activity was retained with vitamin C-treated[115] or *Jak2*-deficient T_{reg} cells[118], despite markedly increased levels of FoxP3 expression in these cells compared with untreated or wild-type T_{reg} cells, which is consistent with a higher level of T_{reg} cell stability.

Insufficient published clinical data exist to discuss the effects of IL-10, IL-5, IFNγ and TGFβ producing $CD4^+$ $FoxP3^-$ T_R1 cells on GvL responses. However, in preclinical studies, T_R1-like cells generated by engineering allospecific $CD4^+$ T cells to overexpress IL-10 were cytolytic in a granzyme B-dependent but antigen-independent manner that also required intracellular adhesion molecule 1 (ICAM-1) and nectin-2 (also known as CD112, a modulator of T cell signalling) expression on $CD13^+$ myeloid leukaemia cells[119]. When transplanted together with peripheral blood mononuclear cells in humanized mouse models, these T_R1 cells suppressed xenogeneic GvHD while retaining and contributing to GvL responses[119].

On the basis of these findings, it is would highly premature to conclude that T_{reg} cells have no adverse effects on GvL efficacy. Indeed, GvL activity is not uniformly retained upon adoptive transfer of T_{reg} cells in preclinical models[87]; however, preliminary clinical results with this approach provide encouraging evidence of GvHD suppression without demonstrable evidence thus far of increased relapse rate[85,98].

NK cells in GvT responses

NK cells were originally characterized as a radioresistant lymphoid cell population mediating the spontaneous rejection of bone marrow but not solid tissue allografts in lethally irradiated, unsensitized or naive mice[120]. This rejection occurred even in strain combinations in which irradiated F1 hybrid recipients rejected parental bone marrow cell allografts in a phenomenon termed ‘hybrid resistance’, which was at variance with the classical laws of transplantation, owing to co-dominant expression of MHC[121]. Subsequently, NK cells were found to be components of the innate immune system that notably mediate spontaneous MHC-unrestricted killing of transformed or virally-infected cells and to lack classical antigen-receptor gene rearrangements with associated diversity and specificity. Unlike T cells, NK cells do not seem to expand clonally and are not considered long-lived, being generated in the bone marrow throughout life, also contrary to the declines in T cell generation in the ageing thymus[122,123]. The features distinguishing NK cells (innate immunity) from T cells (adaptive immunity) have become blurred owing to their use of common pathways and receptors, as well as the recent identification of potentially long-lived adaptive or memory-like NK cell populations[122,124]. Human NK cells lack expression of CD3 but express CD56 as well as multiple NK cell-associated receptors, including members of the NKG2 C-type lectin receptor and killer-cell immunologlobulin-like receptor (KIR) families, that can directly bind to MHC and MHC-like molecules, resulting in potent stimulation or inhibition of NK cell activity depending on the nature of the receptor signalling motifs[123,125]. NK cells also express low-affinity immunoglobulin γ Fc region receptor III (FCγRIII, also known as CD16) and, therefore, participate in antibody-dependent cellular cytotoxicity (ADCC)[123,126]; whether this characteristic can be exploited

clinically is currently being tested by administering NK cells in combination with tumour-targeting mAbs, such a cetuximab (NCT02507154 and NCT03319459) or rituximab (NCT02782546 and NCT0038994) (BOX 1). After NK cells have become activated either through exposure to stimulatory cytokines, such as IL-2, IL-12, IL-15, IL-21 and others, or by engagement of activating receptors such as NKG2D, their cytotoxicity mechanisms are augmented[123]; NK cells might also undergo a transition to 'memory-like' or 'adaptive' phenotypes resulting in increased longevity and enable prolonged and rapid-recall responses with the production of a wide array of cytokines and/or chemokines, notably IFNγ and GM-CSF[122,124]. This memory phenotype is most associated with the role of $NKG2C^+$ NK cells in responses to virally infected cells in individuals with CMV[126,127], and the generation and use of memory NK cell populations might result in greater efficacy after adoptive transfer (BOX 1). Given the sizable population of poorly cytolytic $CD56^{hi}$ NK cells in lymph nodes, the dominant effector functions of NK cells might actually be mediated through immunoregulatory cytokines or direct killing of T cells[123,127]. Paradoxically, NK cells with inhibitory receptors to 'self' MHCs develop with increased activation thresholds, possibly as a means to protect against (termed 'licensing', 'education' or 'arming')[125], which has stimulated intense investigation on whether this difference in activation is important in responses against cancer in the context of allo-HSCT or after extensive ex vivo activation and expansion.

NK cells seem to be prime candidates to mediate GvT responses after allo-HSCT, owing to their principal localization in haematological tissues, their limited ability to directly attack solid tissue allografts thus being unlikely to initiate GvHD, the fact that they are the first lymphoid cell to reconstitute after HSCT, and their well-described preclinical ability to directly target and kill leukaemia and metastatic tumour cells[128]. In mouse models of allo-HSCT, additional engraftment of IL-2-activated allogeneic donor NK cells inhibited GvHD, reflecting competition with and suppression or even killing of activated T cells from the graft[128,129], or possibly suppression of recipient DCs that fuel GvHD reactions of the donor T cells[130], whilst also providing a GvL effect[128,130]. On extrapolation to clinical studies, adoptive NK cell transfer before or after allo-HSCT caused no marked exacerbation of GvHD[131,132], except in one study with donor memory-phenotype NK cells transfer following T cell-depleted allo-HSCT[133], suggesting that NK cell culture and activation conditions might be pivotal in determining GVHD effects[133–136]. Owing to the ability of NK cells to produce numerous pro-inflammatory cytokines, exacerbation of GvHD in T cell-replete grafts will remain a concern associated with infusion of donor NK cells (especially if cytokines such as IL-2 or IL-15 are also administered), whether or not they induce or simply amplify the T cell-driven pathological processes. To date, adoptive cell therapy with donor-type or third-party activated NK cells administered either prior to or following allo-HSCT has been associated with encouraging antitumour effects in paediatric patients with MDS or AML, resulting in both objective responses and reductions in the abundance of high risk clones[132]; other studies in adults have demonstrated objective responses with negligible toxicities or GvHD[131,137,138]. Questions still surround the limited persistence of the NK cells after transfer in HSCT, which might in part be attributed to both their cytokine dependence and limited clonal expansion capabilities[136], as well as whether the GvT responses occurring after HSCT are solely due to the transferred NK cells.

Preclinically, important species-specific differences in NK cell biology preclude direct extrapolation of findings in mouse models to humans. Notably, the MHC-binding molecules in humans (KIR) and mice (Ly49) are structurally distinct and, as opposed to the dominant presence of $CD56^{hi}$ NK cells in human lymph nodes, mouse NK cells (which also lack expression of CD56) are absent from lymph nodes unless activated[123,126]. However, an important similarities exist: NK cells of both species are highly diverse, have similar MHC recognition and functional pathways and belong to multiple subpopulations capable of exerting diverse immunological effects[123,126].

With regard to clinical application in cancer and HSCT, similar to highly activated T cells, NK cells are capable of mediating considerable 'off-target effects' and toxicities, particularly after prior activation. For example, findings from preclinical studies suggest that vascular leak syndrome associated with high-dose IL-2 is attributed, in part, to NK cell responses against endothelial cells expressing NKG2D ligands[139]. NK cells constitutively express NKG2D, a major activation and recognition receptor for induced self-proteins of the MIC and RAET1/ULBP families that are often upregulated on stressed, rapidly proliferating, infected or neoplastic cells. NKG2D is also expressed by activated T cells and, in this context, has been implicated as mediator of GvHD, but this pathway also likely contributes to GvT effects[140]. When used to target cancers via NKG2D-CAR T cells, off-target toxicities resembling cytokine-release syndrome occurred in mice[139]. Thus, broad antitumour effects might be achieved by exploiting NKG2D or even NK cells themselves, although potentially severe toxicities might occur and warrant caution in clinical studies particularly when given with immunostimulatory cytokines and after cytoreductive conditioning of the recipient.

Considerable variability in the contribution of NK cell to the GvT effect has been noted across different haematological malignancies. Among leukaemias, AML has by far the strongest evidence of NK cell-mediated GvL activity, and allo-HSCT is common in patients with this disease, thus generating intense interest in exploiting NK cells in this setting (BOX 1). A pivotal study galvanized the field with the observation that donor–recipient KIR ligand-mismatching during allo-HSCT resulted in substantially greater protection from disease relapse in patients with AML but not adult patients with ALL[130]. Subsequently, multiple clinical studies revealed similar associations of KIR and KIR ligand incompatibility (that is, the presence or absence of activating KIR ligands on the recipient cells that are capable of binding inhibitory donor NK cell KIRs) can result in greater donor NK cell alloreactivity and GvT[141–145]. The complexity of these processes are attributable to the inherent complexity of NK cell themselves: NK cells exist as multiple subsets expressing different KIRs, with some being activating and others inhibitory. In addition to KIR on NK cells, other inhibitory molecules, notably NKG2A which is present on activated T cells and resting NK cells, binds HLA-E, impacting NK licensing as well as anti-tumor activities and likely will be an important therapeutic target[146]. With regard to allo-HSCT, intense interest has been focus on delineating the optimal KIR expression pattern in donors to generate the greatest GvT benefit. Certain KIR combinations do result in improved outcomes, as highlighted in studies using donors with a KIR B haplotype and/or KIR2DS1 genotype, which resulted delays in disease relapse in patients with AML, as well as patients with MM, CLL, lymphomas or even paediatric ALL[141–144,147]. The not insignificant differences in

allo-HSCT protocols between studies have made comparisons difficult, not least because the impact of such differences on the effectiveness of donor NK cell reconstitution can be considerable; however, the associations indicate that application of KIR–KIR ligand-typing protocols to allo-HSCT could be beneficial (BOX 1). Surprisingly, efforts to build on and further augment GvT activity in appropriately mismatched donor–recipient pairs by concurrently promoting or augmenting donor NK cell recovery and/or activation following allo-HSCT have largely been unsuccessful[148], and thus the mechanisms underlying the reduced relapse with KIR–KIR ligand mismatching, and how to further exploit this process, are unclear. The complexity in expression of different KIRs in various NK cell subsets and the use of highly activated NK cell products, which might over-ride the effects of the inhibitory KIRs, raise questions regarding the overall 'net' impact that these receptors have on GvT responses, especially considering that downmodulation of MHC expression often occurs during cancer progression. Interestingly, similar to the observations relating to KIR expression and GvT effects, intriguing associations exist between with KIR expression status and the efficiency of ADCC, notably with KIR B haplotype donors. For example, differences in KIR expression have been associated with differences in the efficacy of NK cell-mediated ADCC with dinutuximab therapy for neuroblastoma[149] indicating that multiple effector functions may be impacted by KIR/KIR ligand interactions. These observations suggest that KIR–KIR ligand typing combined with use of expanded adaptive or memory-like NK cell populations could enhance responses to cell therapies, especially if combined with mAb to induce ADCC (BOX 1). This approach is under clinical investigation using antibodies to a range of targets, including cetuxmimab, transtuzumab, nimotuzumab and rituximab (NCT0398097, NCT02507154, NCT03554889 and NCT00383994).

In contrast to haematopoietic malignancies, clinical NK cell-mediated GvT responses to solid tumours are less robust. NK cell responses to glioblastoma[150] and neuroblastoma[151] have been correlated with better disease outcomes. However, these associations might be contingent on not only the tumour type and the compatibility of the tumour sites with NK cell trafficking and access, but also how stringently the NK cells were assessed and characterized (NK cells share many markers and properties of T cells). In addition, tumour cell subpopulations might have differential sensitivity to NK cells. For example, multiple reports indicate that NK cells can mediate selective cytotoxicity against various solid cancer cells with a cancer stem cell (CSC) or tumour-initiating cell phenotype, owing to low levels of MHC expression with concurrent NKG2D ligand expression within these cells[152,153], which could potentially be exploited clinically. This preferential targeting pattern does not hold true for all cancers, however, given a report that the CSC phenotype in AML is associated a lack of NKG2D ligand expression, which was also correlated with a lower remission rate and an increased risk of relapse in patients with AML[154].

Evolutionarily, NK cells have a strong association with protection against viruses, particularly CMV and other herpes viruses; viruses and NK cells have evolved specific evasion and recognition strategies, respectively, highlighting the importance of this relationship[123]. Clinical studies have revealed that CMV reactivation following allo-HSCT results in increased numbers of activated and memory-like, $NKG2C^+$ NK cells that are correlated with a low risk of disease relapse[155,156]; however, the mechanisms underlying the effect of CMV reactivation on NK cell recovery and possible GvT responses mediated by

these cells remain unclear. Similar to CMV reactivation, greater expansion of memory or adaptive-phenotype NK cell populations, defined either by expression of NKG2C or by transcriptional profiles similar to memory T cells, after allo-HSCT of CMV-negative grafts has been associated with a reduced relapse risk and seems to be determined by host factors (that is, the frequency of these cells mirrors the pattern of NK cell diversity in the recipient prior to transplantation)[157]. Whether adoptive transfer of adaptive NK cells generated ex vivo, or promotion of their de novo development following allo-HSCT, results in similar effects on cell persistence and GvT activity remains to be determined and is currently under clinical evaluation (NCT01898793 and NCT0289092).

IL-15 is a key cytokine necessary for the development and survival of NK cells[123]. Importantly, IL-15 is unique in its requirement for trans-presentation, usually by a myeloid cell, for optimal triggering of IL-15 receptor signalling in the NK cell, explaining why optimal signalling and improved efficacy of systemically administered IL-15 is typically achieve though its conjugation with regions of the IL-15 receptor alpha subunit (creating IL-15 ‘superagonists’). Given the relative rarity of NK cells among the haematopoietic cell populations used for HSCT, various ex vivo culture expansion regimens are used needed for clinical application of NK cells and usually involve combinations of cytokines (for example, IL-21, IL-15, IL-2, IL-12 or IL-7) and/or feeder cell lines comprising IL-21-transduced tumour cells[126]. Unfortunately, upon continuous activation and expansion, like T cells, NK cells upregulate various inhibitory molecules and can possibly become anergic[158]. For example, increased expression of the immune-checkpoint proteins TIGIT, PD-L1 and lymphocyte activation gene 3 protein (LAG3) results in inhibition of NK cell function, which can potentially be reversed through ICI[13,159,160,161], thus, these mechanisms are attractive targets for combination therapies (BOX 1). Many of these proteins are also expressed by T cells and, therefore, ICI following allo-HSCT might result in exacerbation of GvHD; nevertheless, such approaches to circumventing NK cell exhaustion and/or anergy in order to enhance their persistence and function have reached clinical testing[162] (NCT03937895, NCT039588097 and NCT02118285). In addition, use of Toll-like receptor agonists (for example, targeting TLR9) is currently under clinical investigation (NCT02452697). Targeting of other pathways that directly augment NK cell function is also being pursued. For example, SLAMF7 targeting with elotuzumab has been demonstrated to not only inhibit MM cells but also activate NK cells[163], and is under clinical investigation (NCT03003728).

NK cells can be suppressed extrinsically by T_{reg} cells and MDSCs; strategies to overcome these immunosuppressive interactions enhance NK cell-mediated antitumour effects in patients or patient cells ex vivo[164,165]. Similarly, the use of exogenous IL-15 or an IL-15 super-agonist, such as ALT-803, constructed to mimic physiological trans-presentation is being tested in patients with AML after allo-HSCT, with or without donor NK cell transfer[166] (NCT01898793). Continuous IL-15 signalling has been reported to result in NK cell transformation[167]; therefore, the use of continuous administration of IL-15, or NK cells genetically engineered to express this cytokine in order to induce autocrine signalling, raises the potential requirement for engineering of donor effector cells with ‘suicide’ vectors to enable termination of their activity.

Blocking interactions between inhibitory KIRs and their ligands using antibodies was viewed as a promising strategy based on preclinical data demonstrating that targeting of inhibitory Ly49 family receptors, which recognize MHC I molecules to distinguish self-cells from non-self-cells, results in heightened NK cell-mediated anti-leukaemia effects in mice[168]. KIR-blocking antibodies also increase the activity of human NK cells in vitro, but a clinical trial revealed that the anti-KIR2D mAb IPH2101 increased MM progression in association with impairment of NK cell activity attributable to either removal of KIR2D from NK cells by trogocytosis or effects of this agent on NK cell development[169]. Thus, the effects of any therapeutic intervention to enhance the GvT activity of NK cells — whether via increased stimulatory signaling or blockade of inhibitory pathways — on the overall development and function of NK cells needs to be considered, especially in the setting of lymphopenia and immune reconstitution after allo-HSCT that could adversely affected the developmental pathways.

Next steps in adoptive NK cell immunotherapy.

Several key factors can stifle the antitumour efficacy of NK cells therapies and these revolve around infusing enough NK cells to ensure sufficient engraftment, homing to the tumour, and long-term survival and function. NK cells constitute only a small pool of lymphocytes and need to be extensively expanded in culture in order to generate sufficient numbers of activated cells, although third-party or off-the-shelf sources of NK cells can partially obviate this issue. Pre-allo-HSCT cytoreductive conditioning treatment induces factors such as IL-7 and/or IL-15 that, in a lymphopenic environment, can result in successful, albeit short-term NK cell engraftment[166]. Considerable research interest has been placed on different sources of NK cells other than bone marrow-derived or peripheral blood mononuclear cell (PBMC)-derived stem cells or progenitors, which might enable enhancements of cell expansion and/or function, particularly as an off-the-shelf, third-party product; however, careful comparisons and assessments of these different approaches have not been performed. The alternative stem cell sources include cord blood[170] (NCT00354172), placenta-derived cells (NCT029550) or induced pluripotential stem cells (iPSC)[171] (NCT04106167), that might not only have greater expansion and antitumour capabilities but also be more ameniable to genetic manipulation (BOX 1); however, the exact developmental, phenotypic and functional properties of these products relative to those of adult HSC-derived or PBMC-derived NK cells remain unclear. Clinical use of established immortalized NK cell lines (such as NK92 cell) has resulted in transient anticancer effects[172,173], and could enable improved access, homogeneity and genome-modification success rates; however, owing to their immortalized nature and sometimes substantial deviations in receptor expression and functional profiles relative to ‘normal’ NK cell, these cells must be irradiated prior to clinical use, which severely limits their in vivo persistence (measured in days) and is likely to substantially alter their biology and function. Such third-party NK cell approaches also facilitate attempts to promote activation of the cell product, for example, through genetic deletion of inhibitory signals or transduction with growth factors such as IL-15, and to improve tumour cell-targeting through CAR expression[126] (BOX 1). With regard to the latter approach, whether CAR expression in either NK cells or T cells is most advantageous remains unknown, but warrants consideration of the broad reactivity and extensive inflammatory cascade of NK cells as well as their limited persistence. Whether third-party CAR T cells can induce GvHD

is a crucial question for the field, because a primary rationale for using donor or third-party CAR NK cells is their presumed inability to induce GvHD.

Regardless, several CAR NK cell products are under clinical investigation to target not only cancer cells directly[174], but also mediators of tumour immune evasion, such as MDSCs[175]. In addition, disrupting inhibitory factors, such as indolamine (IDO), together with NK cell transfer (NCT02118285), and/or engendering NK cell products with expression of IL-15 to promote sustained autocrine signalling are also being assessed (NCT03056339), with increases in activity and/or persistence being reported in preclinical studies[139,170,171,176]. Impressive effects of third-party, cord blood-derived, anti-CD19 CAR NK cells transduced with IL-15 and a suicide vector have also been demonstrated in patients with refractory lymphoma, with an overall response rate (ORR) of 73%, no observable CRS or GvHD and long-term CAR NK cell engraftment (for >9 months)[177]. However, important caveats of this study include the use of secondary treatments in the majority of patients, which might have contributed to the ORR, and the small size of the patient cohort. Additionally, the use of PCR to ascertain product engraftment is particularly useful in patients in whom the level of the abundance of CAR T cells is at or below the limits of sensitivity of flow cytometry-based quantification. Nevertheless, the findings do lend promise to the further development of CAR NK cell products, whether they be autologous or third-party. As with other NK cell products, stringent characterization of the reproducibility of the generated product is needed. Other questions relate to the homogeneity, stability and activity of the cell product, given the complex interactions of the different receptors systems on NK cells and that the cell sources or culture conditions used might not accurately mirror the normal developmental paradigms. Regardless of the source and manipulations, any activated NK cell product used after HSCT must circumvent the issues of cell engraftment and persistence whilst retaining or enhancing antitumour activities and avoiding possible off-target toxicities, including effects on haematopoietic and immune reconstitution. This consideration is particularly germane when using third-party NK cells, which have the potential to target a range of both HSC-donor and recipient cell types.

Increasing the GvT potential of NK cells.

The immunomodulatory activities of currently used anti-neoplastic agents can be exploited to augment NK cell-mediated GvT activity. Lenalidomide, a thalidomide-derivative IMiD, results in increased NK cell activity and ADCC in patients with MM, but also increased GVHD[178], possibly owing to effects on MDSCs and PD-1–PD-L1 signalling[179]. As discussed for T cells, the proteasome inhibitor bortezomib also sensitizes tumour cells, including CSCs, to NK cell-mediated cytotoxicity, in part through upregulation of death receptors and suppression of MHC expression[152], and is under clinical investigation in combination with autologous NK cell transfer for CML (NCT00720785)[169] (BOX 1). However, immunosuppressive therapies are used after allo-HSCT, and data demonstrate that GvHD can also suppress NK cell function (owing to competition by T cells for IL-15)[180]; these variables complicate efforts to determine the effects of agents such lenalidomide and bortezomib on NK cell-mediated GvT activity, in addition to the fact that many agents have NK cell-independent antitumour effects.

A new generation of novel molecules that can simultaneously retarget NK cells towards tumour cells and promote NK cell activation are currently under investigation. Originally developed as BiTEs comprising CD19-targeting and CD3-targeting antibody moieties to redirect T cells towards B cells, such constructs have proven clinical activity in patients with ALL[181]. Variations on this approach, including bi-specific killer cell-engagers (BiKEs) and trispecific killer cell-engagers (TriKEs), are now being pursued to promote NK cell targeting of various tumour-associated markers (such as EGFR in glioblastoma, GD2 in neuroblastoma, CD33 in AML and CD19 in CLL, among others), provide cytokine and/or stimulatory signals (for example, via IL-15 or agonistic anti-FcγRIII moieties, respectively, with the latter being highly specific for NK cells), and/or block inhibitory molecules (such as PD-1, PD-L1 or TGFβ), thereby increasing NK cell GvT effects[182–186] (NCT03214666) (BOX 1). The advantages of such agents include their potential to engage and activate both endogenous and engrafted donor NK cell populations, as well as any transferred third-party NK cell products. Thus, approaches to optimize NK cells as an adoptive cell immunotherapy and to augment NK cell reconstitution and activation following HSCT are both likely to improve GvT efficacy.

NKT cells and γδT cells to augment GvT

NKT cells are subset of T cells that express both NK (CD56) and T cell (CD3 and TCR) markers but have restricted TCR diversity and are endowed with prominent immunoregulatory properties. This cell type has been under increased investigation, in part, owing to their reported role in suppressing GvHD in preclinical models and antitumour effects in multiple models[187,188]. The activity of NKT cells in suppressing GvHD in mice has been shown to be dependent on production of IL-4 by these cells and their migration to germinal centres, which are associated with expansion of T_{reg} cell populations and resultant suppression of germinal centre reactions[187]. Conditioning regimens that foster the induction of NKT cells, such as total lymphoid irradiation (TLI), are also being applied, as well as adoptive transfer of purified NKT cell populations[187]. CAR NKT cells are also being assessed in various models of cancer, including solid tumours[189]. While the limited abundance of NKT cells remains an issue, this cell type might offer advantages in cell therapy compared with broadly lytic NK cell populations, owing to the immunoregulatory effects of NKT cells in suppressing GvHD when used post-allo HSCT as well as their potentially greater clonal expansion capabilities[187]. Clinical studies involving adoptive transfer of NKT cells are underway (NCT00631072). In preclinical models, NKT cell activation using G-CSF analogues results in potent anti-leukaemia effects via promotion of DC and $CD8^+$ T cell responses[190], suggesting indirect pathways leading to suppression of GvHD and enhancement of GvT effects.

γδT cells are another arm of T cell-mediated immunity, and these cells link adaptive and innate responses owing to their MHC-independent specificity for a limited range of target antigens, leading to antitumour effects with lesser GvHD risk[188], similar to NK cells. Owing to the positive associations between the abundance of γδT cells and clinical outcomes following HSCT[191–195] as well as the ability of activated γδT cells to kill leukaemic cells[196], the use of this cell type as an immunotherapy to mediate GvT without exacerbating GvHD is garnering increasing attention[193]. Similar to NK and NKT cells, the relative rarity

of these γδT cells necessitates extensive ex vivo expansion, use of other cell sources (such as iPSCs) and/or genetic manipulation to obtain sufficient numbers for clinical use[192]. In contrast to NK cells, which rapidly reconstitute after HSCT, the delayed recovery of both NKT and γδT cells likely places more emphasis on the optimizing the characteristics of the donor cells to maximize their persistence and antitumour efficacy, for example, by removing αβT cells from the donor graft[197].

Conclusions

Understanding of the immunological mechanisms governing effective GvL responses remains in its infancy. GvL — or GvT — effects can result from the allo-HSCT process itself, tied to GvHD responses by infused donor T cells or selected effector populations, or from the generation of a more balanced and better functioning immune system. Clearly, more robust preclinical models are needed that better reflect the ever-changing clinical allo-HSCT scenario and that enable focus to be placed on GvT processes independently of GvHD. With increased characterization of cancer cells upon disease relapse, new insights can be gleaned on immune evasion pathways and thus the effector cells that are important for GvT efficacy. MHC II-restricted GvL responses, which seem to be highly relevant according to findings from clinical studies, are important to more fully understand, as is GvHD and the contributions of alloreactivity to persistent GvL responses. Currently, the success of allo-HSCT is judged by the absence of marked acute or chronic GvHD, and modern immunosuppression therapies have enabled considerable advances in this regard. Thus, pinpointed focus on inducing immunity against leukaemia and haematopoietic antigens, rather than broad immune responses to alloantigens, will become increasingly vital for prevention of disease relapse. In some malignancies in which GvT responses are limited, such as MM, immunotherapy might be best applied after auto-HSCT. After allo-HSCT, adoptive cell therapy approaches will likely necessitate genome modification of the cell products or the use of novel agents to heighten the specificity and sustainability of antitumour responses. The increasing application of third-party or off-the-shelf cell therapies opens new opportunities to enhance GvT responses, but limited cell persistence and off-target toxicities remain paramount concerns. These advances, which have served to minimize GvHD and transplant-related mortality, thus now seem likely to usher in a new era wherein therapeutic modulation of anticancer immunity becomes pre-eminent and presents the potential to expand the clinical utility of HSCT to cancers other than haematological malignancies.

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Box 1 |

Potential approaches to augment and sustain GvT responses after allo-HSCT

T cell-focused therapeutic strategies

- Target malignant cells directly and/or enhance their susceptibility to graft-versus-tumour (GvT) responses, for example, using ibrutinib, venetoclax, sorafenib, 5-azacytidine or JAK1 and/or JAK2 inhibitor (such as ruxolitinib, baricitinib and INCB18424).
- Promote T cell recognition of the recipient's leukaemic cells and/or haematopoietic progenitor cells, for example, by infusing isolated cytotoxic $CD8^+$ T lymphocytes (CTLs) specific to leukaemia-associated or haematopoietic cell-restricted minor antigen, such as Wilms tumour protein (WT1) or minor histocompatibility antigen HA-1, respectively.
- Prevent T cell exhaustion via immune-checkpoint inhibition, for example, with monoclonal antibodies to programmed cell death 1 (PD-1), cytotoxic T lymphocyte protein 4 (CTLA-4), T cell immunoreceptor with Ig and ITIM domains (TIGIT) and/or lymphocyte activation gene 3 protein (LAG3), although with the potential increased risk of graft-versus-host disease (GvHD) in allogeneic haematopoietic stem cell transplantation (allo-HSCT), but not autologous HSCT (auto-HSCT).
- Enhance antigen presentation and recognition using IFNα or IFNβ.
- Deplete, incapacitate, destabilize or neutralize immunosuppressive immune cells, including host regulatory T (T_{reg}) cells (for example, using the IL-2–diphtheria toxin conjugate, denileukin diftitox), myeloid-derived suppressor cells (MDSCs, using gemcitabine) and/or monocytes or macrophages (using anti-colony-stimulating factor 1 receptor antibodies).
- Counteract cell-mediated immunosuppressive mechanisms in the tumour microenvironment, for example, using inhibitors of indoleamine 2,3-dioxygenase enzymes, transforming growth factor-β (TGFβ) or nitric oxide production, anti-IL 18 antibodies, or cyclooxygenase-2 inhibitors to suppress prostaglandin E_2 signalling.
- Selective depletion of α/βT cells from the donor graft to enrich donor γ/δT cells.
- Infuse CTLs transduced with specific T cell recepters (TCRs) or chimeric antigen receptors (CARs) targeting particular tumour-associated antigen (for example, anti-CD19 CAR T cells for patients with B cell malignancies).
- Infuse anti-viral CTLs with cross-reactivity to virus-induced malignancies, such as Epstein–Barr virus (EBV)-associated lymphoma

- Administer bi-specific T cell engagers (BiTEs), such as the anti-CD19–CD3 BiTE blinatumomab
- Vaccination with tumour-associated antigens, such the PR1 peptide derived from proteinase 3 and neutrophil elastase that are highly expressed in myeloid leukaemia blasts
- Taper or stop treatment with GvHD prophylaxis agents, such as cyclosporin A

NK cell and NKT cell-focused therapies

- Augment and/or accelerate donor natural killer (NK) cell reconstitution and recovery after allo-HSCT, especially via killer cell immunoglobulin-like receptor (KIR) and KIR ligand profiling of the donor and host, respectively, to ensure optimal mismatch in graft-versus-recipient direction, but also by using IL-15 superagonist or Toll-like receptor agonists that stimulate production of IFNα and IFNβ to augment NK cell function.
- In conjunction with adoptive NK cell infusions, increase target recognition or sensitivity to cytolysis (for example, using bortezomib, sorafenib or lenolidamide), or through upregulation of NKG2D stress ligands and/or decreased MHC expression.
- Disrupt tumour-intrinsic immunosuppressive pathways affecting NK cell function, for example, using immune-checkpoint inhibitors (as above outlined for T cells)
- Genetically modify donor or third-party NK cells, for example, to delete MHC (*HLA*) class I/II genes and/or overexpress *HLA-E*, in order to reduce their immunogenicity and thus rejection, thereby leading to sustained engraftment.
- Determine the optimal source of NK cell progenitor populations (immortalized NK cell lines, cord blood-derived NK cells, placenta-derived NK cells, induced pluripotent stem cell-derived NK cell or peripheral blood mononuclear cell-derived NK cells) resulting greater engraftment, survival and function.
- Determine importance of inducing a memory phenotype ($NKG2C^{+}$) to the activity of transferred NK cells and whether cytomegalovirus reactivation affects the development of memory NK cell and this protection from disease relapse.
- NK cell engineering for expression CARs or TCRs to increase tumour targeting.
- Use of tumour-targeting antibodies to mediate antibody-dependent cellular cytotoxicity (ADCC); use of NK cell engineering or use of enzymatic inhibitors, particularly deletion or inhibition of ADAM17, to render activated NK cells resistant to Fc receptor cleavage and thus more efficacious in ADCC.

- Infuse natural killer T (NKT) cell subsets, either enriched or ex vivo expanded populations, or molecules that support NKT cells, such as α-galactosylceramide.
- Administer bi-specific antibodies or tri-specific killer engagers (TRiKEs) to promote interactions between specific activatory receptors on NK cells (such as CD16) and particular antigens on tumour cells (such as CD19 and/or CD22)
- In general, select patients with cancer types most susceptible to NK cell-mediated killing, such as AML and glioblastoma, and those with metastatic versus primary disease based on the inherent ability of NK cells to target and kill metastatic tumour cells.

Key points

- Autologous haematopoetic stem cell transplantation (HSCT) migh present the optimal platform for immunotherapies, particularly for myeloma and lymphoma. Future approaches to improve allogeneic HSCT will require the introduction of innovative immune and/or targeted therapies to prevent relapse, following effective prevention of graft-versus-host disease (GvHD).
- In initial clinical trials, freshly isolated or in vitro expanded donor regulatory T (T_{reg}) cell infusions have been associated with low GvHD rates; in preclinical models and in preliminary clinical studies, donor T_{reg} cell infusion does not seem to increase leukaemia relapse rates as had been feared, although more extensive, controlled trials are needed.
- Immune system-targeted approaches to treat disease relapse after autologous or allogeneic HSCT now include pharmacological agents that block pathways essential for tumour cell survival and at the same time, for allogeneic HSCT, suppress GvHD.
- Immune-checkpoint inhibitors targeting inhibitory signalling pathways in T cells or infusion of T cells genetically modified to express chimeric antigen receptors (CARs) or T cell receptors (TCRs) reactive with tumour cells might prove advantageous in autologous HSCT, but the risk of GvHD warrants careful consideration in allogeneic HSCT.
- Preclinical models used to delineate graft-versus-tumour (GvT) from GvHD responses as well as assess potential therapeutic interventions often use inbred laboratory mouse strains, which offer many advantages mechanistically, but crucial caveats beyond immunological differences between species need to be acknowledged during clinical extrapolation.
- Nature killer (NK) cells are increasingly being examined for their ability to mediate GvT effects while having minimal effects on GvHD after HSCT, including the use of third-party CAR NK cell products and use of targeted biologic agents to exploit the ability of NK cells to mediate antibody-dependent cellular cytotoxicity.

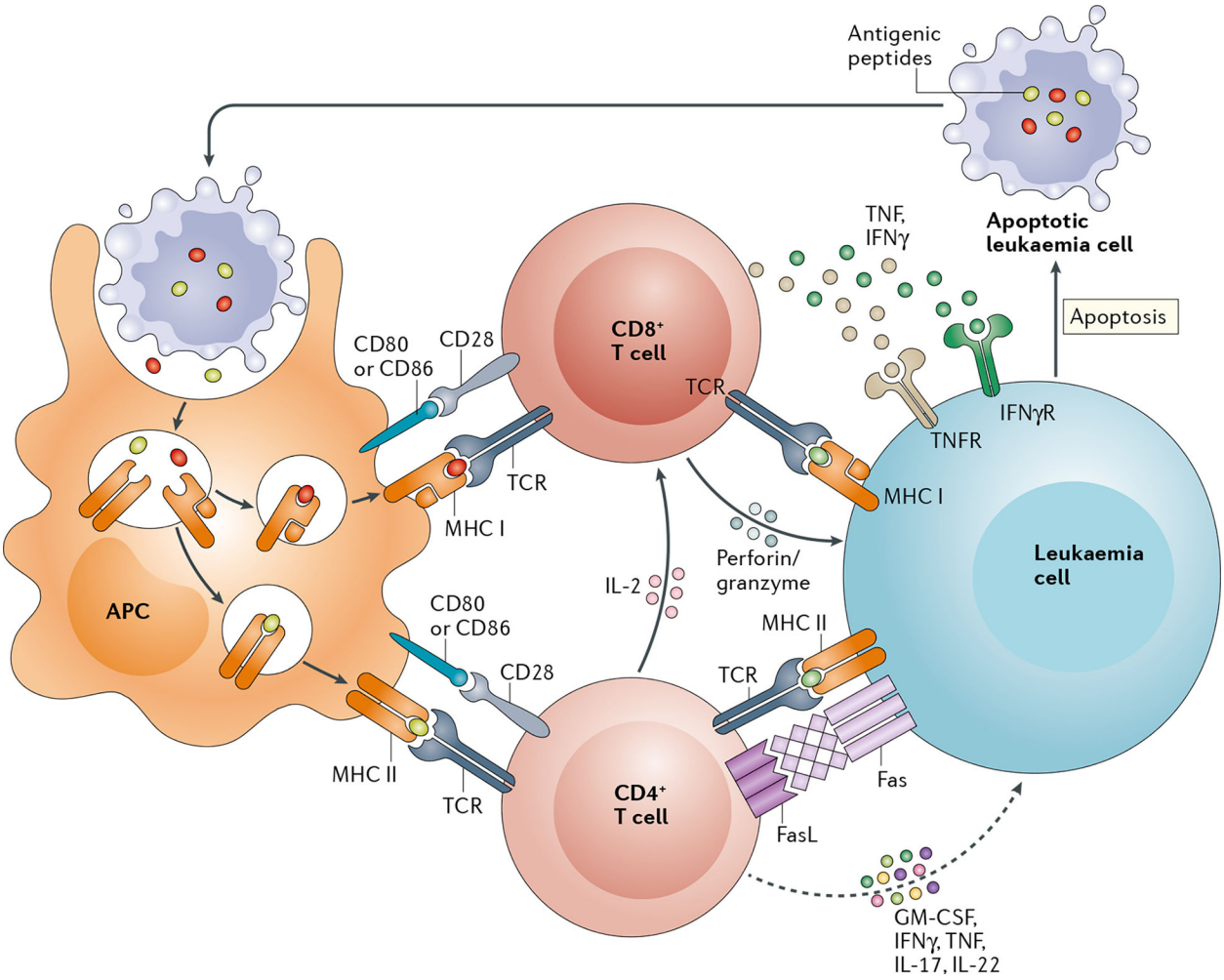

Fig. 1 |. Mechanisms of leukaemia cell recognition and killing after haematopoietic stem cell transplantation.

Early after transplantation, allogeneic and leukaemia antigens derived from apoptotic cells of the recipient are presented by host antigen-presenting cells (APCs) in the context of MHC class I (MHC I) and MHC II molecules. Interaction of these peptide–MHC complexes with T cell receptors (TCRs), in conjunction with CD80/CD86–CD28 co-stimulatory signalling, induces the activation and differentiation of donor $CD8^+$ T cells and $CD4^+$ T cells to generate effector T cell responses. These effector T cells mediate MHC I-dependent and MHC II-dependent graft-versus-leukaemia (GvL) effects via cytolytic pathways that involve perforin and granzyme and tumour necrosis factor (TNF) superfamily members, including Fas–Fas ligand (FasL) and TNF–TNF receptors (TNFRs). Secretion of IFN**γ** by effector T cells enhances antigen presentation by leukaemia cells and thus their recognition during the GvL response. GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN**γ**R, IFN**γ** receptor.

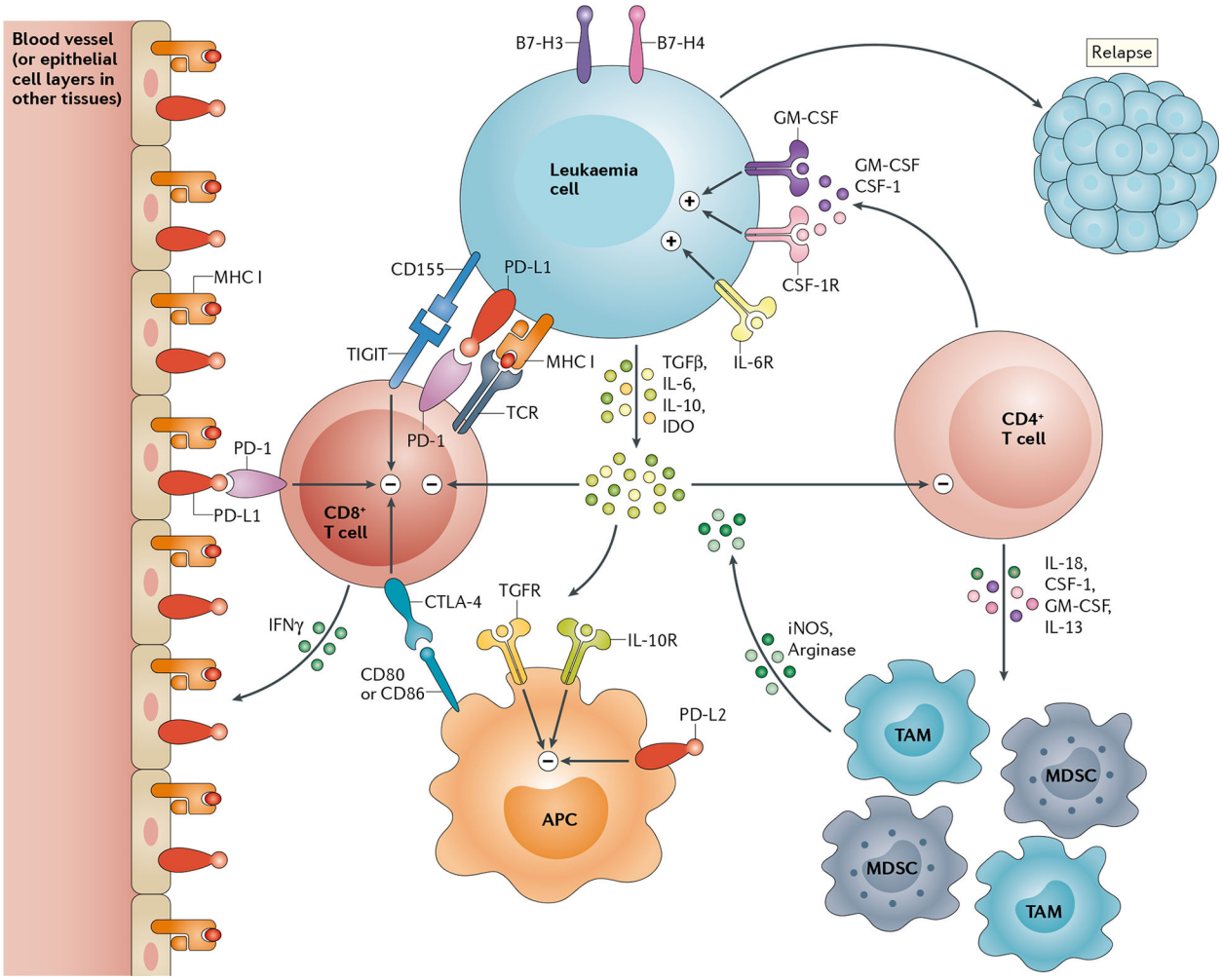

Fig. 2 |. Mechanisms of immune escape after haematopoietic stem cell transplantation.
After transplantation, leukaemia cells can lose expression of MHC class II (MHC II) molecules and upregulate expression of inhibitory immune-checkpoint ligands, including programmed cell death 1 ligand 1 (PD-L1), CD155 (also known as poliovirus receptor) and B7–H3 and/or B7–H4. Leukaemia cells can also secrete soluble suppressive factors, such as transforming growth factor-β (TGFβ), IL-10 and produce indoleamine 2,3-dioxygenase (IDO) enzymes resulting immune suppressive metabolites that inhibit donor T cell and antigen-presenting cell (APC) function. Inflammation during an alloimmune response (particularly that mediated by IFNγ) induces MHC I-dependent antigen presentation and expression of immune-checkpoint ligands (such as PD-L1) on non-haematopoietic tissue (such as the endothelium) thus contributing to T cell exhaustion, manifested by broad activation of suppressive immune-checkpoint receptors (such as T-cell immunoreceptor with Ig and ITIM domains (TIGIT), programmed cell death 1 (PD-1) and cytotoxic T lymphocyte protein 4 (CTLA-4)) on these cells, and loss of effector function that prevent cytolysis of leukaemia cells. Inflammatory molecules secreted by stromal cells and $CD4^+$ T cells, such as IL-6, IL-13, IL-18, granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (CSF-1), promote leukaemia growth directly and indirectly via the expansion of myeloid-derived suppressor cells (MDSCs) and tumour-

associated macrophages (TAMs). These myeloid cells produce arginase and induce the secretion of nitric oxide synthase (iNOS) that further suppress T cell function and antigen presentation, culminating in immune escape and leukaemia relapse. CSF-1R, macrophage colony-stimulating factor receptor; GM-CSFR, granulocyte–macrophage colony-stimulating factor receptor; IL-6R, IL-6 recepter; IL-10R, IL-10 recepter; PD-L2, programmed cell death 1 ligand 2; TGFR, transforming growth factor-β receptor.

Table 1 |

Key differences between preclinical modelling of allo-HSCT and the clinical paradigms

Feature	Mice	Canines	Non-human primates	Humans
Genotype	Inbred (genetically homogenous), although genetic drift occurs within colonies and between vendors. Limited strain combinations exist or used, and grafts are rarely haploidentical and usually full MHC mismatched	Outbred, with substantial genetic variation between breeds. Haematopoietic stem cell transplantation (HSCT) studies usually involve laboratory beagles. Client-owned canines are used in studies of cancer, but these rarely involve HSCT.	Outbred, although colonies are often have some degree of inbreeding.	Outbred, requiring extensive HLA typing, with a preference for the use of related haploidentical donors.
Age	Predominantly very young (8–12 weeks old, equivalent to early adolescence in humans)	Variable age, often adult	Predominantly young adult	Variable age (heavily skewed to >50 years), depending on cancer type
Environment	Highly restricted and specific-pathogen free (SPF) in most facilities.	Similar environmental exposures to those of humans in client-owned canines, but more restricted in laboratory beagles.	Colonies are highly controlled but have similar environmental and pathogen exposures to those of humans. Cytomegalovirus-positive, unless from SPF colony.	Numerous pathogen exposures (acute, chronic and latent) and pre-existing diseases and morbidities
Recipient body mass index (BMI)	Predominantly young and very lean mice (20–25g) are used	Variable, but often lean in HSCT studies.	Variable, but not obese	Highly variable, with an increasing obesity population (BMI >30), including in paediatric patients
Cancer development and modelling conditions	Established rapidly growing tumor cell lines injected into healthy cancer-naive mice, often presenting with minimal tumour burden at the time of HSCT.	Spontaneous cancer (including lymphoma, sarcoma and melanoma) occurs, with age with breed variations; however, no reliable cancer models exist in laboratory beagles that are typically used for HSCT studies	Minimal cancer occurrence within colonies and often in old age, precluding ability to perform cancer treatment efficacy studies. Accordingly, allogeneic HSCT (allo-HSCT) studies in non-human primates are primarily used to investigate graft-versus-host disease (GvHD) and/or engraftment and immune reconstitution outcomes.	Progressive heterogeneous cancers patients with different tumour burdens, various prior treatments and diverse immune statuses
HSCT conditioning regimens	Predominantly total body radiation (lethal single or split doses)	Reduced-intensity chemotherapy conditioning regimens for HSCT studies in laboratory beagles. Client-owned cancer treatments nonmyeloablative as most veterinary facilities are outpatient.	Chemotherapy with or without total body irradiation, including non-myeloablative regimens, with anti-viral and GvHD prophylaxis	Chemotherapy with or without total body irradiation, including non-myeloablative regimens
HSCT use	Modelling of HSCT for human cancers, including xenogeneic transplant models (human into mouse)	Use of laboratory beagles to model allo-HSCT. HSCT is used in client-owned canines for treatment of spontaneous cancers.	Studies of HSCT, with particular regard to engraftment and immune reconstitution, as well as GvHD	Treatment of a variety of disease states ranging from cancer (predominantly leukaemias and lymphomas) to haematopoietic disorders.
GvHD development	Donor bone marrow used as source of haematopoietic stem cells (HSCs) but additional T cell sources and infusions are required to induce GvHD in SPF mice due lack of infectious agents that augment the GvHD processes.	Donor HSCs are sufficient to cause GvHD, depending on the degree of dog leukocyte antigen (DLA) compatibility; Donor lymphocyte infusion involving DLA-identical siblings could result in no GvHD.	Donor HSCs are sufficient to cause GvHD depending on MHC compatibility and recipient conditioning.	Adult HSC sources, even from related donors or cord blood, are sufficient to cause GvHD depending on the level of HLA compatibility and the conditioning regimen used, although non-myeloablative regimens reduce the risk of GvHD.

Feature	Mice	Canines	Non-human primates	Humans
GvHD type	Usually either acute or chronic, crucially dependent on strain combination used (MHC mismatch or minor MHC mismatch model) and the type of conditioning. Restricted organ involvement in most models.	Acute, chronic or mixed (acute and chronic)	Acute, chronic or mixed, but predominantly severe acute GvHD, depending on MHC compatibility, the conditioning regimen used and the intensiveness of post-HSCT immunosuppressive treatment.	Various immunosuppressive prophylaxis strategies are used and GvHD can be mixed (acute and chronic), with delayed chronic GvHD becoming more prevalent.
Graft-versus-tumour (GvT) effect	Numerous studies of approaches to enhance GvT and limit GvHD, although the emphasis is on GvHD prevention or treatment and only short-term results.	Not evaluated	Minimal investigation; however, the immune and myeloid effects and possible off-target toxicities of regimens that could potentially be used to enhance GvT effects have been assessed	Emphasis has been to improve GvT either through adoptive cell therapies or improved immune reconstitution following HSCT, without exacerbating GvHD