Year: 2021

Anti-CD117 immunotherapy to eliminate hematopoietic and leukemia stem cells

Russkamp, Norman F ; Myburgh, Renier ; Kiefer, Jonathan D ; Neri, Dario ; Manz, Markus G

Abstract: Precise replacement of diseased or dysfunctional organs is the goal of regenerative medicine and has appeared to be a distant goal for a long time. In the field of hematopoietic stem cell transplantation, this goal is now becoming tangible as gene-editing technologies and novel conditioning agents are entering the clinical arena. Targeted immunologic depletion of hematopoietic stem cells (HSCs), which are at the very root of the hematopoietic system, will enable more selective and potentially more effective hematopoietic stem cell transplantation in patients with hematological diseases. In contrast to current conditioning regimes based on ionizing radiation and chemotherapy, immunologic conditioning will spare mature hematopoietic cells and cause substantially less inflammation and unspecific collateral damage to other organs. Biological agents that target the stem cell antigen CD117 are the frontrunners for this purpose and have exhibited preclinical activity in depletion of healthy HSCs. The value of anti-CD117 antibodies as conditioning agents is currently being evaluated in early clinical trials. Whereas mild, antibody-based immunologic conditioning concepts might be appropriate for benign hematological disorders in which incomplete replacement of diseased cells is sufficient, higher efficacy will be required for treatment and elimination of hematologic stem cell malignancies such as acute myeloid leukemia and myelodysplastic syndrome. Antibody-drug conjugates, bispecific T-cell engaging and activating antibodies (TEAs), or chimeric antigen receptor (CAR) T cells might offer increased efficacy compared with naked antibodies and yet higher tolerability and safety compared with current genotoxic conditioning approaches. Here, we summarize the current state regarding immunologic conditioning concepts for the treatment of HSC disorders and outline potential future developments.

DOI: https://doi.org/10.1016/j.exphem.2021.01.003

Originally published at:
Russkamp, Norman F; Myburgh, Renier; Kiefer, Jonathan D; Neri, Dario; Manz, Markus G (2021). Anti-CD117 immunotherapy to eliminate hematopoietic and leukemia stem cells. Experimental Hematology, 95:31-45.

DOI: https://doi.org/10.1016/j.exphem.2021.01.003

Experimental Hematology

Experimental Hematology 2021;95:31–45

PERSPECTIVE

Anti-CD117 immunotherapy to eliminate hematopoietic and leukemia stem cells

Norman F. Russkamp[a], Renier Myburgh[a], Jonathan D. Kiefer[a,b], Dario Neri[b], and Markus G. Manz[a]

[a]*Department of Medical Oncology and Hematology, University Hospital Zurich and University of Zurich, Comprehensive Cancer Center Zurich (CCCZ), Zurich, Switzerland;* [b]*Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH Zürich), Zurich, Switzerland*

(Received 23 November 2020; revised 18 January 2021; accepted 18 January 2021)

Precise replacement of diseased or dysfunctional organs is the goal of regenerative medicine and has appeared to be a distant goal for a long time. In the field of hematopoietic stem cell transplantation, this goal is now becoming tangible as gene-editing technologies and novel conditioning agents are entering the clinical arena. Targeted immunologic depletion of hematopoietic stem cells (HSCs), which are at the very root of the hematopoietic system, will enable more selective and potentially more effective hematopoietic stem cell transplantation in patients with hematological diseases. In contrast to current conditioning regimes based on ionizing radiation and chemotherapy, immunologic conditioning will spare mature hematopoietic cells and cause substantially less inflammation and unspecific collateral damage to other organs. Biological agents that target the stem cell antigen CD117 are the frontrunners for this purpose and have exhibited preclinical activity in depletion of healthy HSCs. The value of anti-CD117 antibodies as conditioning agents is currently being evaluated in early clinical trials. Whereas mild, antibody-based immunologic conditioning concepts might be appropriate for benign hematological disorders in which incomplete replacement of diseased cells is sufficient, higher efficacy will be required for treatment and elimination of hematologic stem cell malignancies such as acute myeloid leukemia and myelodysplastic syndrome. Antibody–drug conjugates, bispecific T-cell engaging and activating antibodies (TEAs), or chimeric antigen receptor (CAR) T cells might offer increased efficacy compared with naked antibodies and yet higher tolerability and safety compared with current genotoxic conditioning approaches. Here, we summarize the current state regarding immunologic conditioning concepts for the treatment of HSC disorders and outline potential future developments.

Biology of HSCs and HSC disorders

Adult hematopoiesis takes place in designated areas of the bone marrow, one of the most proliferative and metabolically active organs of the human body. The average daily output of blood cells is estimated to be in the range of $4–5 \times 10^{11}$ total nucleated cells (reviewed by Nombela-Arrieta and Manz [1]). A sophisticated hierarchical arrangement relieves the rare hematopoietic stem cells (HSCs) from the majority of the mitotic burden, which is carried by diverse multipotent and lineage-restricted progenitor cells [2–8]. In this way, HSCs can remain dormant for extended periods and are less vulnerable to damage and mutagenesis

Offprint requests to: Markus G. Manz, Department of Medical Oncology and Hematology, University of Zurich and University Hospital Zurich, Raemistrasse 100, CH-8091 Zurich, Switzerland.; E-mail: markus.manz@usz.ch

https://doi.org/10.1016/j.exphem.2021.01.003

during cell divisions for self-renewal [9]. During a human life span — under the constant stress of inflammation and aging — mutations do occur in HSCs, and certain genetic abnormalities provide a clonal advantage [10]. The presence of mature nucleated blood cells of clonal origin in peripheral blood without overt hematological disease has been coined *clonal hematopoiesis of indeterminate potential* (CHIP). This condition is strongly associated with aging and can be detected in more than 10% of individuals aged 70 years and older [11–14]. CHIP has been linked to several hematopoietic and non-hematopoietic disorders [11,15]. Recently, the presence of clonal myeloid cells in peripheral blood was found to precede the onset of acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) by years to decades [16,17]. AML and MDS are malignant diseases characterized by uncontrolled proliferation and maturation arrest of hematopoietic progenitor cells, which derive from leukemia-initiating cells (LICs) [18,19].

To our current understanding, the consecutive accumulation of mutations, especially in genes encoding for intracellular signaling proteins, transcription factors, or epigenetic regulators, as well as chromosomal rearrangements, lead to profound aberrations in intracellular signaling cascades of hematopoietic stem and progenitor cells [20,21]. These aberrations are not connected to major recurring changes of the surface proteome on malignant hematopoietic cells when compared with healthy counterparts. Hence, the search for aberrant antigen expression profiles or universal neoantigens that could be exploited for selective cell surface-directed immunotherapy of malignant cells has remained futile to date. Combinatorial targeting of leukemia-associated surface antigens might be one approach to circumvent the lack of truly leukemia-specific surface targets [22,23]. An alternative solution would be the use of immunotherapy directed at surface antigens, which are shared between malignant and healthy hematopoietic cells such as CD117, CD123, and CD135 [24,25]. Currently approved immunotherapies for the treatment of B-cell and plasma cell neoplasias are based on this approach as they are lineage-directed and not malignancy-directed. Here, collateral damage to healthy cells is taken into account and is attenuated by appropriate measures, such as replacement of immunoglobulins. Immunotherapies directed at HSCs will cause severe bone marrow aplasia as fundamentally all healthy, HSC-derived hematopoiesis will be depleted alongside malignant cells. Therefore, replacement of hematopoiesis by hematopoietic stem cell transplantation (HSCT) after immunotherapy would be required.

In summary, HSC disorders such as CHIP, MDS, and AML are considered a consequence of multiple mutations that are acquired by HSCs or early progenitor cells and that ultimately interfere with the production of normal blood cells. Despite the major impact on homeostasis of the individual patient, the phenotypic cellular changes are minor and heterogeneous, and no broadly targetable leukemia- or MDS-specific target antigen has yet been discovered, hence restricting the potential of surface-directed immunotherapy in AML.

Hematopoietic stem cell transplantation for elimination of AML and MDS stem cells

Current first-line strategies in the treatment of younger patients with AML are aimed at remission induction, that is, the reduction of leukemia cells in the bone marrow below a threshold of 5%, followed by HSCT in most cases. Continuous administration of cytarabine in combination with an anthracycline ("7+3 chemotherapy") induces temporary remissions with regeneration of healthy hematopoiesis in more than 85% of cases [26]. Sensitivity to cytotoxic agents varies substantially, and specific mutations have only limited predictive power [20,21]. Despite successful initial reduction of leukemia burden, disease relapses are common without consolidation therapy. These relapses are thought to originate from leukemia stem cells (LSCs), that is, malignantly transformed HSCs with a slow cycling rate and pluripotent, albeit blocked differentiation potential [19,27,28]. LSCs, as well as HSCs, are relatively refractory to cytotoxic agents and irradiation and are thus difficult to eradicate by means of conventional therapy. Therefore, not only specific mutations, but depth of remission (i.e., minimal or measurable residual disease [MRD]) indicates the response to therapy and is a valid indicator of long-term relapse-free survival [29]. Allogeneic HSCT is the most effective treatment for many patients with AML and can provide long-term disease control in up to 50% of the cases [30]. This favorable outcome is attributed in large part to the graft-versus-leukemia effect (GvL), that is, immunologic elimination of host-derived hematopoiesis by donor-derived lymphocytes, most importantly T cells and NK cells [31]. This effect, in essence, can be regarded as graft-versus-host disease (GVHD) against hematopoietic cells.

To enable engraftment of allogeneic HSCs, three indispensable prerequisites need to be fulfilled: (1) freeing of space for incoming HSCs in the bone marrow microenvironment (the so-called stem cell "niche"); (2) immunosuppression of the host to prevent rejection of the graft; (3) reduction of remaining leukemia cells [32]. To achieve these, transplant recipients are conditioned with a combination of cytotoxic agents including antimetabolites (e.g., cytarabine, fludarabine), alkylating agents (busulfan, cyclophosphamide), and

irradiation [33]. Additionally, alloreactive host and donor T cells are often ablated by use of antibodies. A hallmark and a major disadvantage of conventional conditioning regimens is the lack of specificity as major damage is inflicted to mature and immature, healthy and diseased hematopoietic and non-hematopoietic cells. Advantages of the currently used conditioning regimes include the decades of experience that have been accumulated as well as comparably inexpensive drug costs. Importantly, laborious customization in the sense of personalized medicine is not required. However, the risks of hematopoietic stem cell transplantation with conventional conditioning regimes outweigh the benefits in almost all use case scenarios, with few exceptions such as malignant hematological diseases and certain rare inherited or acquired immune or blood disorders. Although conditioning regimes have been gradually attenuated in past decades, they still cause relevant toxicity in several organ systems. Toxicity in the bone marrow microenvironment leads to prolonged cytopenia in peripheral blood and release of pro-inflammatory mediators, which in turn alter the immunologic state of the incoming graft. The development of chronic GVHD is a severe consequence of this damage-associated immune response [34]. In addition, cytotoxic regimens can cause other severe acute or delayed complications such as seizures, damage to kidney or liver, hemorrhagic cystitis, and secondary malignancies [33]. More refined conditioning techniques are therefore needed and could enable HSCT with substantially reduced toxicity. In the setting of AML and MDS, this could bring the prospects of HSCT to groups of patients that are currently excluded because of the high risk of adverse effects at advanced age or in the presence of comorbidities.

Current state of immunotherapies for AML

Despite an ever-extending list of successful immunotherapies for hematological cancers and also some solid tumors, AML and MDS have remained difficult targets, as reviewed elsewhere [24,35–37]. To date, the anti-CD33 antibody–drug conjugate (ADC) gemtuzumab–ozogamicin is the only immunotherapy approved for clinical application in a subset of AML patients in combination with classic “7+3 chemotherapy" [38]. More recently, the anti-CD70 antibody cusatuzumab has exhibited promising activity in combination with hypomethylating agents (HMAs) [39]. Other immunotherapeutic efforts have failed or are in early clinical stages, such as the combination of HMA with anti-CD47 antibodies or immunotherapeutic agents directed at the surface antigens CD33, CD123, and CXCR4 [24,25,40–43].

Because of the phenotypic similarity between HSCs/progenitor cells and LSCs, the alternative concept, based on radical and complete immunologic elimination of both healthy and diseased hematopoiesis, followed by HSCT from an allogeneic donor, is gaining momentum. In the absence of specific leukemia-defining antigens in AML and MDS, this approach could eventually bring the advantages of immunotherapy to patients with stem cell disorders [44]. In our view, the most promising target antigen to date is CD117 (c-Kit), the cognate receptor for stem cell factor (SCF).

Identification of CD117 as a promising target for immunologic conditioning

A long list of surface antigens and potential (intracellular) HLA-presented neo-antigens have been explored for immunologic targeting of HSCs and leukemic blasts. All of these antigens have peculiar advantages and disadvantages, as reviewed elsewhere [45]. In general, targets that are more highly expressed on maturing cells, such as CD33 and CD45, may be associated with unwanted adverse effects such as myelosuppression [46]. CD33 is additionally vulnerable to slow receptor internalization and not essential for cell survival [47,48]. Hence, the clinical activity of anti-CD33 agents such as gemtuzumab–ozogamicin is limited. Targets that are essential for survival of HSCs and blasts, for example, CD117, CD123 (interleukin-3 [IL3] receptor α), and CD135 (FLT3), might be more attractive as dependence on the respective growth factors SCF, IL3, and FLT3-ligand is difficult to compensate for, even by malignant cells. CD117 is the most widely explored target for immunologic conditioning of healthy HSCs and, thus, also a promising target for immunologic depletion of malignant HSCs/LSCs.

Physiological functions of SCF/CD117 in non-hematopoietic tissues

CD117 (also c-Kit or stem cell factor receptor [SCFR]) is an evolutionary ancient membrane receptor tyrosine kinase (RTK) that is crucial for ontogenesis and homeostasis of the individual [49]. SCF is the only cognate ligand and induces diverse functions in various organs: SCF/CD117 signaling is mostly known for its role in hematopoiesis, but other features such as development of the CNS, germ cell maturation, cell migration, and intestinal motility equally rely on CD117 signaling [49–51]. SCF is produced by various cell types as a glycosylated transmembrane protein and released by proteolytic cleavage. Alternative splicing results in a separate isoform that lacks the cleavage site and remains membrane bound. Soluble SCF generates gradients that are involved in migration of different cells, such as melanocytes. Cell surface–bound SCF expressed by endothelial cells of the bone marrow or perivascular stroma cells of the bone marrow is required for maintenance/self-renewal of HSCs [52].

Major insights into the function and significance of SCF/CD117 signaling have been gained from the study of mice with naturally occurring mutations in the respective genes [53–55]. Homozygous SCF or CD117-deficient mice are not viable because of severe perinatal anemia. Similarly, there are no known cases of humans with homozygous SCF or CD117 deficiency. Mice with heterozygous null mutations or homozygous variants in the *kit* gene can be viable and fertile; however, they present with phenotypic changes such as abnormalities in fur pigmentation and reduced blood cellularity [56]. In humans, the syndrome of piebaldism is connected to heterozygous missense mutations in the human *KIT* gene [57,58].

Physiological functions of SCF/CD117 in hematopoiesis

High levels of CD117 expression are characteristic of mature mast cells, where SCF is responsible for maintenance, migration, and activation [59]. In addition, SCF/CD117 signaling is crucial for hematopoietic stem and progenitor cells in fetal and adult hematopoiesis [60]. HSCs express high levels of CD117, and maintenance of adult HSCs depends on expression of SCF in the bone marrow microenvironment, specifically by endothelial and perivascular stromal cells [52]. HSCs and progenitor cells with CD117 loss-of-function variants have a competitive disadvantage compared with wild-type cells, even of xenogeneic origin [55,56]. Functionally, SCF/CD117 signaling is involved in the regulation of self-renewal, survival, proliferation, and differentiation of HSCs and stemness of progenitor cells in general [61,62]. Even minor changes in signaling through the SCF/CD117 axis have a major impact on cell cycling and differentiation patterns. In mice, phenotypically defined HSCs exhibit differential CD117 expression within a log scale range. Of these cells, higher CD117 expression is linked to increased cycling rate, higher cell output, lower long-term differentiation potential, and megakaryocyte lineage bias [63,64].

Expression patterns in mice and humans

The data on CD117 expression in human tissues are inconsistent. Whereas CD117 is required for organogenesis of different organs, presumably functioning as a guiding path for migrating cells such as melanocytes, only a few mature cell types consistently express high levels of CD117 (Figure 1) [49,65]. In addition to hematopoietic stem cells and mast cells, interstitial cells of Cajal (ICCs) and melanocytes, epithelial cells, and certain cells in the reproductive organs are highly positive for CD117 [66–68]. In a recent publication, we used immunohistochemistry to compare CD117 expression patterns in several vital organs of humans and mice [69]. In summary, we found that low-level CD117 expression is more common than generally acknowledged and is, for example, present in tubular epithelial cells of the kidney or ovarian follicular cells. Expression patterns in the central nervous system are

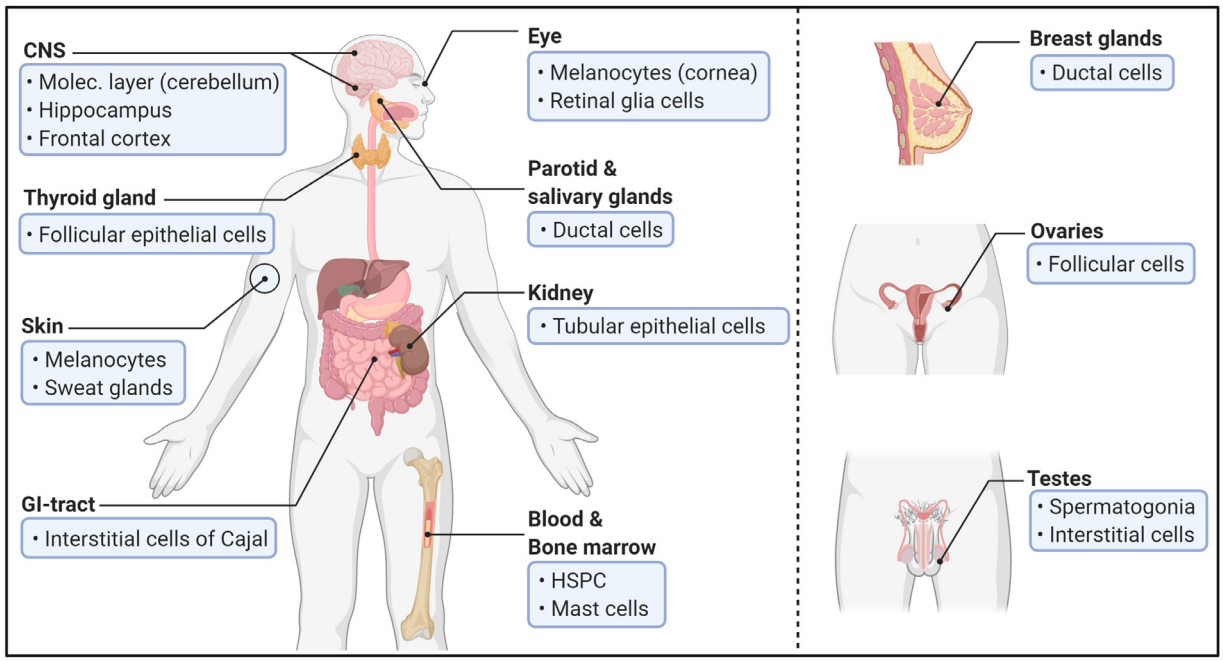

Figure 1. CD117 expression as demonstrated by immunohistochemistry or immunofluorescence microscopy in healthy adult human tissue [65–71].

not entirely elucidated, but findings indicate the presence of rare cerebellar CD117-positive cells and some cells in the hippocampus and eye [65,70,71]. Furthermore, tissue expression patterns in mice and humans are generally comparable and therefore provide evidence for the mouse as a likely valid model to study on-target, off-tumor toxicity of anti-CD117–directed immunotherapies.

CD117 in cancerogenesis

Because of the inherent functions in regulation of stemness, proliferation, and cell cycling, CD117 is a proto-oncogene and involved in cancerogenesis of different tumor entities [72]. Oncogenic driver mutations in CD117 may occur in cell types that inherently express CD117. In this way, gastrointestinal stroma tumors (GISTs) develop from ICCs, mastocytosis from mast cells, melanoma from melanocytes, and acute myeloid leukemia from HSPCs. Under physiological conditions, soluble or membrane-bound SCF binds to the distal extracellular domains of CD117 and induces dimerization and autophosphorylation of CD117 [73]. Mutational hotspots include a tyrosine kinase domain (e.g., D816V, exon 17) or the intracellular (exon 11) or extracellular (exons 8 and 9) juxtamembrane domains [49,74,75]. Mutations in exon 9 and 11 increase ligand-independent receptor dimerization and/or reduced suppression of the kinase domain [49,76]. Mutations in the tyrosine kinase domain are thought to increase the affinity of the receptor to ATP and limit inhibitory effects of the juxtamembrane domain in a monomeric state [77,78]. There are predilections for specific mutations between different cancer types, however, oncogenic mutations typically result in gain-of-function phenotypes and permanently active CD117 signaling [49,74,75]. Only recently, CD117 was described as a dependence receptor with pro-apoptotic functions in the absence of SCF [79]. This observation might help to explain why loss of CD117 expression confers a survival advantage in some cancer types such as neuroblastoma [79]. Importantly, mutations influence subcellular localization of the receptor and can reduce CD117 surface expression levels [49]. For an in-depth analysis of the pathophysiological functions of specific CD117 mutations in diverse human malignancies, we refer to detailed reviews on the topic [49,72,74,75].

Human CD117 was first identified in blasts of an AML patient, and expression of CD117 in acute myeloid leukemia occurs in about 85% of AML cases as determined by flow cytometry [80,81]. Mutations in CD117 are prognostically relevant in the group of core-binding factor (CBF) leukemias with t(8;21) or inv(16) [82]. Despite the absence of direct experimental proof, it is highly likely that LSCs are CD117 positive, at least in cases of CD117-positive AML. Moreover, paracrine and autocrine expression of SCF by tumor cells, including AML blasts, has been reported, and experimental data indicate that SCF signaling might be an essential survival factor for LSCs [83–86]. These properties position CD117 as a promising target for immunotherapy of AML as it might be an unlikely candidate for antigen-negative escape.

Anti-CD117 conditioning: preclinical data from rodent models

After the concept of antibody-based immunologic conditioning was originally conceived, feasibility was analyzed in animal models, mostly in rodents. In preclinical studies, spearheaded by the groups of Weissman and Shizuru at Stanford University, CD117 has emerged as a most promising target. In mice, the transplantation of HSCs into unconditioned immunocompromised recipients depends on the availability of HSC niche space as larger numbers of transplanted HSCs do not result in a linear increase of donor chimerism [87,88]. SCF/CD117 signaling constitutes a crucial aspect of the HSC niche in bone marrow [52,62,89,90]. With the generation of the anti-CD117 antibody ACK2, a novel experimental tool became available that specifically blocks ligation of SCF to CD117 and hence enables analysis of the physiological effects of CD117 signaling in vivo [91,92]. Other anti-mouse CD117 antibodies such as ACK4, 2B8, and 3C11 bind to different epitopes and do not block SCF-induced receptor dimerization [92–94]. Importantly, the blocking antibody ACK2 is not cross-reactive with human CD117. In mice, administration of ACK2 induced temporary depletion of HSPCs in the bone marrow and subsequent reduction of mature myeloid and erythroid cells in the peripheral blood [92]. When used in a transplantation setting, blocking of CD117 by ACK2 caused an increased availability of HSC “niche” space, which facilitated engraftment of donor-derived HSCs and resulted in substantially higher donor chimerism [94]. ACK2 was initially described as a purely blocking antibody without depleting functionality [91,92]. However, in a more recent publication, ACK2 Fab fragments that were engineered to lack the IgG stalk were less efficient in facilitating donor HSC engraftment compared with the full IgG2b antibody. This indicates that blockade of the SCF/CD117 interaction is not the only mode of action but that immune effector functions such as complement-mediated cell death and antibody-dependent cytotoxicity contribute as well [95]. With ACK2 as sole conditioning agent, stable donor chimerism was achieved only in immunocompromised recipients (e.g., *Rag1-*, *FancA-*, or *Fancd2*-deficient animals) and in a syngeneic setting [87,94,96]. The presence of adaptive host immunity prevents engraftment even across minor immunologic barriers (e.g., CD45.1 to CD45.2) [87,95]. To enable transplantation in immunocompetent mice, different combination strategies have been employed. Low-dose irradiation in combination with ACK2 proved to efficiently decrease HSC numbers and permit transplantation of bulk bone marrow cells into immunocompetent congeneic mice [97]. However, combination with irradiation nullifies one of the main advantages of immunologic conditioning, that is, the reduction of toxicity and inflammatory stimuli.

An elegant concept was established by combination of ACK2 with blockade of the CD47-SIRPα axis. The "don't eat me signal" CD47 is ubiquitously expressed to prevent attacks on healthy cells by phagocytes. Importantly, HSCs are not strictly resident in the bone marrow niche, and a certain fraction is found circulating in peripheral blood at any given time. During the transition of HSCs from the bone marrow to peripheral blood, CD47 is upregulated to prevent loss of rare HSCs to attacks from phagocytes [98]. Similarly, upregulation of CD47 occurs in AML, and blockade with anti-CD47 antibodies mediates phagocytic elimination of LSCs but not HSCs [99,100]. Concomitant blockade of CD47 potentiates the activity of ACK2 by enhancing IgG-receptor–mediated phagocytosis of HSCs, hence enabling immunologic conditioning of immunocompetent animals [95]. In a separate study, administration of anti-CD47 antibodies increased the efficacy of in utero HSC depletion and newborn transplantation [101,102]. When T cells are depleted in addition, transplantation across minor immune barriers can be accomplished [95]. The CD47–SIRPα axis is a subject of intensive research, and our understanding of its role in immunity is rapidly expanding [103]. Therefore, other yet undefined mechanisms might contribute to the efficacy of CD47 blockade in immunotherapy.

In a more refined experimental setup, the Weissman group recently developed an all antibody-based conditioning regime, which is based on blockade of CD117 and CD47 in combination with antibody-mediated lymphocyte depletion. This six-antibody cocktail allowed for haplo-identical or even fully MHC-mismatched transplantation between different immunocompetent mouse strains [104]. Despite the immunologic differences, no signs of GVHD occurred, and central immunologic tolerance was induced by removal of host-derived T cells with reactivity against donor antigens. Consequently, HLA-mismatched solid-organ transplantation was feasible, and mice were able to mount an adequate humoral immune response 6 weeks after transplantation. With this concept, stable chimerism of more than 50% was reproducibly achieved.

Anti-human CD117 conditioning: preclinical data

All of the data summarized above were generated with the anti-mouse CD117 antibody ACK2, which is not cross-reactive to human CD117. The mouse anti-human CD117 antibody SR-1 (IgG2a) has comparable properties as it blocks binding of human SCF and is a weak inducer of antibody-dependent cytotoxicity (ADCC) [105]. SR-1 was developed into a humanized, aglycosylated IgG1 antibody with reduced effector functionality and favorable pharmacokinetic properties, subsequently termed AMG191 and currently licensed as JSP191 [106]. Similar to ACK2 in the mouse setting, JSP191 transiently depletes HSPCs in fully immunocompetent cynomolgus monkeys after a single intravenous injection [107]. When administered to macaques in a different non-human primate model, SR-1 exhibited only limited efficacy in combination with busulfan or total-body irradiation [108]. Importantly, no effects were observed on circulating mature white blood cells or non-hematopoietic organs. Likewise, in xenografted humanized mice, a single injection of JSP191 caused subtotal depletion ($< 5\%$) of human $CD45^+$ cells in most animals and enabled engraftment of HSCs from an unrelated donor [107]. Importantly, mouse SCF is cross-reactive to human CD117, albeit with lower biological activity [109–113]. Nevertheless, endogenous mouse SCF levels are sufficient for engraftment of human cells in immunodeficient mice [114,115]. In view of these promising experimental data, it seems probable that antibody-based conditioning regimens will change HSC transplantation in benign hematological disorders in the near future.

Taken together, the cumulative data indicate that purely antibody-based conditioning approaches lead to stable mixed chimerism and immune tolerance between donor and host immune cells. However, complete eradication of the host hematopoietic system is not achieved, in part possibly because of lack of activation of donor leukocytes in the absence of an inflammatory microenvironment. Therefore, the potency required for conditioning in hematopoietic malignancies will likely not be sufficient, as depletion of virtually all malignant stem cells will be required for long-term disease control. Indeed, in a patient-derived xenograft (PDX) model, poor-risk MDS cells were mostly resistant to treatment with JSP191, while lower-risk MDS cells were partly susceptible [83]. These data might also indicate that external survival signals are less important for higher-grade myeloid malignancies, which are therefore less susceptible to mere blockade of CD117. To modulate the efficacy of CD117-targeting agents, different concepts have been devised.

Anti-CD117 IgG antibodies

The CD117 expression profile is reason for concern for highly effective immunotherapeutic agents. The initial development of JSP191 from the mouse antibody SR-1 was therefore aimed at generating an agent with optimized pharmacokinetics, high blocking activity, and minimal effector functionality in the sense of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). The unmodified humanized SR-1 IgG1 isoform (hSR-1 IgG1) was discarded because of the high affinity to activating Fc-

receptors and partly agonistic properties on CD117. Prototypes of humanized SR-1 antibodies in IgG2 (hSR-1 IgG2) format exhibited partly activating properties and induced clumping of cells, while SR-1 in the human IgG4 framework (hSR-1 IgG4) had adverse biochemical properties as it aggregated during purification [106]. Deglycosylation of the humanized IgG1 antibody (hSR-1 aIgG1) resulted in a molecule with reduced effector functionality and desired pharmacodynamic and pharmacokinetic properties [106]. However, residual activation of macrophages occurs with aglycosylated antibodies and might contribute to the effects of JSP191. More recently, several separate human anti-CD117 antibodies were generated and found to have preclinical efficacy in blockade of SCF /CD117 signaling [116–119].

Anti-CD117 immunotoxins/antibody–drug conjugates

Following the favorable toxicity profile of blocking antibodies, the next generation of anti-CD117 targeted therapies was designed with increased effector functionality. Immunotoxins and ADCs deliver a cytotoxic payload specifically to antigen-positive cells. Two different cytotoxic agents were linked to anti-CD117 antibodies and tested in preclinical trials. First, the anti-mouse CD117 antibody 2B8, linked to the protein saporin, efficiently cleared HSCs from the bone marrow compartment and enabled engraftment of allogeneic mouse stem cells, resulting in up to 80% donor chimerism [120]. In the experimental system tested (i.e., single injection, readout at day +8), functional HSCs were subtotally reduced (>99%). Interestingly, other $CD117^+$ progenitor cells were only mildly affected (<50% reduction of total $CD117^+$ cells/femur). Expectedly, mature myeloid cells and lymphocytes were resistant to this therapy, and immunity to viral infection remained intact. Saporin-enhanced anti-CD117 antibodies were also used for conditioning of mice with Fanconi anemia and enabled immunologic tolerance to skin allografts in separate studies [121,122]. Importantly, anti-human CD117-saporin immunotoxins eliminated human HSCs in humanized mice in a proof-of-principle experiment [120]. A second anti-human ADC was generated by linkage of the RNA polymerase inhibitor amanitin to an anti-CD117 IgG. Even though definitive results are not published yet, early data proved the effectiveness of depletion of functional HSCs in humanized mice and non-human primates [123].

Bispecific T cell–engaging and –activating antibodies

The bispecific T-cell engager (BiTE) blinatumomab (CD19 × CD3) was the first clinically approved product of its class and has had a major impact on the treatment of acute B-cell neoplasias [124]. A high-affinity single-chain variable fragment (scFv) for a given lineage marker is connected by a short linker peptide to a lower affinity, activating scFv for CD3, resulting in a tandem scFv [125]. In this way, cytotoxic T lymphocytes are redirected (independently of TCR-activation) against antibody-covered target antigen-positive malignant cells, where they induce T cell–mediated killing. Because of the absence of the Fc region, conventional antibody-mediated functions such as complement activation, facilitation of phagocytosis, and long half-life caused by cellular re-cycling are lacking [125]. T cell–mediated immunity against LSCs is the fundamental mode of action of the graft-versus-leukemia effect in allogeneic stem cell transplantation and donor-lymphocyte infusions. Therefore, the concept of exclusively redirecting T cells against HSCs by use of a tandem scFv format is appealing. A major advantage of this drug class is the short in vivo half-life of approximately 90 min as this property enables immediate cessation of anti-HSC activity when clinically desired, for example, for reasons of toxicity or subsequent transfusion of the donor graft [125,126]. Other bispecific formats, such as dual affinity-retargeting antibodies (DARTs) and tetravalent bispecific tandem antibodies (tandAbs), exhibit different pharmacokinetic properties and might convey benefits in other clinical scenarios [127]. Importantly, not only T cells can be recruited to a given target cell by use of bispecific diabodies. Promising reports exist for redirection of natural killer (NK) cells by targeting the surface antigen CD16 or NKp46 [128,129]. Several bispecific diabodies are in clinical development for the treatment of AML and target CD33 and CD123 among others (reviewed in Guy and Uy [130]). A CD117 × CD3 bispecific diabody in tandem scFv format was recently developed by us and found to have biological activity against healthy HSCs and AMLs (Kiefer et al., unpublished data). More specifically, CD117-positive cells were selectively eliminated by T cells in presence of the CD117 × CD3 antibody while CD117-negative cells were spared. In conclusion, bispecific diabodies combine advantages of antibody-based therapies, such as comparably simple large-scale production, storage, and controllability, with the efficacy of T-cell immunotherapy and might therefore be ideal candidates for use in immunologic conditioning regimes [131].

Anti-CD117 CAR T cells

Yet another revolution in the treatment of B-cell malignancies was the approval of gene-engineered, patient-derived, chimeric-antigen receptor (CAR) T cells in 2017 [132]. CAR T cells are genetically engineered to carry an antibody domain on their surface, which is linked to an intracellular signaling moiety. On ligation

of the target antigen, CAR T cells are activated, which causes proliferation and target cell lysis. The translation of this approach for the treatment of AML has several limitations and pitfalls as discussed below. However, several concepts have emerged that exhibit convincing preclinical or early activity [43]. Building on the fundamental studies that established CD117 as a potential target for immunologic conditioning, mouse anti-CD117 CAR T cells were developed in a proof-of-concept study [133]. After lymphodepleting chemotherapy, immunocompetent mice were treated with anti-CD117 CAR T cells, which were co-transduced with CXCR4 for increased homing to the bone marrow. CAR T cells were depleted after 10 days, and transplantation of HSPCs from a congenic mouse strain ensued. This treatment protocol enabled stable mixed chimerism of approximately 40% with no relevant reported side effects. However, there are many inherent limitations to this study (e.g., depletion of CAR T cells after 10 days, inherently lower activity of mouse CAR T cells compared with those from humans) that might lead to underestimation of the potency of anti-CD117 CAR T cells in the treatment of AML.

We recently developed anti-human CD117 CAR T cells that exhibited near-total depletion of healthy HSCs and AML blasts in vitro and in vivo in humanized patient-derived xenograft systems [69]. As discussed below, we anticipate that CAR T cells might provide the highest effector activity.

Risk–benefit estimation of different anti-CD117 modalities

An ideal compound for immunologic conditioning would combine the following characteristics: (1) high cytotoxic efficacy for profound depletion of target cells; (2) a favorable toxicity profile resulting from a lack of expression of the target antigen by non-hematopoietic cells; (3) the option to control and terminate cytotoxic activity when clinically desired; and (4) the option for bulk production without the need for individualization. Bare humanized IgG immunoglobulins are on one side of the spectrum and are relatively easy to produce, store, and administer (Figure 2A). However, the serum half-life of 21 days is a disadvantage if side effects occur or subsequent transplantation with antigen-positive donor cells is desired. CAR T cells, on the other hand, are on the opposite end of the spectrum. As "living drugs," they are highly effective and expand in vivo until clearance of target cells is accomplished. Inherent disadvantages are the lack of clinically solid-proven means to control and terminate CAR T-cell activity. Although different approaches have been devised, such as antibody-based clearance via transgenic expression of surface molecules (e.g., RQR8, CD19) or the caspase-9–based suicide system, clinical proof of high efficacy is lacking [134–136]. Another obvious disadvantage is the high demand of personalization that is required for autologous CAR T-cell production.

Timing of immunologic conditioning in relation to standard-of-care chemotherapy will be critical in early clinical trials of patients with AML and MDS. In our view, as outlined in Figure 2B, immunologic conditioning should be applied at an early time point, for example, in first complete remission (CR1), possibly in an MRD-positive situation. Sampling of autologous patient T cells at first clinical presentation might be challenging, and production time of CAR T cells might exceed the available time window before relapse. Possibly, progress in the development of universal donor CAR T cells might mitigate these problems. Modified immunoglobulin-based therapies such as ADC and bispecific tumor and T cell–engaging antibodies are between those two poles. In our view, T cell–engaging antibodies combine good pharmacodynamic properties (i.e., high cytotoxic activity) with advantageous pharmacokinetics (i.e., good controllability) because of the short half-life. Obviously, patients will require continuous infusion of the drug for a treatment duration in the range of days to weeks; however, this is already routinely performed in clinics with blinatumomab. The number of ADCs approved for clinical application is expanding, especially for hematological malignancies, in combination with conventional chemotherapy. Mechanistically, the cytotoxic payload amanitin or saporin interferes with protein biosynthesis. The susceptibility of slowly cycling and metabolically inactive HSCs/LSCs to these toxins needs to be determined.

SCID as a "model bridge" to clinics

Building on growing evidence emphasizing the role of mast cells in inflammatory processes as wells as on the success of the tyrosine kinase inhibitor imatinib, Amgen developed a humanized aglycosylated version of the mouse anti-human CD117 antibody SR-1 as indicated before. Development of the compound, initially intended for depletion of mast cells in inflammatory diseases, was halted after a phase 1 trial. Shizuru et al., who were behind the preclinical trials demonstrating immunologic niche clearance by anti-CD117 IgG in mice, realized the clinical potential of the antibody. Accordingly, a first clinical trial is ongoing in which children with severe combined immunodeficiency (SCID) received JSP191 as sole conditioning agent prior to allogeneic stem cell transplantation [137]. These children are the predestined population for immunologic conditioning for several reasons: (1) the severity of adverse effects of irradiation- or chemotherapy-based conditioning prohibits use in these highly immunocompromised patients; (2) absence of an

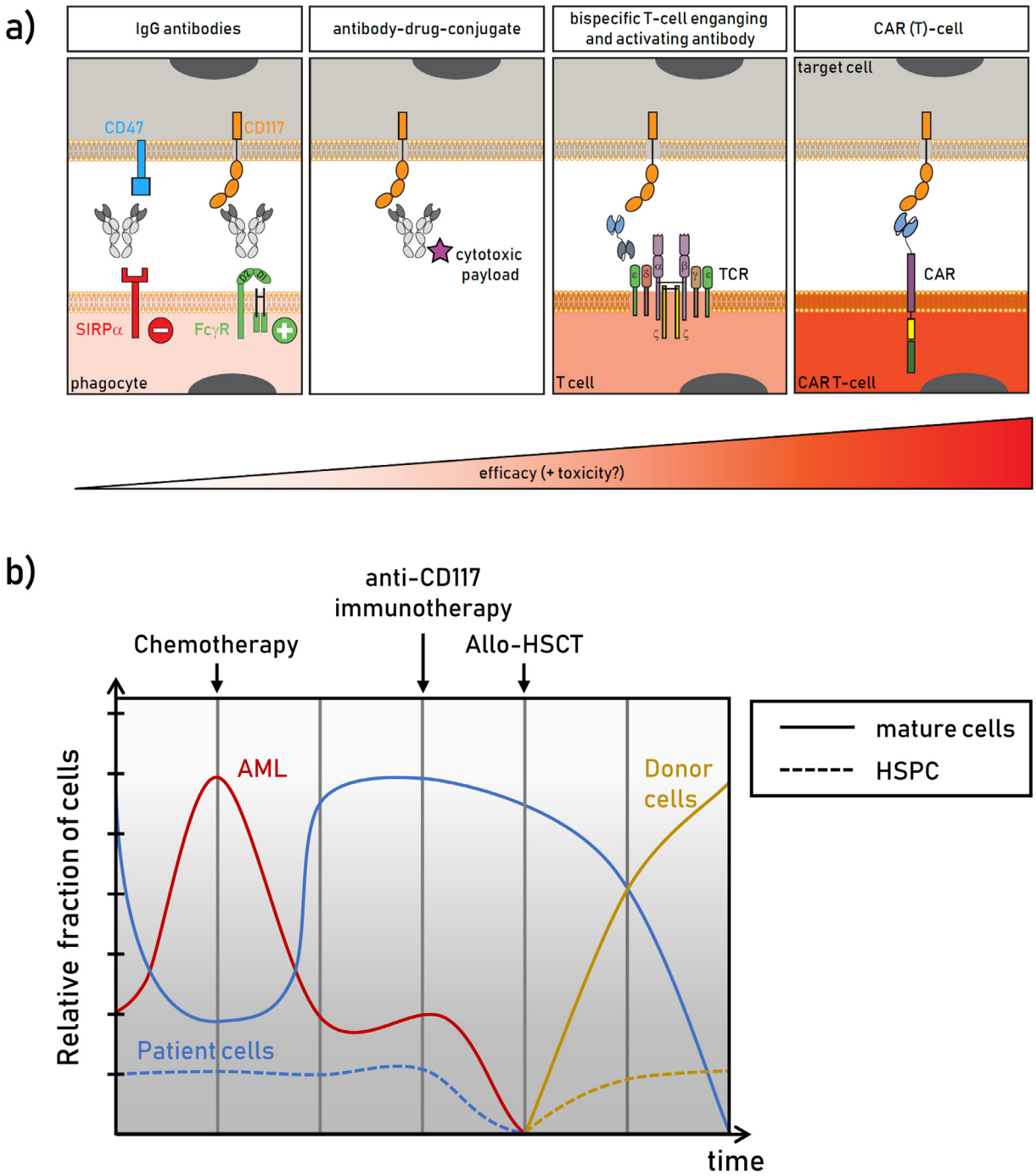

Figure 2. Cell population kinetics in bone marrow and effector strength of anti-CD117 immunotherapies. (A) Function, effector strength, and toxicity of different anti-CD117 immunotherapies. From left to right, anti-CD117 antibodies in combination with anti-CD47 antibodies lead to phagocytosis of target cells (i.e., leukemia cells and healthy HSPCs). Antibody–drug conjugates cause direct toxicity of target antigen-positive cells. Bispecific T cell-engaging antibodies redirect cytotoxic T cell toward target cells. CAR T cells are gene-engineered to express a synthetic chimeric receptor, which enables a surface antigen-directed attack of target cells. (B) Fraction of bone marrow cells during treatment of an AML patient with conventional chemotherapy, anti-CD117 conditioning, and allogeneic stem cell transplantation. AML cells are shown in *red*, healthy patient hematopoiesis in *blue*, donor-derived hematopoiesis in *yellow*. *Solid lines* represent mature cells; *dashed lines* represent progenitor cells.

immune barrier facilitates engraftment of donor cells; and (3) low-level chimerism might likely be sufficient for restoration of immune function. According to early reports, stable donor-derived HSC chimerism has been achieved in these patients with very limited toxicity. With this proof of safety and feasibility, the product was licensed to Jasper Therapeutics, which aims to advance the antibody to broader patient populations. Currently, patients with AML or MDS will receive JSP191 in addition to a conventional conditioning regime prior to allogeneic stem cell transplantation [138].

Outline of a trial for anti-CD117 immunotherapy and subsequent HSCT

All anti-CD117–directed immunotherapies will have significant toxicity against healthy HSCs. Therefore, the inclusion in pretransplant conditioning regimes is the ideal setting to evaluate safety and toxicity, as was done in the Jasper trial [138]. For bispecific antibodies or CAR T cells, we envision a first clinical application in AML and MDS patients with high risk of relapse who have achieved a complete morphologic remission with detectable MRD after standard therapy. Standard chemotherapy allows for debulking of the disease on the one hand and for production of CAR T cells or regeneration of autologous lymphocytes on the other. In this particular setting, less than 5% of blasts are present in bone marrow of patients, allowing for a favorable effector-to-target ratio [139]. Yet, direct response to anti-CD117 immunotherapeutic agents can be assessed by molecular quantification of MRD. Infusion of anti-CD117 CAR T cells or bispecific antibodies would therefore be carried out with a sufficient time gap prior to conventional conditioning chemotherapy. This would allow for analysis of potential toxicity and efficacy. We estimate that a window of 1–4 weeks will be required for sufficient activity of either CAR T cells or bispecific antibodies.

Figure 2B illustrates the application of anti-CD117 immunotherapies in the context of a potential treatment concept. Subsequent trials could be aimed at decreasing the standard conditioning regimens if the approach proves to be sufficient to eliminate healthy HSCs.

Potential limitations

Some inherent limitations of anti-CD117 conditioning are evident. The data on unarmed IgG antibodies and ADCs indicate depletion of the majority of CD117-positive cells; however, a significant fraction remains. Additionally, more aggressive stem cell malignancies such as high-risk MDS and AML evade immune mechanisms and might require higher effector strength. Anti-CD117 CAR T cells harbor that potency; however, they might take too much time. Preclinical data in myeloid and clinical data in B-cell malignancies indicate that CAR T cells require a longer time frame from 1 to 6 months to achieve their full potential [140,141]. B-Cell aplasia is a surrogate parameter for CD19 CAR T-cell activity, and B-cell aplasia of less than two months correlates with high relapse rates, indicating the requirement for extended presence and immunologic activity of CAR T cells. Prolonged HSC aplasia, for obvious reasons, is not a desirable endpoint and might limit the applicability of CAR T cells in myeloid malignancies. Another aspect of concern for CD117-directed T cell–mediated immunotherapy is the potential impairment of quantity and fitness of patient-derived T cells after AML/MDS and intensive chemotherapy. Patients who do not achieve complete remission might be an even more challenging patient cohort because of the larger number of target cells and the inflammatory perturbations in the bone microenvironment.

Also, given the broad expression of CD117 in non-hematopoietic tissues (Figure 1), the absence of systemic side effects in preclinical models and in the clinical trials seems astonishing. Whereas JSP191 merely blocks binding of SCF, both SR-1 and ACK2 do have Fc-mediated effector functions [91,92,95,105,106]. However, no serious side effects were observed in mice and non-human primates [83,94,95,97,102,107,108]. Also, mouse anti-CD117 ADC and CAR T cells were tolerated in immunocompetent mice without off-target effects [120–122,133]. The CD117 expression patterns in mice and humans are comparable; however, the respective antibody or antibody fragments differ [69]. A mouse model with expression of human CD117 is currently not reported. Therefore, in clinical trials with anti-CD117 CAR T cells or bispecific antibodies, there will be a special emphasis on potential on-target, off-tumor side effects in, for example, the central nervous system, gonads, skin, and kidneys. For entirely non-genotoxic conditioning concepts in immunocompetent humans, additional obstacles need to be considered. Host-versus-graft immunity against transplanted HSCs needs to be overcome. It remains to be seen if purely antibody-based concepts for depletion of T/NK cell–mediated immunity are readily translatable from mice to humans. For CAR T cell–mediated conditioning, definitive eradication of the anti-CD117 effector cells prior to infusion of the graft is another concern that has not yet been solved. Additionally, with the fast-moving pace of biological and medical discoveries, other treatment concepts, such as targeting of the CD70/CD27 axis, might lead the way.

Scenarios of use outside AML treatment

Immunologic conditioning could not only revolutionize the treatment of rare hematological malignancies. The range of potential applications of safer hematopoietic stem cell transplantation is wide, as reviewed elsewhere [142–145]. In particular, the combination with current and future gene modification technologies will enable transplantation with autologous gene-corrected hematopoietic stem cells for the treatment of genetic disorders such as sickle cell disease, chronic

granulomatous disease (CGD), and thalassemia. Furthermore, allogeneic stem cell transplantation could be used to generate mixed donor chimerism and might enable solid organ transplantation independent of HLA barriers. Lastly, aggressive systemic mastocytosis is inherently positive for CD117 and might be within the scope of CD117 immunotherapies [146].

Summary and outlook

About 30 years ago, CD117 was discovered as the receptor for stem cell factor [111,147,148]. One generation later, specific binders have been developed and optimized, and potential clinical applications have been identified and are being evaluated in clinical trials. At the end of this first generation, the feasibility of immunologic conditioning has been demonstrated, and the future potential seems vast. However, efficacy needs to be demonstrated in immunocompetent humans. Five years from now, we will have learned lessons from the current clinical trials using first-generation anti-CD117 targeting agents. It remains to be seen if efficacy of unarmed IgG antibodies is sufficient for depletion of malignant hematopoietic cells. If malignant hematopoietic diseases are resistant, it will be a challenge to increase cytotoxic efficacy, for example, by application of anti-CD117 CAR T cells or bispecific antibodies, without increasing inherent toxicity. Most likely, evidence from clinical trials will lead to another round of fine-tuning of anti-CD117 agents and their combinatorial use in basic research and preclinical models. At the same time, progress in gene editing technology will broaden the scope of diseases that can be tackled by transplantation of gene-edited autologous HSC. In 10 years‘ time, we might see the second round of clinical translation of optimized anti-CD117 agents for treatment of malignant hematological diseases, as well as for conditioning prior to autologous transplantation. It will take time and effort before anti-CD117 agents will make traditional conditioning regimens obsolete.

In conclusion, the demand for optimized and less toxic conditioning concepts is obvious and diverse approaches targeting CD117 on HSCs are among the frontrunners of this development. If progress in the field continues at the current pace, we might reach an era of regenerative medicine in which targeted and specific “precision replacement and regeneration” of diseased HSCs with allogeneic or gene-edited autologous HSCs becomes reality.

Conflict of interest disclosure

The authors filed a patent application for a bispecific, T cell-engaging and -activating diabody targeting CD117 and CD3.

Acknowledgments

This work is in part supported by the Clinical Research Priority Program “ImmunoCure” of the University of Zurich to MGM and DN; a University Research Priority Project Translational Cancer Research grant to RM; and a Pioneer Fellowship from ETH Zürich to JDK. Figure 1 was created with Biorender.com.

References

1. Nombela-Arrieta C, Manz MG. Quantification and three-dimensional microanatomical organization of the bone marrow. Blood Adv. 2017;1:407–416.
2. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell. 2008;132:631–644.
3. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. Cell Stem Cell. 2012;10:120–136.
4. Zhang Y, Gao S, Xia J, Liu F. Hematopoietic hierarchy—an updated roadmap. Trends Cell Biol. 2018;28:976–986.
5. Busch K, Klapproth K, Barile M, et al. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. Nature. 2015;518:542–546.
6. Sun J, Ramos A, Chapman B, et al. Clonal dynamics of native haematopoiesis. Nature. 2014;514:322–327.
7. Schoedel KB, Morcos MNF, Zerjatke T, et al. The bulk of the hematopoietic stem cell population is dispensable for murine steady-state and stress hematopoiesis. Blood. 2016;128:2285–2296.
8. Sheikh BN, Yang Y, Schreuder J, et al. MOZ (KAT6A) is essential for the maintenance of classically defined adult hematopoietic stem cells. Blood. 2016;128:2307–2318.
9. Trumpp A, Essers M, Wilson A. Awakening dormant haematopoietic stem cells. Nature Rev Immunol. 2010;10:201–209.
10. Boettcher S, Wilk CM, Singer J, et al. Clonal hematopoiesis in donors and long-term survivors of related allogeneic hematopoietic stem cell transplantation. Blood. 2020;135:1548–1559.
11. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–2498.
12. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477–2487.
13. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20:1472–1478.
14. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. Science. 2019;366:eaan4673.
15. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377:111–121.
16. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559:400–404.
17. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24:1015–1023.
18. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373:1136–1152.
19. Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. Blood. 2017;129:1577–1585.
20. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood. 2016;128:686–698.

21. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–2221.
22. Perna F, Berman SH, Soni RK, et al. Integrating proteomics and transcriptomics for systematic combinatorial chimeric antigen receptor therapy of AML. Cancer Cell. 2017;32:506–519. e505.
23. Haubner S, Perna F, Kohnke T, et al. Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. Leukemia. 2019;33:64–74.
24. Beyar-Katz O, Gill S. Novel approaches to acute myeloid leukemia immunotherapy. Clin Cancer Res. 2018;24:5502–5515.
25. Masarova L, Kantarjian H, Ravandi F, Sharma P, Garcia-Manero G, Daver N. Update on immunotherapy in AML and MDS: monoclonal antibodies and checkpoint inhibitors paving the road for clinical practice. Adv Exp Med Biol. 2018;995:97–116.
26. AML Collaborative Group. A systematic collaborative overview of randomized trials comparing idarubicin with daunorubicin (or other anthracyclines) as induction therapy for acute myeloid leukaemia. Br J Haematol. 1998;103:100–109.
27. Maccalli C, Tasian SK, Rutella S. Targeting leukemia stem cells and the immunological bone marrow microenvironment. In: Maccalli C, Todaro M, Ferrone S, eds. Cancer Stem Cell Resistance to Targeted Therapy, Cham: Springer; 2019:153–172.
28. Pollyea DA, Jordan CT. Therapeutic targeting of acute myeloid leukemia stem cells. Blood. 2017;129:1627–1635.
29. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2018;131:1275–1291.
30. Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA. 2009;301:2349–2361.
31. Sweeney C, Vyas P. The graft-versus-leukemia effect in AML. Front Oncol. 2019;9:1217.
32. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006;354:1813–1826.
33. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124:344–353.
34. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373:1550–1561.
35. Lichtenegger FS, Krupka C, Haubner S, Kohnke T, Subklewe M. Recent developments in immunotherapy of acute myeloid leukemia. J Hematol Oncol. 2017;10:142.
36. Tasian SK. Acute myeloid leukemia chimeric antigen receptor T-cell immunotherapy: how far up the road have we traveled? Ther Adv Hematol. 2018;9:135–148.
37. Liu Y, Bewersdorf JP, Stahl M, Zeidan AM. Immunotherapy in acute myeloid leukemia and myelodysplastic syndromes: the dawn of a new era? Blood Rev. 2019;34:67–83.
38. Appelbaum FR, Bernstein ID. Gemtuzumab ozogamicin for acute myeloid leukemia. Blood. 2017;130:2373–2376.
39. Riether C, Pabst T, Hopner S, et al. Targeting CD70 with cusatuzumab eliminates acute myeloid leukemia stem cells in patients treated with hypomethylating agents. Nat Med. 2020;26:1459–1467.
40. Sallman DA, Asch AS, Al Malki MM, et al. The first-in-class anti-CD47 antibody magrolimab (5F9) in combination with azacitidine is effective in MDS and AML patients: ongoing phase 1b results. Blood. 2019;134:569.
41. Cancilla D, Rettig MP, DiPersio JF. Targeting CXCR4 in AML and ALL. Front Oncol. 2020;10:1672.
42. Valent P, Sadovnik I, Eisenwort G, et al. Immunotherapy-based targeting and elimination of leukemic stem cells in AML and CML. Int J Mol Sci. 2019;20:4233.
43. Mardiana S, Gill S. CAR T cells for acute myeloid leukemia: state of the art and future directions. Front Oncol. 2020;10:697.
44. Manz MG, Russkamp NF. Selective CD117(+) HSC exchange therapy. Blood. 2019;133:2007–2009.
45. Anguille S, Van Tendeloo VF, Berneman ZN. Leukemia-associated antigens and their relevance to the immunotherapy of acute myeloid leukemia. Leukemia. 2012;26:2186–2196.
46. Walter RB, Press OW, Bernstein ID. Antibody-based therapeutics targeting CD33, CD45, and CD66. In: Andreeff M, ed. Targeted Therapy of Acute Myeloid Leukemia, New York: Springer; 2015:531–555.
47. Kim MY, Yu KR, Kenderian SS, et al. Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. Cell. 2018;173:1439–1453. e1419.
48. Walter RB. The role of CD33 as therapeutic target in acute myeloid leukemia. Expert Opin Ther Targets. 2014;18:715–718.
49. Lennartsson J, Ronnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev. 2012;92:1619–1649.
50. Ward SM, Burns AJ, Torihashi S, Sanders KM. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J Physiol. 1994;480:91–97.
51. Russell ES. Hereditary anemias of the mouse: a review for geneticists. Adv Genet. 1979;20:357–459.
52. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature. 2012;481:457–462.
53. Nocka K, Tan JC, Chiu E, et al. Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. EMBO J. 1990;9:1805–1813.
54. Galli SJ, Zsebo KM, Geissler EN. The kit ligand, stem cell factor. Adv Immunol. 1994;55:1–96.
55. Waskow C, Paul S, Haller C, Gassmann M, Rodewald HR. Viable c-Kit(W/W) mutants reveal pivotal role for c-kit in the maintenance of lymphopoiesis. Immunity. 2002;17:277–288.
56. Cosgun KN, Rahmig S, Mende N, et al. Kit regulates HSC engraftment across the human−mouse species barrier. Cell Stem Cell. 2014;15:227–238.
57. Giebel LB, Spritz RA. Mutation of the KIT (mast/stem cell growth factor receptor) protooncogene in human piebaldism. Proc Natl Acad Sci USA. 1991;88:8696–8699.
58. Oiso N, Fukai K, Kawada A, Suzuki T. Piebaldism. J Dermatol. 2013;40:330–335.
59. Caslin HL, Kiwanuka KN, Haque TT, et al. Controlling mast cell activation and homeostasis: work influenced by Bill Paul that continues today. Front Immunol. 2018;9:868.
60. Broudy VC. Stem cell factor and hematopoiesis. Blood. 1997;90:1345–1364.
61. Kent D, Copley M, Benz C, Dykstra B, Bowie M, Eaves C. Regulation of hematopoietic stem cells by the steel factor/KIT signaling pathway. Clin Cancer Res. 2008;14:1926–1930.
62. Domen J, Weissman IL. Hematopoietic stem cells need two signals to prevent apoptosis: BCL-2 can provide one of these, Kitl/c-Kit signaling the other. J Exp Med. 2000;192:1707–1718.

63. Shin JY, Hu W, Naramura M, Park CY. High c-Kit expression identifies hematopoietic stem cells with impaired self-renewal and megakaryocytic bias. J Exp Med. 2014;211:217–231.
64. Grinenko T, Arndt K, Portz M, et al. Clonal expansion capacity defines two consecutive developmental stages of long-term hematopoietic stem cells. J Exp Med. 2014;211:209–215.
65. Lammie A, Drobnjak M, Gerald W, Saad A, Cote R, Cordon-Cardo C. Expression of c-kit and kit ligand proteins in normal human tissues. J Histochem Cytochem. 1994;42:1417–1425.
66. May AJ, Cruz-Pacheco N, Emmerson E, et al. Diverse progenitor cells preserve salivary gland ductal architecture after radiation-induced damage. Development. 2018;145:dev166363.
67. Miliaras D, Karasavvidou F, Papanikolaou A, Sioutopoulou D. KIT expression in fetal, normal adult, and neoplastic renal tissues. J Clin Pathol. 2004;57:463–466.
68. Kato N, Honma K, Hojo H, Sasou S, Matsuzaki O, Motoyama T. KIT expression in normal and neoplastic renal tissues: immunohistochemical and molecular genetic analysis. Pathol Int. 2005;55:479–483.
69. Myburgh R, Kiefer JD, Russkamp NF, et al. Anti-human CD117 CAR T-cells efficiently eliminate healthy and malignant CD117-expressing hematopoietic cells. Leukemia. 2020;34:2688–2703.
70. Miettinen M, Lasota J. KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. Appl Immunohistochem Mol Morphol. 2005;13:205–220.
71. Too LK, Gracie G, Hasic E, Iwakura JH, Cherepanoff S. Adult human retinal Müller glia display distinct peripheral and macular expression of CD117 and CD44 stem cell-associated proteins. Acta Histochem. 2017;119:142–149.
72. Pittoni P, Piconese S, Tripodo C, Colombo MP. Tumor-intrinsic and -extrinsic roles of c-Kit: mast cells as the primary off-target of tyrosine kinase inhibitors. Oncogene. 2011;30:757–769.
73. Yuzawa S, Opatowsky Y, Zhang Z, Mandiyan V, Lax I, Schlessinger J. Structural basis for activation of the receptor tyrosine kinase KIT by stem cell factor. Cell. 2007;130:323–334.
74. Ashman LK, Griffith R. Therapeutic targeting of c-KIT in cancer. Expert Opin Investig Drugs. 2013;22:103–115.
75. Lennartsson J, Jelacic T, Linnekin D, Shivakrupa R. Normal and oncogenic forms of the receptor tyrosine kinase kit. Stem Cells. 2005;23:16–43.
76. Reshetnyak AV, Opatowsky Y, Boggon TJ, et al. The strength and cooperativity of KIT ectodomain contacts determine normal ligand-dependent stimulation or oncogenic activation in cancer. Mol Cell. 2015;57:191–201.
77. Laine E, Chauvot de Beauchene I, Perahia D, Auclair C, Tchertanov L. Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region: implications for dimerization and activation mechanisms. PLoS Comput Biol. 2011;7:e1002068.
78. Gajiwala KS, Wu JC, Christensen J, et al. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci USA.. 2009;106:1542–1547.
79. Wang H, Boussouar A, Mazelin L, et al. The proto-oncogene c-Kit inhibits tumor growth by behaving as a dependence receptor. Mol Cell. 2018;72:413–425. e415.
80. Ashman LK, Roberts MM, Gadd SJ, Cooper SJ, Juttner CA. Expression of a 150-kD cell surface antigen identified by monoclonal antibody YB5.B8 is associated with poor prognosis in acute non-lymphoblastic leukaemia. Leuk Res. 1988;12:923–928.
81. Tsao AS, Kantarjian H, Thomas D, et al. C-kit receptor expression in acute leukemias—association with patient and disease characteristics and with outcome. Leuk Res. 2004;28:373–378.
82. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B study. J Clin Oncol. 2006;24:3904–3911.
83. Pang WW, Czechowicz A, Logan AC, et al. Anti-CD117 antibody depletes normal and myelodysplastic syndrome human hematopoietic stem cells in xenografted mice. Blood. 2019;133:2069–2078.
84. Guan Y, Gerhard B, Hogge DE. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). Blood. 2003;101:3142–3149.
85. Pietsch T. Paracrine and autocrine growth mechanisms of human stem cell factor (c-kit ligand) in myeloid leukemia. Nouv Rev Fr Hematol. 1993;35:285–286.
86. Fatrai S, van Schelven SJ, Ubink I, et al. Maintenance of clonogenic KIT(+) human colon tumor cells requires secretion of stem cell factor by differentiated tumor cells. Gastroenterology. 2015;149:692–704.
87. Bhattacharya D, Rossi DJ, Bryder D, Weissman IL. Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. J Exp Med. 2006;203:73–85.
88. Takizawa H, Regoes RR, Boddupalli CS, Bonhoeffer S, Manz MG. Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation. J Exp Med. 2011;208:273–284.
89. Crane GM, Jeffery E, Morrison SJ. Adult haematopoietic stem cell niches. Nat Rev Immunol. 2017;17:573–590.
90. Waskow C, Madan V, Bartels S, Costa C, Blasig R, Rodewald HR. Hematopoietic stem cell transplantation without irradiation. Nat Methods. 2009;6:267–269.
91. Nishikawa S, Kusakabe M, Yoshinaga K, et al. In utero manipulation of coat color formation by a monoclonal anti-c-kit antibody: two distinct waves of c-kit-dependency during melanocyte development. EMBO J. 1991;10:2111–2118.
92. Ogawa M, Matsuzaki Y, Nishikawa S, et al. Expression and function of c-kit in hemopoietic progenitor cells. J Exp Med. 1991;174:63–71.
93. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. Proc Natl Acad Sci USA.. 1992;89:1502–1506.
94. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. Science. 2007;318:1296–1299.
95. Chhabra A, Ring AM, Weiskopf K, et al. Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. Sci Transl Med. 2016;8:351ra105.
96. Chandrakasan S, Jayavaradhan R, Ernst J, et al. KIT blockade is sufficient for donor hematopoietic stem cell engraftment in Fanconi anemia mice. Blood. 2017;129:1048–1052.
97. Xue X, Pech NK, Shelley WC, Srour EF, Yoder MC, Dinauer MC. Antibody targeting KIT as pretransplantation conditioning in immunocompetent mice. Blood. 2010;116:5419–5422.
98. Jaiswal S, Jamieson CH, Pang WW, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell. 2009;138:271–285.
99. Majeti R, Chao MP, Alizadeh AA, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138:286–299.
100. Theocharides APA, Jin L, Cheng PY, et al. Disruption of SIRPα signaling in macrophages eliminates human acute

myeloid leukemia stem cells in xenografts. J Exp Med. 2012;209:1883–1899.
101. Derderian SC, Togarrati PP, King C, et al. In utero depletion of fetal hematopoietic stem cells improves engraftment after neonatal transplantation in mice. Blood. 2014;124:973–980.
102. Witt RG, Wang B, Nguyen QH, Eikani C, Mattis AN, MacKenzie TC. Depletion of murine fetal hematopoietic stem cells with c-Kit receptor and CD47 blockade improves neonatal engraftment. Blood Adv. 2018;2:3602–3607.
103. Dai H, Friday AJ, Abou-Daya KI, et al. Donor SIRPα polymorphism modulates the innate immune response to allogeneic grafts. Sci Immunol. 2017;2:eaam6202.
104. George BM, Kao KS, Kwon H-S, et al. Antibody conditioning enables MHC-mismatched hematopoietic stem cell transplants and organ graft tolerance. Cell Stem Cell. 2019;25:185–192. e183.
105. Broudy VC, Lin N, Zsebo KM, et al. Isolation and characterization of a monoclonal antibody that recognizes the human c-kit receptor. Blood. 1992;79:338–346.
106. Ng G, Shen W. Humanized C-kit antibody (US Patent No. 8436150B2), U.S. Patent and Trademark Office. Available at: https://rb.gy/q00vhe.
107. Kwon HS, Logan AC, Chhabra A, et al. Anti-human CD117 antibody-mediated bone marrow niche clearance in nonhuman primates and humanized NSG mice. Blood. 2019;133:2104–2108.
108. Chandrasekaran D, Nakamoto B, Watts KL, Kiem HP, Papayannopoulou T. Modeling promising nonmyeloablative conditioning regimens in nonhuman primates. Hum Gene Ther. 2014;25:1013–1022.
109. Manz MG. Human-hemato-lymphoid-system mice: opportunities and challenges. Immunity. 2007;26:537–541.
110. Lev S, Yarden Y, Givol D. Dimerization and activation of the kit receptor by monovalent and bivalent binding of the stem cell factor. J Biol Chem. 1992;267:15970–15977.
111. Zsebo KM, Williams DA, Geissler EN, et al. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell. 1990;63:213–224.
112. Martin FH, Suggs SV, Langley KE, et al. Primary structure and functional expression of rat and human stem cell factor DNAs. Cell. 1990;63:203–211.
113. Rongvaux A, Takizawa H, Strowig T, et al. Human hemato-lymphoid system mice: current use and future potential for medicine. Annu Rev Immunol. 2013;31:635–674.
114. Brehm MA, Racki WJ, Leif J, et al. Engraftment of human HSCs in nonirradiated newborn NOD-scid IL2rgamma null mice is enhanced by transgenic expression of membrane-bound human SCF. Blood. 2012;119:2778–2788.
115. Takagi S, Saito Y, Hijikata A, et al. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. Blood. 2012;119:2768–2777.
116. London CA, Gardner HL, Rippy S, et al. KTN0158, a humanized anti-KIT monoclonal antibody, demonstrates biologic activity against both normal and malignant canine mast cells. Clin Cancer Res. 2017;23:2565–2574.
117. Le Gall M, Crepin R, Neiveyans M, et al. Neutralization of KIT oncogenic signaling in leukemia with antibodies targeting KIT membrane proximal domain 5. Mol Cancer Ther. 2015;14:2595–2605.
118. Lebron MB, Brennan L, Damoci CB, et al. A human monoclonal antibody targeting the stem cell factor receptor (c-Kit) blocks tumor cell signaling and inhibits tumor growth. Cancer Biol Ther. 2014;15:1208–1218.
119. Reshetnyak AV, Nelson B, Shi X, et al. Structural basis for KIT receptor tyrosine kinase inhibition by antibodies targeting the D4 membrane-proximal region. Proc Natl Acad Sci USA. 2013;110:17832–17837.
120. Czechowicz A, Palchaudhuri R, Scheck A, et al. Selective hematopoietic stem cell ablation using CD117-antibody–drug-conjugates enables safe and effective transplantation with immunity preservation. Nat Commun. 2019;10:617.
121. Li Z, Czechowicz A, Scheck A, Rossi DJ, Murphy PM. Hematopoietic chimerism and donor-specific skin allograft tolerance after non-genotoxic CD117 antibody–drug-conjugate conditioning in MHC-mismatched allotransplantation. Nat Commun. 2019;10:616.
122. Srikanthan MA, Humbert O, Haworth KG, et al. Effective multi-lineage engraftment in a mouse model of Fanconi anemia using non-genotoxic antibody-based conditioning. Mol Ther Methods Clin Dev. 2020;17:455–464.
123. Pearse BR, McDonough SM, Proctor JL, et al. CD117-amanitin antibody drug conjugates effectively deplete human and non-human primate HSCs: proof of concept as a targeted strategy for conditioning patients for bone marrow Transplant. Blood. 2018;132:3314.
124. Kantarjian H, Stein A, Gokbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376:836–847.
125. Brinkmann U, Kontermann RE. The making of bispecific antibodies. MAbs. 2017;9:182–212.
126. Klinger M, Brandl C, Zugmaier G, et al. Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. Blood. 2012;119:6226–6233.
127. Reusch U, Harrington KH, Gudgeon CJ, et al. Characterization of CD33/CD3 tetravalent bispecific tandem diabodies (TandAbs) for the treatment of acute myeloid leukemia. Clinical Cancer Research. 2016;22:5829–5838.
128. Gauthier L, Morel A, Anceriz N, et al. Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity. Cell. 2019;177:1701–1713. e1716.
129. Wiernik A, Foley B, Zhang B, et al. Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 × 33 bispecific killer cell engager and ADAM17 inhibition. Clin Cancer Res. 2013;19:3844–3855.
130. Guy DG, Uy GL. Bispecific antibodies for the treatment of acute myeloid leukemia. Curr Hematol Malig Rep. 2018;13:417–425.
131. Slaney CY, Wang P, Darcy PK, Kershaw MH. CARs versus BiTEs: a comparison between T cell–redirection strategies for cancer treatment. Cancer Discov. 2018;8:924–934.
132. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359:1361–1365.
133. Arai Y, Choi U, Corsino CI, et al. Myeloid Conditioning with c-kit-Targeted CAR-T Cells Enables Donor Stem Cell Engraftment. Mol Ther. 2018;26:1181–1197.
134. Philip B, Kokalaki E, Mekkaoui L, et al. A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. Blood. 2014;124:1277–1287.
135. Marin V, Cribioli E, Philip B, et al. Comparison of different suicide-gene strategies for the safety improvement of genetically manipulated T cells. Hum Gene Ther Methods. 2012;23:376–386.
136. Straathof KC, Pule MA, Yotnda P, et al. An inducible caspase 9 safety switch for T-cell therapy. Blood. 2005;105:4247–4254.
137. Stanford University, California Institute for Regenerative Medicine. AMG191 conditioning/CD34+CD90 stem cell transplant study for SCID patients. 2020. Trial Identifier: NCT02963064, JAS-BMT-CP-001.

138. Jasper Therapeutics, Inc. JSP191 antibody targeting conditioning in MDS/AML patients Undergoing hematopoietic cell transplantation. 2020. ClinicalTrials.gov Identifier: NCT04429191. Available at: https://ClinicalTrials.gov/show/NCT04429191.
139. Gökbuget N, Dombret H, Bonifacio M, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood. 2018;131:1522–1531.
140. Tasian SK, Kenderian SS, Shen F, et al. Optimized depletion of chimeric antigen receptor T cells in murine xenograft models of human acute myeloid leukemia. Blood. 2017;129:2395–2407.
141. Finney OC, Brakke H, Rawlings-Rhea S, et al. CD19 CAR T cell product and disease attributes predict leukemia remission durability. J Clin Invest. 2019;129:2123–2132.
142. Logan AC, Weissman IL, Shizuru JA. The road to purified hematopoietic stem cell transplants is paved with antibodies. Curr Opin Immunol. 2012;24:640–648.
143. Czechowicz A, Weissman IL. Purified hematopoietic stem cell transplantation: the next generation of blood and immune replacement. Immunol Allergy Clin North Am. 2010;30:159–171.
144. Abadir E, Bryant C, Larsen S, Clark GJ. Targeting the niche: depleting haemopoietic stem cells with targeted therapy. Bone Marrow Transplant. 2019;54:961–968.
145. Abadir E, Gasiorowski RE, Silveira PA, Larsen S, Clark GJ. Is hematopoietic stem cell transplantation required to unleash the full potential of immunotherapy in acute myeloid leukemia? J Clin Med. 2020;9:554.
146. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood. 2017;129:1420–1427.
147. Witte ON. Steel locus defines new multipotent growth factor. Cell. 1990;63:5–6.
148. Williams DE, Eisenman J, Baird A, et al. Identification of a ligand for the c-kit proto-oncogene. Cell. 1990;63:167–174.