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The road to purified hematopoietic stem cell transplants is paved with antibodies

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Abstract

Hematopoietic progenitor cell replacement therapy remains a surprisingly unrefined process. In general, unmanipulated bone marrow or mobilized peripheral blood grafts which carry potentially harmful passenger cells are administered after treating recipients with high-dose chemo- and/or radiotherapy to eradicate malignant disease, eliminate immunologic barriers to allogeneic cell engraftment, and to “make space” for rare donor stem cells within the stem cell niche. The sequalae of such treatments are substantial, including direct organ toxicity and non-specific inflammation that contributes to the development of graft-versus-host disease and poor immune reconstitution. Passenger tumor cells that contaminate autologous hematopoietic grafts may contribute to relapse post-transplant. Use of antibodies to rid grafts of unwanted cell populations, and to eliminate or minimize the need for non-specifically cytotoxic therapies used to condition transplant recipients, will dramatically improve the safety profile of allogeneic and gene-modified autologous hematopoietic stem cell therapies.

Introduction

The fundamental goal of hematopoietic cell (HCT) and stem cell (HSCT) transplantation — both autologous and allogeneic — is to replace defective, malignant, or chemotherapy-damaged stem cells. For most patients undergoing this type of stem cell replacement therapy, recipient conditioning has traditionally involved high doses of cytotoxic and/or immunosuppressive chemotherapy, with or without adjunctive radiation to all or part of the body. Hematopoietic rescue or cell replacement is currently achieved by infusion of

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Conflict of interest

ILW co-founded SyStemix, Inc., which performed the $CD34^{+}CD90^{+}$ HSC selection in the trials discussed in this review, but currently has no financial interest in the technology as SyStemix, Inc., is now a wholly owned subsidiary of Novartis, Inc. ACL and JAS have no relevant conflicts of interest to disclose.

unmanipulated hematopoietic cell products carrying passenger cells with the potential to cause harm to the recipient. Given the diversity of conditions that are treated with HCT/HSCT, a uniform approach to conditioning is neither practical nor desirable. Rather, a balance between targeted disease eradication, graft manipulation, and immunosuppression tailored to individual malignant and non-malignant indications for HSC transplantation will prevail.

The primary directive of autologous HCT/HSCT is to regenerate stem cell reservoirs damaged by a malignancy such as lymphoma or myeloma or by the chemotherapy used to treat these conditions. In this setting, the use of antibodies during conditioning may primarily be focused on improving disease control or decreasing regimen toxicity. In the case of lymphoma, a monoclonal antibody (mAb) has also been used to purge autografts of lymphoma progenitors [1]. Since the advent of clinical antibody therapy with OKT3, an immunosuppressant murine anti-human CD3ε mAb [2], and the widespread use of Rituximab, a mouse/human chimeric mAb directed at the human CD20 antigen expressed on B lineage lymphomas and leukemias [3], therapeutically useful antibodies to targets in several other malignancies have been developed [4**]. These agents may be employed to eradicate malignant cells in patients receiving autologous transplants; however, it is critically important to develop a strategy that ensures passenger tumor cells are not reinfused with the HCT product.

Antibody selection using technologies to sort purified HSC by immunomagnetic beads and/or fluorescence activated cell sorting (FACS) are alternative and perhaps preferable methods for providing autologous HSC grafts free of contaminating tumor cells. This approach is relevant to several malignant diseases treatable with myeloablative chemotherapy and rescue with autologous HCT, including lymphomas, multiple myeloma, germ cell tumors and carcinomas. Administration of antibody-purified, cancer depleted HSC grafts may prevent the reinfusion of circulating tumor cells.

When malignant or immunogenetically defective stem cells and hematopoietic populations are targeted for replacement by allogeneic HCT, the requirements of the conditioning regimen are more substantial. Lethality to endogenous stem cells is required, but, in addition, sufficient immunosuppression must be achieved to prevent host-versus-graft (HVG) mediated immunologic graft rejection [5*]. Furthermore, ongoing immunosuppression is required post-transplant to attenuate graft-versus-host disease (GVHD) caused by donor T cells in unmanipulated HCT grafts [6].

Here we provide a discussion about use of mAbs for: 1) improving conditioning regimens by facilitating host stem cell depletion, thus removing physical barriers to engraftment into the stem cell niche, 2) facilitating HSC graft purification, and 3) enhancing immunosuppression to enable engraftment of stem cells across histocompatibility barriers.

Conditioning strategies: Radioimmunoconjugates

Antibodies conjugated with radionuclides have been shown to effectively deliver radiotoxicity to tumors. This technology is adaptable to use in radiation-mediated

myeloablation of bone marrow stem and progenitor cells. To date, most approaches have utilized non-HSC-specific targets that are present in the bone marrow, such as CD45, a pan-leukocyte antigen. When antibody-bound radionuclides concentrate in the marrow due to affinity to such targets, the HSC are subjected to genotoxic radiation either by virtue of the fact that they also express the antigen, or via a bystander effect (so-called “cross-fire effect”), in which case HSC are physically situated in close proximity to other cells with the cognate antigen to which the antibody binds [7,8*]. The rationale for intensifying radiotoxicity to the marrow with this strategy is based upon experience using total body irradiation (TBI) to condition patients for allotransplantation. Increasing the dose of TBI reduced relapse and ensured achievement of full donor chimerism (ie, higher level and longer lasting multi-lineage donor engraftment), but escalating doses are associated with increased regimen-related mortality and GVHD [9,10]. Radioimmunoconjugates enable the delivery of higher radiation exposures specifically to hematopoietic tissues, thus sparing exposure to other critical organs.

The safety of ^{131}I-labelled monoclonal antibodies to CD45 was originally demonstrated in mice [7], non-human primates [11], and then humans [8,12], by investigators at the Fred Hutchinson Cancer Research Center. This approach has since been adapted to clinical use by other investigative groups. Other non-HSC-specific antigens which have been targeted include CD33 [13–15] and CD66 [16]. Original studies of this approach utilized monoclonal antibodies conjugated with beta particle emitters such as ^{131}I [12,17], ^{188}Re [16], and ^{90}Y [18]. These radionuclides are not entirely suited to this application due to deep tissue penetration of beta particles, which may be associated with undesirable toxicities. As an alternative, alpha emitters such as ^{213}Bi [19,20–21] and ^{211}At [19,22], which have tissue penetration path lengths of 40 to 90 μm, are being investigated to limit off-site toxicities and increase the therapeutic index of these treatments [23*]. Nevertheless, comprehensive elimination of endogenous HSC with these short-range reagents will likely depend on targeting antigens that are present on HSC, due to decreased by-stander effect.

A further refinement of the radioimmunoconjugate approach is the development of pre-targeting strategies, in which a streptavidin conjugated antibody is administered and time is permitted for its accumulation in the target tissue (e.g., bone marrow) [23*,24]. A biotin-bound radionuclide is then administered, and because the radionuclide-bound protein is rapidly excreted in urine if it is not retained by binding to the pre-targeted antibody-streptavidin complex, off-site radiotoxicity is minimized.

Conditioning regimens: Unconjugated antibodies

As an alternative to using mAbs to deliver toxic payloads, unconjugated mAbs can be effectively applied to targeted elimination of specific cell populations. The therapeutic effects of these antibodies rely on functional characteristics of the specific immunoglobulin molecule produced by the mAb-generating hybridoma. Potential mechanisms to facilitate stem cell depletion include lysis of target cells by complement fixation, apoptosis induction by surface antigen cross-linking or growth factor deprivation, antibody-mediated cellular cytotoxicity, and antibody-dependent phagocytosis.

France and colleagues used two rat anti-human CD45 mAbs which work synergistically to fix complement and lyse cells via assembly of a membrane attack complex [25**]. Although HSC express abundant CD45, as do all hematopoietic cells in the bone marrow, treatment with these mAbs was not associated with significant depletion of marrow progenitor cells or enhancement of engraftment in syngeneic mice [26], but did show some activity in clearance of leukemic blasts from the bone marrow in humans [27]. These mAbs were recently employed in a reduced-intensity conditioning regimen for congenital immunodeficiencies, in conjunction with fludarabine, cyclophosphamide, and alemtuzumab (anti-CD52) [28**]. Although the regimen was well-tolerated and high-level donor chimerism was achieved, the contribution of the lytic anti-CD45 mAb treatment in the context of these other agents remains to be further clarified.

Another way mAb targeting may be used to deplete normal and malignant cells involves deprivation of protective barriers between these cells and components of the innate immune system. For instance, the cell surface protein CD47 prevents phagocytosis of migrating stem cells by providing inhibitory signals to macrophages via binding to SIRPα and is upregulated on HSC and all human leukemias [29]. When CD47:SIRPα interactions are blocked, pro-phagocytic signals mediated by another surface protein, calreticulin, predominate [30*]. Blocking mAbs to CD47 have been developed which enable phagocytosis of otherwise protected cell populations such as mobilized stem cells and malignant progenitors [31**]. Normal HSC express lower levels of calreticulin as compared to malignant cells explaining why this approach has been demonstrated to eliminate cancer stem cells in xenogeneic models of acute myelogenous leukemia [32**] acute lymphoid leukemia [33*], and several solid tumors [34**,35] while sparing normal hematopoiesis. Anti-CD47 based therapies are rapidly moving toward clinical application and may ultimately play a role in clearing stem cell niches of malignant progenitors.

Monoclonal antibodies to cell surface receptors that inhibit interactions with critical ligands represent a potentially powerful mechanism to induce specific stem cell depletion. Targeting HSC surface antigens which are linchpins to their survival or required for the maintenance of pluripotency is a goal of this research. Czechowicz and colleagues demonstrated the feasibility of using mAbs to CD117 (c-kit) that block interactions with its ligand stem cell factor (aka, kit ligand or steel factor), as an approach for facilitating host HSC depletion and donor HSC engraftment in histocompatible mouse strains [36**]. Anti-CD117, when administered to immunodeficient $Rag2^{-/-}$ or $Rag2^{-/-}IL2R\gamma c^{-/-}$ mice — which are models for human severe combined immunodeficiency (SCID) variants lacking B and T cells (as well as natural killer [NK] cells in the case of $IL2R\gamma c^{-/-}$) — leads to deep depletion of long-term HSC and all downstream c-kit-expressing progeny [36**]. When adequate time is permitted for antibody catabolism, allogeneic HSC engraft at significantly higher levels in anti-CD117-treated animals than observed in unconditioned animals. We have since found a clinical-grade humanized anti-human CD117 mAb that capably depletes human HSC in a xenograft model (unpublished) and this approach will soon be evaluated as an allotransplant conditioning method in patients with SCID.

Interestingly, when used as a monotherapy, anti-CD117 mAbs do not adequately deplete endogenous HSC or facilitate allogeneic HSC engraftment in immunocompetent animals

[36**]. Via mechanisms which have yet to be fully elucidated, cells not present in SCID animals, but present in immunocompetent hosts, appear to partially protect HSC from depletion in the setting of c-kit blockade. Nevertheless, Xue and colleagues demonstrated engraftment of congenic hematopoietic progenitors in immunocompetent mice treated with anti-CD117 and low dose radiation [37]. Even low dose radiation, however, has the potential to confer long-term risks, particularly in children, so it remains an important goal to develop regimens that permit anti-CD117-mediated HSC depletion without adding significant additional toxicity.

Graft preparation: Antibody-purified autologous HSC

A misnomer in the field of cellular therapy is the practice commonly called autologous “stem cell” transplantation. Autografts comprised of unmanipulated G-CSF mobilized peripheral blood (MPB) cells are routinely used to rescue hematopoiesis following high-dose chemotherapy, which as a side effect causes myeloablation. Antibody-purified HSC grafts can substantially reduce the likelihood of reinfusing circulating tumor cells in this context. Monoclonal antibody reagents may be used to either deplete tumor cells based on their expression of specific antigens, or to sort pure stem cell populations away from other cells in MPB, as is commonly practiced when HSC are enriched by CD34 expression [38–40,41*]. It is important to recognize, however, that CD34 is not expressed exclusively on HSC, and indeed some non-hematologic malignancies may be $CD34^+$ [41*,42*], so this practice does not yet offer a satisfactory solution for purifying HSC grafts of circulating tumor cells. This fact necessitates the development of other mAb reagents of good manufacturing practice (GMP) quality to achieve better HSC purification.

$CD34^+CD90^+$ ($Thy1^+$) selected MPB HSC sorted by FACS were evaluated for use in autografting for multiple myeloma after it was demonstrated that this phenotype excluded myeloma progenitor cells [43*]. Sorting on the additional parameter of CD90 expression eliminates roughly 50–60% of $CD34^+$ cells in G-CSF MPB that do not have long-term engraftment capacity, and facilitates purification of HSC from malignant cells. The first study using these highly enriched HSC in myeloma treated 9 patients and demonstrated a delay in neutrophil and platelet engraftment; however, the study was not designed to identify a threshold cell dose required for adequate engraftment [44]. A contemporaneous study by another group using the same $CD34^+CD90^+$ selection method treated 23 myeloma patients with a similar conditioning regimen and observed four engraftment failures (four patients failed to recover adequate platelet counts, and three remained dependent on G-CSF to prevent neutropenia) [45*]. This study defined a minimum $CD34^+CD90^+$ HSC dose of $8x10^5$ cells per kilogram recipient weight, above which no engraftment failures were noted and no delays to neutrophil or platelet recovery were observed [45*].

Vose and colleagues autografted 20 non-Hodgkin lymphoma (NHL) patients with $CD34^+CD90^+$ HSC after high-dose chemotherapy [46]. Autograft products sorted for this phenotype were assayed for contaminating tumor cells by PCR quantification of lymphoma-specific targets, such as mutated bcl-1 or bcl-2. The sort strategy depleted circulating NHL cells by a factor of 3–5 fold. Rapid neutrophil engraftment (day 12) and platelet transfusion

independence (day 12) was observed with a median CD34+CD90+ cell dose of $5x10^5$ cells per kilogram recipient weight. No engraftment failures were observed.

$CD34^+CD90^+$ HSC were also used in a single trial of high-dose chemotherapy for metastatic breast cancer [42*]. Micro-metastases to bone marrow have been reported in up to 82% of patients with stage IV breast cancer [47], substantiating a risk for reinfusion of circulating tumor cells in unmanipulated grafts in this non-hematopoietic malignancy. The $CD34^+CD90^+$ phenotypic sorting strategy eliminated circulating breast tumor cells to a level less than 0.0001%, which compared favorably with unmanipulated HCT that were often contaminated with cytokeratin positive adenocarcinoma cells [42*]. No engraftment failures or delays to neutrophil or platelet engraftment (days 10 and 14, respectively) were observed in 22 patients treated on this clinical trial. Long-term follow-up of these patients revealed a higher than expected fraction not only remained free of cancer, but maintained normal hematopoiesis and peripheral blood counts for as long as 14 years after autologous G-CSF mobilized $CD34^+CD90^+$ HSC administration [48**]. These findings verify that cells of the $CD34^+CD90^+$ phenotype rapidly rescue long-term blood formation, and that the efficacy of this approach for metastatic breast cancer and potentially other malignancies deserves further investigation in clinical trials.

Graft preparation: Purified allogeneic HSC transplantations

In the future, some indications for allogeneic transplantation will likely be supplanted by gene-modified autologous HSC replacement. Nevertheless, many malignant and non-malignant diseases will remain curable only with allogeneic transplantation, the primary toxicity of which is GVHD. Using positive selection for $CD34^+CD90^+$ cells as discussed above can reduce the T cell content of MPB grafts by 1,000,000-fold to a level at which the risk of GVHD is substantially reduced. Such antibody-purified allogeneic HSC transplants may not be a viable approach for treatment of malignancies until methods for complete eradication of all host tumor progenitor cells is achieved, thus eliminating the need for graft-versus-malignancy (GVM) effects. It is possible, however, that graft manipulation strategies, such as provision of T cells with known targets and sortable by tetramer reagents, will obviate the need to give crude T cell grafts. Co-transplantation with purified HSC and T cell subpopulations with targeted anti-tumor or anti-pathogen activity may be a useful strategy for providing stem cell replacement and rapid disease-specific functional immunity to improve outcomes. As a proof-of-concept example of this approach, Müller and colleagues recently demonstrated transplantation of mice with purified allogeneic HSC plus tetramer-selected T cells directed against murine cytomegalovirus (MCMV) [49*]. Massive expansion of functional anti-MCMV specific T cells with enhanced anti-viral immunity was observed in comparison with recipients of HSC plus unselected T cells. *De novo* T cell immunity arising from the donor HSC was also significantly improved [49*], indicating this strategy may lead to better qualitative immune recovery than with unmanipulated grafts that involve crude whole T cell adoptive transfer.

Combining antibody conditioning and antibody-purified allogeneic HSC grafts

The area of most immediate promise for the use of antibody purified allogeneic HSC and targeted host conditioning is in the treatment of non-malignant disease. Graft T cells are thought to both confer GVM effects as well as facilitate the engraftment of donor cells aiding in the conversion of recipients to fully donor-derived hematopoiesis. Even low amounts of adoptively transferred allogeneic T cells are associated with overt GVHD, however [39,40,50–52]. Furthermore, subclinical GVHD severely impairs immune reconstitution [53*,54*], even when destruction of non-immune tissues is not observed. Since neither GVM nor complete donor chimerism are required for effective treatment of non-malignant disease, GVHD should be avoidable without compromising the efficacy of stem cell replacement.

The multitude of non-malignant disorders curable by partial hematopoietic chimerism ranges from congenital immune deficiencies to hemoglobinopathies, lysosomal storage diseases, and other marrow failure states. In most of these conditions, low-level mixed donor chimerism (ie, 5–30%) is sufficient to correct the hematologic disorder and prevent disease-related sequalae. Another clinical scenario in which mixed donor chimerism achieved with HSCT following reduced-intensity conditioning may be useful is the induction of immune tolerance in patients undergoing solid organ transplantation. As proof of concept for this latter scenario, Scandling and colleagues recently demonstrated in a clinical study that mixed chimerism can be routinely achieved in patients undergoing combined human leukocyte antigen (HLA)-matched kidney and hematopoietic cell transplantation thereby permitting cessation of immune suppressive drugs with long-term kidney allograft survival [55]. Mixed chimerism and immune tolerance was achieved using conditioning with total lymphoid irradiation and anti-thymocyte globulin followed by CD34-enriched MPB hematopoietic precursors, a regimen utilizing antibody-based immunoablation with administration of antibody-enriched HSC.

Another nascent field which will likely be advanced by the availability of antibody-based conditioning and purified HSC grafts is the treatment of severe autoimmune disorders. Evidence that transplantation of purified allogeneic HSC and establishment of mixed hematopoietic chimerism can successfully inhibit autoimmune disease pathogenesis has been demonstrated in mice. Beilhack and colleagues showed that transplantation of either major histocompatibility complex (MHC)-mismatched or MHC-matched allogeneic HSC blocked development of hyperglycemia in diabetes-prone NOD mice by attenuating auto-reactive peripheral T cells responsible for pancreatic islet destruction [56,57]. Diabetes was prevented in NOD mice treated with non-marrow ablative radiation and establishment of multi-lineage mixed donor/host chimerism. In a mouse model of systemic lupus erythmatosus (SLE), Smith-Berdan and colleagues similarly showed that induction of mixed chimerism by transplantation of purified allogeneic HSC after nonmyeloablative conditioning reversed the symptoms of established disease, including proteinuric lupus nephritis, and lowered the frequency of circulating immune complexes or autoantibodies

[58]. These studies support further clinical study of purified HSCT in patients with severe autoimmune disorders.

Use of antibodies to overcome allogeneic immune barriers

Although promising and technically within reach, the proposed use of purified allogeneic HSC grafts for the routine treatment of non-malignant diseases will require a paradigm shift in the practice of preparing patients for stem cell transplants. Regimen-related toxicities that may have an acceptable benefit-risk ratio and confer therapeutic effects against malignancies can be made unnecessary for non-malignant diseases if alternative approaches to permit engraftment are established. Given that the major barriers to allogeneic HSC engraftment are host immune cells (primarily T and NK cells) and resident HSC it is possible to target and eliminate these populations with antibodies.

Attempts have been made to develop T cell specific mAb reagents such as anti-TCRαβ in murine [59] and canine [60,61] HSC transplant models; however, no such reagents have yet been employed therapeutically in human trials. Rather, polyclonal antibody preparations such as anti-thymocyte globulin (ATG) and non-T cell specific monoclonal antibodies such as alemtuzumab, an antibody to CD52, which leads to depletion of T, B, NK, and some myeloid cells, are increasingly being used clinically to facilitate immunosuppression of allotransplant recipients, with conflicting results regarding benefit [62–64].

Alternatively, mAb reagents targeting T cell co-stimulatory molecules are being advanced to clinical trials to facilitate development of peripheral immunologic tolerance and prevent graft rejection [65]. CTLA4-Ig, which interrupts CD28:CD80/CD86 (B7-1/B7-2)-mediated costimulation, demonstrated promising activity for development of immunologic tolerance to hematopoietic and other grafts in mice [66], but has not yet translated to use in human HSC transplantation. Antibodies to CD154 (CD40 ligand), which mediates antigen-presenting cell activation when ligated to CD40, were shown to facilitate the development of mixed chimerism in mice receiving fully MHC mismatched bone marrow grafts without other conditioning [67*]. Unfortunately, attempts to translate this clinically were halted when anti-CD154 mAbs were found to precipitate arterial and venous thromboemboli, likely due to aggregation of CD154-expressing activated platelets [68]. Combination of CTLA4-Ig, a non-depleting mAb to CD40, and sirolimus, which blocks T and B cell response to IL-2, was recently shown to facilitate development of mixed donor chimerism and immunologic tolerance in a non-human primate model of unmanipulated bone marrow transplantation, and may represent a clinically translatable approach for induction of immune tolerance to purified HSC grafts [69].

The combination of these lymphodepleting or lymphosuppressive reagents, together with antibodies that target endogenous marrow and/or HSC populations (as discussed above), will be required to overcome host resistance and allow the engraftment of purified allogeneic HSC. A similar need for deep immunosuppression of the recipient is also needed when gene-modified autologous HSC are given, since the product of the corrected gene, in most cases, represents a neo-antigen to the recipient and thus a target for HVG immune rejection [70–72].

Concluding remarks

Monoclonal antibody reagents are increasingly important in the design of clinical trials addressing methods for improving patient outcomes in autologous and allogeneic transplantation by reducing regimen-related toxicities and improving eradication of diseased or damaged hematopoietic stem cells. Immunosuppression required for engraftment of cells with minor (and, potentially, major) histocompatibility differences from the recipient will increasingly rely on targeted immune therapies. Antibody reagents will also play an increasingly critical role in the isolation and clinical separation of stem cell populations from other contaminating cells. These approaches to eradication of stem cells, malignant or otherwise, using functionally active mAbs in combination with immunodepleting mAb therapies, will enable minimally-toxic stem cell replacement with material from multiple potential sources, including gene-modified autologous cells, allogeneic stem cells, and potentially embryonic stem cells and induced pluripotent stem cells.

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patients, in fact, exhibited CD34-expressing cytokeratin-positive cells, suggesting CD34 selection would not be an adequate mechanism for tumor cell depletion. Furthermore, 36% of patients were found to have cytokeratin positive cells in the apheresis product or CD34-enriched fraction thereof. The grafts administered to patients were further refined by positive sorting for expression of CD34 and CD90 (Thy-1), and this additional cell selection eliminated cytokeratin-positive cells from all 22 grafts administered to patients. Long-term outcomes from this trial are reported in Ref. 48. [PubMed: 10871151]

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Highlights (for review)

- We discuss different methods for using monoclonal antibodies to improve conditioning regimens for hematopoietic stem cell transplantation
- We discuss methods for using antibodies to purify hematopoietic stem cells to remove potentially harmful passenger cells
- We discuss use of antibodies to enhance immunosuppression to enhance engraftment of allogeneic hematopoietic stem cells