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INVITED PERSPECTIVE

# Metcalf Lecture Award: Applying niche biology to engineer T-cell regenerative therapies

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**The processes generating cells of adaptive immunity render them less amenable to the single cytokine signals used so effectively to regenerate myeloid cells. T-cell neogenesis begins in the bone marrow, where specific sets of late osteolineage cells govern the specification of hematopoietic cells capable of migrating to the thymus where differentiation is completed. Osteocalcin-expressing bone marrow stromal cells producing Dll4 serve as a progenitor niche enabling this T-competent cell production. Biocompatible alginate-based cryogels containing bone morphogenetic proteins (BMP-2) and the Notch ligand Dll4 were engineered to recapitulate the endogenous niche. These cryogels are highly pliable and can be injected under the skin of animals undergoing bone marrow transplantation. The result in mice is an ectopic niche fostering T-competent progenitor generation that results in improved T-cell numbers and receptor diversity. The recipients can generate neoantigen vaccine responses while having improved tolerance manifest by reduced graft-versus-host disease upon allogeneic transplant. Through emerging details of niches in the bone marrow, therapeutics more complex than those necessary for myeloid reconstitution are possible. Niche biology-guided bioengineered design offers the possibility of regenerative therapies for T lymphoid cells.** 

Immunity depends on the orderly transition of stem/progenitor cell populations to the mature effector cells conducting host defense. Understanding and manipulating that process in myeloid cells through the use of single cytokines have provided invaluable medical therapies. This is the legacy of Dr. Donald Metcalf, whose pioneering work on myeloid growth factors has transformed hematology. However, a similar paradigm cannot be meaningfully applied to lymphoid cells where late-acting cytokines increase the abundance of existing clones, but not the clonal diversity on which adaptive immunity depends. Earlier populations of progenitors must be targeted, but primitive cells are generally not responsive to single cytokine triggers. Perhaps because more primitive cells pose existential threats by virtue of self-renewal capacity or exhaustion, they are often dependent on complex cues to guide their differentiation and proliferation. Dependence on multiple signals can add both resilience against a dysfunction in any one pathway and nuance through integration of independent signals.

Studies examining the bone marrow microenvironment have indicated that there are indeed multiple cell and molecular components to the hematopoietic stem cell (HSC) niche. These range from highly integrative cell populations—neural cells that convey signals based on systemic inputs and endothelial cells that communicate inputs from systemwide and local events—to local cells—hematopoietic cells that reflect cell production and resident mesenchymal cells that provide maintenance signals [1–3]. Among the mesenchymal cells,

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osteolineage cells have a range of activities, some controversial. These include sites of hematopoietic stem and progenitor cell (HSPC) engraftment after transplantation, enhancement of granulocyte colony-stimulating factor (G-CSF) mobilization, production of stress responsive cytokines and lymphoid progenitor support [4–9]. Further, osteocalcin production by osteoblastic cells has been directly implicated in broad-based systemic regulation of energy utilization, cognition, reproduction, and muscle function [10–13]. Elegant studies by the Karsenty laboratory have demonstrated how osteocalcin is central to issues of organismal function. Decline in osteocalcin-producing cells is thought to play a key role in the aging phenotype broadly and osteoporosis specifically. It may also contribute to the decline in adaptive immune cell production with age.

## Bone marrow cells governing T-lymphopoietic competence

Testing whether specific subpopulations such as those expressing osteocalcin have an impact on aspects of hematopoiesis beyond stem cell regulation, we conducted a series of stromal cell depletion studies. This "take one out" strategy involved using inducible differentiation stage-specific promoters such as those for *Osterix* or *Osteocalcin* (*Ocn*), genes expressed sequentially in osteolineage differentiation, driving a *Diptheria Toxin Receptor* (*DTR*) gene [14,15]. Delivering diptheria toxin (DTx) could then acutely deplete the subset of cells with reasonable efficiency (~70% for osteocalcin+ cells).

It should be noted that prior studies had indicated an impact of osteolineage cells on lymphoid cells. For example, bone cells were reported to provide CXCL12 for B-cell differentiation by the Nagasawa laboratory [16], and subsequent studies found that G-$\alpha$-s signaling or CXCL12 expression in osteolineage cells was important for early B-cell development including common lymphoid progenitors (CLPs) in the CXCL12 study [8,9]. Deletion of early osteolineage cells through osterix-driven expression of the *DTR* and selective cell depletion impaired B-cell differentiation [15]. Deletion of osterix-expressing cells or deletion of *IGF-1* in osterix-expressing cells resulted in impaired transition of pro-B to pre-B cells. Therefore, a role for the osteolineage in lymphoid differentiation is well established.

However, the importance of specific cells within the bone marrow for the formation of T-lineage–competent common lymphoid progenitors (CLPs) has been less well defined. Hematopoietic cell subtypes in the bone marrow that emigrate to and engraft in the thymus have been characterized [17–22]. The stromal cells guiding their production were not. Deleting more mature osteoblastic cells expressing osteocalcin, we noted a defect in the production of T-competent cells by both immunophenotype and function [14]. Animals with *Osteocalcin* promotor-driven expression of the DTR and exposure to DTx had reduced skeletal growth and reduced CLPs. They had normal bone marrow cellularity and HSCs and myeloid progenitors. However, notably distinct from the osterix+ cell-depleted animals, they had a decrease in the T-competent CLPs as defined by Ly6D- or c-kit(lo)Thy1(hi) [15,19,20]. B-cell populations were also decreased, but we prioritized the unanticipated finding of particular loss of the T-competent CLPs. This finding was accompanied by a decrease in all stages of thymocytes from the earliest thymic progenitor to single positive CD4- or CD8-expressing cells [14].

Because the thymus is known to express many genes otherwise thought to be tissue specific, it is possible that osteocalcin-expressing cells could have been deleted in the thymus to account for the decrease in thymocyte subpopulations with DTx treatment. To control for this, bone marrow cells were directly injected into the thymi of animals with or without DTx treatment and found that the numbers of thymocytes generated were equivalent: there was no evidence for dysfunction of the thymus in the setting of adequate input cells. We concluded that the impairment of thymocytes in the setting of the DTx-treated *Ocn*-DTR mice was indeed caused by decreased production of the T-competent progenitors moving from bone marrow to thymus. Therefore, the osteocalcin-expressing cells of bone appear to provide a niche that enables specification of T-competent CLPs.

## Factors in the niche driving T-competent CLPs

Assessing what might be produced by the osteocalcin-expressing cells compared with other osteolineage subsets, we created a triple transgenic mouse with distinctive *Osterix*- and *Osteocalcin* promoter-driven fluorophores. Cells could then be isolated based on the promoter being active and we examined the transcriptome of each. As osterix-expressing cells affect B lineage without clear T-lymphopoietic defects, emphasis was placed on those genes differentially expressed in the osteocalcin+ cells, particularly those predicted to encode cell surface or secreted gene products.

Notch ligands were notable in that all were comparably expressed in osterix- and osteocalcin- expressing cells except Delta-like ligand-4 (Dll4) [14]. Immunohistochemistry confirmed that the Dll4 protein was markedly reduced in the osteocalcin+ cell-depleted cell mouse bone marrow. Further, intracellular (activated) Notch was reduced in hematopoietic cells from the osteocalcin+ cell-depleted mice.

To confirm the role of Notch activation in specification of the T-cell–competent CLPs, a number of genetically engineered mice were used. To affect the so-

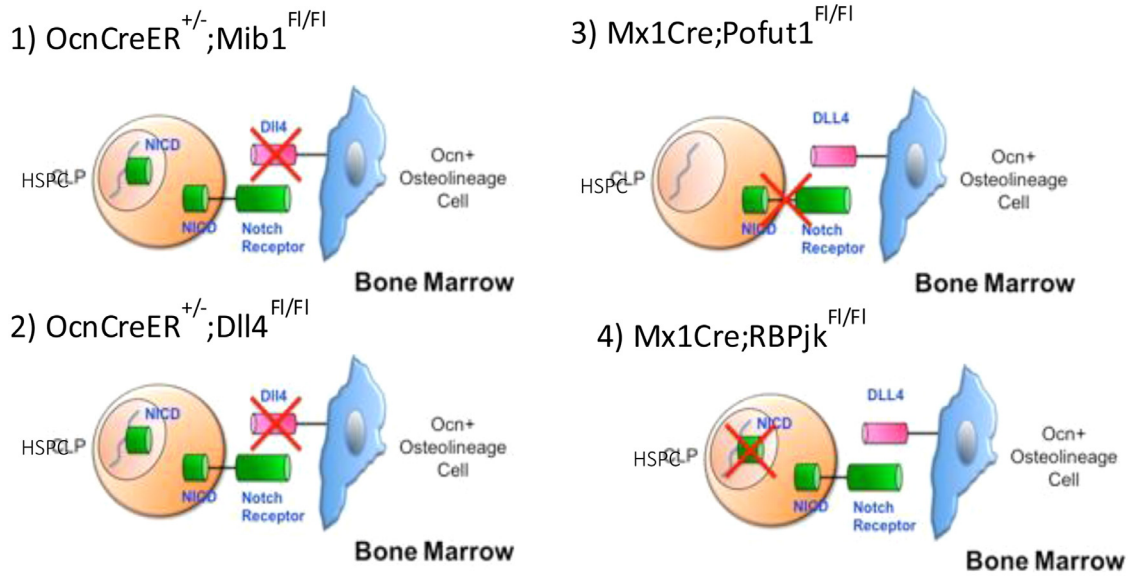


**Figure 1.** Studies confirming a role for Notch signaling in T-cell generation. Genetic models used to assess whether the osteocalcin+ cell depletion effect on T-cell generation included those that genetically depleted the Notch signaling molecules *Mindbomb* (Mib1) and *Dll4* on the "sending" osteocalcin-expressing cell (OcnCreER) as depicted in models 1 and 2 and complementary studies modifying Notch receptor pathway genes *ProteinO-fucosyltransferase 1* (Pofut1) and *RBPjk* on "receiving" hematopoietic cells (Mx1Cre) depicted by models 3 and 4. The results that the effects on T-competent CLP generation in mice were comparable with those seen with osteocalcin+ cell depletion.

called "sending" cells on the osteoblast side, we took advantage of the *Osteocalcin* promoter driving a Cre-ER that could inductively deplete a loxP flanked gene in osteocalcin-expressing cells selectively. Both the Notch ligand processing gene, *Mindbomb*, and the *Dll4* gene were deleted in this manner. The phenotype in each such animal was very comparable to the osteocalcin+ cell-depleted animal in generating reduced T-competent CLPs. Reciprocal deletion of molecular machinery to process Notch receptors or their signals on the "receiving" cells, HSPCs, was inductively accomplished with Mx-1-Cre activation (Figure 1). The same phenotype in hematopoietic cells in the bone marrow was observed. Therefore, it is likely that the direct interaction of Dll4 on osteocalcin-expressing cells in the marrow with Notch on the hematopoietic cells leads to specification of cells with T-lymphocyte competence. We also observed that lower levels of CCR7 and PSGL1, previously defined by others as relevant to thymus homing [23,24], were found on the T-competent CLPs that did form. Specific bone marrow stromal cells expressing osteocalcin are therefore guiding the generation of cells that can emigrate to the thymus and become thymocytes.

## Do limited thymic emigrants restrict T-cell generation?

The issue of limited T-cell neogenesis in adults has long been ascribed to the atrophy of thymus after achieving puberty. De novo T-cell production can be measured by assaying the T-cell excision circles (TRECs) that result following T-cell receptor (TCR) recombination [25]. The excised portion of the TCR locus religates similarly to the endogenous chromosomal DNA, but does so to itself, creating a circular DNA. The TREC does not replicate with cell division and so is a quantitative means of determining the number of newly minted T cells; daughter cells do not contain it. TRECs decline after adulthood, and the thymus becomes atrophic [26].

Age-related atrophic changes in the thymus are linked to androgen production [27]. Sutherland and colleagues have reported that with androgen depletion, the thymus can regenerate [28]. The thymus remains competent for regeneration even in men over the age of 60 who have undergone androgen ablation therapy for prostate cancer [28]. In that context, TRECs increased by greater than twofold in five of the 10 men studied. Similarly, some patients with AIDS undertaking potent anti-retroviral therapy undergo increased thymus volume and TREC production [26,29,30].

Testing whether part of the atrophic state of the thymus may be attributable to the substrate of cells entering it, Awong and colleagues found that by transferring wild-type hematopoietic cells into an NSG mouse (incapable of making T and B cells on a hematopoietic cell autonomous basis), the thymus changed markedly. In particular, the epithelial subsets normalized. Normal thymus has both cytokeratin-8–expressing cortical epithelia and cytokeratin-5–expressing medullary epithelia. The medullary component is virtually absent in the NSG animal, but when wild-type hematopoietic cells were

added, the architecture and representation of medullary cytokeratin-5–positive cells normalized [31].

Others also found that human T-cell progenitors generated from cord blood CD34+ cells ex vivo on immobilized Dll4 could populate the thymus and enhance T-cell regeneration when transferred into NSG mice [32]. Notably, they found that transferring T-cell progenitors also enhanced thymopoiesis from the stem cell graft. Awong et al found that T progenitors expressing CD5 particularly augmented thymus receptivity to cells coming from the stem cell transplant. Therefore, the thymus is responsive to particular hematopoietic cell-based inputs that can augment its function [33]. Persistence of improved thymus function and thymic epithelial cycling requires the ongoing presence of T progenitors [34]. Collectively, these data indicate that the thymus can regenerate. It can do so when a suppressive signal such as androgens or chronic HIV infection is reduced or in response to increased hematopoietic cell substrate.

## Settings of reduced osteocalcin-expressing cells

Because the Osteocalcin+ cells enable T-cell specification among HSPCs in the bone marrow, their reduction may contribute to reduced T-cell neogenesis with age and disease. It is clear that osteoblastic cells do decrease with age in humans as does their production of osteocalcin [35]. This is associated with, if not causative of, osteoporosis. Osteolineage and osteocalcin+ cells have also been reported to be sensitive to the conditioning regimens associated with hematopoietic stem cell transplantation (HSCT) [4,36–38]. Further, when we assessed the triple transgenic mice that we created to allow us to discriminate early osterix-expressing osteoprogenitors from osteocalcin-expressing cells [14], abrupt declines in osteocalcin+ cells were noted with particular stresses. Stresses that might occur in HSCT, such as graft-versus-host disease (GvHD), granulocyte colony-stimulating factor (G-CSF) treatment, or interferon $\gamma$ treatment all resulted in marked decreases in osteocalcin+ cells. Therefore, it is likely that multiple aspects of HSCT conspire to reduce the stromal population needed to generate T-competent hematopoietic progenitors. The acute need for T-cell production and education in the thymus that accompanies HSCT occurs in settings that impair generation of T-competent progenitors in the bone marrow.

## Bioengineering devices to augment T-competent progenitors

The confluence of a compromised marrow microenvironment with age-related thymic atrophy creates a setting of maximally suppressed T-cell production. Transplant patients experience that lack of T-cell production and education through failure of the immune system to control infections and to modulate activity against the new host, GvHD. To assess whether improved bone marrow production of T-competent progenitors could influence T-cell neogenesis, we collaboratively developed a device designed to supplement the marrow stromal contribution to T-cell production. David Mooney's laboratory has extensive experience developing biocompatible matrices that incorporate instructive proteins [39–41]. A shared postdoctoral fellow between our labs, Nisarg Shah, led the effort to determine if a bioengineered construct could be made that enhances generation of an ectopic bone marrow capable of generating T-competent progenitors. Using the information gained from the studies of the mouse bone marrow T-competent progenitor niche, a cryogel was synthesized using alginate cross-linked to polyethylene glycol and Dll4 [42]. The polymerized cryogel also included BMP-2, although this was not covalently bound to the matrix to facilitate its gradual elution into surrounding soft tissue. The concept was to foster the migration of local host mesenchymal cells and endothelial cells into the cryogel through the BMP-2 and to enforce osteolineage differentiation of the mesenchymal cells by BMP-2. The cryogel was formed to achieve a fairly consistent pore size (Figure 2A) and was highly malleable and compressible once formed. A $1 \times 1 \times 0.2$-cm cryogel could be readily aspirated into a 16-g syringe needle and would readily resume its shape when injected either into liquid or under the skin. Various concentrations of growth factors and Dll4 were tested in the cryogel to achieve the goal of generating an ossicle in vivo.

When subcutaneously injected into mice, the cryogels with BMP-2 gradually increased in size, forming a firm nodule. These ossified and were vascularized, eventually becoming invested with bone marrow [42]. The bone marrow cryogel (BMC) was injected concurrently with lineage-depleted, green fluorescent protein (GFP)–labeled, bone marrow–derived hematopoietic cells into lethally irradiated recipient mice. This resulted in GFP+ bone marrow engrafting the BMC containing either BMP-2 alone or BMP-2 plus Dll4. The majority of the hematopoietic cells in the cryogel were myeloid, not dissimilar from endogenous bone marrow. The BMC with BMP-2 plus Dll4, however, was uniquely able to generate more T-competent CLPs than the BMP-2–only BMC (Figure 2B). This was accompanied by greater numbers of GFP+ cells in the thymus [42]. To test whether cells from the BMC trafficked to the thymus, BMC that had been in GFP+-transplanted animals were excised and implanted into sublethally irradiated non-GFP recipients who did not receive any third-party bone marrow cells. The only GFP+ cells transferred were those that were in the BMC. GFP+ cells were detected in the thymus of the recipient animal [42]. Therefore,

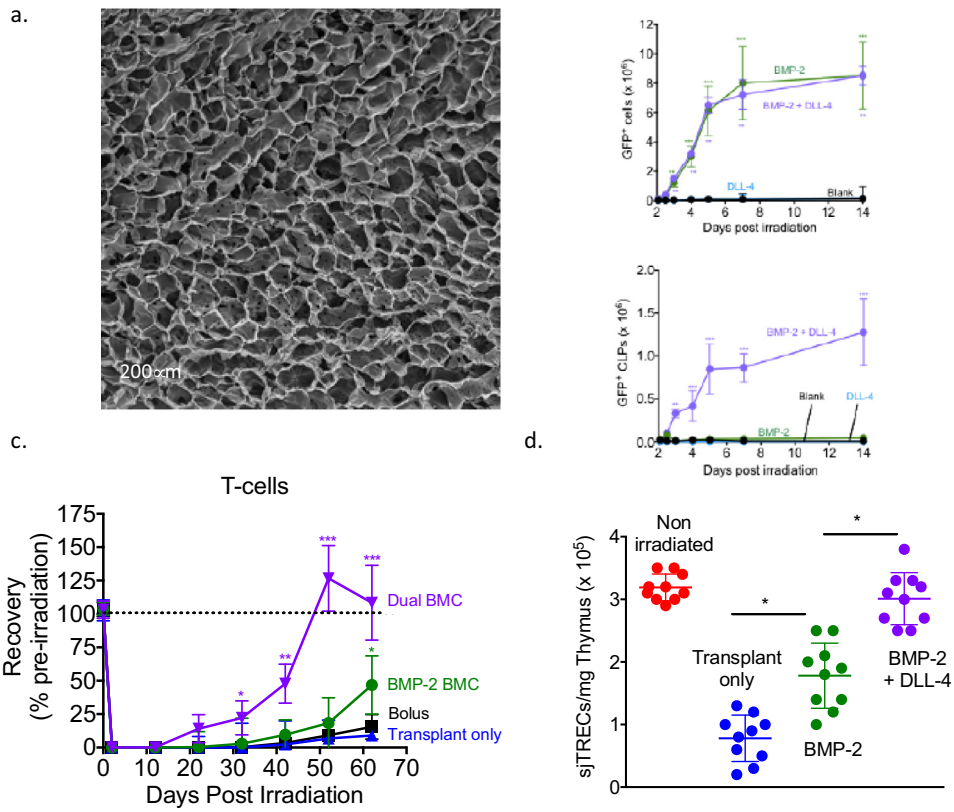


**Figure 2.** Features of a bioengineered T progenitor niche. **(A)** Photomicrograph of the bone marrow cryogel prior to subcutaneous injection. **(B)** The hematopoietic cells found in the subcutaneous cryogel on the indicated days after the concurrent injection of the cryogel and bone marrow transplantation of GFP+ lin− donor cells in a lethally irradiated recipient mouse. Blank refers to no proteins in the cryogel. **(C)** Recovery of peripheral blood T cells in animals receiving the cryogel and lin− bone marrow. Transplant only refers to no bone marrow cryogel (BMC) being administered. Bolus refers to the BMC without proteins. Dual refers to BMP-2 and Dll4 in the BMC. **(D)** Signal joint T-cell excision circles (sjTREC) per milligram of thymus measured 1 month after transplant. $*p < 0.05$. All experiments shown were repeated at least twice with at least five mice per group.

the cryogel was able to generate a bone and bone marrow that was able to enhance T-competent progenitors capable of trafficking to the thymus.

### Improved T-cell immune regeneration after HSCT

The ability of the BMC to generate T-competent CLPs migrating to the thymus was confirmed, and the impact on thymocyte subsets, T cells, and immune function assessed. Animals transplanted with autologous whole bone marrow mononuclear cells do not manifest apparent T-cell compromise presumably because of transplanted T-cell expansion. However, if mature cells are depleted from the graft and only lineage cells are transplanted, T-cell reconstitution is delayed. This setting allowed evaluation of whether the BMC could augment T-cell generation. In that context, the presence of the BMC with both BMP-2 and Dll4 (called “dual” BMC) increased the subsets of thymocytes that are of intermediate stages of differentiation and single positive CD4 or CD8 mature cells [14]. As other cell types in the marrow also produce Dll4, it may be that they also contribute to the impact of the BMC [43,44]. Notably, both thymocytes and circulating T cells were increased in the context of the dual BMC (Figure 2C).

The number of T cells is physiologically meaningful if there is a repertoire of T-cell receptors present within the T-cell population that can provide immunity. It is possible to define whether new T cells were being made or existing T cells were being expanded through the use of the TREC assay mentioned previously. This polymerase chain reaction (PCR)−based assay can be performed on varying tissues; we conducted it on thymus and spleen to determine if cells were moving from the thymus to sites of immune activity in the spleen. In both locations, the number of TREC+ cells was markedly increased in transplanted animals receiving the dual BMC compared with either transplant alone or transplant with control BMC (Figure 2D). The levels were statistically not different from those of untransplanted animals in the thymus.

In addition to measuring the new T-cell generation by measuring TREC, the complexity of the recombined

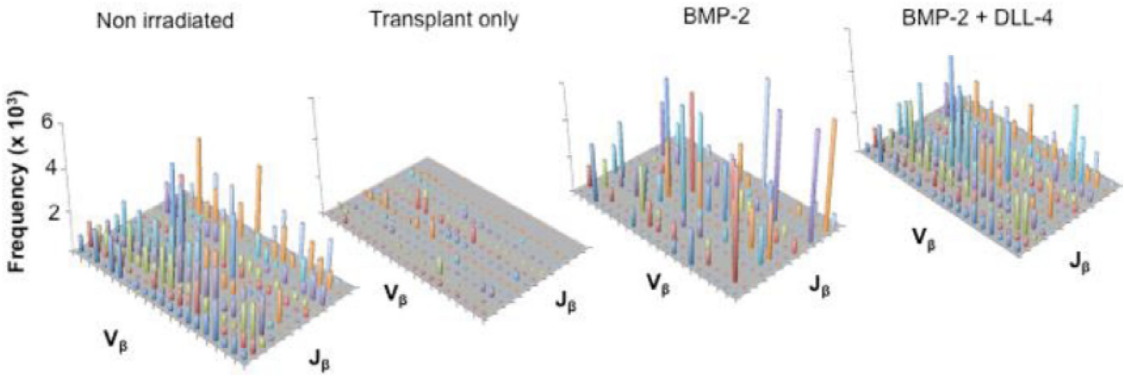


**Figure 3.** T-cell receptor diversity generated after transplantation with the bone marrow cryogel in vivo. The diversity in antigen receptors of T cells as analyzed by the sequenced V and J segments of the CDR3 beta chain in the BMC and transplant mice. Each bar represents a single clone. The plot provides depth (length of bar) and diversity (number of bars) of T cells in the mice. Samples were pooled from five mice for each group, and the combined data are represented. BMP-2 and BMP-2 + DLL-4 indicate the proteins included in the BMC.

TCRs can be quantified using TCR sequencing. The relative abundance of specific subtypes of recombination events can be evaluated and was distinctly suppressed in the animals that had undergone transplant compared with controls (Figure 3). The number of cells, although not the TCR diversity, was increased by the presence of the control cryogel. However, in the presence of the dual BMC, complexity and amounts of recombined TCR closely resembled that of untransplanted controls. Therefore, the dual BMC can foster new T-cell generation and do so productively to achieve a diverse repertoire of TCR-expressing T cells.

The presence of a recombined TCR does not ensure immune reactivity and cell function, however. To assess these parameters, we conducted two types of functional analysis. One was the response of the T-cell system to a neoantigen, and the second, a measure of tolerance by evaluating cells in the context of a histocompatibility-mismatched bone marrow transplant. For neoantigen response, we vaccinated wild-type transplanted BMC-engrafted or control animals with ovalbumin and evaluated T-cell responses using the SIINFEKL tetramer assay (Figure 4A) [45]. This assay quantifies the production of T cells bearing a TCR that recognizes a well-characterized peptide within ovalbumin that is processed and presented on the major histocompatibility complex. The results for the dual BMC-receiving transplanted animals were not comparable with those for untransplanted control animals but were markedly increased compared with those of controls.

GvHD is a disease characterized by imbalanced activation and tolerization of host-responsive T cells. In the experimental situation of a BALB/c mouse transplanted with C57BL/6 mouse bone marrow, GvHD is severe and lethal. In advance of the experiment, we were not sure whether the presence of the dual BMC might worsen GvHD by increasing the T-cell repertoire. However, the animals transplanted and receiving the dual BMC had improved survival with a significantly delayed time to morbidity and mortality (Figure 4B). The presence of more T cells, more rapidly at a balanced CD4:CD8 ratio (2:1 as seen in untransplanted controls) and with more Tregs (Figure 4C), may have blunted the GvHD. The known ability of Dll4 to enhance Treg development may be particularly relevant to the effects of the BMC on GVHD [46].

## Potential for humans

Human xenografted immunodeficient mice have been helpful in modeling some aspects of human immunity. This model suffers from the lack of cytokines or adhesion molecules cross-reacting with human cells. With that caveat in mind, the BMC was evaluated in an NSG mouse transplanted with human thymus and fetal liver. The mice with the dual BMC had a less prominent increase in T cells compared with what was seen in the mouse cells–into–mouse transplant experiments. However, the CD4:CD8 ratio closely approximated the 2:1 ratio of control animals, Tregs were increased over controls, and the dual BMC-receiving animals again had a prolonged survival compared with animals transplanted without the BMC (Figure 5). These data suggest that the dual BMC has potential for providing medical benefit in patients, particularly those undergoing allogeneic transplantation.

Translating the dual BMC to a human context clearly requires that several issues be addressed. The first is whether the BMC construct can be scaled and be tolerably implanted in patients. Allometric scaling estimates by the Mooney laboratory suggest that the sizes needed will be in the range ($\sim$25 $cm^3$) of other devices that have been routinely implanted subcutaneously in patients, such as vascular ports and older-type cardiac pacemakers. Ideally, the BMC would be self-limited in time, leading to resorption by the body after

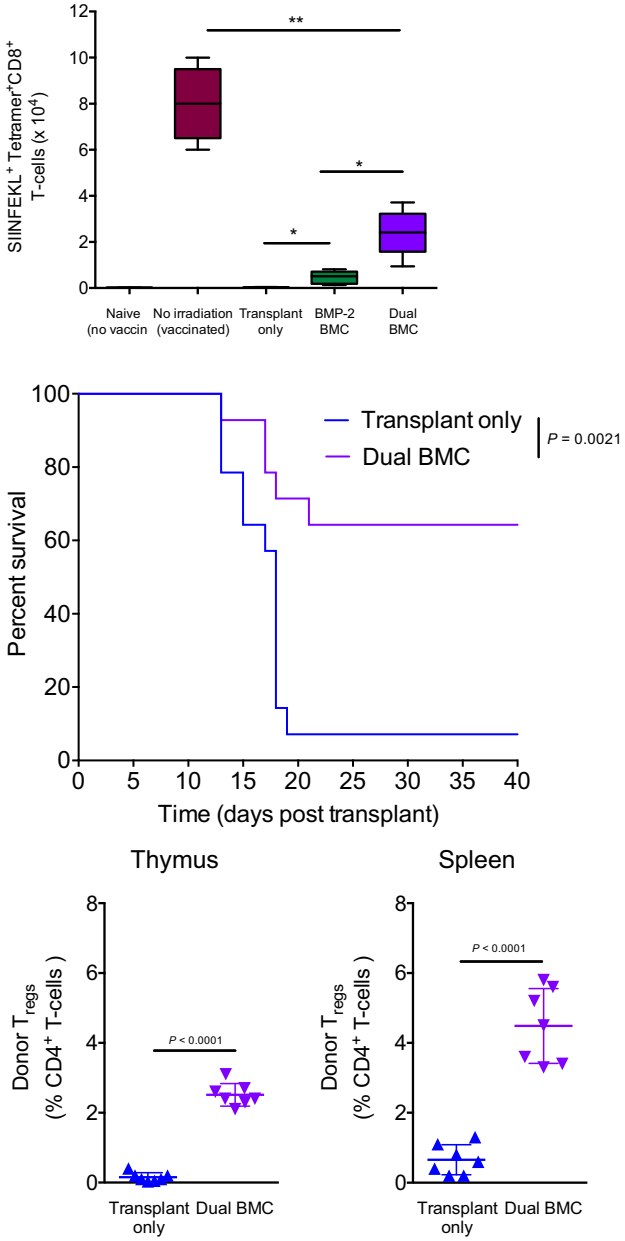


**Figure 4.** Immunologic function after transplantation with the bone marrow cryogel. **(A)** Donor SIINFEKL+CD8+ T cells enumerated in ovalbumin-vaccinated mice 30 days after syngeneic hematopoietic stem cell transplantation. **(B)** Kaplan–Meier survival of BALB/cJ recipient mice that received 850 cGy of total body irradiation. Within 48 hours postradiation, mice were transplanted with allogeneic GFP $5 \times 10^5$ lineage-depleted GFP bone marrow cells and $10^6$ GFP splenocytes. One group was simultaneously treated with the dual BMC. $n = 10$ mice. **(C)** Percentage of CD4+ cells with a Treg immunophenotype in the indicated tissues 30 days after transplant with or without the dual BMC. All experiments are representative of at least two independent experiments.

an interval during which T-cell neogenesis is likely to be most critical. However, these experiments are still underway in the Mooney laboratory. In the interim, the nodule generated by the presence of the dual BMC

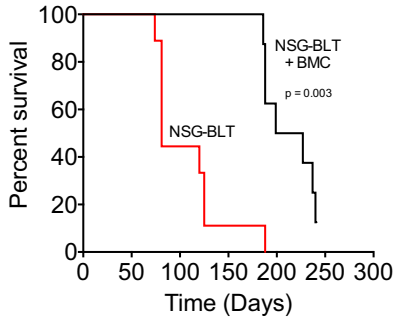


**Figure 5.** Effect of the bone marrow cryogel on survival of mice after human xenograft transplantation. Humanized BLT mice (10 per group) receiving transplantation alone or with the dual BMC as indicated. The experiment was repeated twice.

does not appear to insinuate calcified processes into local soft tissue. This facilitates removal, which in the mouse has required simple incision and removal of the ossified BMC with forceps; no extensive dissection of regional or distant calcified tissue was required.

A second issue is whether an older adult could benefit from the generation of pro-T cells given the atrophic state of the thymus. Maintenance of T-cell neogenesis capability is evident in at least some people. This is manifest in the setting of allogeneic HSCT, where a slow, but progressive expansion of the TCR repertoire is observed. It is further supported by the data previously mentioned on AIDS patients who go on antiretroviral therapy and elderly men undergoing androgen ablation therapy for prostate cancer. It is therefore likely that at least some patients will be able to restore thymopoiesis. Whether that is substantially incremented by the presence of the dual BMC will require clinical testing to determine.

## Perspectives on hematopoietic regeneration

The ability to therapeutically manipulate blood cell production is one of the triumphs of medical research. It was accomplished by the dedicated basic investigation of blood cell production by pioneers like Donald Metcalf, Leo Sachs, and Eugene Goldwasser, who provided the paradigmatic work enabling characterization and isolation of hormonal factors controlling myeloid cells. The subsequent basic and clinical efforts of innumerable academics and inspired employees of biotechnology firms have given clinicians hematopoietic growth factors as life-saving therapeutics benefiting the lives of thousands.

Yet finding methods of increasing the number of stem cells has remained extremely challenging. No single factor is capable of accomplishing HSC expansion. Many combinations have been reported to increase HSCs in vitro, and some are in advanced clinical testing. However, unlike erythropoietin or G-CSF, none of

them works as a single agent; they are combined with multiple other growth factors. This likely reflects the underlying biology of stem cells. The stem cell is the point source of the hematopoietic cascade and its extreme capacity to generate new cells. Faulty governance of the stem cell poses the possibility of either exhaustion through excess activation or malignancy because of unrestrained self-renewal. It is not surprising that the regulation of such a cell may depend on multiple positive and negative regulatory inputs: it is multidetermined.

The lymphoid system is also distinctive among lineage-restricted hematopoietic cells for its extensive ability to self-renew even after maturing well beyond the stem cell state. It has the capacity to generate de novo reactivity based on the recombination events that characterize its early developmental programs. It may also engender particular risks for the organism in terms of controlling reactivity and controlling overgrowth. When lymphoid cells get to the stages of full maturation, they do have clear responsiveness to single triggers like interleukin (IL)-2, IL-15, and IL-4. However, other growth modulators in addition to the early acting Flt-3L or IL-7 are necessary for early events in lymphoid differentiation [47,48]. The absence of IL-7 does not preclude CLP formation, and animals overexpressing IL-7 and Flt-3L have reduced thymocytes [48,49]. IL-7 also adversely affects bone [50]. Notch ligands like Dll4 and Dll1 induce increases in early T progenitors but are too pleiotropic in their effects to be used systemically in vivo and need support cells in vitro [51–53]. Regenerating early T-cell progenitors is therefore not likely to follow the single cytokine administration intervention that myeloid cells have with granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, erythropoietin, and thrombopoietin. The exception may be with a cytokine that is directed at the microenvironment, as has been observed with IL-22 [54]. That epithelial cell affecting cytokine may sufficiently change a diverse set of signals from the thymic epithelial niche to improve T cell regeneration.

Increasing diverse populations of multidetermined cells like HSCs and early lymphoid progenitors have medical importance. This is particularly true in acute depletion contexts like that of hematopoietic stem cell transplantation. However, other contexts of lymphoid cell depletion are more chronic and more common. It is the case that although total T cells do not decline with age, TCR repertoire complexity does, in a linear manner [55]. This matches common experience as evident in the phenomenon of a chronic virus like herpes zoster remaining under immunologic control for decades but breaking through in older age as shingles. A declining T-cell repertoire may also incur risks for other age-associated diseases such as neoplasms and autoimmune disorders like giant cell arteritis, primary biliary cirrhosis, and inflammatory bowel disease [56,57]. Studies defining clonal hematopoiesis also suggest that oligoclonal HSC outgrowths occurring increasingly with age carry with them risks of myeloid malignancies, cardiovascular disease, and all-cause mortality [58–60]. Whether these can be attributed to limited diversity or a rogue clone overgrowing is not clear, but in many biologic systems, diversity constrains aberrant cell or organism overgrowth [57,61,62].

Achieving increased populations of primitive cells may therefore be more broadly applicable than just in the setting of iatrogenic, acute depletion like transplant. Durable increased production would be optimal and not practical with intravenous growth factor [54] or ex vivo expansion or organoid-based methods [63] that are patient specific. Scaling and delivering such interventions at an economically sustainable scale would be very challenging. Whether a potentially off-the-shelf device requiring no patient matching and no cells, like the BMC, can provide a solution remains to be seen. However, it offers a relatively simple construct to address a biologic problem that relies on the complex biology of the niche. The BMC does not engineer a niche; it prompts the body to generate an adaptive niche and that approach may offer a way forward.

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## Conflict of interest disclosure

The author is a founder, director, and consultant for Magenta Therapeutics; a founder and consultant for Fate Therapeutics; a Director for Agios Pharmaceuticals and Editas Medicine; a consultant for FOG Pharma and VCanBio; and a founder and director for Red Oak Medicines, Clear Creek Bio, and LifeVault Bio. He received sponsored research funds from Novartis.

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