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60 Years Young: The Evolving Role of Allogeneic Hematopoietic Stem Cell Transplantation in Cancer Immunotherapy

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Abstract

The year 2020 marked the thirtieth anniversary of the Nobel Prize in Medicine awarded to E. Donnall Thomas for the development of allogeneic hematopoietic stem cell transplantation (allo-HSCT) to treat hematologic malignancies and other blood disorders. Dr. Thomas, "father of bone marrow transplantation", first developed and reported this technique in 1957, and in the ensuing decades, this seminal study has impacted fundamental work in hematology and cancer research, including advances in hematopoiesis, stem cell biology, tumor immunology, and T cell biology. As the first example of cancer immunotherapy, understanding the mechanisms of anti-tumor biology associated with allo-HSCT has given rise to many of the principles used today in the development and implementation of novel transformative immunotherapies. Here we review the historical basis underpinning the development of allo-HSCT as well as advances in knowledge obtained by defining mechanisms of allo-HSCT activity. We review how these principles have been translated to novel immunotherapies currently utilized in clinical practice and describe potential future applications for allo-HSCT in cancer research and development of novel therapeutic strategies.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has paved the way for three of the most exciting areas of cancer research: stem cell therapies, immune-modulating techniques and the individualization of cancer therapeutics. After more than 60 years from the first attempted HSCT and with more than one million performed worldwide since (1), allo-HSCT remains the only form of stem cell therapy that is widely clinically available, and the most common form of cellular immunotherapy.

The era of allo-HSCT began in the wake of the first atomic bomb, with the landmark observations that mice could be protected from the lethal effects of radiation by shielding

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their spleens or femurs with lead (2), or rescued by intravenous infusions of bone marrow (3). While many scientists initially postulated that protection was granted by some “humoral” factor in the spleen or bone marrow able to stimulate recovery, different groups independently demonstrated that radiation protection was due to the transplanted stem cells (4–6). The discovery that protection from radiation could be conferred by stem cell transplant had exciting implications for cell biology and for the therapy of patients with life-threatening hematologic malignancies: it was now possible to treat patients with high doses of chemotherapy or radiotherapy that would be otherwise limited by toxicity to the hematopoietic system. The first suggestion of a donor immune response against leukemia contributing to the overall effect of allogeneic transplantation came in 1956: in murine HSCT attempts, leukemia relapses appeared to be reduced following infusion of allogeneic bone marrow as compared with the syngeneic marrow (7). The following year, the first human allogeneic bone marrow transplantations were reported by E. Donnall Thomas (8). Six patients were treated with myeloablative chemotherapy and radiotherapy, followed by an infusion of bone marrow from a healthy donor. Only two of six patients showed evidence of engraftment and, unfortunately, all died within 100 days of transplantation. Donors and patients were not matched for histocompatibility, as little was known about human histocompatibility antigens at that time. This disappointing result contributed to the general view that the barriers to allogeneic transplantation would never be overcome, causing many researchers, but not Thomas, to abandon the idea that allo-HSCT could be used to treat cancer.

A decade later the discovery of the human leukocyte antigen (HLA) system by Dausset and Van Rood (9) allowed a more careful matching of donor and recipient HLAs, reigniting interest of the scientific community in stem cell transplantation and setting the stage for renewed, and ultimately successful, efforts to treat patients with hematological diseases. In 1965, Mathé reported histocompatibility testing of bone marrow donors for a patient with acute lymphoblastic leukemia (10). The patient was transplanted with a graft derived from six family members (mother, father, 3 brothers and 1 sister) and developed a “secondary syndrome”, later to be recognized as graft versus host disease (GvHD), involving the skin, gastrointestinal tract, and liver. Six months after the transplant, to consolidate an antileukemic effect, an additional bone marrow infusion was performed. To select the donor least likely to exacerbate the secondary syndrome, an elemental histocompatibility test was performed by giving the patient skin grafts from each donor. The donor whose graft survived was selected, but still the allogeneic boost led to a recrudescence of the “secondary syndrome”, which was steroid-responsive.

The development of HLA typing techniques for donor-recipient pairs in the mid/late 1960s enabled E. Donnall Thomas to open an allogeneic bone marrow transplantation program in Seattle using HLA-matched donors for patients with acute leukemia, thus configuring the first patient-individualized cancer therapy program. In 1977, they reported 100 transplants from HLA-identical related donors for relapsed and refractory acute leukemia (11). Although only 13 patients were leukemia-free after 1–4.5 years follow-up in this case series, administering transplantation earlier in the course of disease resulted in a cure rate of 50% in patients transplanted in first remission (12). Crucially, Thomas appreciated that the donor immune system played a key role in eliminating residual leukemic cells: although survival

was reduced in patients with severe GvHD, most patients did not die of relapse. In 1990, E. Donnall Thomas won a Nobel Prize for his discoveries concerning cell transplantation in the treatment of human disease, laying the foundations of modern cancer immunotherapy (Figure 1). The history of allo-HSCT is one of trials and tribulations, of triumphs, and of perseverance. Here we celebrate E.D. Thomas's legacy, through five fundamental lessons allo-HSCT has taught us about cancer immunotherapy (Figure 2).

The ABCs of allo-HSCT

The basic principle underpinning allo-HSCT is that of infusing the hematopoietic stem cells (HSCs) and the full immunologic repertoire from a normal donor into a patient to establish donor-derived hematopoiesis and immunity. Originally designed as a stem cell rescue technique, allo-HSCT in its infancy relied on intensive myeloablative conditioning regimens, which limited its applicability to young patients without comorbidities. In the early days of this field, only 25% of patients younger than 35 years of age could be feasibly transplanted with the requirement of using exclusively HLA-identical siblings. In the present day, almost all adult patients in need of a transplant up to 70 years of age may be offered an allo-HSCT, due to several refinements in the transplant procedure. First, while early transplants relied on bone marrow grafts (requiring hospitalization and sedation of the donor), the use of G-CSF to mobilize HSCs to peripheral blood has markedly simplified graft collection (13), such that it is now a minimally-invasive outpatient procedure and provides similar overall survival compared to bone marrow grafts, albeit with a slightly higher incidence of chronic GvHD (14, 15). Second, the increasing appreciation of the graft versus leukemia properties of allo-HSCT allowed the development of reduced-intensity conditioning (RIC) transplants, using lower doses of chemotherapy, which has extended this potentially life-saving treatment to older and frailer patients (16).

Third, we witnessed the expansion of alternative sources of allogeneic HSCs, including unrelated volunteer donors, umbilical cord blood (UCB), and most recently T-replete haploidentical donors. Since the first unrelated donor allo-HSCT in 1979 (17), national donor registries have been successfully established to facilitate registration and identification of potential unrelated donors, as well as collection and transportation of stem cell products across national and international lines. Despite over 10 million HLA-typed registered volunteers worldwide, the chance of finding a suitable donor still poses challenges, particularly for underrepresented populations and for patients in need of a more immediate source of HSCs (18). UCBs can be easily collected after birth, without risks for mothers or donors, banked in dedicated institutions, and available for immediate use. Drawbacks of UCB remain the limited cell dose and slower kinetics of immune reconstitution. Alternatively, for nearly any patient, a first degree haploidentical relative (e.g. parent, child, or partially matched sibling) can be identified. Historically, haploidentical transplants were hampered by high rates of graft failure and severe GvHD, but the introduction of post-transplant cyclophosphamide as an *in vivo* T cell depletion strategy for GvHD prevention has dramatically improved outcomes of this mode of allo-HSCT (19). It remains unclear whether any of these alternate donor approaches – cord blood, haploidentical, mismatched unrelated – is better than another, and results from ongoing randomized trials are eagerly awaited.

Immunologic impairment is universal after allo-HSCT and commonly persists for several months after transplant, with faster recovery of innate immunity (monocytes, granulocytes, NK cells), followed by a slower recovery of B and T cells, which can take up to 2 years to reconstitute (20). The extent of immunodeficiency and subsequent kinetics of immune reconstitution depend upon many variables, including patient and donor characteristics, graft source, HLA disparity, graft manipulation, and development of GvHD.

Although not the focus of the present review, allo-HSCT represents a curative treatment option also for non-malignant conditions, such as autoimmune diseases and genetic disorders, including hemoglobinopathies and immunodeficiency syndromes, and has set the foundations for the development and clinical application of gene therapy approaches, as comprehensively reviewed elsewhere (21).

Fundamental Lessons and Fruits From HSCT

HSCT as the first consistent human model of effective antitumor immunotherapy

Within a decade of the first successful bone marrow transplants in humans, HLA was identified and its role in typing and histocompatibility began to emerge (10, 22). With this advance in technology, it became possible to HLA type potential bone marrow donors to ensure compatibility. In a study of 100 patients transplanted for acute leukemia, patients who developed GvHD but survived were less likely to die of relapse, and if they did relapse, this tended to occur later in the disease course (11). This provided the first evidence that donor immune responses driving GvHD may also be driving an antileukemia, or graft versus leukemia (GvL) effect (23, 24) and was the first demonstration that the immune system engages in anti-tumor responses, making allo-HSCT the first cancer immunotherapy applied to patients. Additional evidence for an immune-mediated GvL effect included the observation that disease relapse rates increased if donor bone marrow grafts were depleted of T cells in an effort to reduce GvHD (25), that withdrawal of immunosuppressive agents could lead to remission in patients who relapsed after transplant (26), and that some degree of genetic difference between donor and recipient appeared to be protective against relapse (27).

Perhaps the most convincing demonstration of the GvL effect was made in 1990 with the implementation of donor leukocyte infusions (DLI) for treatment of relapsed leukemia following transplant (28). In a landmark study for the field of adoptive immune therapy, three patients with relapsed chronic myeloid leukemia (CML) were infused with interferon-α along with buffy coat preparations from the original bone marrow stem cell donor, resulting in remission of their disease without the use of additional chemotherapy or radiation. Since that time, the use of DLI has become widespread and established for the treatment of relapse after transplant across several hematologic diseases. The GvL effect of DLI is more pronounced in some hematologic malignancies than others, but the underlying mechanisms for these differences remain elusive. CML appears to be the most sensitive to DLI with response rates of up to 90–100% for cytogenetic relapse (29, 30). Unfortunately, however, not all hematological malignancies display the same sensitivity to DLI as CML. However, acute myeloid and lymphoblastic leukemias (AML and ALL, respectively), demonstrate lower response rates to DLI (remission rates of 15–20% and

10–20%, respectively) (31, 32), while aggressive lymphomas and myelomas respond in approximately 30% of cases (33–36).

While DLI is by now an established post-HSCT therapy, the precise mechanisms of its anti-tumor activity remain elusive. Studies in CML have provided the most informative platform thus far for studying how DLI mediates its effects. Analyses of immune reconstitution following DLI have revealed its ability to stimulate both cellular and humoral immunity by leading to increased T and B cell neogenesis, enhanced TCR repertoire diversity, and production of tumor-directed antibodies (37, 38). Furthermore, there is some evidence that DLI stimulates the expansion of pre-existing donor-derived tumor-specific $CD8^+$ T cell responses (39). More recently, we and others, using approaches such as bulk and single cell transcriptome analysis, have demonstrated that a T cell exhaustion phenotype is associated with relapse after transplant, and that DLI is capable of reversing this exhausted phenotype, an effect associated to response to treatment (40–43). Innate immune responses also appear to be critical to an effective anti-tumor response and are stimulated by DLI. Post-DLI plasma from patients with relapsed CML have been shown to have highly upregulated inflammatory cytokines, stimulated by TLR 8/9 activation mediated through tumor-specific immunoglobulin-nucleic acid complexes (44). These data support a critical role for DLI-mediated responses in the arsenal of stem cell therapy tools. However, there remains substantial work to be done in more precisely delineating its mechanisms of activity in harnessing anti-tumor immune responses and defining the molecular determinants of the different susceptibility to DLI of distinct hematologic malignancies to enhance the anti-tumor effect of these therapies.

Other controversial cellular players in the transplant GvL effect are NK cells, innate immune cells capable of distinguishing “self” from “non-self” through killer immunoglobulin-like receptors (KIRs) interacting with KIR ligands on leukemic cells (45). To date, NK cell alloreactivity has proven protective against AML relapse in T cell depleted haploidentical HSCT (45), but definitive proof of NK alloreactivity effectiveness in the context of T cell replete transplants is lacking, although encouraging results from adoptive transfer of donor-derived cytokine-activated NK cells in AML patients relapsing after allo-HSCT have been recently reported (46).

How to tame immune overactivation – the Graft vs Host phenomenon

In the early days of experimentation with transfer of immune cells from one host to another, it was observed that transfer of bone marrow to a lethally irradiated murine host of a different strain led to a clinical syndrome of diarrhea, skin lesions, and wasting within a few weeks after irradiation, and that this process was dependent on genetic differences between donor and host (47, 48). This phenomenon was termed “runt disease” when it was observed that injection of adult lymphoid cells into newborn mice of a different strain led to defects in growth as well as changes in pathology of the spleen, liver, and other organs. The process was determined to result from an immune reaction against the recipient cells by adult donor cells given that: 1) transfer of donor cells did not replicate the disorder, 2) transfer of isologous (genetically identical) cells failed to replicate the phenotype, and 3) the degree of severity of the phenotype was related to the degree of genetic difference between

the donor and the host strains (49). Later termed the “secondary syndrome” and now known as graft versus host disease (GvHD), this phenomenon was observed repeatedly in animal models of bone marrow transfer after lethal irradiation (50–54), as well as early attempts at human bone marrow transfer after lethal irradiation gained some early success (55, 56). The study of GvHD, its pathogenesis, and mechanisms of prevention was of utmost interest in the infancy of bone marrow transplantation, as the syndrome frequently led to death of the recipient (10, 57), and continues to be a major area of clinical and basic research.

GvHD is now recognized to encompass two distinct pathologic forms: an acute form that typically occurs early after transplantation and a chronic form that tends to appear later, and the two phenomena are driven by distinct mechanisms (58). Acute GvHD is inflammatory in nature and presents clinically as skin rash, diarrhea, and/or liver inflammation and dysfunction, likely exacerbated by tissue damage and inflammatory changes induced by conditioning chemotherapy regimens peri-transplant. This inflammatory milieu leads to translocation of gut flora and expression of pathogen associated molecular products (PAMPs), resulting in activation of host antigen presenting cells (APCs), expression of inflammatory cytokines, and activation of donor-derived cytotoxic T cells which mediate downstream end-organ damage in the recipient (59–66). This process is thought to be driven primarily by T helper 1 (T_H1) and T_H17 type immune reactions (67, 68). In contrast, chronic GvHD is more fibrotic in nature, has clinical features more suggestive of autoimmune disorders, and is thought to be primarily mediated by T_H2-type cytokine responses. Activation of these cells leads to production of fibrogenic cytokines and is associated with dysregulation of B cell function and skewing toward autoreactive B cell activation (69–72).

The role of immune cells, and of T cells in particular, in mediating pathogenesis of GvHD was recognized early on, as evidenced by two critical observations: first, that matching histocompatibility between donor and recipient resulted in long-term survival of transplanted animals and second, that treating recipient animals with immunosuppressive agents ameliorated GvHD and improved survival (73–77). Methotrexate and cyclosporine were two agents frequently used in early studies of prevention of GvHD in animals. These same techniques were soon applied to transplantation in humans and found to have important clinical benefit in the prevention of GvHD (78–80), with many of these same regimens continuing to be the standard of care. Nevertheless, GvHD continues to be a major cause of post-transplant morbidity and mortality, resulting in death in nearly 15% of transplant patients (81), indicating that there is substantial room for improvement in our current understanding of and strategies for prevention and treatment of GvHD. While treatments for GvHD have made some advances in recent years, the mainstay of therapy continues to be corticosteroids for their anti-inflammatory and immunosuppressive properties (82, 83). There is much interest in developing novel therapeutics and repurposing of agents approved for other inflammatory diseases for treatment of GvHD (84–86). Not surprisingly, the most common complication of DLI is exacerbation of GvHD (87). Methods of manipulating the DLI product to preserve the GvL effect while mitigating the GvHD effect have included depletion of $CD8^+$ T cells in the DLI product, selective depletion of alloreactive T cells (defined by expression of activation markers), and dose escalation of serial DLI infusion, with each strategy achieving modest clinical efficacy (88–91).

Administration of immunosuppressive agents after DLI has been shown to significantly reduce GvHD without an appreciable impact on the GvL effect of DLI (92).

Mechanistic insights gleaned from the study of GvHD pathogenesis have made major contributions to basic understanding of the immune system. In particular, studies of T cell mechanism of activity in both acute and chronic GvHD have given rise to an appreciation of the dynamic interplay between cytotoxic T cell responses, tolerogenic versus immunostimulatory roles for both T cells and APCs, T and B cell priming, and remodeling of the immune microenvironment after transplantation leading to autoimmune-like pathophysiology. Several of the inflammatory symptoms associated with GvHD, particularly the acute form, are echoed by toxicities to newer cancer immunotherapies. Common side effects from immune checkpoint blockade (ICB) therapy include colitis, autoimmune hepatitis, and skin rashes, thought to be due to hyperactivated T cells leading to robust cytokine production and cytotoxic T cell activity, similar to what is known about the mechanism of acute GvHD (93, 94). Corticosteroids continue to be the mainstay of treatment for these ICB-associated toxicities, and mechanistic studies in both efficacy and toxicity of ICB therapy reinforce the close relationship between autoimmunity, T cell hyperactivation, and GvHD (95).

Minor Histocompatibility Antigens: the first genomically defined immune targets for personalized cancer immunotherapy

The early observation that GvL and GvHD still occur in patients whose stem cell donors are fully HLA-matched led to the hypothesis that an additional antigen system besides MHC shaped post-transplant immunological reactions. These antigens were originally designated as minor Histocompatibility Antigens (mHAgs) (96), but their nature remained elusive until the mid-nineties, when, fueled by the advances in the understanding of mechanics of T cell recognition (97), pioneering studies demonstrated that mHAgs were polymorphic peptides presented in the context of HLA (98). Today, we know that any non-synonymous variation in the coding region of the genome can potentially result in an immunogenic mHAg after allo-HSCT. Single nucleotide polymorphisms (SNPs) generating amino acid substitutions are the commonest source of known mHAgs (98–101), however base-pair insertions and deletions (indels) (102), as well as frameshifts (103) or copy number variations (104) have been shown to contribute to the mHAg portfolio. There is, however, an upper limit to the number of possible mHAgs for any given donor-recipient pair: first, of the more than least 660,000,000 SNPs and indels in the human genome (105), less than 1% are non-synonymous; second, only non-synonymous SNPs that give rise to mismatches with the correct directionality (recipient homozygous positive or heterozygous, donor homozygous negative for the immunogenic allelic variant) and able to be presented on the available HLA alleles, contribute to the GvL effect. As such, the resulting mHAgs hold the record as the first genomically-identified as well as the first patient-individualized targets for immunotherapy.

Over the decades, the study of mHAgs, initially using laborious and time-consuming T cell expression cloning approaches or biochemical strategies, has been instrumental to our current understanding of the mechanistic basis of the curative potential of allo-HSCT and

of the potential source of its toxicities. Indeed, the GvL effect, at least in the HLA-matched transplants, can be conceptualized as the result of the donor-mediated immune responses against mHAgs expressed, though not necessarily limited to hematopoietic cells, while detrimental GvHD depends on the recognition of mHAgs expressed on GvHD-targeted tissues (106).

The discovery of mHAgs encoded by genes preferentially expressed by hematopoietic lineages has long been recognized as a foundation for generating mHAg-based immunotherapy, with the long-sought aim of separating GvL from GvHD effects. Indeed, mHAgs expressed only by hematopoietic cells would allow the selective targeting of the residual or recurrent hematologic malignancies, while the newly reconstituting donor-derived hematopoietic system (i.e. mHAg negative) would remain unharmed. For broad therapeutic application, however, it has been proposed that hematopoietic-specific mHAgs presented by common HLA alleles and with a balanced population prevalence should be prioritized in order to maximize the chances of finding targetable disparities between donor and patient pairs (107). Disappointingly though, the quest for such a panel of ideal mHAgs to date has resulted only in the identification of a handful of targets, some of which have been tested in clinical trials. These have resulted in diverse mHAg-directed immunotherapeutic approaches to either prevent or treat post-HSCT relapse, and have included the infusion of *ex vivo* expanded (108, 109) or TCR-redirected (110, 111) mHAg-specific T cells, as well as vaccination using dendritic cells loaded with mHAg peptides (112, 113) or mRNAs (NCT02528682). In general, until now, the exploitation of mHAgs in the clinical arena has revealed itself to be more challenging than anticipated due to the inherent difficulty in finding donor-recipient pairs not only suitably mHAg-mismatched but also carrying the appropriate HLA restrictions; thus, studies to target mHAgs have generally resulted in slow enrollment or even premature termination due to poor accrual (NCT00943293).

The discovery of targetable mHAgs is currently undergoing reinvigoration due to the expansion of innovative genomic capabilities available to dissect human samples. The understanding that genetic alterations could result in immunogenic epitopes, illustrated already more than 2 decades ago by mHAgs, was one of the early contributing sources of evidence that encouraged the development of genomics-based approaches for the discovery of tumor neoantigens which are now increasingly the subject of immunotherapeutic targeting for cancers (114). Indeed, the innovations in genomic technology to predict and identify neoantigens are now circling back and fueling new interest in more systematically identifying mHAgs from donor-recipient pairs. While neoantigens are the immunological byproduct of tumor-specific mutations (115), mHAgs are the immunological end result of germline genetic polymorphisms. Hence, the only substantial difference between them is that, at the genetic level, mHAgs are inherited while neoantigens are somatic events; both otherwise conform to the same rules for gene expression, antigen processing and HLA presentation. From the perspective of donor T cells, mHAgs and neoantigens are both sensed as foreign, and therefore there is no thymic negative selection for high-affinity T cell clones (116), and hence mHAg-encoded epitopes, like neoantigens, would be expected to be highly immunogenic. Among the *in silico* tools available for analysis of genomic data to discover mHAg are included tools to systematically identify germline differences between

DNA sequences from donor and recipient, tools for tissue expression profiling (single cell expression atlas (117), GTEx (118, 119), the human Proteome Atlas (120)), algorithms for peptide processing and binding, such as IEDB (121), netMHC (122) and HLAthena (123), and tools for the multidimensional detection of antigen-specific T cells (124). Altogether, these capabilities have, in recent years, all contributed to the establishment of robust pipelines for not only the prediction and selection of neoantigen-derived HLA class I epitopes, which have formed the basis for personal neoantigen-targeting vaccines (125–127), but also for mHAg discovery, with growing attempts at their large-scale prediction (128, 129). Of the predicted mHAgs, only a few have been validated thus far, demonstrating that these approaches require more development in order to fully realize the potency of these candidate targets (130). Assuredly, however, such methods will allow the future identification of novel mHAgs, and as in an analogous fashion as for tumor neoantigens, will form the basis for systematic personalized immunotherapy following allo-HSCT.

From GvL to novel cellular and immunomodulatory therapies to unleash the full power of T cells

Inspired by the GvL effect first recognized in the setting of stem cell transplant, innovative adoptive T cell therapies have made major strides in recent years, with many novel therapeutics demonstrating clear clinical benefit in a variety of malignancies (131–133). Strikingly, most of these advances have been in diseases where transplantation is not the mainstay of treatment.

These new cellular therapies have included the bi-specific T cell engagers (BiTEs) and the chimeric antigen receptor (CAR) T cells. BiTEs directly link CD3 on T cells with a tumor-specific antigen in order to overcome an immunosuppressive tumor microenvironment. This results in direct coupling of tumor cells with T-cells, leading to T-cell activation, proliferation, and anti-tumor cytotoxicity (134). Blinatumomab is a BiTE recognizing CD3 and CD19, a marker specific for B-lineage cells and commonly found on B cell malignancies. Clinical studies have shown considerable efficacy blinatumomab in maintaining remission in B cell acute lymphoblastic leukemia after relapse or in the presence of minimal residual disease (135, 136). Many novel therapeutics extending and enhancing features of BiTEs are currently under development, including bifunctional checkpoint-inhibitory T cell engagers (CiTEs), trispecific killer engagers (TriKEs), and bispecific constructs utilizing T cell receptor based moieties (137).

CAR-T cells employ *ex vivo* manipulation of autologous or allogeneic T cells engineered with T cell receptor (TCR) specificity to tumor antigens. This specificity allows CAR-T cells to bypass the MHC restriction required for endogenous anti-tumor T cell activity, and to recognize a specific tumor antigen, resulting in CAR-T cell expansion, direct cytotoxicity, and the potential for long-lasting memory responses (133, 138, 139). While first generation CAR-T cell therapy was limited in efficacy, the subsequent addition of co-stimulatory domains in second- and third-generation CAR-T constructs has resulted in impressive clinical responses in a variety of B-cell malignancies (140). CD19 targeting CAR-T cells have been the first targeted cellular therapy to demonstrate significant clinical impact, first in CLL, then B-ALL (138, 141), and ultimately gaining approval for clinical

use for refractory large B-cell lymphoma and B-ALL in children and young adults (142–144). There is certainly intense interest in adapting similar therapies to be used for myeloid malignancies, but for now, the optimal design of such therapeutics remains elusive.

The study of T cell anti-tumor biology in HSCT has further contributed to the investigations into the many regulatory molecules expressed on T cells. Immune checkpoint blockade has long been recognized to play a role in GvHD and GvL effects in animal models of bone marrow transplant. Studies in mice have shown an important role for cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and its homologous T cell co-stimulatory protein CD28 in mediating both GvL and GvHD effects after bone marrow transplantation (145, 146). Inhibition of CD28 was shown to effectively reduce GvHD mortality and augment anti-tumor T cell responses, and selective blockade of CTLA-4 enhanced the GvL effect in a model of delayed donor lymphocyte infusions while accelerating mortality due to GvHD. (146). Further, blockade of programmed death-1 (PD-1) was also shown to augment lethality due to GvHD (147). Despite the potential for checkpoint blockade to exacerbate GvHD suggested by these preclinical studies, several lines of evidence have also pointed to the potential beneficial role of checkpoint inhibition in hematologic malignancies. Murine leukemia cell lines demonstrate upregulated expression of PD-L1, the cognate ligand for PD-1, *in vivo*, and mice treated with anti-PD-L1 have enhanced anti-tumor T cell responses (148). Studies in a murine model of CML showed that disease-specific cytotoxic T cells with an exhausted phenotype upregulate PD-L1, leading to disease progression (149). Inhibition of PD-1/PD-L1 interaction reversed this phenotype and restored function of anti-tumor cytotoxic T cells.

Such promising preclinical work combined with the established role for PD-1 and CTLA-4 blockade across human cancer types has led to recent clinical investigations exploring the role of checkpoint inhibition in the treatment of relapsed hematologic malignancies after HSCT. In this setting, blockade of CTLA-4 with ipilimumab has been shown to result in objective disease response without development of significant GvHD or other treatment related toxicity (150). A subsequent phase I/Ib trial found that ipilimumab led to some durable responses of relapsed hematologic disease after allo-HSCT although with some immune-mediated toxicity and GvHD (151). Responses to ipilimumab treatment were associated with infiltration of cytotoxic $CD8^+$ T cells into the tumor microenvironment, reduced activity of regulatory T cells, and expansion of effector T cells in peripheral blood samples of patients. Optimal use of checkpoint blockade in hematologic malignancies before, during, and after stem cell transplant remains an area of active investigation, aided significantly by a basic understanding of the role of these pathways in mediating anti-tumor immune responses.

The immunological pressure exerted by the donor-derived immune system could also trigger immune evasion mechanisms ultimately leading to disease relapse. Several mechanisms of post-transplant immune escape have been described, including genomic loss of mismatched HLA after mismatched HSCT. In this setting, recurrent leukemic cells lose, through acquired uniparental disomy of chromosome 6p, the mismatched HLA alleles, therefore abrogating recognition by alloreactive donor T cells (152). Initially described in the haploidentical setting, where it accounts for a third of relapses (153), this mechanism has been documented

also for unrelated donor transplants (154–156). Non genomic mechanisms of immune evasion include, among others, transcriptional silencing of HLA class II expression and deregulation of costimulatory molecules (157, 158) or production of lactic acid (159). Intriguingly, metabolic reprogramming through the administration of sodium bicarbonate restored GvL activity, suggesting that metabolic fine-tuning of donor T cells may provide antileukemia benefit. Certainly, much remains to be explored in defining and honing the GvL effect of donor immune cells to prevent and treat relapsed disease.

HSCT as a springboard for the rational design of combinatorial immunotherapies

Allogeneic HSCT has extended the lives of innumerable hematologic malignancy patients worldwide, yet relapse of the original malignancy remains the most frequent cause of treatment failure and mortality (160). In efforts to prevent or treat recurrent disease, numerous therapeutic avenues have been explored with the aim of either directly modifying the tumor or indirectly altering the microenvironment to sensitize resistant disease to allogeneic immune elements. In many respects, allo-HSCT can be considered one of the first examples of effective combination immunotherapy – which by now is also a central tenet of cancer immunotherapy in the non-transplant setting – bringing together cytoreduction with coordinated humoral and cellular immunity. At the same time, this complex therapy also serves as an inviting launching point for additional interventions. Indeed, the post-transplant setting represents a versatile platform for immune intervention, with the allogeneic background offering several advantages for reinvigorating the anti-tumor immune response. A crucial aspect of this setting is the fact that the reconstituted donor T cells have not been exposed to the immune suppressive tumor microenvironment, nor to chemotherapy, thereby resulting in the possibility that they are more amenable to *in vivo* manipulation or *ex vivo* gene modification (161, 162). In addition, the T cell homeostatic cytokine milieu immediately after stem cell transplantation has been shown to provide favorable conditions for T cell expansion (163, 164).

With the remarkable expansion of the arsenal of mechanistically driven therapeutic options for hematologic malignancies, it has become evident that maintenance therapies might be crucial to sustain and boost the immunotherapeutic effects of allo-HSCT (165, 166). These can include the sequential administration of antigen-specific or whole tumor cell cancer vaccines (167–170), monoclonal antibodies (such as Inotuzumab (NCT03104491), Blinatumumab (171), or gemtuzumab ozogamycin (172, 173), cell-based therapeutics including but not limited to DLI (162, 174, 175) or additional use of targeted drugs. In the last two decades, drug development has taken huge steps forward, with many new molecules developed and marketed for the blood malignancies (176). Some of these have broader activity, such as hypomethylating agents (175, 177, 178) or venetoclax (179), while others possess a narrower scope, such as FLT3 inhibitors for AML (which notably displayed a potent synergistic effect with DLI in the treatment of post-HSCT relapse) (180–183), JAK2 inhibitors for myeloproliferative disorders (184), Tyrosine Kinase inhibitors (TKIs) for Philadelphia positive ALL (185) or Bruton Tyrosine Kinase inhibitors for CLL (186, 187), as a few examples. With the notable exception of TKIs for CML, none of the new drugs have been shown to fully eliminate the need for allo-HSCT and its immunotherapeutic effects, but rather improve bridging to transplantation for high-risk patients, and the optimal

timing of allo-HSCT in the setting on these novel agents remains the focus of ongoing clinical investigation.

A major challenge to the success of targeted therapies is development of resistance, as documented for most small molecules that have entered the clinical arena (188). Indeed, clonal heterogeneity and evolution render the tumor a moving target which might be difficult to combat with just one “weapon”, no matter how precise. In this respect, allo-HSCT could complement the selectivity of targeted drugs with the broader, though less precise, effects of polyclonal T cell alloreactivity, potentially improving disease control, as shown by the promising results of dual targeting of FLT3 mutated AML with sorafenib and DLI (180). This could represent a dual approach model for precision cancer medicine, laying the foundation for delineating which therapeutic agents might have the best synergistic effects when used in combination.

Conclusions

As the success of more sophisticated cellular therapies such as CAR-T cells continues to expand, one may wonder whether the genetically engineered precision of CAR-T therapy will one day obviate the need for the nonspecific alloreactivity of traditional allo-HSCT. Despite the unprecedented remission rate and controllable side effects, disease relapse after CAR-T cell therapy remains a considerable hurdle (189, 190). Early loss or exhaustion of CAR-T cells, selection of antigen-negative clones or downregulation of target expression, lineage switching of leukemia, and tumor microenvironment are all important factors now identified as contributing to relapse after CAR-T cell therapy (191). With this respect, CAR-T cell therapy could be envisioned as an effective and safe method to induce complete responses – especially in the challenging context of refractory disease – which could then be consolidated with allo-HSCT. Notably, several studies have affirmed that ALL patients receiving consolidative allo-HSCT have longer leukemia-free survival than those receiving CAR-T cell therapy alone (192–194). Whether the current surge of CAR-T therapy in other disease settings will herald the decline of the more than 60 years of allo-HSCT practice remains to be seen. Indeed, allo-HSCT and CAR-T are tightly intertwined, with lessons being constantly translated from one platform to the other, such as the management of cytokine release syndrome, the benefits of lymphodepletion, and the potential for “off-the-shelf” allogeneic universal CAR-T cells (195). Perhaps, the best testament to the potential for synergic use of CAR-T and HSCT comes from the AML setting, where researchers are trying to overcome the lack of suitable CAR targets by genetically engineering the allograft to remove a candidate surface antigen, such as CD33, from the normal hematopoietic system and transplanting this allograft in sequence with donor-derived CAR-T cells against CD33 (196). Although the coupling of CAR-T with next generation engineered allo-HSCT is still in the preclinical phase of development, it is incredibly exciting that we have reached a point where we can even envision such innovative combination strategies, and we like to think that E.D. Thomas would share our enthusiasm for the unrelenting progress of the HSCT field.

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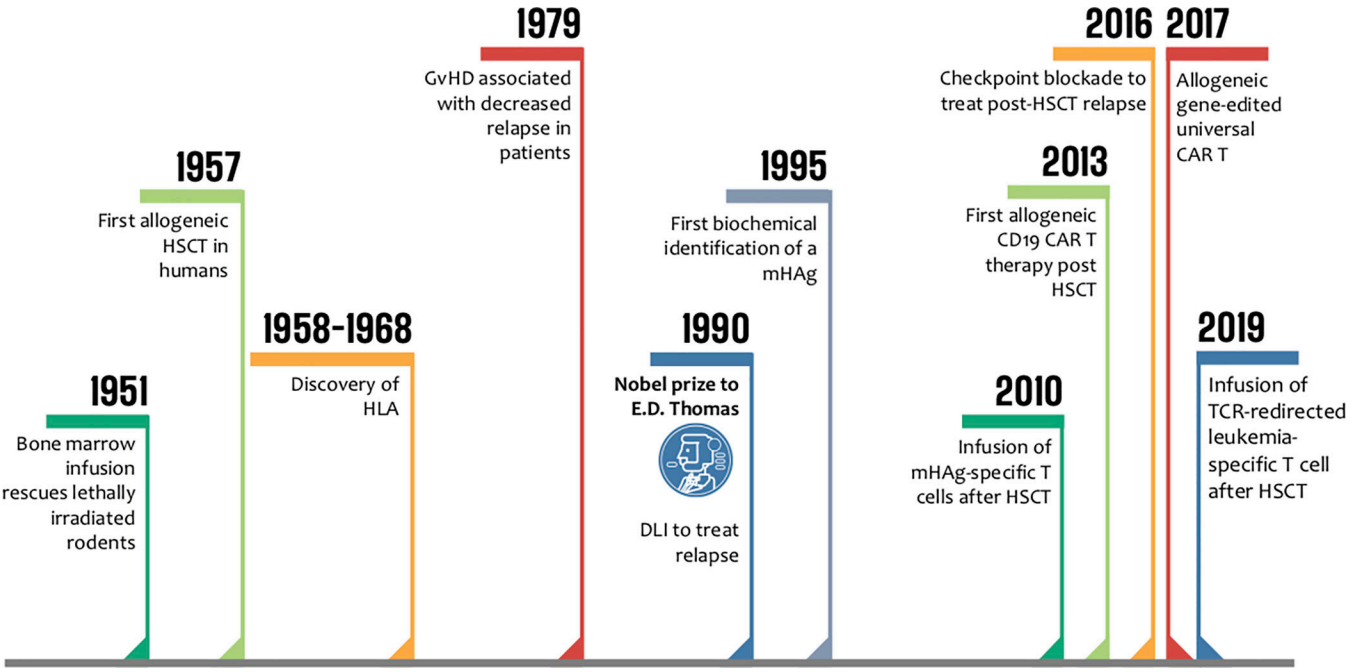

Figure 1: 60 years young: the history of allogeneic HSCT.

Timeline summarizing major milestones in the history of allo-HSCT. HLA: human leukocyte antigen; DLI: donor lymphocyte infusion; mHAg: minor histocompatibility antigen; CAR: chimeric antigen receptor; TCR: T cell receptor.

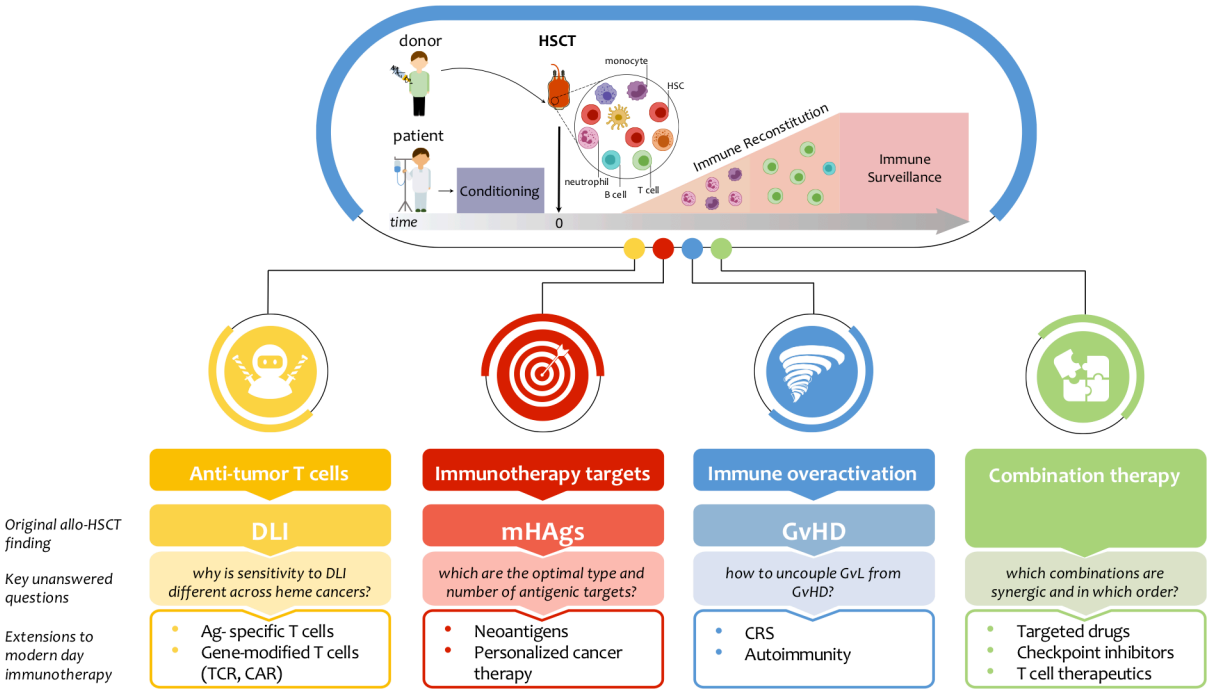

Figure 2: Fundamental lessons and fruits from allo-HSCT.

Top panel: allogeneic HSCT has been the first form of cancer immunotherapy to enter the clinical arena. Hematopoietic stem cells (HSCs) are collected from a donor, while the recipient undergoes conditioning therapy. Upon infusion into the patient, HSCs begin to proliferate and differentiate to repopulate the hematopoietic compartment in a process termed immune reconstitution. The donor-derived immune system is then responsible for long-term immune surveillance against disease recurrence. *Bottom panel*: allo-HSCT has profoundly influenced cancer immunotherapy in several aspects: for each of them, from top to bottom we report the original allo-HSCT finding, the key unanswered questions in the field and the subsequent extensions to modern day immunotherapy (bullet points). DLI: donor lymphocyte infusion; Ag: antigen; mHAg: minor histocompatibility antigen; GvL: graft vs. leukemia; GvHD: graft vs. host disease; CRS: cytokine release syndrome.