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Targeting minor histocompatibility antigens in graft versus tumor or graft versus leukemia responses

Xin Feng¹, Kwok Min Hui¹, Hashem M. Younes¹, and Anthony G. Brickner^{1,2}

¹ Department of Medicine and University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA

² Department of Immunology, University of Pittsburgh, Pittsburgh, PA 15213, USA

Abstract

Allogeneic hematopoietic cell transplantation (alloHCT) represents the only curative therapy for several hematologic malignancies, and shows promise as a nascent treatment modality for select solid tumors. Although the original goal of alloHCT was hematopoietic reconstitution after sub-lethal chemoradiotherapy, recognition of a profound donor lymphocyte-mediated graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect has shifted the paradigm from pre-transplant cytoreduction to tumor control via donor lymphocytes. In human leukocyte antigen (HLA)-compatible alloHCT, GVL and GVT reactions are induced primarily by donor T-cell recognition of minor histocompatibility antigens (mHAg). Here we review the literature regarding mHAg-specific T cells in GVL and GVT reactions, and discuss the prospects of exploiting mHAg as immunotherapeutic targets.

Minor histocompatibility antigens are key targets of GVT effects and GVHD

Allogeneic hematopoietic cell transplantation (alloHCT) is well established as a potentially curative therapy for individuals with hematologic malignancies [1]. Although the original goal of alloHCT was to reconstitute the hematopoietic compartment after sub-lethal chemoradiotherapy, subsequent recognition of a profound donor lymphocyte-mediated graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect has shifted the treatment paradigm of alloHCT from pre-transplant cytoreduction to tumor eradication and relapse prevention via donor lymphocytes. In the human leukocyte antigen (HLA)-compatible setting, the curative capacity of alloHCT results from a potent GVL or GVT effect mediated largely by mature donor CD4⁺ and CD8⁺ T cells normally resident within the stem cell graft. The GVL effect in hematologic malignancies is the most extensively studied and clinically validated example of immunologic anti-tumor responses after alloHCT [2]. The increased incidence of leukemic relapse observed after autologous, syngeneic (identical twin) or T-cell depleted alloHCT (reviewed in Ref. [1]), and the effectiveness of donor lymphocyte infusion (DLI) in inducing remissions of hematologic malignancies after post-alloHCT relapse [3–6] provide compelling evidence for an immune-mediated allogeneic GVL effect driven chiefly by donor T cells. These observations also illustrate that the potential advantage of alloHCT over syngeneic or autologous immunotherapy is the ability to exploit immunological non-identity (histoincompatibility) for the induction of allogeneic T cells; that is, the barrier of self-tolerance to non-mutated tumor antigens and differentiation antigens [7,8] inherent during the autologous response is completely eliminated during alloHCT [9]. Furthermore, the eligible alloHCT patient population has expanded considerably with the advent of reduced-intensity pre-transplant conditioning, which is designed to prevent rejection and facilitate donor stem cell

engraftment, while minimizing toxicity and damage to normal host tissues. Rather than directly reducing tumor burden, reduced intensity conditioning relies extensively on GVT effects to induce durable complete remissions [10,11]. The expansion of the eligible alloHCT patient population might also continue with the emergence of alloHCT as a potential treatment modality for select solid tumors [12,13]. Unfortunately, the advantages conferred by alloHCT are countered by the considerable morbidity and mortality of graft-versus-host disease (GVHD), which remains a crucial obstacle in alloHCT [14] (Box 1).

Major histocompatibility complex (MHC) molecules (HLA in humans) bind and present potentially antigenic peptides to T-cell receptors (TCRs) that distinguish immunological self from non-self. In alloHCT, if patient and donor HLA molecules differ, the principal antigenic targets of the T cells of the donor are the host HLA or ‘major’ histocompatibility antigens, and GVHD onset and severity is typically dramatic [1]. But even if donor and recipient are HLA-identical siblings, their repertoire of HLA-presented peptides differs because of allelic polymorphisms in the genome, often single nucleotide polymorphisms (SNPs), which result in amino acid sequence differences or differences in the expression of normal cellular proteins [2]. These polymorphisms can result in the processing of immunogenic peptides, termed minor histocompatibility antigens (mHAg), which can be presented by MHC molecules. In MHC-matched alloHCT, when an mHAg is presented on the cells of the recipient but not on the cells of the donor, CD8⁺ or CD4⁺ donor T cells can recognize the mHAg epitope as a foreign antigen and induce donor-anti-host GVT and GVHD reactions [2,15]. This occurs when the recipient is homozygous or heterozygous for the immunogenic mHAg-encoding (i.e. mHAg-positive) allele, and the donor is homozygous for the alternate non-immunogenic (i.e. mHAg-negative) allele and, therefore, not tolerant to the mHAg (Figure 1). mHAg-specific alloreactivity is, thus, the combined effect of recognition by numerous mature donor T cells of different mHAg as foreign antigens on normal and malignant recipient cells. CD8⁺ cytotoxic T lymphocytes (CTL) recognize mHAg presented by class I MHC molecules and directly lyse target cells via perforin or granzyme secretion and the TRAIL cytotoxic pathway [16]; they also produce inflammatory cytokines that can be tumoricidal and recruit additional effector cells (reviewed in Refs [17,18]). CD4⁺ T cells recognize mHAg presented by class II molecules and can directly lyse targets via perforin [19] and Fas-FasL interactions, license antigen presenting cells (APC) via CD40-CD40L interactions and produce cytokines that orchestrate immune responses [18].

Donor-derived effector cells that mediate GVL in HLA-matched alloHCT include T cells specific for tumor-associated antigens or mHAg that are expressed on normal or leukemic hematopoietic cells of the recipient [7]. However, because donor T cells recognize some mHAg that are presented on both the non-hematopoietic cells of the recipient and on normal and malignant hematopoietic cells, GVL is strongly associated with GVHD [4]. Despite this strong association, GVL or GVT can occur in the absence of GVHD [1,2,4,20], indicating that some antigens that are recognized by T cells on malignant or normal hematopoietic cells are not presented by cell types that are GVHD targets. This concept and its potential clinical implications are discussed in further detail in the following section.

All mHAg arise as a consequence of normal genetic variation between individuals, but there are diverse mechanisms by which they are generated and become immunogenic when donor and recipient are genetically discordant [21]. The observed mHAg-generating allelic variations have ranged from a homozygous gene deletion in the donor [22] to single nucleotide polymorphisms, and have resulted in differences in mRNA splicing [23], protein translation [24,25], proteasomal cleavage [26] or protein splicing [27], peptide transport [28], MHC binding [29,30] and T-cell receptor contact [31,32]. One or more of these mechanisms renders the antigenicity of the mHAg and hence the tendency of it to be recognized by the donor immune system. As described earlier, most autosomal mHAg have a corresponding

immunogenic and non-immunogenic allele. In some instances, however, both of the mHAg allelic peptides are capable of being generated, presented by MHC and recognized as immunogenic by T cells of the discordant allelic genotype. In humans, this bi-directional or reciprocal allelic mHAg recognition is exemplified by the mHAg HB-1, in which either allelic peptide is immunogenic to the T cells of a discordantly homozygous individual [33].

Although it is well-accepted that T cells are primary mediators of both GVL and GVHD, recent studies in the setting of sex-mismatched alloHCT with male recipients and female donors have shown that antibodies are also capable of recognizing the proteins from which the human Y chromosome-encoded (H-Y) mHAgs are derived [34]. These antibodies can distinguish between (male) recipient and (female) donor derived peptides in soluble form [35], and the presence of H-Y protein-specific antibodies has been associated with chronic GVHD [34]. The human H-Y mHAg DBY has been shown to elicit a coordinated B- and T-cell response after alloHCT [35]. The role of antibodies specific for autosomal mHAg-generating proteins and soluble peptides in GVHD or GVT effects is presently unknown but highly plausible, given the frequency and profundity of normal structural genetic variation (e.g. copy number variation, gene deletion, protein truncation or frame shift, etc.) between any two individuals, even siblings [21,36–38].

Box 1. GVHD: description, pathophysiology and control

GVHD is a potentially life-threatening immune attack against the tissues of an alloHCT recipient by mature donor T cells contained within the graft. The manifestations of GVHD vary over its course, and clinical GVHD has an acute form and a chronic form.

- **Acute GVHD** typically appears within several weeks after alloHCT and is characterized by damage to the skin, gastrointestinal tract mucosa and liver. It is responsible for 15%–40% of mortality and is the major cause of morbidity after alloHCT. In MHC-mismatched alloHCT the incidence of acute GVHD is directly related to the degree of MHC mismatch. In HLA-identical alloHCT, despite HLA identity between patient and donor, 40% of patients develop acute GVHD because of in large part to mHAg disparities.
- **Chronic GVHD** is defined as GVHD that occurs after day 100 post-transplantation and is histopathologically and clinically distinct from acute GVHD. Chronic GVHD resembles a chronic autoimmune disorder and can have multiple manifestations.

The pathophysiology of acute GVHD

Although GVHD does not occur in a step-wise and sequential manner, the development and evolution of acute GVHD can be conceptualized in three sequential phases:

- APCs of donor and host origin become activated. This occurs when host tissues damaged from alloHCT conditioning regimens (i.e. irradiation and chemotherapy) respond by secretion of a proinflammatory ‘cytokine storm’, which in turn increases expression of costimulatory molecules, adhesion molecules, MHC and chemokine gradients that act as ‘danger signals’ to activate APCs.
- Infused donor T cells interact with, and are activated by, the primed APCs, inducing the T cells to proliferate, differentiate and migrate out of lymphoid tissues and traffic to target organs and cause tissue damage.
- A complex cascade of cellular effectors (e.g. CD8⁺ CTL and CD4⁺ T cells), inflammatory cytokines and chemokines lead to the damage of GVHD target organs.

GVHD prophylaxis and immunosuppression

Immunosuppression administered early after alloHCT represents the standard of care for GVHD prophylaxis. Despite prophylaxis, ~50% of alloHCT patients develop severe acute GVHD, and require additional immunosuppression. Intensive immunosuppressive regimens and/or T-cell depletion can dramatically reduce the incidence and severity of GVHD, but this comes at the expense of increased incidence of fatal post-transplant infections and tumor relapse.

Characteristics of mHAg potentially applicable to immunotherapy via GVL and GVT

The ultimate goal of alloHCT-based immunotherapy is to maximize the GVL or GVT response while mitigating collateral damage to normal tissues by GVHD; therefore, dissecting the role of mHAg in the elicitation of these effects has been a key impetus for research. A first indication that mHAg could be used as immunotherapeutic targets in alloHCT was the observation that CTL specific for several different mHAg can differentially recognize various cell types; that is, mHAg can be presented preferentially on hematopoietic cells, or more broadly on non-hematopoietic cell types [39,40]. Although ubiquitously expressed mHAg are presented on leukemic cells and can mediate anti-leukemic activity, for the most part it is believed that targeting ubiquitous mHAg will not promote separation of GVHD from GVL. This suggests that mHAg presented preferentially on normal or malignant hematopoietic cells could potentially serve as immunotherapeutic targets to enhance GVL activity with absent or minimal GVHD.

In support of this assumption, several ubiquitously or broadly expressed human mHAg have been associated with GVHD [22,41–43]; conversely, T cells specific for individual hematopoietic cell-restricted mHAg have been isolated from blood samples after alloHCT and have exhibited *in vitro* and *in vivo* anti-leukemic activity with minimal recognition of non-hematopoietic cells [40,44–47] (Table 1). Lending further credence to this paradigm, an *ex vivo in situ* skin explant assay model was reported in which incubation with human CTLs specific for the ubiquitously expressed male mHAg H-Y mediated severe histological graft-versus-host (GVH) reactions, whereas CTLs specific for the hematopoietic system-selective human mHAg HA-1 and HA-2 exhibited either no or very mild tissue injury [48]. Although there are conflicting data concerning a role for HA-1 in GVHD [43,49,50], evidence is accumulating that both HA-1 and HA-2 are targets for a GVL effect after alloHCT. One study found that HA-1 positive recipients with an HA-1-negative donor had a lower incidence of leukemic relapse than HA-1 compatible pairs [51]. Additional evidence has come from the analysis of patients who received DLI to treat post-transplant leukemic relapse. Longitudinal analyses have shown an association of the emergence or dramatic expansion of functional hematopoietic mHAg-specific CD8⁺ T cells in peripheral blood following DLI (as detectable by tetramers) concomitant with the decline or disappearance of leukemic cells [25,32,46,47, 52]. Preclinical studies of the efficacy of human mHAg-specific CTL clones in eradicating leukemia have been performed in immunodeficient mice models, and have demonstrated GVL effects via the recognition of some hematopoietic mHAg on leukemic stem cells [45,53]. Taken together, these data strongly indicate that donor T cells specific for hematopoietic-restricted mHAg on recipient cells can be involved in the induction and/or maintenance of remission of hematologic malignancies after alloHCT. Thus, the primary goal of current mHAg discovery and characterization efforts is to ultimately dissect GVT effects from GVHD via mHAg-targeted immunotherapy.

In murine models of mHAg immunotherapy, the adoptive transfer of CTLs specific for a single immunodominant mHAg that is ubiquitously expressed, H7^a, can eradicate mHAg-positive implanted malignant murine hematopoietic cell lines [54] or murine melanoma cell lines (as an established tumor) [55] without GVHD. The observation in this model that GVHD occurred only when mHAg-specific CTL were co-infused with naïve T cells indicates that precursor T cells reactive to other host mHAg were activated through ‘epitope spreading’ via antigens leaked from the cells initially targeted by the infused CTL [54]. Although these data provide insight into potentially segregating GVHD from GVT, their extrapolation to human alloHCT must be tempered with caution because humans are a highly outbred population and multiple mHAg disparities exist even between HLA-identical siblings.

The prospect of utilizing mHAg tissue distribution or immunodominance as a basis for segregating GVL from GVHD, although compelling, is oversimplified, and there are important caveats to consider when drawing conclusions from the earlier-mentioned studies. Numerous variables can strongly influence GVHD or GVT reactions and sway the dominance of CD4⁺ or CD8⁺ effector cells in GVHD or GVT. These include, but are not limited to, the conditioning regimen, immunological disparity between donor and recipient from genetic loci outside the MHC and mHAg, the influence of regulatory T (Treg) cells, age of donors and recipients, graft tissue source and number of donor cells infused (reviewed in Ref. [56]). Indeed, CD4⁺CD25⁺ Treg cells have potent suppressive activity *in vitro* and *in vivo* on immune effector cells reactive to host antigens (reviewed in Ref. [57]) and have been shown in mice to suppress acute GVHD while retaining GVL against a lymphoid malignancy [58]. Depletion of CD4⁺CD25⁺ Treg cells from the graft or in the recipient immediately after alloHCT promotes acute and chronic GVHD in various mouse studies [59,60].

At present, fourteen proteins have been identified which generate mHAg that are selectively presented on hematopoietic cells (Table 1). Interestingly, several of the genes encoding these mHAg exhibit expression that, although hematopoietic cell type-specific or preferential, is profoundly up-regulated within certain hematopoietic lineages, subtypes of malignancies or states of differentiation, maturation, proliferation or activation [24,31,61]. mHAg of this category could perhaps be exploited in alloHCT-based immunotherapeutic strategies targeting very specific subsets of hematologic malignancies, for example, acute lymphocytic leukemia [31], multiple myeloma [61], chronic lymphocytic leukemia and subsets of non-Hodgkins lymphoma [24] while minimizing the extent of collateral damage to non-cancerous hematopoietic cells. Additionally, several of the Y chromosome-encoded HY mHAg exhibit hematopoietic cell type-specific recognition by their cognate CTL, although at the level of mRNA their expression is ubiquitous [62–65]. Finally, because several of the tissue-restricted mHAg in Table 1 also exhibit high-level expression in and presentation on select solid malignancies, it will be important to assess whether a known or novel mHAg is expressed aberrantly in various solid tumors, and whether T-cell responses to these mHAg are associated with documented tumor regression after alloHCT. Interestingly, CTL specific for HA-1 and other mHAg (e.g. HA-3, HA-8) [66], including a novel mHAg broadly presented on normal tissues and several tumor cell lines [67], have been isolated from renal cell carcinoma patients who showed partial tumor responses or stable disease after alloHCT, further supporting a role for mHAg in GVT effects.

Although the human mHAg previously mentioned represent potentially promising immunotherapeutic targets for a variety of hematopoietic and solid malignancies, several caveats must be considered. First, the anti-mHAg TCR specificities and cognate patterns of mHAg tissue distribution (using T-cell reactivity as a readout) reported thus far have, in most cases, been generated *in vitro* using only a single mHAg-specific T-cell clone with a certain avidity to the relevant mHAg. It is thus difficult to assess whether the ignorance of non-hematopoietic cell types demonstrated by the TCR of a given T-cell clone specific for a tissue-

restricted mHAg can also be extrapolated to all other TCRs specific for the relevant mHAg. Additionally, it is presently unclear whether tissue-restricted mHAgS will be expressed at high levels in the parenchymal tissues affected by GVHD under certain conditions *in vivo* (e.g. variability in pre-transplant conditioning regimens, degree of inflammatory ‘cytokine storm’, post-transplant immunosuppression, etc). A strategy of selecting T-cell clones solely on the basis of *in vitro* assays will, therefore, not be sufficient for ensuring safety in each and every patient [68].

Several key characteristics of the T-cell response to mHAgS might contribute to its anti-tumor efficacy. MHAgS, as alloantigens, are strongly immunogenic, which is reflected by the fact that donor T cells cause GVHD and mediate GVT despite the administration of immunosuppressive drugs to block alloreactivity. Additionally, the T-cell response to mHAgS after alloHCT involves both CD8⁺ and CD4⁺ T-cell subsets. Although the available experimental data are strongly skewed towards CD8⁺ T cell-mediated GVT based on the dominant role of this effector population in most mouse GVT models, CD4⁺ T cells can directly mediate GVT [69–71], and can also participate in the GVT effect by augmenting and sustaining CD8⁺ T-cell responses. This has been demonstrated for mHAg-specific CTL and helper T-cell clones [62]. Finally, the T-cell response to mHAgS can be directed to multiple determinants. This provides a potentially broader spectrum of antigen recognition with which to prevent the outgrowth of tumor cells that have lost or reduced levels of antigen expression, and is more effective than immunotherapy that targets only a single determinant [72]. It is possible, however, that in humans there might exist a hierarchy of mHAg immunodominance, such that some mHAgS will be more powerful in eliciting immune responses than others, as has been demonstrated in congenic mice. The most crucial challenge for alloHCT is to take advantage of these attributes, but design approaches that will permit separation of GVHD from the GVT or GVL effect or abrogation of GVHD after tumor eradication is complete [2].

Possibilities for mHAg-specific immunotherapy of cancer

Currently, several potential avenues exist for the therapeutic exploitation of mHAgS in alloHCT to elicit GVL or GVT effects [15]. These include: adoptive cellular immunotherapy with mHAg-specific T cells; mHAg peptide, protein, mRNA or DNA vaccination (including vaccination with antigen-loaded or mRNA-transfected dendritic cells); selective immunodepletion of GVHD-instigating T cells; and mHAg-specific TCR gene transfer. Although adoptive cellular therapy with mHAg-specific T cells is presently feasible, the procedure is laborious and expensive, and the process of culturing T cells can potentially alter their effector capability or their capacity to survive *in vivo* [73]. Cloned mHAg-specific CTL have induced responses, although the apparent lack of persistence of these lines *in vivo* has limited their efficacy [68]. Amelioration of GVHD by the selective depletion of CD4⁺ or CD8⁺ T cells alloreactive specifically with mHAgS expressed primarily on epithelial cells [74,75] might represent another approach to augment the latter strategy, but is limited by the paucity of knowledge regarding human mHAgS associated with GVHD. The transfer of mHAg-specific TCRs circumvents the requirement for donor or recipient mHAg allelic disparity, but has been employed only in the experimental phase in preclinical models [76,77]. Presently, perhaps the most feasible and efficient approach is vaccination of post-alloHCT patients with defined mHAg peptides in addition to longer mHAg polypeptides or whole proteins, which could be pulsed onto dendritic cells (DC) to induce both CD8⁺ and CD4⁺ T-cell responses. Although vaccination approaches have been tested quite extensively in solid tumors, particularly in the case of melanoma-associated tumor antigens, tolerance and autoimmunity still present imposing obstacles to the success of self-antigen based tumor immunotherapy in the endogenous or autologous setting (reviewed in Ref. [78]). By contrast, because mHAgS are alloantigens and immunogenicity is based on mHAg histoincompatibility, tolerance is circumvented. Investigation of more effective adjuvants (e.g. DC and cytokines), forms of

antigens (e.g. peptides, whole protein, mRNA and DNA), and routes of vaccination administration (e.g. intra-nodal, intra-tumor and subcutaneous, etc.) might improve anti-tumor effects in alloHCT.

GVT-associated mHAgS could be used to vaccinate either the selected mHAg alloHCT donor or to boost the recipient post-alloHCT or post-DLI. Vaccination of a healthy donor, unless performed *ex vivo* using donor DC, would involve obvious ethical issues, therefore, the administration of mHAg peptides, proteins or mRNA to the patient currently represents the most feasible method of vaccination. This could be accomplished by administration to the patient of the patient's self-mHAgS after alloHCT, with or without mHAg-pulsed donor-derived DC. The mHAg-specific immune response of T cells already primed by recipient-derived antigen presenting cells (APCs) might then be boosted [15].

Despite the inclusion of the known GVL or GVT-biased mHAgS in the potential arsenal for mHAg-targeted immunotherapy, the number of patients that could be treated (based on the frequency with which any given donor and recipient pair will be genetically disparate in the appropriate donor-anti-recipient direction) presently remains quite low because of the phenotypic frequencies of the mHAgS and their cognate HLA restriction molecules. The paucity of molecularly identified mHAgS seriously limits their current clinical potential. There is thus a clear need to enlarge the pool of GVT-relevant mHAgS with common HLA restriction and with allelic frequencies conducive to frequent donor-recipient disparity. To meet this need, the pace of human mHAg discovery and characterization must be accelerated via novel high-throughput antigen identification methodologies that will complement the existing antigen identification techniques (Box 2).

Clinical trials utilizing mHAgS have thus far suffered from the limitation of small patient numbers because of the challenges described earlier. However, several phase I clinical trials utilizing adoptive therapy with mHAg-specific T cells post-alloHCT are presently in progress or planned, as are the first phase I and II mHAg peptide vaccination trials, although the results are, as yet, unpublished.

Box 2. 'Traditional' and nascent mHAg identification techniques

'Traditional' techniques for human mHAg identification:

- Direct antigen identification: peptides are eluted from purified class I molecules of a mHAg-positive cell line, fractionated by high-performance liquid chromatography, then exogenously pulsed on mHAg-negative (e.g. donor) target cells to assay for CTL cytotoxicity. Active fractions are subjected to further fractionation, and candidate peptides are analyzed and sequenced via mass spectrometry, then tested for their immunogenicity [92].
- cDNA expression cloning: a cDNA library is constructed from a mHAg-positive cell line, then co-transfected with the cDNA of the relevant HLA molecule into mHAg-negative cells (e.g. COS-1 cells). Cells are transfected with pools of the cDNA library then assessed for their ability to stimulate cytokine secretion by a mHAg-specific CTL. The process is repeated with individual cDNAs from biologically active pools, positive cDNAs are sequenced and peptides encoded by these cDNAs are evaluated for their immunogenicity [31].
- Genetic linkage analysis: transformed B-cell lines derived from individuals in large multi-generation pedigrees from the Centre d'Etude du Polymorphisme Humain reference family collection have been densely mapped for genetic markers suitable for linkage analysis. CTL cytotoxicity (phenotype) for a given mHAg can be

analyzed in cell lines from multiple families, correlated statistically with marker loci and validated via transfection of candidate DNA sequences [93].

- Exogenous transfer of mHAg-specific genes into mHAg-negative cells via retroviral transduction: this methodology was used to identify the first HLA class II-restricted human mHAg, an HLA-DQ5-restricted peptide derived from the DBY protein, via trans-duction of an HLA-DQ5⁺ female B-cell line with a set of Y-specific genes [86].

Nascent techniques for the identification of human mHAg:

- The delivery of exogenous antigens (from recombinant bacteria cDNA expression libraries) using endogenous HLA class II molecules: this method has recently been employed for the identification of the first human autosomal class II mHAg [84]. Class II mHAg have historically proven particularly recalcitrant to identification.
- Reverse immunology' approaches: *in silico* mHAg prediction that evaluates peptide sequences encoded by known non-synonymous SNPs for their ability to bind particular HLA molecules and be appropriately handled by the antigen processing machinery [94].
- Phenotype-genotype correlation studies: high-density genotyping (genome-wide analysis) arrays of human SNPs and copy number variation polymorphisms are available, permitting a genotype-phenotype utilizing large panels or pools of genomic DNA from CTL-phenotyped mHAg-positive versus mHAg-negative cells [95]. Such strategies might be augmented by analysis of HapMap data in conjunction with genetic linkage analysis.

Conclusions

Toxicity, particularly GVHD, and donor availability remain formidable impediments to alloHCT, and much remains to be discovered about the factors governing the susceptibility of specific hematologic malignancies and particularly solid tumors to GVT. Despite these limitations and the advent of powerful novel modalities such as the tyrosine kinase inhibitor imatinib, the alkylating agent bendamustine and the antibodies rituximab and alemtuzumab for the treatment of hematologic malignancies, alloHCT remains the sole therapy offering cure to patients with advanced hematologic malignancies. The eligible patient population has expanded considerably via the introduction of reduced-intensity conditioning regimens, which rely extensively on GVT effects to induce durable complete remissions and have permitted alloHCT to be used in older patient populations. Given the prominent role that mHAg have in GVL and GVT effects, it is inevitable that mHAg-mediated immunotherapy will have a growing role in the control of cancer, and for several malignancies might tip the balance from palliative to curative therapy.

The incremental translational progress towards clinical trials for the evaluation of mHAg-based immunotherapy has been partly because of the limited number of potentially therapeutic mHAg identified to date (and in turn because of the difficulty in identifying mHAg) and also because of the relatively small number of patients receiving alloHCT. Further efforts and innovative techniques are needed to accelerate the discovery of new mHAg epitopes to enable immunotherapeutic coverage of the majority of patients. After establishment of the safety of any mHAg-based immunotherapy, preventive immunotherapy can be utilized for patients with high risk of relapse post-alloHCT, via mHAg vaccination strategies or adoptive transfer of mHAg-specific T cells at appropriate times post-transplant. Immunotherapy targeted to mHAg selectively expressed in hematopoietic cells or solid tumors, combined with safer alloHCT through reduced-intensity conditioning, holds great promise as a new treatment

modality for high-risk patients. Additionally, a more thorough understanding of the molecular targets and mechanisms of the anti-mHAg response should augment the development of more effective approaches to induce autologous tumor immunity. The net result should be a profound positive impact in clinical outcomes, not only for traditional alloHCT patients but also those of older age, with advanced hematologic malignancies, and those with select solid tumors susceptible to GVT effects.

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Figure 1.

Induction of graft-versus host reactivity, including GVL and GVT effects. In order for donor-anti-recipient T-cell ($CD4^+$ or $CD8^+$) effector activity to occur during alloHCT in response to a given mHAg, recipient cells must express the polymorphic allele which encodes the mHAg peptide, whereas donor cells (indicated in blue) must not express the allele that encodes the mHAg. Thus, to elicit GVL or GVT reactivity, the recipient must be genetically homozygous or heterozygous for the mHAg-encoding allele (i.e. mHAg-positive), and the donor must be homozygous for the alternate polymorphic allele (or lack it altogether), which does not encode the mHAg. Frequently, the mHAg allelic disparity between donor and recipient is the result of a single nucleotide polymorphism, which can in turn result in the substitution of a single amino acid residue in an MHC-bound peptide, as represented by a single red circle within a blue peptide epitope. Upon recognition by the donor T-cell receptor of a genetically disparate 'non-self' mHAg peptide-MHC complex, the donor CTL can eradicate the mHAg-positive recipient cell via cytolytic activity. Donor-derived normal hematopoietic cells which reconstitute the graft after alloHCT are spared from donor T-cell mediated cytotoxicity because they are immunogenetically 'self' and do not express mHAg epitopes.

Table 1

Presently identified human mHAg epitopes

mHAg	Gene/chromosome	HLA restriction	Peptide sequence	Tissue distribution	Refs
HA-1	<i>HMHA1</i> /19p13	A*0201 B60 A*0206	VLHDDLLEA KECVLHDDL VLHDDLLEA	Hematopoietic, select solid tumors	[29] [79] [80]
HA-2	<i>MYOG1</i> /7p13-p11.2	A*0201	YIGEVLVS $\bar{V}$	Hematopoietic	[81]
HA-3	<i>AKAP13</i> /15q24-25	A1	VTEPGTAQY	Ubiquitous	[26]
HA-8	<i>KIAA0020</i> /9p24.2	A*0201	RTLDKVLEV	Ubiquitous	[28]
HB-1	<i>HB-1</i> /5q32	B44	EEKRGS $\bar{L}$ HW EEKRGS $\bar{L}$ YVW	Hematopoietic (B-ALL and EBV-BLCL)	[31] [33]
ADIR	<i>TOR3A</i> /1q25.2	A*0201	SVAPALALEPA	Ubiquitous, but highly expressed in activated hematopoietic cells including myeloma; select solid tumors	[61]
BCL2A1	<i>BCL2A1</i> /15q24.3	A24 B*4403	DYLYV $\bar{L}$ QI KEFEDD $\bar{I}$ INW	Hematopoietic, select solid tumors	[82]
C19orf48	<i>C19orf48</i> /19q13	A*0201	CIPPD $\bar{S}$ LLFPA	Ubiquitous, select solid tumors	[67]
CTSH	<i>CTSH</i> /15q24-25	A*3101 A*3303	ATLPLLCAR WATPLLCAR	Ubiquitous	[83]
ECGF1	<i>ECGF1</i> /2q13.33	B*0702	RPHAIRRPLAL	Hematopoietic, select solid tumors	[32]
HMSD	<i>HMSD</i> /18q21.33	B44	MEIFEVFSHF	Hematopoietic	[23]
LB-P14K2B-1S	<i>P14K2B</i> /4p15.2	DQB1B*0603	SRSSSAELDRSR	Ubiquitous protein expression, but hematopoietic- restricted T-cell recognition	[84]
LRH-1	<i>P2RX5</i> /17p13.2	B*0702	TPNQ $\bar{R}$ QNV	Hematopoietic (lymphoid)	[25]
PANE1	<i>CENPM</i> /22q13.2	A3	RVVDLPGVLK	Hematopoietic (resting B cells, B-CLL, NHL subtypes)	[24]
SP110	<i>SP110</i> /2q37.1	A3	SLPRGTSTPK	Hematopoietic	[27]
UGT2B17	<i>UGT2B17</i> /4q13	A*2902, B44	AELLNIPLY	Ubiquitous	[22,85]
DBY	<i>DDX3Y</i> /Yq11	DQ5 DRB1*1501 B*2705	HIE $\bar{N}$ FSDIDMGE ASTASKGRYIPPHLRNKEA SRDSRGKPGY	Hematopoietic	[86] [35] [87]
DFFRY	<i>USP9Y</i> /Yq11.2	A*0101	IVDCLTEMY	Ubiquitous	[88]
RPS4Y	<i>RPS4Y1</i> /Xp11.3	DRB3*0301 B*5201	VJKVNDTVQI TIRYPDPVI	Ubiquitous protein expression, but hematopoietic- restricted CTL recognition	[62] [63]
SMCY	<i>JARID1D</i> /Yq11	B*0702	SPSVDKARAE $\bar{L}$	Ubiquitous	[89]
TMSB4Y	<i>TMSB4Y</i> /Yq11.22	A*0201 A*3303	FIDS $\bar{Y}$ ICQV EVLRLPGLHFR	Ubiquitous	[90] [91]
UTY	<i>UTY</i> /Yq11	B8	LPHNHTDL	Ubiquitous protein expression, but hematopoietic- restricted CTL recognition	[64]

mHA _g	Gene/chromosome	HLA restriction	Peptide sequence	Tissue distribution	Refs
		B60	<u>RESEEE</u> <u>SY</u> <u>SL</u>		[65]

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; B-CLL, B-cell chronic lymphocytic leukemia; EBV-BLCL, Epstein-Barr virus-transformed B cells; NHL, non-Hodgkins lymphoma.