

## REVIEW

# Exploiting T cells specific for human minor histocompatibility antigens for therapy of leukemia

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Minor histocompatibility (H) antigens are major targets of a graft-versus-leukemia (GVL) effect mediated by donor CD8<sup>+</sup> and CD4<sup>+</sup> T cells following allogeneic hematopoietic cell transplantation (HCT) between human leukocyte antigen identical individuals. In the 15 years since the first molecular characterization of human minor H antigens, significant strides in minor H antigen discovery have been made as a consequence of advances in cellular, genetic and molecular techniques. Much has been learned about the mechanisms of minor H antigen immunogenicity, their expression on normal and malignant cells, and their role in GVL responses. T cells specific for minor H antigens expressed on leukemic cells, including leukemic stem cells, can be isolated and expanded *in vitro* and infused into allogeneic HCT recipients to augment the GVL effect to prevent and treat relapse. The first report of the adoptive transfer of minor H antigen-specific T-cell clones to patients with leukemic relapse in 2010 illustrates the potential for the manipulation of alloreactivity for therapeutic benefit. This review describes the recent developments in T-cell recognition of human minor H antigens, and efforts to translate these discoveries to reduce leukemia relapse after allogeneic HCT.

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Allogeneic hematopoietic stem cell transplantation (HCT) is the only potentially curative therapy for many patients with high-risk or recurrent acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). The success of the procedure is now understood to be due in large part to a graft-versus-leukemia (GVL) effect mediated by donor immune cells.<sup>1–7</sup> However, leukemia recurs following HCT in many patients, and strategies to augment the GVL effect to prevent and treat leukemic relapse continue to be a focus of research. Immune effector cells that have been implicated in the GVL response include CD8<sup>+</sup> and CD4<sup>+</sup> T cells, and natural killer (NK) cells, each of which may contribute more or less to the GVL effect depending on the type of HCT. After T-cell-depleted haplo-identical HCT, donor NK cells can mediate a potent GVL effect against AML in the small subset of recipients who do not express donor class I human leukocyte antigen (HLA) molecules required to engage donor NK-inhibitory receptors.<sup>8–12</sup> After haploidentical HCT in which small numbers of donor T cells are administered to the patient, T cells that recognize major HLA differences participate in the GVL effect.<sup>13</sup> In allogeneic HLA-identical HCT either from related or unrelated donors, the GVL effect is thought to be mediated primarily by T cells that recognize recipient minor histocompatibility (H) antigens, which are distinct HLA-binding peptides encoded by polymorphic genes that differ between the donor and recipient. Specifically, an HLA-matched individual who is homozygous for the minor H antigen ‘negative’

allele may have T cells in their repertoire that recognize recipient cells that are homozygous or heterozygous for a polymorphism that encodes a minor H antigen. A key feature of minor H antigen recognition is that the tissue expression of the antigen(s) determine whether the alloreactive T-cell responses that develop after HCT cause graft-versus-host disease (GVHD), in which cells in the skin, gastrointestinal tract and other tissues are targeted, a GVL effect, or both. This review will focus on recent developments in T-cell recognition of human minor H antigens, and efforts to translate these discoveries to reduce leukemia relapse after allogeneic HCT.

## THE BASIS FOR IMMUNOGENICITY OF MINOR H ANTIGENS

Minor H antigens are by definition non-self peptides; therefore, the donor T-cell repertoire is not subject to tolerance mechanisms that limit self-reactivity, and as a result the T-cell responses that are elicited to these determinants are typically of high avidity (Figure 1).<sup>2–7</sup> Most commonly, immunogenicity originates from one or more nucleotide polymorphisms in the coding sequences of homologous donor and recipient genes that alter peptide-major histocompatibility complex (MHC) binding and/or T-cell receptor (TCR) recognition of the peptide–MHC complex. There are at least 90 000 non-synonymous single-nucleotide polymorphisms (SNPs) in the human genome,<sup>14</sup> providing a potentially large number of minor H antigens, based on minor alterations in protein sequence. Copy number variation,

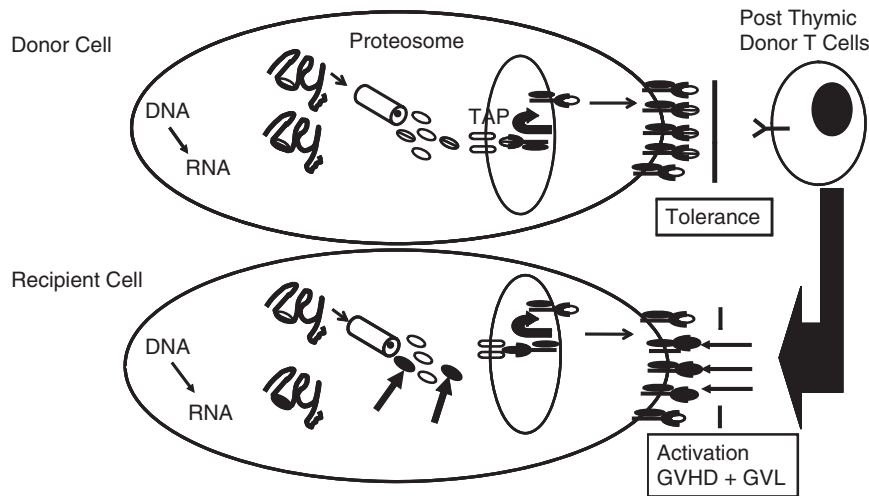
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**Figure 1** The cartoon illustrates the pathways of generation and presentation of minor H antigens, and the recognition of minor H antigens by donor T cells during allogeneic HCT. Minor H antigens arise as a consequence of the normal cellular mechanisms for processing and presenting foreign antigens to T cells. Polymorphic genes are transcribed and translated and short peptide sequences are generated by proteolytic digestion of longer precursors in the proteasome. The peptides are transported into the endoplasmic reticulum by the peptide transporter (TAP) and loaded onto MHC class I molecules. The peptide-MHC complex is subsequently presented on the cell surface. In the setting of allogeneic HCT, polymorphisms in the recipient genome can result in the expression of proteins and peptides that are distinct from those in donor cells. Donor T cells fail to recognize self-peptides presented on the cell surface of donor cells due to thymic and peripheral tolerance mechanisms. However, there are high-avidity T cells in the donor repertoire that can recognize recipient minor H antigens and these cells become activated following allogeneic HCT and contribute to GVHD and to the GVL effect.

specifically homozygous gene deletion of a member of a polymorphic gene family (UGT2B17) in the donor, has also been shown to result in T-cell recognition of peptides derived from this protein in recipient cells that express one or both copies of the gene.<sup>15–18</sup> For the majority of minor H antigens, only unidirectional recognition has been shown, either because the corresponding donor peptide is not generated<sup>15,19</sup> or transported by the antigen processing machinery,<sup>20–21</sup> does not bind stably to MHC molecules<sup>22–25</sup> or is not recognized by T cells<sup>22–23,26–27</sup> (Table 1).

#### RATIONALE FOR TARGETING LINEAGE-RESTRICTED MINOR H ANTIGENS USING T-CELL IMMUNOTHERAPY FOLLOWING ALLOGENEIC HCT

The potency of the GVL effect mediated by donor T cells is illustrated by the analysis of outcome data after allogeneic HCT, which shows a dramatically lower relapse rate in patients who develop acute and/or chronic GVHD.<sup>5</sup> There is also a reduction in relapse in recipients of allogeneic HCT that do not develop GVHD compared with recipients of syngeneic HCT, suggesting that the GVL effect may be separated from GVHD. One strategy to accomplish this separation has been shown in murine models in which leukemia was eradicated by the adoptive transfer of CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) specific for a single recipient minor H antigen without GVHD.<sup>42</sup> After allogeneic HCT in humans, both CD8<sup>+</sup> and CD4<sup>+</sup> T cells that recognize minor H antigens on recipient cells are activated *in vivo* and can be isolated *in vitro*, suggesting a similar approach to that taken in mice would be feasible in man.<sup>7,43–47</sup> Human CD8<sup>+</sup> minor H antigen-specific T-cell clones have been shown to lyse primary AML and ALL leukemic cells and inhibit the growth of AML colonies *in vitro*. Moreover, these T cells prevent the engraftment of AML in immunodeficient mouse models, showing that the earliest leukemic progenitors are targets for minor H antigen-specific T cells.<sup>47–50</sup> Unfortunately, most minor H antigen-specific T cells that have been isolated from patients and screened for recognition of cells derived from non-hematopoietic tissues also recognize at least some non-hematopoietic cells, raising

concern that they would cause toxicity if adoptively transferred. A small subset of minor H antigens discovered are predominantly or exclusively on hematopoietic cells and several of these are being evaluated as targets for immunotherapy to facilitate a selective GVL effect (Table 2).<sup>43,51–53</sup> The potential for a few of these ‘hematopoietic-restricted’ minor H antigens described below to serve as targets for immunotherapy to augment the GVL effect is supported by the analysis of T-cell responses in patients who have responded to allogeneic HCT, or to a donor lymphocyte infusion (DLI) to treat relapse.

#### HA-1 and HA-2

HA-1 was the first molecularly characterized autosomal human minor H antigen and has been comprehensively investigated as a potential target for GVL therapy. HA-1 is a nonamer peptide (VLHDDLLEA, called HA-1<sup>H</sup>). The bold and underlined letter represent the polymorphic amino acids within the minor H antigen peptide sequence) that arises as a consequence of an SNP in the *HMHA1* gene and is presented by HLA-A\*0201. The corresponding non-immunogenic sequence or ‘null allele’ is VLRDDLLEA (called HA-1<sup>R</sup>), and two groups have studied the basis for differential immunogenicity of these peptides in depth.<sup>22–24</sup> The proteasomal cleavage and transport of the two peptides into the endoplasmic reticulum via TAP is similar, and both variants can bind to the HLA-restricting allele. However, the arginine (R) variant has lower affinity and less stable binding to HLA-A\*0201, likely related to the relatively large size of the arginine molecule that results in steric and electrostatic hindrance with HLA-A\*0201 D pocket residues.<sup>22–24</sup> The interaction between soluble HA-1<sup>H</sup>-specific TCRs and the HA-1<sup>H</sup> and HA-1<sup>R</sup> peptides has also been analyzed, and it was observed that the TCR bound the HA-1<sup>H</sup> as expected, albeit with relatively low affinity (35  $\mu$ M) and rapid dissociation, but completely failed to bind HA-1<sup>R</sup>.<sup>23</sup> Thus, differences in both MHC and TCR binding account for the immunogenicity of HA-1<sup>H</sup>. The immunogenicity of HA-2 (sequence YIGEVLVSV, called HA-2<sup>V</sup>), which is also presented in association with HLA-A\*0201, arises from

**Table 1 Mechanisms of immunogenicity of human minor histocompatibility antigens**

	Minor H antigen (gene)	HLA allele	Immunogenic peptide epitope	Genetic mechanisms	Molecular mechanisms preventing recognition of the alternative allelic peptide
<i>Altered peptide binding to MHC and/or TCR</i>					
Altered peptide binding to MHC	AAC-4 <sup>R</sup> ( <i>cathepsin H</i> ) <sup>25</sup>	A*3101	ATPLLCAR	Exonic nsSNP	Δ peptide MHC binding
	AAC-5 <sup>R</sup> ( <i>cathepsin H</i> ) <sup>25</sup>	A*3303	WATPLLCAR	Exonic nsSNP	Δ peptide MHC binding
	HA-2 <sup>V</sup> ( <i>MYO1G</i> ) <sup>28–29</sup>	A*201	YIGEVLSV	Exonic nsSNP	Δ peptide MHC binding (+possible additional factors)
Altered TCR recognition of MHC–peptide complex	HA-1 <sup>H</sup> ( <i>HMHA1</i> ) <sup>22,30</sup>	A*0201 A*0206	VLHDDLLEA	Exonic nsSNP	Δ peptide MHC binding Δ T-cell recognition of MHC–peptide complex
	HA-1 <sup>H</sup> ( <i>HMHA1</i> ) <sup>31</sup>	B*60 B*40012	KECVLHDDL(L)	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	HB-1 <sup>H</sup> ( <i>HMHB1</i> ) <sup>32–33a</sup>	B*4402 B*4403	EEKRGSLHVW	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	HB-1 <sup>Y</sup> ( <i>HMHB1</i> ) <sup>34a</sup>	B*4402 B*4403	EEKRGSLYVW	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	ACC-1 <sup>Y</sup> ( <i>BCL2A1</i> ) <sup>35a</sup>	A*2402	DYLQYVLQI	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	ACC-2 <sup>D</sup> ( <i>BCL2A1</i> ) <sup>35</sup>	B*4403	KEFEDDIINW	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	ACC-1 <sup>C</sup> ( <i>BCL2A1</i> ) <sup>36a</sup>	A*2402	DYLQCVLQI	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex
	LB-ECGF1-1 <sup>H</sup> ( <i>ECGF1</i> ) <sup>26</sup>	B*0702	RPHAIRPLAL	Exonic nsSNP in alternative ORF	Δ T-cell recognition of MHC–peptide complex
	SP110 <sup>R</sup> ( <i>SP110</i> ) <sup>37</sup>	A*0301	SLPRGTSTPK	Exonic nsSNP	Δ T-cell recognition of MHC–peptide complex, reverse splicing non-contiguous peptides
	LB-ADIR-1F ( <i>ADIR/TOR3A</i> ) <sup>27</sup>	A*0201	SVAPALALFPA	Exonic nsSNP in alternative ORF	Δ T-cell recognition of MHC–peptide complex
<i>Altered protein transport and/or processing</i>					
Altered transport in TAP	HA-8 <sup>R</sup> ( <i>KIAA0020</i> ) <sup>20</sup>	A*0201	RTLDKVLV	Exonic nsSNP	Δ peptide transport TAP leading to absent peptide presentation in donor cells
Proteosomal cleavage	HA-3 ( <i>Lbc/AKAP13</i> ) <sup>21</sup>	A*0101	VTEPGTAQY	Exonic nsSNP	Proteosomal cleavage leading to precursor peptide destruction
Unknown	SLC1A5 <sup>A16</sup>	B*4002	AEATANGGLAL	Exonic nsSNP	Possible Δ TAP transport
<i>Altered transcription</i>					
Gene deletion	UGT2B17 <sup>15,17</sup>	A*2902 B*4403	AELLNIPFLY	Gene deletion	No transcribed sequence
	UGTB17 <sup>16</sup>	A*0206	CVATMIFMI	Gene deletion	No transcribed sequence
Frameshift mutation	LRH-1 ( <i>P2X5</i> ) <sup>38–40</sup>	B*0702	TPNQQRQNV	Frameshift mutation	Major difference in transcribed sequence
Nonsense mutation	PANE1 ( <i>CENPM</i> ) <sup>19</sup>	A*0301	RVWDLPGVLK	Nonsense mutation	Mutation-related stop codon, transcription aborted, truncated transcript
mRNA splicing	ACC-6 <sup>V</sup> ( <i>HMSD</i> ) <sup>41</sup>	B*4402 B*4403	MEIFIEVFSHF	Intronic SNP	Δ mRNA splicing leads to a different transcript
			HMSD variant		

Abbreviations: HLA, human leukocyte antigen; MHC, major histocompatibility complex; nsSNP, non-synonymous single nucleotide polymorphisms; TAP, transporter associated with antigen processing; TCR, T-cell receptor.

The bold and underlined letters represent the polymorphic amino acids within the minor H antigen peptide sequence.

<sup>a</sup>Biallelic recognition has been shown.

an SNP in the *MYO1G* gene that results in altered binding of the encoded peptides to HLA-A\*0201. Like HA-1, HA-2 has a hematopoietic-restricted tissue distribution,<sup>52</sup> although it has been less extensively studied than HA-1 and has a less favorable phenotypic distribution in the population.

It is well established that HA-1- and HA-2-specific CTL can kill leukemic blasts and prevent the growth of leukemic progenitors *in vitro*.<sup>48–49, 57–59</sup> The first example of an *in vivo* effect of HA-1-specific T cells was reported by Kircher *et al.*,<sup>57</sup> in which a patient with a chemotherapy-refractory relapse of BCR-ABL-positive ALL 1 year after HCT received a DLI from the original HLA-identical sibling HCT donor. The patient achieved a complete cytogenetic and molecular remission, and clonal analysis of the specificity of a minor H antigen-specific CTL line established from patient peripheral blood

mononuclear cells revealed sole specificity for HLA-A2<sup>+</sup> HA-1<sup>H+</sup> target cells, and recognition of primary leukemic cells that endogenously presented the HA-1<sup>H</sup> minor H antigen. The Goulmy group performed an analysis of three additional patients who received DLI to treat post-transplant relapse using MHC/peptide tetramers to quantify HA-1- and/or HA-2-specific T cells.<sup>60–61</sup> They observed an expansion of CD8<sup>+</sup> T cells specific for HA-1 and/or HA-2 in the blood after DLI, and the emergence of HA-1- and HA-2-specific T cells coincided with remission of the malignancies (chronic myeloid leukemia (CML) and multiple myeloma) and restoration of complete donor hematopoietic cell chimerism. HA-1- and HA-2-specific T cells were also isolated from these patients and shown to lyse recipient leukemic cells and inhibit leukemic colony formation *in vitro*.<sup>60–61</sup> In one additional report, the emergence of HA-1<sup>H</sup>-specific CTL in patients treated with

**Table 2** Minor H antigens selectively expressed in hematopoietic cells

Minor H antigen	Gene/chromosome	HLA allele	Polymorphism	Immunogenic peptide epitope	Genotype frequencies (%)	Estimated disparity MSD (%)	Estimated disparity MUD (%)
<i>Hematopoietic</i>							
HA-1 <sup>H22,30</sup>	<i>HMHA1</i> /19p13.3	A*0201 A*0206	rs1801284	<u>VL</u> <u>H</u> DDLLEA	HH=13, HR=45.8, RR=41.2	6.4 +A*0206<1	11.6 +A*0206<1
LRH-1 <sup>38</sup>	<i>P2X5</i> /17p13.3	B*0702	rs5818907	<u>TPNQRQ</u> <u>NVC</u>	+/-=4, +/-=-50, -/-=46	4.9	7.5
ACC-2 <sup>D35</sup>	<i>BCL2A1</i> /15q24.3	B*4403	rs3826007	KEFED <u>D</u> IINW	DD=6.4, DG=38.1, GG=55.5	3.6	6.7
HEATR1 <sup>E54</sup>	<i>HEATR1</i> /1q43	B*0801	rs2275687	ISKERA <u>E</u> AL	E/E=10, E/G=45, G/G=45	3.1	5.6
ACC-1 <sup>Y35</sup>	<i>BCL2A1</i> /15q24.3	A*2402	rs1138357	DYLQ <u>Y</u> VLQI	YY=6.7, YC=39.5, CC=53.5	2.8	5.2
ACC-6 <sup>V41</sup>	<i>HMSD</i> /18q21.3	B*4402 B*4403	rs9945924	<u>MEIFIEV</u> <u>FSHF</u> <i>HMSD variant</i>	V/V=10, V/wt=23, wt/wt=66.7	2.3	5.9
HA-2 <sup>V28,29</sup>	<i>MYO1G</i> /7p13-p11.2	A*0201	rs61739531	YIGEVLS <u>V</u>	VV=56.8, VM=37.7, MM=5.5	1.8	2.5
SP110 <sup>R37</sup>	<i>SP110</i> /2q37.1	A*0301	rs1365776	SLP <u>R</u> GTSTPK	RR=37.0, RG=48.3, GG=14.7	1.6	2.5
LB-LY75-1K <sup>55</sup>	<i>LY75</i> /2q24	DRB1 *1301	rs12692566	GITYRN <u>K</u> SLM	K/K=6.7, K/N=33, N/N=60	1.2	2.4
HA-1 <sup>H31</sup>	<i>HMHA1</i> /19p13.3	B*60 B*40012	rs1801284	KECVL <u>H</u> DDL(L)	HH=13, HR=45.8, RR=41.2	<1	<1
ACC-1 <sup>C36</sup>	<i>BCL2A1</i> /15q24.3	A*2402	rs1138357	DYLQ <u>C</u> VLQI	CC=53.8, YC=39.5, YY=6.7	<1	<1
<i>B cell</i>							
CD19 <sup>L56</sup>	<i>CD19</i> /16p11.2	DQ A1*05B1*02	rs2904880	WEGEP <u>P</u> CLP	L/L=3.4, L/V=50, V/V=46.6	10.4	15.9
HB-1 <sup>Y34</sup>	<i>HMHB1</i> /5q31.3	B*4402 B*4403	rs161557	EEKRGS <u>L</u> YVW	YY=5.2, HY=41.2, HH=53.7	3.9	6.8
HB-1 <sup>H32,33</sup>	<i>HMHB1</i> /5q32	B*4402 B*4403	rs161557	EEKRGS <u>L</u> HVW	HH=53.7, HY=41.2, YY=5.2	1.2	1.3

Abbreviations: HLA, human leukocyte antigen; MSD, matched sibling donor; MUD, matched unrelated donor.  
The bold and underlined letters represent the polymorphic amino acids within the minor H antigen peptide sequence.

DLI for relapse of CML appeared to coincide with the elimination of the CML.<sup>23</sup>

HA-1-specific CTL have been identified in peripheral blood mononuclear cell samples obtained from patients who have received DLI without experiencing severe GVHD.<sup>30,57,60-61</sup> However, analysis of transplant outcome based on donor/recipient disparity of HA-1 has provided conflicting data concerning a potential role of HA-1 in GVHD.<sup>62-64</sup> This conflicting data could reflect the inherent limitations of analyzing GVHD outcomes in relation to a few known minor H antigen differences in population studies of highly outbred individuals. HA-1 is widely accepted to be selectively expressed on hematopoietic cells, and multiple publications have documented very low to absent HA-1 gene expression in non-hematopoietic cells,<sup>65-67</sup> and lack of recognition of HA-1<sup>H</sup>-positive non-hematopoietic cells by HA-1<sup>H</sup>-specific CTL in cellular assays.<sup>52</sup> Furthermore, HA-1<sup>H</sup>-specific CTL induce little or no specific tissue damage when co-cultured with HA-1<sup>H</sup>-positive skin biopsy specimens in a skin explant model of GVHD.<sup>68</sup>

#### BCL2A1/ACC-1 and ACC-2

*BCL2A1* is a member of the bcl-2 family of antiapoptotic genes and encodes the minor H antigens, ACC-1<sup>Y</sup>, ACC-1<sup>C</sup> and ACC-2<sup>D</sup>. Biallelic recognition of ACC-1 has recently been shown such that both ACC-1<sup>Y</sup> (sequence DYLQYVLQI. The bold and underlined letter represent the polymorphic amino acids within the minor H antigen peptide sequence) and ACC-2<sup>C</sup> (DYLQCVLQI) are processed, bind the HLA-A\*2402-restricting allele and are immunogenic.<sup>36</sup> ACC-2<sup>D</sup> results from a different nucleotide polymorphism in the *BCL2A1* gene and is presented by HLA-B\*4403.<sup>35</sup> The *BCL2A1* gene is highly expressed in hematological malignancies and may contribute to the survival of malignant cells, making minor H antigens encoded by this gene attractive for antileukemic immunotherapy.<sup>69</sup> The immunogenicity

of ACC-1<sup>Y</sup>, ACC-1<sup>C</sup> and ACC-2<sup>D</sup> have been attributed to physical differences in the peptide-MHC complex for TCR discrimination. ACC-1<sup>Y</sup>- and ACC-2<sup>D</sup>-specific T cells were isolated from transplant recipients and lysed primary leukemic cells *in vitro*.<sup>35</sup> Gene expression analysis by northern blot,<sup>35</sup> quantitative PCR<sup>67</sup> and database microarray data suggests a predominantly hematopoietic-restricted distribution (<http://biogps.gnf.org>), suggesting that ACC-1<sup>Y</sup>, ACC-1<sup>C</sup> and ACC-2<sup>D</sup> may be useful targets for segregating the GVL effect from GVHD.<sup>70</sup>

#### P2X5/LRH-1

The *P2X5* gene is a member of the P2X purinergic ATP-gated non-selective cation channels and encodes the HLA-B\*0702-restricted LRH-1 minor H antigen.<sup>38</sup> A frameshift mutation resulting from an insertion/deletion SNP underlies the immunogenicity of LRH-1. The cytosine deletion in exon 3 leads to the production of a truncated protein in the donor cells and a sequence disparity between the recipient and donor in the transcribed region, including a major partial disparity between recipient and donor for the amino-acid sequence that gives rise to the minor H antigen peptide in recipient cells (TPNQRQNVC in the recipient, TPTSGRTSV in the donor. The bold and underlined letters represent the polymorphic amino acids within the minor H antigen peptide sequence).<sup>38</sup> The fate of the donor transcript is not known.

Quantitative PCR data show that *P2X5* gene is expressed in normal lymphocytes, B and T lineage ALL, lymphoma and multiple myeloma, and the CD34<sup>+</sup> fractions of CML and AML. *P2X5* is expressed at lower levels in the brain and skeletal muscle, but there is minimal if any expression in the target tissues of GVHD (small intestine, colon, liver, lung, skin),<sup>38</sup> and skin fibroblasts that are positive by LRH-1 genotyping, are not recognized in cellular assays.<sup>38</sup> Although *P2X5* is predominantly expressed in lymphocytes and there is little expression in

normal myeloid cells, LRH-1-specific minor H antigens kill CD34<sup>+</sup> ALL, AML and CML cells, and CD138<sup>+</sup> multiple myeloma cells *in vitro*.<sup>38–40</sup> Dolstra's group studied seven HLA-B7<sup>+</sup> LRH-1<sup>+</sup>-positive patients who received HCT for a hematological malignancy and subsequently DLI from an HLA-B7<sup>+</sup> LRH-1<sup>–/–</sup> donor, and detected LRH-1-specific responses coinciding with clinical, molecular or cytogenetic responses in three of the seven patients.<sup>38,40</sup>

### PROGRESS IN THE DEVELOPMENT OF IMMUNOTHERAPY TARGETING MINOR H ANTIGENS FOLLOWING ALLOGENEIC HCT

Despite the discovery of at least 14 hematopoietic-restricted minor H antigens and observational data suggesting that certain minor H antigen-specific T cells may have the potential to induce a potent selective GVL effect, the translation of these discoveries into clinical trials that prospectively evaluate strategies such as adoptive T-cell therapy or vaccination to augment specific T-cell responses has been challenging. The challenges that have impeded clinical translation of minor H antigen-directed immunotherapy and the progress in the field that provides optimism that the potential of this approach will be realized are described below.

### MOLECULAR IDENTIFICATION OF MINOR H ANTIGENS

#### The challenges

A rational assumption for the clinical investigation of minor H antigen-directed immunotherapy has been that minor H antigens that have a predominantly hematopoietic-restricted tissue distribution should be selected as targets. Analysis of the genotype frequency of the minor H antigens shown in Table 2 reveals an inherent difficulty, which is that the current list will only be relevant for a minor proportion of the transplant population, and that any study targeting a single minor H antigen would have difficulty accruing sufficient number of patients to be informative. Ideally, a large panel of minor H antigens would be available from which to select appropriate targets for the treatment of individual HCT recipients because of the need to have the appropriate HLA-restricting molecule and the correct directional disparity between HCT recipient and donor.

The success in assembling an adequate selection of minor H antigens for clinical immunotherapy trials is likely to depend on the stringency of the criteria used to define 'hematopoietic restricted', as the proportion of human genes that are expressed absolutely exclusively in hematopoietic cells is very small. It is uncertain as to whether it is essential that the expression of minor H antigens be absolutely exclusive to hematopoietic cells to prevent toxicity in the context of adoptive immunotherapy or vaccination, or what level of expression in non-hematopoietic cells might be tolerated. However, this issue is difficult to approach experimentally, thus there remains a strong case

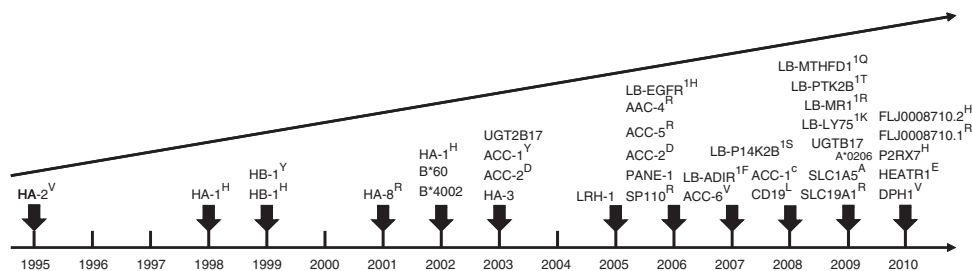
for selecting highly hematopoietic-restricted minor H antigens as targets for the initial immunotherapy clinical trials until safety is established.

#### The progress

At the present time, 25 and 40% of recipients of HLA-identical related and unrelated donor HCT, respectively, would be eligible for immunotherapy targeting one of the molecularly characterized minor H antigens expressed predominantly on hematopoietic cells (Table 2).<sup>71</sup> The discovery of minor H antigens is accelerating as a result of novel approaches for isolating minor H antigen-specific CTL clones that can be used for gene discovery, and the development of databases of human genetic polymorphisms and cell lines with characterized genotypes that facilitate dissection of T-cell recognition (Figure 2). How these developments are being applied to minor H antigen gene discovery is discussed briefly below.

**Cellular tools: isolation of minor H antigen-specific CTL clones by primary *in vitro* stimulation.** Minor H antigen-specific T cells provide essential reagents for the molecular identification and characterization of the polymorphic genes that encode the antigens, and such T cells have in the past been isolated from post-transplant blood obtained from allogeneic HCT recipients. This approach was cumbersome and often unsuccessful, perhaps due to the immunosuppressive drugs the patients were taking to impede the development of GVHD. We developed an approach for isolating minor H antigen-specific CD8<sup>+</sup> T-cell clones that can be stably propagated for antigen discovery, which relies on the stimulation of naïve CD8<sup>+</sup> T cells from unprimed donors with monocyte-derived dendritic cells (DC) from the HLA-identical sibling.<sup>54</sup> The generation of minor H antigen-specific CTL by primary *in vitro* stimulation required interleukin (IL)-12 and  $\gamma$ -chain cytokines in the culture. With this approach, a large panel of CTL clones was obtained from granulocyte colony-stimulating factor mobilized stem cell products of 10 consecutive HCT donors. This panel included CTL clones specific for novel minor H antigens that were presented in association with common HLA alleles, encoded by polymorphisms with a balanced phenotype frequency (between 26 and 78% of the population), and expressed on hematopoietic cells, including leukemia.<sup>54</sup> As proof of principle, a minor H antigen-specific CTL clone from this panel was used to discover a new minor H antigen encoded by the *HEATR1* gene that is presented on AML stem cells and minimally in non-hematopoietic tissues.<sup>54</sup> Additional minor H antigens have been identified using CTL clones derived by primary *in vitro* stimulation and these are currently being fully characterized.

**Genetic and molecular tools: genome-wide association and advances in cDNA library screening.** Approaches that have been used previously to identify the polymorphic genes and peptide sequences that provide



**Figure 2** The accelerating pace of minor H antigen discovery. Each year in which a molecularly characterized autosomal chromosome-associated human minor H antigen was discovered is marked on the horizontal time line by the vertical arrows. The name(s) of one or more minor H antigen identified in each discovery year is listed above the corresponding arrows.



minor H antigens include cDNA library screening, genetic linkage analysis and peptide elution followed by high-performance liquid chromatography to identify immunogenic peptides and mass spectrometry to determine their sequence. These approaches have been reviewed elsewhere,<sup>7</sup> but their broad application has been restricted to a few laboratories with the technical expertise to perform this work. The application of new genetic and molecular techniques that incorporate advances in human genomics reduces technical complexity and is accelerating the pace of minor H antigen discovery.

**Genome-wide association studies and HapMap screening:** An important development in the field is the application of genome-wide association studies (GWAS) and HapMap screening for minor H antigen identification. GWAS with HapMap scanning takes advantage of the publically available genotyping data from the Human HapMap project and involves analyzing the relationship between patterns of expression of the minor H antigen phenotype (as determined by *in vitro* cytotoxicity or cytokine secretion assays) of B-cell lymphoblastoid cell lines (B-LCL) and their patterns of SNP genotype downloaded from databases. In contrast to genetic linkage analysis, the association studies in GWAS are performed simultaneously for SNPs across the whole genome making it a potentially rapid and powerful technique. GWAS has now been used to discover three novel minor H antigens, and to retrospectively identify two others.<sup>16,56,72</sup> The Akatsuka group published the first report of the use of GWAS for discovering novel minor H antigens.<sup>16</sup> They screened minor H antigen-specific CTL clones for recognition of up to 72 HapMap B-LCL, and stratified the B-LCL into those that were clearly positive for expression of the minor H antigen and those that were clearly negative. Association scores were then calculated using the purpose-designed computer program, and chromosomal regions that contained SNPs that were significantly associated with T-cell recognition were identified. Candidate polymorphisms were then evaluated using synthetic peptides to identify the precise epitope. In one example reported by the Akatsuka group,<sup>16</sup> the actual SNP that corresponded to the antigenic allele was not identified in the original GWAS because of genotyping errors for that SNP in the database, but was subsequently identified and confirmed on re-sequencing of the candidate gene. The GWAS approach is more rapid than traditional genetic linkage analysis and requires less technical expertise. However, there can be difficulties with the identification of multiple polymorphisms located in disparate regions, even different chromosomes, representing a mixture of false and true positive associations.<sup>16,56,72</sup>

**Bacterial cDNA libraries for the identification of class II MHC-restricted minor H antigens:** Most published work on minor H antigen discovery describes the identification of class I MHC-restricted minor H antigens recognized by CD8<sup>+</sup> T cells. There has been renewed interest in a role for HLA class II minor H antigens in a selective GVL effect because HLA class II is not expressed on most non-hematopoietic tissues under non-inflammatory conditions, and therefore expression of a minor H antigen in non-hematopoietic tissue in the absence of its HLA-restricting allele is less likely to cause GVHD. It remains to be determined whether there is sufficient HLA class II expression on leukemic cells, including leukemic stem cells to be recognized by CD4<sup>+</sup> T cells, and if so whether leukemic cells will be susceptible to death pathways invoked in target cells by CD4<sup>+</sup> T cells. These issues are best studied with T cells specific for molecularly characterized class II-restricted minor H antigens.

Several class I MHC-restricted minor H antigens were discovered by screening cells co-transfected with pools of a cDNA library prepared from minor H antigen-positive cells and with a plasmid encoding the class I MHC-restricting allele. Conventional cDNA library screening

by transfection into COS7 or 293T cells is not as easily applied for the discovery of class II minor H antigens, as these cells are not professional antigen-presenting cells. This issue can be resolved by constructing the cDNA libraries such that the invariant chain is fused to each cDNA to direct the transport of translation products to class II processing compartments, and co-transfecting the COS cells with essential components of the class II antigen presentation pathway.<sup>73</sup> A subsequent improvement for discovery of class II-restricted antigens relates to the expression of cDNA libraries in bacteria, and loading of bacteria containing the expressed human protein into class II MHC-positive B-LCL.<sup>55,74–75</sup> The first example of the discovery of a minor H antigen using a bacterial cDNA library is the minor H antigen LB-P14K2B-1S encoded by the phosphatidylinositol 4-kinase type II  $\beta$  (*PI4K2B*) gene.<sup>75</sup> A recombinant cDNA library was constructed from patient B-LCL by cloning randomly primed cDNAs into a pKE-1 vector, which encodes the glutathione-binding domain of GST under the control of an isopropyl  $\beta$ -D-thiogalactoside promoter. Protein expression was induced by IPTG, and individual pools of 50 bacteria were then opsonized with complement and loaded onto aliquots of donor B-LCL. The B-LCLs were then screened for T-cell recognition by enzyme-linked immunosorbent assay to detect interferon- $\gamma$ . One bacterial pool induced a positive response and sub-cloning of this pool identified a single cDNA that was identical to the *PI4K2B* gene. An SNP in *PI4K2B* was subsequently confirmed to be the polymorphism relevant for recognition.

Several other autosomal minor H antigens have now been discovered using bacterial cDNA libraries, including LB-LY75-1K, LB-MR1-1R, LB-PTK2B-1Y and LB-MTHFD1-1Q.<sup>55,75</sup> A minor H antigen of particular interest is LB-LY75-1K (sequence GITYRNKSLM), which is encoded by the lymphocyte antigen 75 (*LY75*) gene, and arises from a non-synonymous SNP (rs12692566, G/T, lysine/asparagine) in exon 29. *LY75* is also known as *DEC205* and functions as a scavenger receptor in DC.<sup>76,77</sup> *LY75* is expressed in both normal lymphoid and myeloid hematopoietic cells, with the highest level of expression in DC. LB-LY75-1K-positive AML cells but not fibroblasts (a representative non-hematopoietic cell) induced interferon- $\gamma$  release by the LB-LY75-1K-specific CD4<sup>+</sup> T-cell clone, suggesting that targeting LB-LY75-1K could potentially induce a GVL effect without GVHD.<sup>55</sup> One caveat for targeting LB-LY75-1K to induce a selective GVL effect is that it is also expressed on cortical thymic epithelial cells that, unlike most non-hematopoietic cells, do express HLA class II molecules.<sup>78</sup> Thymic damage is considered to be a factor in the development of chronic GVHD by allowing thymocytes to escape deletion of T cells with self-reactive TCRs. Cortical thymic epithelial cells contribute to tolerance, and thus the elimination of recipient thymic epithelial cells by targeting LB-LY75-1K could theoretically contribute to chronic GVHD.

**Revisiting old tools: screening candidate polymorphic peptides using reverse immunology.** There has been renewed interest in using reverse immunology for minor H antigen discovery with the wealth of available data on human genetic polymorphism. Reverse immunology involves screening candidate peptides selected from the sequence of a gene product of interest often based on computer algorithms that predict binding to HLA molecules. This approach has the potential for high-throughput analysis and allows the selective analysis of peptides encoded by genes that are known to be preferentially expressed in hematopoietic cells. Reverse immunology has been used extensively to discover tumor-associated antigens, and in the past has facilitated the identification of additional epitopes in genes such as *HMHA-1*, and *HB-1* that encode minor H antigens previously discovered by 'forward' immunology.<sup>34</sup> Reverse immunology has recently been used by the Ritz group to

identify potentially immunogenic novel Y-chromosome-associated minor H antigens after HLA-identical transplant between sex-mismatched individuals. A large panel of Y-chromosome-derived peptide sequences was screened for recognition by T cells obtained from short-term cultures of peripheral blood mononuclear cells obtained from patients following allogeneic HCT using interferon- $\gamma$  release as a readout. T-cell responses to a subset of the peptides were observed, although only a small number of patients responded to any given peptide. Studies showing that these putative novel HY antigens are processed and presented by leukemic cells have not yet been reported.<sup>79</sup>

Our lab has performed an *in silico* analysis that incorporated gene expression and SNP databases, and peptide prediction algorithms to assemble a library of candidate minor H antigen peptides derived from genes that are predominantly expressed in hematopoietic cells. This library of peptides will enable screening for T-cell responses that might develop in allogeneic HCT recipients who are appropriately discordant at the putative antigenic allele with the donor, and can be used for *in vitro* priming of T-cell responses from the donor-naïve T-cell repertoire.

## ADOPTIVE T-CELL THERAPY TARGETING MINOR H ANTIGENS

### The challenges

The adoptive transfer of donor T cells specific for viral antigens is effective for preventing cytomegalovirus and Epstein-Barr virus disease after allogeneic HCT without causing GVHD,<sup>80–84</sup> and the transfer of autologous tumor-reactive T cells has been employed successfully in a subset of patients with melanoma- and Epstein-Barr virus-related malignancies.<sup>85–89</sup> The molecular characterization of minor H antigens that are expressed by leukemic cells but not on non-hematopoietic tissues provides the opportunity to use adoptive transfer of minor H antigen-specific T cells to augment the GVL effect after HCT without GVHD. There are several significant challenges to be met before this complex approach can be routinely employed. These include the need to develop efficient methods for isolating and expanding rare donor T cells that are specific for relevant minor H antigens, to define the appropriate cells and conditions that promote persistence of transferred T cells *in vivo*, and to implement strategies to ensure that transferred T cells do not cause toxicity to normal tissues.

### The progress

Steady progress has been made in defining principles for isolating, expanding and re-infusing T cells to treat human infections and malignancy,<sup>84,90–91</sup> including the use of gene transfer techniques to introduce TCR  $\alpha\beta$  genes and confer specificity for a target antigen, and to introduce genes that will enhance safety.<sup>92–93</sup> Another important advance is the recognition that cell intrinsic qualities of effector T cells ( $T_E$ ) that are selected for adoptive transfer play a critical role in their ability to persist *in vivo* and establish long-lived memory responses. Here, we discuss how these advances are being applied to adoptive T-cell therapy targeting minor H antigens.

**Isolation and expansion of minor H antigen-specific T cells.** Donor T cells specific for minor H antigens, including hematopoietic-restricted antigens such as HA-1<sup>H</sup>, ACC-1<sup>Y</sup> and ACC-2<sup>D</sup> and LRH-1, are already amplified in some HCT recipients,<sup>23,30,35,38,40,47,57,60–61</sup> although their ability to eliminate leukemia may be compromised by the administration of immunosuppressive drugs to prevent GVHD. Such T cells can be isolated post transplant and expanded *in vitro* for adoptive transfer to magnify the endogenous response and potentially improve the GVL effect. T-cell clones rather than polyclonal T cells are preferred for

immunotherapy in the allogeneic HCT setting to avoid infusing other potentially alloreactive T cells, and to facilitate the analysis of safety and efficacy. A phase I clinical trial of adoptive immunotherapy has been performed by our group in which T-cell clones that were specific for minor H antigens that exhibited preferential expression on hematopoietic cells by *in vitro* assays were adoptively transferred to patients with leukemia relapse.<sup>94</sup> Before infusion, the T-cell clones were expanded to several billion cells using culture methods that employ antibodies to the CD3 signaling complex to activate T cells, 'feeder' cells to provide co-stimulation and IL-2 to promote T-cell proliferation and survival. This study showed that generating minor H antigen-specific T cells for therapy was feasible in a significant subset of patients, and showed that the transferred T cells infiltrated the bone marrow and mediated antileukemic activity *in vivo*. However, some treated patients experienced reversible pulmonary toxicity at high T-cell doses that was subsequently shown to reflect the unexpected expression of the targeted minor H antigen in pulmonary epithelial cells. This 'on-target' toxicity emphasized the need to focus on immunotherapy targeting molecularly characterized minor H antigens with a well-defined tissue distribution for future clinical trials.<sup>94</sup> A second problem observed in this trial was the short duration of persistence of transferred T cells, which is commonly observed in adoptive T-cell therapy trials for malignancy and correlates with lack of a sustained antitumor effect.<sup>95</sup> The basis for poor T-cell persistence is beginning to be elucidated and strategies to improve cell persistence are discussed later in this review.

An alternative approach is to isolate minor H antigen-specific T cells directly from the donor before transplant and administer them either as part of the stem cell graft or early post transplant when the tumor burden is low. Early therapy may be especially important after non-myeloablative HCT because of the limited antitumor activity of the conditioning regimen. Specialized culture conditions employing DC that are either pulsed with immunogenic peptides, transfected with the gene encoding the antigen or derived from monocytes isolated from the HCT recipient (and thereby naturally expressing the relevant minor H antigens) have been developed to isolate donor T cells specific for leukemia-associated minor H or non-polymorphic antigens.<sup>54,96–99</sup> Techniques have been established for the isolation of human T-cell clones and involve plating T cells directly from polyclonal cultures at limiting dilution or after selection using tetramers or cytokine capture, and screening colonies to identify those with the desired reactivity.<sup>47,100</sup> Potential problems with this approach include the technical difficulty of priming and expanding rare T cells from the naïve repertoire of the donor, and the possibility that  $T_E$  cells derived from naïve T-cell precursors *in vitro* may not be appropriately programmed for survival *in vivo*.

**TCR  $\alpha\beta$  gene transfer to derive T cells for adoptive immunotherapy.** Although culture conditions for the isolation of minor H antigen-specific T cells for GVL therapy have improved, novel strategies to generate T cells may prove more efficient and ultimately more effective. One potential approach is to engineer donor T cells for antigen specificity by the introduction of genes that encode the TCR  $\alpha$ - and  $\beta$ -chains from previously isolated minor H antigen-specific T-cell clones. TCR gene transfer has been successful for generating antigen-specific T cells that are effective in murine models of adoptive immunotherapy,<sup>101–103</sup> and the first human clinical trials of T cells modified by TCR gene transfer to treat metastatic melanoma have been performed with some success.<sup>104–105</sup>

TCR gene transfer has the advantage of providing 'off the shelf' reagents that could be used to target minor H antigens in HCT recipients that are appropriately discordant at specific loci. The

availability of TCR genes for multiple minor H antigens would increase the applicability of minor H antigen-specific immunotherapy, requiring only that the donor and recipient be genotyped before transplant to select the appropriate targets for therapy. The TCRs would need to be inserted into donor T cells that lack the ability to cause GVHD, which could potentially be accomplished by introducing the genes into virus (cytomegalovirus or Epstein–Barr virus)-specific T cells.<sup>103,106–111</sup> For example, the minor H antigen HA-2 has a favorable tissue distribution and CTL specific for HA-2 have been observed in patients with relapsed leukemia responding to DLI. However, 95% of the population expresses the antigenic HA-2<sup>v</sup> allele and therefore naturally mismatched recipient donor pairs are infrequent. The transfer of HA-2-specific TCRs into T cells from HLA-A\*0201-positive, HA-2-negative individuals using retroviral vectors has been shown to successfully redirect cytolytic activity against HA-2-positive target cells, including leukemia.<sup>103</sup> Furthermore, the introduction of an HLA-A\*0201-restricted HA-2-specific TCR into HLA-A\*0201-negative lymphocytes also transferred HA-2-specific anti-leukemic reactivity *in vitro*, and only against HLA-A\*0201-positive target cells.<sup>103</sup> Thus, this approach could also be applied to T-cell therapy in non-HLA-matched HCT where the donor lacks the HLA-restricting allele for a minor H antigen that both the donor and recipient share.

There are potential limitations of the TCR transfer approach, including (a) mispairing of the gene-transferred TCR and native TCR chains leading to the development of dysfunctional or potentially autoreactive TCRs, (b) competition for the CD3 co-receptor between the gene-transferred TCR with the native TCR or mixed TCR dimers leading to reduced function of the introduced antigen-specific TCR, and (c) inefficient gene transfer and unstable transgene expression. Potentially harmful neoreactivity, including HLA class I and II alloreactivity and autoreactivity, has been observed in human T cells *in vitro* as a consequence of mispairing of the gene-transferred minor H antigen or viral-specific TCR  $\alpha$ - or  $\beta$ -chains and native TCR chains,<sup>112</sup> and a lethal GVHD syndrome ('TCR gene transfer-induced GVHD') has been observed in murine models as a result of mispairing of endogenous and introduced TCRs.<sup>113</sup> Efforts to make TCR transfer safer include modifications of the introduced  $\alpha$ - and  $\beta$ -chains to increase correct pairing,<sup>112,114–117</sup> and/or to prevent expression of the endogenous TCR by the introduction of a zinc-finger nuclease specific for the endogenous TCR  $\beta$ -chain or the use of small interfering RNA, thereby avoiding the opportunity for mispairing.<sup>118,119</sup> A further option to prevent mispairing of the introduced and endogenous  $\alpha$ - and  $\beta$ -chains is to introduce the TCR  $\alpha\beta$  genes together with the requisite CD4 and CD8 co-receptors into  $\gamma/\delta$  T cells, which lack endogenous  $\alpha$ - and  $\beta$ -chains. The  $\gamma/\delta$  T-cell approach has been studied using the HA-2 minor H antigen TCR as a model and the HA-2  $\alpha\beta$ -transduced  $\gamma/\delta$  T cells showed good functional activity *in vitro*.<sup>107,120</sup>

*Promoting persistence of adoptively transferred antigen-specific T cells.* The optimal regimen for promoting persistence of transferred T cells in humans has not yet been defined. Several factors have been suggested to interfere with T-cell persistence, including terminal differentiation from prolonged culture of T cells before infusion, the use of excessive cytokines *in vitro* and the absence of a CD4<sup>+</sup> helper T-cell response *in vivo*.<sup>81, 121</sup> Strategies for improving *in vivo* survival of transferred T cells that have been evaluated in animal models include the addition of exogenous cytokines, such as IL-2 or IL-15, or cytokine transduction of the CTL; co-stimulation via CD28 or 4-1BB; and transferring T cells when the recipient is lymphopenic and homeostatic regulatory mechanisms that promote T-cell expansion and

survival are invoked.<sup>85–86,122–125</sup> The use of IL-2 or IL-15 is likely to be difficult in the allogeneic HCT setting as these cytokines may provoke GVHD by inducing the proliferation of donor alloreactive T cells present in the stem cell graft.

The importance of heritable cell intrinsic qualities of transferred T cells is increasingly being recognized as a factor for the persistence of transferred T cells. T cells for adoptive transfer could be isolated from the naïve (T<sub>N</sub> CD45RA<sup>+</sup> CD45RO<sup>−</sup> CD62L<sup>+</sup>), central memory (T<sub>CM</sub> CD45RO<sup>+</sup> CD62L<sup>+</sup>) or the effector memory (T<sub>EM</sub> CD45RO<sup>+</sup>, CD62L<sup>−</sup>) T-cell subsets.<sup>126</sup> After *in vitro* activation and expansion, T cells acquire a uniform T<sub>E</sub> phenotype and lack most memory T-cell markers.<sup>127</sup> Studies in our lab in a non-human primate model have shown that clonally derived T<sub>E</sub> cells obtained from T<sub>CM</sub> precursors have a greatly superior capacity to persist *in vivo* after adoptive transfer compared with those derived from T<sub>EM</sub> precursors, and reacquire memory markers, and respond to antigenic challenge *in vivo*.<sup>128</sup> This finding is consistent with studies in murine models showing that T<sub>CM</sub> confer superior antitumor protection after adoptive transfer compared with T<sub>EM</sub>.<sup>129</sup> These results suggest that the transfer of TCRs specific for minor H antigens to target leukemia would best be accomplished by purifying T<sub>CM</sub> cells with a native TCR specific for a persistent virus to facilitate their survival and function after adoptive transfer. A recent study performed in a transgenic mouse model suggests that T<sub>E</sub> cells derived from T<sub>N</sub> are also superior to those derived from T<sub>EM</sub> cells in their ability to persist *in vivo* after adoptive T-cell transfer, provided they are not extensively cultured.<sup>130</sup>

*Preventing toxicity associated with adoptive T-cell transfer:* The adoptive transfer of minor H antigen-specific T cells to augment the GVL effect has the potential to cause GVHD or toxicity to normal tissues that express the target antigen, as illustrated by the results of the first clinical trial of this approach.<sup>94</sup> The use of gene transfer to engineer T-cell specificity would impose an additional risk of insertional oncogenesis.<sup>131</sup> If the therapy is effective in eliminating leukemia, some limited toxicity may be acceptable. However, it would be advantageous to be able to eliminate transferred T cells if serious toxicity developed. The introduction of a conditional suicide gene, such as the HSV thymidine kinase (TK) gene, has been effective for controlling GVHD after polyclonal DLI to treat relapse or Epstein–Barr virus lymphoproliferation in HCT recipients, but this approach can be limited by the immunogenicity of the viral TK, which can result in premature elimination of transferred effector cells.<sup>93,132–134</sup> Alternative suicide genes based on the expression of chimeric human proteins such as Fas or caspase 9 that can be activated by a synthetic non-toxic drug to induce cell death are being investigated by several groups.<sup>135–136</sup> Another approach is to express a surface molecule such as CD20 that can be targeted by a monoclonal antibody. Preliminary studies indicate that virus (cytomegalovirus)-specific T cells co-transduced with CD20 and the minor H antigen HA-2 TCR exhibit high-level HLA-restricted cytotoxicity against HA-2<sup>+</sup> targets *in vitro* and can be efficiently destroyed with the  $\alpha$ CD20 monoclonal antibody Rituximab by complement-dependent cytotoxicity.<sup>137</sup> These developments suggest that more sophisticated approaches to augmenting GVL activity by the adoptive transfer of minor H antigen-specific T cells will be feasible in the future.

## FUTURE DIRECTIONS—VACCINATION AGAINST MINOR H ANTIGENS

Vaccination of HCT recipients against minor H antigens could represent an alternative approach to adoptive immunotherapy, or a complementary strategy for augmenting the GVL effect. However, despite considerable effort to elicit tumor-reactive T-cell responses in



cancer patients, most studies have yielded poor results. Additional challenges to priming or boosting immune responses in HCT recipients are posed by the pharmacological immunosuppression that is administered to prevent or treat GVHD, and by the delayed immune reconstitution that occurs after HCT. Conversely, there are grounds for optimism about the potential of vaccines targeting minor H antigens to augment the GVL effect. In contrast to solid tumors, hematological malignancies may be more susceptible to vaccine-induced T-cell responses because the tumor environment is more accessible physically and immunologically. Several clinical trials of peptide or DC vaccines targeting non-polymorphic leukemia-associated antigens have now been conducted and immunological and clinical responses have been shown.<sup>138–151</sup> Minor H antigens are foreign to the donor T cells and therefore should have the potential to induce high-affinity responses, in contrast to most non-polymorphic tumor-associated antigens.

Clinical trials of HA-1 or HA-2 vaccination of the transplant recipient to augment the GVL effect following allogeneic HCT have been initiated or are planned at several centers, but results have not yet been reported. Vaccination of the immune-competent transplant donor before transplant is an intriguing alternative approach that could increase the frequency T cells specific for leukemia-associated antigens in the stem cell graft and facilitate the GVL effect. The feasibility of inducing immune responses with vaccination and transferring them with adoptive immunotherapy post transplant in humans has been shown in the autologous HCT setting,<sup>152</sup> and transfer of vaccine-induced T-cell responses to a tumor antigen (idiotype) through the HCT graft has also been shown in HCT between HLA-identical siblings.<sup>153</sup> Donor vaccines targeting minor H antigens may pose a theoretical risk of inducing autoimmunity by virtue of cross-reactivity with the 'non-immunogenic' allele. However, the observation that multiparous women remain healthy in spite of being primed to minor H antigens from the fetus suggests this is unlikely.<sup>154–155</sup>

## CONCLUSIONS

It is now 15 years since the first molecular characterization of human minor H antigens and significant strides in minor H antigen discovery are now being made as a consequence of advances in cellular, genetic and molecular techniques. Much has been learned about the mechanisms of minor H antigen immunogenicity, their expression on normal and malignant cells, and their role in GVL responses. As discussed in this review, the challenges in translating these findings to improve the outcome of allogeneic HCT have been substantial. The first report of the adoptive transfer of minor H antigen-specific T-cell clones to patients with leukemia relapse in 2010 illustrates the potential for manipulation of alloreactivity for therapeutic benefit. Concurrent progress in defining the basic requirements for effective adoptive T-cell immunotherapy, such as the transfer of T-cell subsets with a high intrinsic capacity for prolonged survival, and in the use of gene transfer to confer specificity promise to hasten the clinical translation of cellular therapeutics targeting minor H antigens.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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