Hematological malignancies have long been at the forefront of the development of novel immune-based treatment strategies. The earliest successful efforts originated from the extensive body of work in the field of allogeneic hematopoietic stem cell transplantation. These efforts laid the foundation for the recent exciting era of cancer immunotherapy, which includes immune checkpoint blockade, personal neoantigen vaccines, and adoptive T cell transfer. At the heart of the specificity of these novel strategies is the recognition of target antigens presented by malignant cells to T cells. Here, we review the advances in systematic identification of minor histocompatibility antigens and neoantigens arising from personal somatic alterations or recurrent driver mutations. These exciting efforts pave the path for the implementation of personalized combinatorial cancer therapy.
Personal tumor antigens in blood malignancies: genomics-directed identification and targeting

Livius Penter1,2 and Catherine J. Wu1,3,4,5

1Department of Hematology, Oncology, and Tumor Immunology, Charité – Universitätsmedizin Berlin (CVK), Berlin, Germany. 2Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. 3Broad Institute, Massachusetts Institute of Technology and Harvard University, Cambridge, Massachusetts, USA. 4Harvard Medical School, Boston, Massachusetts, USA. 5Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts, USA.

Hematological malignancies have long been at the forefront of the development of novel immune-based treatment strategies. The earliest successful efforts originated from the extensive body of work in the field of allogeneic hematopoietic stem cell transplantation. These efforts laid the foundation for the recent exciting era of cancer immunotherapy, which includes immune checkpoint blockade, personal neoantigen vaccines, and adoptive T cell transfer. At the heart of the specificity of these novel strategies is the recognition of target antigens presented by malignant cells to T cells. Here, we review the advances in systematic identification of minor histocompatibility antigens and neoantigens arising from personal somatic alterations or recurrent driver mutations. These exciting efforts pave the path for the implementation of personalized combinatorial cancer therapy.

In recent years, the clinical successes of immune checkpoint blockade (ICB) have ignited broad enthusiasm for understanding and utilizing the modulation of immune control in order to meaningfully induce cancer control across diverse solid tumors and blood malignancies (1–6). Investigations into the basis of these dramatic immune responses have yielded numerous insights, including the critical contributions of infiltrating T lymphocytes within the tumor microenvironment and the control and expression of negative immunoregulatory checkpoints in tumors and within their milieu (7–9).

Another key insight from these investigations has been the observation of tumor neoantigens as critical targets driving the effective T cell responses associated with these novel therapies (10, 11). The identification of tumor-specific antigens has always been a high priority, since this focuses efforts toward precise immunological targeting. Tumor neoantigens arising from mutations have long been considered potentially optimal tumor antigens given their exquisite tumor-restricted expression and their high level of immunogenicity due to the lack of central tolerance against them (12). However, until next-generation sequencing technologies became available over the past decade, there were considerable challenges to neoantigen identification on a patient-specific basis. The blood malignancies have been consistently at the forefront of targeted cellular therapy and combinatorial immune-based treatment approaches (13). Here, we review the experience of allogeneic hematopoietic stem cell transplantation (HSCT) for the curative treatment of blood malignancies, which has provided the field with the first evidence that the targeting of antigens arising from patient-specific DNA changes could give rise to clinically meaningful immunological responses (14). We describe the range of antigen candidates that have been identified across blood malignancies through genomic analyses and consider how these can be effectively therapeutically targeted using combinatorial approaches (Table 1).

mHAs: early examples of genomically defined immune targets

To a certain extent, the recent demonstrations of human immune responses against tumor neoantigens across diverse malignancies are not surprising, given the backdrop of long-standing studies in the field of HSCT for blood malignancies (15). These studies, performed almost 30 years ago, demonstrated the immunogenicity of minor histocompatibility antigens (mHAs), which arise from the estimated tens of thousands of differences in SNPs present between each donor and recipient pair (16). mHAs have been fundamental to our current understanding of the mechanistic basis of the curative potential of HSCT as well as of the potential source of its toxicities. Indeed, when considering the classes of antigens targeted by engrafted donor immune cells, the curative graft-versus-leukemia (GvL) effect can be conceptualized as the result of donor immune responses against mHAs expressed on hematopoietic tissue, including, but not limited to, epitopes with hematopoietic tissue restriction. Likewise, the pathogenesis of graft-versus-host disease (GvHD) may be understood as donor-derived immune responses directed against mHAs that are broadly expressed across tissues, or at least on GvHD-affected target tissues (Figure 1A).

The first evidence that T cells directed against mHAs could potently eradicate leukemic cells came from in vitro studies of T cells specific for the HLA-A*02:01-restricted HA-1 and HA-2 epitopes and later in a leukemia mouse model treated with HA-1-specific T cells (17, 18). HA-1, a SNP of the gene encoding Rho GTPase-activating protein 45, was initially believed to be a
Table 1. Ongoing trials targeting neoantigens and minor histocompatibility antigens in blood malignancies

<table>
<thead>
<tr>
<th>Approach</th>
<th>Phase/status</th>
<th>Enrollment</th>
<th>Regimen</th>
<th>Disease</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination</td>
<td>Phase II, recruiting</td>
<td>105</td>
<td>DC/AML fusion vaccine vs. observation</td>
<td>AML achieving CTX-induced CR</td>
<td>NCT03059485</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Phase I/II, recruiting</td>
<td>30</td>
<td>Personalized long-peptide neoantigen vaccine + GM-CSF</td>
<td>Children and young adults with primary/relapsed ALL</td>
<td>NCT03559413</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Phase I, not recruiting</td>
<td>10</td>
<td>CALR exon 9 mutant peptide</td>
<td>CALR mutant MPN</td>
<td>NCT03566446</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Phase I, recruiting</td>
<td>30</td>
<td>Personalized long-peptide neoantigen vaccine</td>
<td>Smoldering multiple myeloma</td>
<td>NCT03631043</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Phase I, not yet recruiting</td>
<td>20</td>
<td>Personalized long-peptide neoantigen vaccine (NeoVax)</td>
<td>Grade I–IIIA follicular lymphoma</td>
<td>NCT03361852</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Phase I, not yet recruiting</td>
<td>10</td>
<td>Personalized long-peptide neoantigen vaccine (NeoVax) + cyclophosphamide</td>
<td>CLL IGHV unmutated, asymptomatic, and treatment-naive</td>
<td>NCT03219450</td>
</tr>
<tr>
<td>ICB</td>
<td>Phase II, recruiting</td>
<td>34</td>
<td>Pembrolizumab</td>
<td>MPN</td>
<td>NCT03065400</td>
</tr>
<tr>
<td>ACT</td>
<td>Phase I, recruiting</td>
<td>12</td>
<td>Autologous T cells immunized ex vivo with personal neoantigens (PACTN)</td>
<td>MDS</td>
<td>NCT03258359</td>
</tr>
</tbody>
</table>

Combinatorial approaches

<table>
<thead>
<tr>
<th>Approach</th>
<th>Phase/status</th>
<th>Enrollment</th>
<th>Regimen</th>
<th>Disease</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination + ICB</td>
<td>Phase II, recruiting</td>
<td>25</td>
<td>DC/myeloma fusion vaccine + nivolumab</td>
<td>Relapsed multiple myeloma</td>
<td>NCT03782064</td>
</tr>
<tr>
<td>Vaccination + ICB</td>
<td>Phase I, recruiting</td>
<td>20</td>
<td>Personalized long-peptide neoantigen vaccine (NeoVax) + nivolumab</td>
<td>Follicular lymphoma</td>
<td>NCT03121677</td>
</tr>
<tr>
<td>Vaccination after HSCT</td>
<td>Phase II, recruiting</td>
<td>152</td>
<td>GM-CSF secreting autologous leukemia cell vaccination (GVAX) vs. placebo</td>
<td>AML/advanced MDS after HSCT</td>
<td>NCT01773395</td>
</tr>
<tr>
<td>Vaccination after HSCT</td>
<td>Phase I/II, recruiting</td>
<td>10</td>
<td>mHA-loaded PD-L1/2-silenced DC vaccine</td>
<td>Hematological malignancies after HSCT</td>
<td>NCT02528682</td>
</tr>
<tr>
<td>Vaccination after HSCT</td>
<td>Phase I, recruiting</td>
<td>45</td>
<td>DC/AML fusion vaccine +/− decitabine</td>
<td>AML after HSCT</td>
<td>NCT03679650</td>
</tr>
<tr>
<td>ICB after HSCT</td>
<td>Phase I, recruiting</td>
<td>55</td>
<td>Ipilimumab and/or nivolumab after HSCT</td>
<td>Relapsed or high-risk AML/MDS after HSCT</td>
<td>NCT03600155</td>
</tr>
<tr>
<td>ICB + HMA</td>
<td>Phase I, recruiting</td>
<td>48</td>
<td>Ipilimumab and decitabine after HSCT or transplant-naive</td>
<td>Relapsed/refractory MDS or AML after HSCT and transplant-naive</td>
<td>NCT02890329</td>
</tr>
<tr>
<td>ACT after HSCT</td>
<td>Phase I/II, not recruiting</td>
<td>20</td>
<td>mHA-specific donor-derived T cells (GLIDE 201/44)</td>
<td>Hematological malignancies after HSCT</td>
<td>NCT03091933</td>
</tr>
<tr>
<td>ACT after HSCT</td>
<td>Phase I, recruiting</td>
<td>24</td>
<td>HA1-specific CD8+ and CD4+ donor memory T cells</td>
<td>Relapsed or refractory acute leukemia after HSCT</td>
<td>NCT03326921</td>
</tr>
</tbody>
</table>

CR, complete remission; CTX, chemotherapy; HMA, hypomethylating agent; IGHV, Ig heavy chain gene; PACTN, patient-specific MDS stem cell neoantigens.

Contributing factor for GvHD and was originally identified after purification by HPLC and tandem mass spectrometry from a patient-derived EBV-transformed B cell line (19, 20). Likewise, HA-2 arises from a SNP in the gene MYO1G (encoding myosin 1G); like HA-1, it is involved in cytoskeletal rearrangement (21, 22). Both mHAs have been the focus of extensive efforts aimed at enhancing GvL because their tissue distribution is restricted to hematopoietic tissue (23). HA-1 and HA-2 differ in MHC binding affinity and in their recognition by T cells compared with their nonimmunogenic variants, which explains why disparity between donor and recipient at these loci mediates GvL effects (24).

Larger retrospective studies have evaluated the association of HA-1 disparity between donor and recipient with clinical outcome: in a cohort of 285 chronic myelogenous leukemia (CML) patients, HA-1 disparity in the presence of acute GvHD correlated favorably with regard to overall survival, relapse-free survival, and risk of relapse (25). Similarly, a multicenter analysis of 849 patients after HSCT across different malignancies demonstrated that mismatch for 10 different mHAs and occurrence of GvHD reduced the likelihood of relapse and increased relapse-free survival as well as overall survival (26).

Given its immunogenicity, various efforts have explored the potential of cellular therapies to target HA-1. Notably, this approach has the potential to be clinically impactful, since 25% of White patients express both HA-1 and HLA-A*02:01. One such early example explored the effects of administering donor lymphocyte infusions (DLIs) in the setting of HA-1 and/or HA-2 incompatibility for treatment of post-HSCT disease relapse. Three such patients, two with CML and one with multiple myeloma (MM), achieved complete donor chimerism and remission following cell infusion (27). Dossa et al. proposed an off-the-shelf approach for targeting mHAs by developing an HA-1–specific HLA-A*02:01–defined T cell receptor (TCR) for adoptive T cell transfer (ACT) (28).

A growing list of other candidate mHAs with expression limited to hematopoietic tissue has been identified (Figure 2 and Table 2). As an example, Akatsuka et al. identified variants of the BCL2A1 gene restricted by HLA-A*24:02 (29). A variant of PANE1 (HLA-A*03:01) was found to be selectively expressed on resting CD19+ B cells and B chronic lymphocytic leukemia (B-CLL) cells and therefore a potential therapeutic target for B cell malignancies (30). As another example, an HLA-B*44–restricted epitope of HB-I, selectively expressed on transformed B cells, was identified in a patient with B cell acute lymphoblastic leukemia (B-ALL) following HSCT, in which HB-I–specific T cells recognized EBV-transformed B cells and B-ALL blasts (31).
To expand mHA-specific T cells and target recipient cells, vaccination strategies have been devised. For example, donor-derived DC vaccines pulsed with mHA peptides of LRH-1, UTA2-1, and HA-1 could induce specific T cell responses in patients with MM (32). To improve the efficacy of mHA-targeting DC vaccines, Hobo et al. developed siRNAs for the in vitro knockdown of the checkpoint ligands PD-L1 and PD-L2, and found that this strategy increased DC-induced mHA-specific T cell expansion (33). A phase I/II trial is currently testing this approach (NCT02528682; ClinicalTrials.gov). Another promising concept has explored the use of an HA-1 vaccine to induce HA-1-specific T cells in HA-1 donors, from whom a vaccine-augmented DLI product targeting mHAs could then be apheresed (34).
Tumor neoantigens: optimal tumor antigen targets

Neoantigens are novel peptides derived from somatic mutations in malignant cells. Conceptually, they represent ideal tumor antigen targets because of their tumor-restricted expression, hence providing the potential to trigger only disease-specific immune responses without the risk of targeting normal tissues (Figure 1C). Neoantigen-specific T cell responses may be part of physiological immune surveillance and may underlie normal strategies to augment immunological tumor control (38). Unlike native proteins overexpressed on malignant cells (e.g., WT1 or survivin), or cancer/testis antigens (e.g., MAGE1, PRAME, or NY-ESO-1) that are only expressed on immune-privileged germ cells, neoantigens are not presented in normal tissue and are therefore not subject to central T cell tolerance (39).

The current extensive investigations into tumor neoantigens in the field of cancer immunotherapy have been preceded by a large body of early anecdotal reports supporting the notion that tumor neoantigens are clinically relevant targets of effective anti-tumor immunity (40–44). However, only with the availability of modern-day sequencing technologies to comprehensively detect the somatic mutations present in primary human cancer specimens and improved epitope prediction, through neural network-based algorithms, has systematic identification of tumor neoantigens become widely possible (Figure 1D). Early work using these modern tools to identify tumor neoepitopes was achieved in a study of resistance mutations to imatinib in the driver BCR-ABL in patients with CML. Cai et al. used in silico epitope prediction methods to screen for immunogenic neoepitopes arising from 26 previously described BCR-ABL resistance mutations identified by targeted sequencing, and demonstrated strong T cell responses against these predicted targets in vitro, including strong responses against these predicted targets in vitro, including strong responses...
arising following effective HSCT (45). Extending the concept that neoantigen-specific antitumor T cell responses could be discovered in the setting of effective tumor immunity in blood malignancies, Rajasagi et al. used systematic evaluation of private somatic mutation profiles of 91 CLL samples, identified by whole-exome sequencing (46). They showed the feasibility of consistently predicting immunogenic epitopes arising from missense mutations in CLL and traced the sustained persistence of circulating T cells with specificity for personal neoantigens in long-term survivors following HSCT.

Targeting driver mutation–derived neoantigens in blood malignancies

Although passenger mutations represent more than 90% of mutation load per cancer (47, 48) and have the potential to be immunogenic (46, 49, 50), the targeting of driver mutations is a highly strategic approach that reduces the likelihood of immune escape, as these events are critical to the fitness and survival of malignant cells. Many examples of this class of targets in blood malignancies hold great therapeutic promise (Figure 2 and Table 2).

### Table 2. Examples from the different classes of personal antigen targets in blood malignancies

<table>
<thead>
<tr>
<th>Class</th>
<th>Disease</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mHAs (A)</td>
<td>MDS, leukemia, MM</td>
<td>HA-1*, HA-2*</td>
<td>27*, 28, 35*</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>HEATR, GRK4</td>
<td>37, 176</td>
</tr>
<tr>
<td></td>
<td>CLL</td>
<td>PAN1</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>CLL, EBV-associated B cell malignancies</td>
<td>HB-1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Hematological malignancies</td>
<td>BCL2A</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LB-ARHGDI{1R}</td>
<td>177, 178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LB-ITG{21}</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMSD, UTA{21}</td>
<td>32, 180, 181</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LRH-1*</td>
<td>32*, 182</td>
</tr>
<tr>
<td>Somatic mutations (B)</td>
<td>CLL</td>
<td>MGA*</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>NPM1*</td>
<td>54, 55</td>
</tr>
<tr>
<td></td>
<td>MPN</td>
<td>CALR*</td>
<td>61, 63, 64, 66*</td>
</tr>
<tr>
<td></td>
<td>B-NHL</td>
<td>MYB81, E2H2*</td>
<td>73, 74</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>CREBBP{485}, MEF2B{183}</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Hematological malignancies</td>
<td>KRAS*</td>
<td>138*, 139</td>
</tr>
<tr>
<td>Gene fusion (C)</td>
<td>CML and ALL</td>
<td>BCR-ABL*</td>
<td>45, 56, 57*, 87*, 88*, 89*, 90*</td>
</tr>
<tr>
<td></td>
<td>ALL</td>
<td>ETV6-RUNX1</td>
<td>86, 91, 92</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>CBFB-MYH11</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PML-RARα</td>
<td>84</td>
</tr>
<tr>
<td>Posttranslational modifications (D)</td>
<td>Phosphopeptides</td>
<td>AML and CLL</td>
<td>LSP1, NCOA-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML</td>
<td>MLL</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>T cell leukemia, CML, MM</td>
<td>Mucin-1</td>
<td>105, 106</td>
</tr>
<tr>
<td></td>
<td>ALL</td>
<td>RNA-binding protein</td>
<td>102</td>
</tr>
<tr>
<td>Alternative splicing (E)</td>
<td>CML and ALL</td>
<td>BCR-ABL</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>B cell malignancies</td>
<td>CD20</td>
<td>98</td>
</tr>
<tr>
<td>Hypervariable Ig regions (F)</td>
<td>MCL, FL, CLL, and DLBCL</td>
<td>IGHV and IGLV</td>
<td>110–112</td>
</tr>
<tr>
<td></td>
<td>FL, MM</td>
<td>Idiotype*</td>
<td>107*, 108*, 109*, 150*</td>
</tr>
</tbody>
</table>

Each class corresponds to mechanistic diagrams in Figure 2, A–F. Asterisks indicate targets that have been clinically tested, along with the respective references. IGLV, Ig light chain variable region gene; MCL, mantle cell lymphoma.

### Acute myeloid leukemia

Approximately 30% of acute myeloid leukemia (AML) patients harbor founding mutations in nucleophosmin (NPM1), making it the most commonly altered gene in this disease (51). NPM1mut gives rise to a 4-bp frameshift mutation in exon 12 with an alternative reading frame at the C-terminus, leading to altered cytoplasmic localization. Two HLA-A*02:01–restricted NPM1mut neoepitopes were first reported to generate clinically relevant T cell responses in an AML patient with molecular relapse who received DLI and subsequently achieved molecular remission (52). In an evaluation of 25 patients, patients displaying NPM1mut-specific T cell responses against these epitopes had superior survival compared with those without (53). Forghieri et al. tracked spontaneous appearance and persistence of NPM1mut-specific T cells in 31 AML patients, and 4 of 5 patients without NPM1mut-specific T cells relapsed eventually (54). As preclinical studies to develop ACT against NPM1mut, van der Lee et al. transduced an HLA-A*02:01 TCR specific for NPM1mut into T cells from healthy donors. These transgenic T cells showed in vitro activity against AML blasts and in a leukemia mouse model (55).
To therapeutically exploit BCR-ABL–specific T cells, Comoli et al. reported the ex vivo expansion of autologous or allogeneic T cells using DCs pulsed with BCR-ABL p190 peptides. Three heavily pretreated Ph+ ALL patients with relapsed disease achieved durable molecular or hematological remission after infusion of such expanded BCR-ABL–specific T cells in combination with tyrosine kinase inhibitors (57), providing demonstration of the activity of such an approach.

Chronic lymphocytic leukemia. Hu, Anandappa, et al. (58) predicted immunogenic mutations in MGA, a known driver recurrently mutated in high-risk CLL (59). HLA-A*02:01 T cells specific for MGA mut could be isolated from healthy donors. A TCR was identified that selectively recognizes mutated MGA, thereby offering a potential basis for a T cell–based therapy directed at MGA mut (58).

Myeloproliferative neoplasms. Myeloproliferative neoplasms (MPNs) often harbor immunogenic driver mutations such as BCR-ABL, JAK2V617F, mutated calreticulin (CALRmut), or MPLW515K/L/A. HSCT has been clinically successful in many JAK2V617F–mutated patients (60). CD8+ T cells with higher binding affinity for JAK2V617F than for JAK2WT, which preferentially lyse cells homozygous for JAK2V617F, have been identified from healthy donors (61). Notably, JAK2V617F–mutated cells in patients with MPNs have been found to express increased levels of PD-L1, suggesting a potential synergy of a T cell–based approach against JAK2V617F in combination with PD-1 blockade (62).

Mutated calreticulin is a driver mutation in 30% of patients with JAK2WT essential thrombocythemia and primary myelofibrosis. Calreticulin exon 9 mutations (CALR mut) have been characterized as 1-bp frameshift mutations that impair peptide loading to MHC I and give rise to HLA II–restricted immunogenic neoepitopes that can be targeted by cytotoxic CD4+ T cells (63–65). Cimen Bozkus et al. demonstrated that CALRmut-specific T cells have increased immune checkpoint expression, thus providing a rationale for PD-1 inhibition in this disease setting, currently under investigation in a phase II trial probing pembrolizumab in advanced MPNs including CALR WT and CALR mut patients (NCT03065400) (66). As for MPL, while up to 17 neoepitopes arising from the W515K/L/A mutation have been predicted (67), it has not yet been demonstrated whether these are truly immunogenic. However, this will be crucial given that Tubb et al. failed to detect processing or presentation of a number of putative HLA I–restricted CALR mut neoepitopes (68).

Non-Hodgkin lymphoma. Among B cell non-Hodgkin lymphomas (B-NHLs), the driver mutations in MYD88 and EZH2 (in diffuse large B cell lymphoma [DLBCL], Waldenström’s macroglobulinemia, follicular lymphoma [FL]) have been pre-
dicted to generate neoepitopes (69–72). Nielsen et al. identified T cells specific for MYD88L265P and EZH2L641W with preferential binding affinity for the mutated protein. However, these T cells have a low prevalence among healthy individuals and were not detectable in two patients with MYD88L265P-mutated lymphoma, suggesting that absence of neoepitope-specific T cell responses may contribute to lymphomagenesis (73). Nelde et al. similarly detected T cell responses against MYD88L265P in only 1 of 22 patients with MYD88L265P-mutated lymphoma. In contrast, T cells specific for MYD88L265P could be induced in vitro using naïve T cells obtained from healthy donors or from one patient with CLL (74). For FL, the immunogenicity of the putative driver mutations CREBBP and MEF2B has been evaluated. Nielsen et al. found mutation-specific T cells in 3 of 13 FL patients at low frequencies in peripheral blood that could be expanded in vitro (75). Taken together, T cell immunity against driver mutations in B-NHL is inducible in some patients, suggesting a window of opportunity for T cell–based immunotherapies.

**Classes of neoantigens not derived from somatic mutations**

In light of the therapeutic success of immune-based therapies in blood malignancies and their low mutational burden, other groups of antigens likely play a central role. This is illustrated by mass spectrometry–based analyses of the HLA ligandome in MM, AML, and CLL, which have identified disease-specific nonmutated peptides as targets of T cell responses (76–78). Models and examples of these targets are given in Figure 2 and Table 2.

**Gene fusions.** Gene fusions have the potential to give rise to immunogenic neoepitopes, as has been recently demonstrated in head and neck cancers (79). Gene fusions often arise from chromosomal translocations and are a hallmark of hematological malignancies (51, 80–83). Although immunogenic neoantigens arising from gene fusions have long been described in hematological neoplasms (45, 84–86), only BCR-ABL has been targeted therapeutically using vaccination approaches, which were able to induce specific T cell responses (87–90). Efforts to develop a T cell–based therapy directed at a particular neoepitope arising from ETV6-RUNXI, the most common fusion gene in childhood B-ALL, were stopped because of a lack of endogenous processing (91). Recently, more immunogenic neoantigens deriving from ETV6-RUNXI have been uncovered (92, 93). Given the central role of gene fusions in the pathogenesis of hematological malignancies, other neoepitopes from this group are likely candidates as therapeutic targets.

**Alternative splicing.** Alternative splicing can lead to entirely novel and disease-specific immunogenic neojunctions found in many cancer entities (94). Since alternative splicing is common among blood malignancies, neoantigens arising from neojunctions may harbor great therapeutic potential (95, 96). The first evidence for neoantigens deriving from alternative splicing was observed in the setting of CML. T cells specific for alternative splice variants of BCR-ABL obtained from CML patients were able to lyse blasts (97). In many B cell lymphomas, a splice variant of CD20 is commonly expressed and T cell responses against CD20D393 are detectable in patients. In a mouse model, Vauchy et al. could induce CD20D393–specific T cells with a vaccination approach. CD20D393 is not found in B cells of healthy individuals and therefore is a promising candidate as a therapeutic target (98).

**Posttranslational modifications.** Aberrant protein phosphorylation leading to novel phosphopeptides and rewired cell signaling is a fundamental mechanism in blood malignancies and the basis for kinase inhibitors such as imatinib in CML or midostaurin in AML (99, 100). Cobbold et al. reported that aberrantly phosphorylated proteins may be immunogenic and can give rise to neoantigens. T cell responses for 95 tumor-specific phosphopeptides were present in healthy individuals, but were reduced in patients with hematological malignancies, hinting at the possibility that phosphopeptide-derived neoantigens play a role in tumor immune surveillance. Consistent with this observation, in 12 patients with AML after HSCT, the reconstituted donor-derived T cell responses against phosphopeptides were increased (101).

**Glycopeptides.** Glycopeptides are proteins characterized by β O-linked N-acetylgalactosamine (O-GlcNAc). Malaker et al. used mass spectrometry to identify 36 O-GlcNAc–modified peptides as candidate neoantigens in primary leukemia samples. T cell responses against these glycopeptides, like those against phosphopeptides, were detectable in healthy donors. T cells selectively lysed cells that presented the O-GlcNAc–modified peptides (102). Mucin-1 is aberrantly glycosylated in solid tumors and hematological malignancies such as MM (103, 104). Chimeric antigen receptor (CAR) T cells targeting glycosylated mucin-1 have been developed that specifically kill malignant cells in experimental leukemia models (105, 106).

**Ig rearrangements.** In B-NHL, neoantigens may arise from productive rearrangement and somatic hypermutation within Ig genes, which may induce specific T cell responses against malignant B cells. Despite this promise, three different phase III trials of disease-specific idiotype (Id) vaccination in FL revealed only modest clinical activity (107–109). Subgroup analyses demonstrated increased progression-free or disease-free survival in patients who received IgM-Id instead of IgG-Id vaccines (107) or displayed an increase of idiotype-specific antibody titers (109).

Idiotype-specific CD4+ T cells able to selectively lyse tumor cells have been isolated from peripheral blood of patients across different B cell malignancies (110). Khodadoust et al. demonstrated that MHC II–restricted presentation of neoantigens arising from Ig rearrangement is common in mantle cell lymphoma. Interestingly, neoantigens from nonsynonymous mutation were not identified in this cohort of 17 patients, possibly reflecting immune editing and low mutation burden in this disease (111). In an analogous fashion, Ig neoantigens were shown to be presented mainly by MHC II in FL, DLBCL, and CLL (112).

**Approaches for targeting mHAs and neoantigens therapeutically**

Given that we are now able to systematically predict or identify personal mHAs or tumor antigen targets, diverse avenues for using this information to rationally design therapy tailored to the individual become feasible. In addition to ICB and HSCT, which are broadly immunomodulatory approaches but not highly targeted to specific epitopes, this can be achieved through antigen-specific approaches such as vaccination or by ACT (Figure 3).

**ICB.** The recent clinical availability and potency of ICB agents for the treatment of diverse cancers, and now FDA approvals
across various indications, have been transformative for the field of cancer immunotherapy (113). Numerous studies in the solid-tumor malignancies have revealed the role of neoantigens as targets of responses achieved in diseases harboring high mutational load (11, 114–116). In the blood malignancies, the responses to IC Figure 5. Adoptive T cell transfer. ACT directly provides high quantities of functional T cells at aiming eliminating malignant cells. This approach relies on T cells specific for targets expressed selectively on malignant cells. The dramatic successes of CART cells directed against CD19 for the B cell malignancies (130), and now against B cell maturation antigen–expressing (BCMA-expressing) MM (131, 132), provide clear demonstration of the cytotoxic potency of T cells when they are linked to tumor-expressed antigens. CART cells act independently of HLA and may be further optimized with costimulatory receptors. However, thus far, their in vivo persistence is limited, remissions are short-lived as a result of antigen downregulation, and on-target toxicities have been common (133). As a promising alternative approach to targeting tumor-expressed antigens, Chapuis et al. expanded allogeneic CD8+ T cells specific for WT-1. In 4 of 11 advanced cases of acute leukemia, durable complete remissions were achieved that correlated with long-term persistence of WT-1–specific T cells (134). Remarkably, in 12 high-risk patients, no relapse was observed 44 months after prophylactic infusion of WT-1–specific T cells after HSCT (135).

Personalized ACT against neoantigen or mHA targets has been proposed and developed either as antigen-specific cells expanded from tumor-infiltrating lymphocytes (TILs) or as T cells engineered to express neoantigen/mHA-specific TCRs. Examples of the former include ACT targeting neoantigens in melanoma (136) and single cases of cholangiocarcinoma (137) or colorectal cancer (138). An ongoing phase I trial in MDS is testing the effects of autologous T cells that are reinfe Figure 4. Personalized ACT against neoantigen or mHA targets has been proposed and developed either as antigen-specific cells expanded from tumor-infiltrating lymphocytes (TILs) or as T cells engineered to express neoantigen/mHA-specific TCRs. Examples of the former include ACT targeting neoantigens in melanoma (136) and single cases of cholangiocarcinoma (137) or colorectal cancer (138). An ongoing phase I trial in MDS is testing the effects of autologous T cells that are reinfe Figure 4. Personalized ACT against neoantigen or mHA targets has been proposed and developed either as antigen-specific cells expanded from tumor-infiltrating lymphocytes (TILs) or as T cells engineered to express neoantigen/mHA-specific TCRs. Examples of the former include ACT targeting neoantigens in melanoma (136) and single cases of cholangiocarcinoma (137) or colorectal cancer (138). An ongoing phase I trial in MDS is testing the effects of autologous T cells that are reinfe Figure 4. Personalized ACT against neoantigen or mHA targets has been proposed and developed either as antigen-specific cells expanded from tumor-infiltrating lymphocytes (TILs) or as T cells engineered to express neoantigen/mHA-specific TCRs. Examples of the former include ACT targeting neoantigens in melanoma (136) and single cases of cholangiocarcinoma (137) or colorectal cancer (138). An ongoing phase I trial in MDS is testing the effects of autologous T cells that are reinfe Figure 4. Personalized ACT against neoantigen or mHA targets has been proposed and developed either as antigen-specific cells expanded from tumor-infiltrating lymphocytes (TILs) or as T cells engineered to express neoantigen/mHA-specific TCRs. Examples of the former include ACT targeting neoantigens in melanoma (136) and single cases of cholangiocarcinoma (137) or colorectal cancer (138). An ongoing phase I trial in MDS is testing the effects of autologous T cells that are reinfe
The post-transplant setting has long been recognized as an advantageous platform for immunotherapy, insofar as donor immune reconstitution overcomes host immunosuppression and can favorably reprogram the immune microenvironment (Figure 3B). The concept that donor-derived leukemia-specific T cells could be generated by HSCT but that transcriptional signatures of T cell exhaustion were present in marrow-infiltrating T cells in the setting of leukemic relapse was demonstrated in studies of patients with CML following HSCT. Furthermore, this phenotype could be reversed in association with effective DLI therapy (144). This work naturally sets the stage for combining HSCT with ICB therapy. As mentioned above, the combination of CTLA-4 blockade with HSCT to effectively treat AML relapse has provided a notable example of responsiveness of hematological malignancies to ICB (120). Ongoing follow-up studies are now aimed at testing ipilimumab in combination with decitabine (NCT02890329) or nivolumab (NCT03600155) for relapsed AML following HSCT. On the other hand, varying rates of excess GvHD-associated toxicity in the same setting point to mechanistic differences among ICB agents and the impact of parameters such as dosage, previous history of GvHD, or time post-HSCT (145, 146).

The early post-transplant setting, with host lymphodepletion and the presence of a favorable homeostatic cytokine milieu for T cell expansion, has been likewise thought to provide an opportunity window for vaccination to induce donor-derived tumor-specific T cells, and thereby enhance GvL (147). Burkhardt et al. observed increased CD8+ T cell reactivity against CLL-associated antigens and effector cytokine production in 18 patients with advanced CLL after challenge with autologous GVAX administered within the first 4 months after allogeneic HSCT (148). Ho et al. similarly detected tumor-specific immune responses in a pilot study of GVAX after HSCT for patients with advanced AML or MDS, now expanded to a randomized phase II follow-up trial (NCT01773395) (149). With the ability to predict neoantigens and mHAs, one could likewise envision the feasibility of developing vaccines targeting these specific antigens following HSCT. As an alternative approach to boosting donor-derived tumor responses through vaccination with HSCT, Foglietta et al. tested the concept of pre-HSCT donor vaccination. Ten HLA-matched sibling donors received recipient-derived MM idiotype vaccines before collection of allografts, and demonstrated that idiotype-specific immune responses can be induced in the donor and transferred into the recipient (150).

In the absence of HSCT, vaccines have been recognized as important adjuncts to ICB, given their ability to induce de novo naive T cell responses, amplify memory T cell responses, and broaden the diversity of antitumor T cells. Indeed, preclinical data have shown the synergistic effects of a DC/myeloma fusion vaccine and PD-1 inhibition (151), with testing of this approach currently under way in a phase II trial (NCT03782064). Given the promising clinical responses to the combination of personal neoantigen vaccination with anti-PD-1 therapy described in a few patients with high-risk melanoma (49, 125), this combination is now being formally tested in a series of clinical trials (NCT02897765, NCT03289962). Early results of these studies have indicated the detection of neoantigen-specific T cell responses beyond the epitopes provided by the neoantigen vaccine, consistent with off-tumor targeting by the therapy (152). The concept of combining neoantigen vaccines with PD-1 inhibition is now under investigation for patients with FL (NCT03121677).

Suboptimal responses to ACT have been linked to exhaustion of effector cells and their inability to expand in vivo, which may be overcome by combination with ICB or vaccination (153, 154). These investigations are active, though still in their infancy. For example, the administration of anti–PD-1 therapy was able to induce clinical responses in 3 of 9 DLBCL and 2 of 4 B-ALL patients refractory or progressive after CAR T cell therapy (155–157). Successful efforts in melanoma combining ACT with vaccinations could be a model for approaches in blood malignancies (158, 159).

Outlook

Personal antigen-directed therapeutic approaches have come a long way since the early days of HSCT and promise to remain a driving force for progress in hematology. The recent breathtaking technological advances have opened doors for a systematic understanding of target antigens (46, 50, 58), the identities and characteristics of subpopulations of TILs (160–162), and immunological aspects of disease biology (163).

In addition to deeper mechanistic investigation and clinical studies about effective combinatorial immunotherapy, we can expect further exciting developments in the realms of antigen discovery and the engineering of immunotherapy. Neoantigen detection pipelines provide novel candidate target antigens and therefore the opportunity to link TILs to their cognate TCRs (164–166). Technologies such as single–T cell paired TCRβ sequencing (167, 168), mass cytometry, or FACS index sorting (169–171) can provide deeper complex understanding of TIL biology and aid in developing fresh therapeutic strategies directed at candidate target antigens. Using these advances, we are now also able to trace the coevolution of hematological malignancies and their host immune system (172). Likewise, the development of CRISPR/Cas9 gene editing (173), the discovery efforts with genome-wide screens (174), and developments in the area of spatial tissue-based characterization (175) will have important implications for the delivery of novel targets and subsequent engineering of immune responses.

Acknowledgments

This work was supported by grants from the NIH, National Cancer Institute (U10CA180861-01, 1R01CA155501, and P01CA229092, to CJW). CJW is a Scholar of the Leukemia and Lymphoma Society. We acknowledge support from the Fishman Family Fund. LP is supported by a research fellowship from the German Research Foundation (DFG, PE 3127/1-1).

Address correspondence to: Catherine J. Wu, Department of Medical Oncology, Dana-Farber Cancer Institute, Dana 520C, 44 Binney Street, Boston, Massachusetts 02115, USA. Phone: 617.632.5943; Email: cwu@partners.org.

41. Forghieri F, et al. Characterization and dynamics of specific T cells against nucleosomine-1


REVIEW SERIES: IMMUNOTHERAPY IN HEMATOLOGICAL CANCERS


