Personal tumor antigens in blood malignancies: genomics-directed identification and targeting

Livius Penter[1,2] and Catherine J. Wu[2,3,4,5]

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

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Table 1. Ongoing trials targeting neoantigens and minor histocompatibility antigens in blood malignancies

Approach	Phase/status	Enrollment	Regimen	Disease	ClinicalTrials.gov identifier
Approaches based on single-treatment modalities					
Vaccination	Phase II, recruiting	105	DC/AML fusion vaccine vs. observation	AML achieving CTX-induced CR	NCT03059485
Vaccination	Phase I/II, recruiting	30	Personalized long-peptide neoantigen vaccine + GM-CSF	Children and young adults with primary/relapsed ALL	NCT03559413
Vaccination	Phase I, not recruiting	10	CALR exon 9 mutant peptide	CALR mutant MPN	NCT03566446
Vaccination	Phase I, recruiting	30	Personalized long-peptide neoantigen vaccine	Smoldering multiple myeloma	NCT03631043
Vaccination	Phase I, not yet recruiting	20	Personalized long-peptide neoantigen cancer vaccine (NeoVax)	Grade I–IIIA follicular lymphoma	NCT03361852
Vaccination	Phase I, not yet recruiting	10	Personalized long-peptide neoantigen vaccine (NeoVax) +/– cyclophosphamide	CLL IGHV unmutated, asymptomatic, and treatment-naive	NCT03219450
ICB	Phase II, recruiting	34	Pembrolizumab	MPN	NCT03065400
ACT	Phase I, recruiting	12	Autologous T cells immunized ex vivo with personal neoantigens (PACTN)	MDS	NCT03258359
Combinatorial approaches					
Vaccination + ICB	Phase II, recruiting	25	DC/myeloma fusion vaccine + nivolumab	Relapsed multiple myeloma	NCT03782064
Vaccination + ICB	Phase I, recruiting	20	Personalized long-peptide neoantigen cancer vaccine (NeoVax) + nivolumab	Follicular lymphoma	NCT03121677
Vaccination after HSCT	Phase II, recruiting	152	GM-CSF secreting autologous leukemia cell vaccination (GVAX) vs. placebo	AML/advanced MDS after HSCT	NCT01773395
Vaccination after HSCT	Phase I/II, recruiting	10	mHA-loaded PD-L1/PD-L2–silenced DC vaccine	Hematological malignancies after HSCT	NCT02528682
Vaccination after HSCT	Phase I, recruiting	45	DC/AML fusion vaccine +/– decitabine	AML after HSCT	NCT03679650
ICB after HSCT	Phase I, recruiting	55	Ipilimumab and/or nivolumab after HSCT	Relapsed or high-risk AML/MDS after HSCT	NCT03600155
ICB + HMA	Phase I, recruiting	48	Ipilimumab and decitabine after HSCT or without HSCT	Relapsed/refractory MDS or AML after HSCT and transplant-naive	NCT02890329
ACT after HSCT	Phase I/II, not recruiting	20	mHA-specific donor-derived T cells (GLIDE 201/44)	Hematological malignancies after HSCT	NCT03091933
ACT after HSCT	Phase I, recruiting	24	HA-1–specific $CD8^+$ and $CD4^+$ donor memory T cells	Relapsed or refractory acute leukemia after HSCT	NCT03326921

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-1*, selectively expressed on transformed B cells, was identified in a patient with B cell acute lymphoblastic leukemia (B-ALL) following HSCT, in which HB-1–specific T cells recognized EBV-transformed B cells and B-ALL blasts (31).

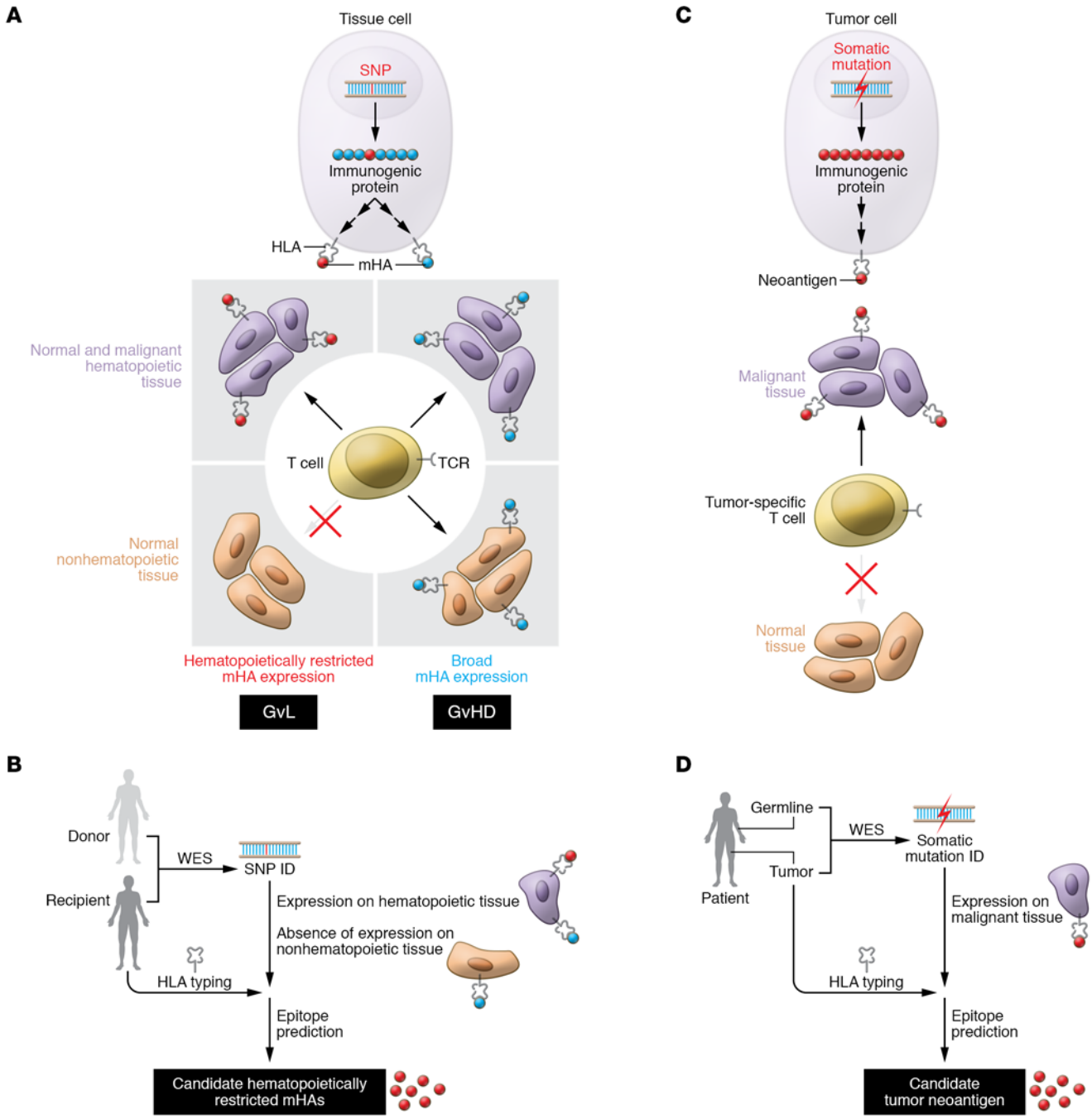

Figure 1. Hematopoietic-restricted mHAs and tumor neoantigens. (**A**) Differences in SNPs between donor and recipient that give rise to immunogenic epitopes are the basis of mHAs in the context of allogeneic HSCT. While mHAs with hematopoietic tissue restriction are targets for GvL effects, mHAs that are broadly expressed serve as basis for GvHD. (**B**) Identification of therapeutically relevant mHAs is based on epitope prediction of SNPs and selection of hematopoietically restricted candidates. (**C**) Tumor-specific neoantigens arise from somatic mutations in the tumor that are immunogenic. Neoantigens are only expressed by tumor cells and therefore are ideal targets for highly specific cellular therapeutic approaches. (**D**) Identification of neoantigens is based on epitope prediction of immunogenic mutations.

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).

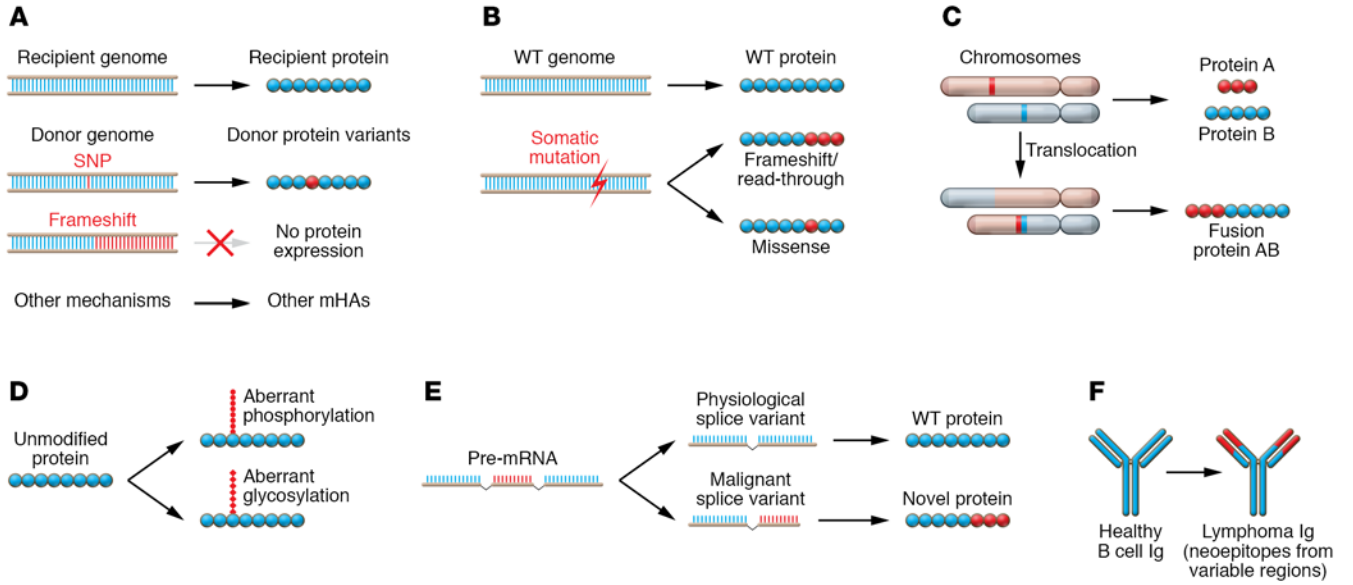

Figure 2. Classes of personal antigen targets in blood malignancies. (**A**) Minor histocompatibility antigens often arise due to SNPs, resulting in differing physiological protein variants between the donor and the host of allogeneic HSCT. Other mechanisms giving rise to mHAs, such as frameshift mutations, have also been described and are reviewed extensively elsewhere (183). (**B** and **C**) Somatic mutations (**B**) and gene fusions due to chromosomal aberrations (**C**) give rise to physiologically nonexistent, tumor-specific protein variants. (**D**) Aberrant posttranslational modifications add neoepitopes to physiological proteins. (**E**) Alternative splicing results in neojunctions due to altered posttranscriptional processes in tumor cells. (**F**) Hypervariable Ig regions can be immunogenic, disease-specific epitopes in B cell malignancies.

In recognition of the unique set of mHAs per donor-recipient pair based on the patient's genetics and HLA expression, Warren et al. prospectively isolated mHA-specific $CD8^+$ T cells personal to each recipient by coculturing donor-derived T cells obtained after HSCT with recipient PBMCs and selecting $CD8^+$ clones with cytolytic activity against recipient hematopoietic cells, but not against recipient fibroblasts or donor hematopoietic cells. Five of seven patients who relapsed after HSCT achieved complete remission after receiving salvage chemotherapy and mHA-specific T cells; notably, in three cases, remission occurred only after infusion of mHA-specific T cells (35). Ultimately, however, all patients relapsed, which was attributed to the short persistence observed of the transferred T cells. In some cases, high-grade GvHD was observed, consistent with the expression of certain targeted mHAs not restricted to hematopoietic tissue.

Moving beyond the classical labor-intensive discovery methods, Granados et al. reported 39 novel candidate mHAs with expression restricted to hematopoietic tissue, identified through proteomic screening of 13 healthy donors and the mining of publicly available RNA-Seq tissue expression data of bone marrow and skin tissue to ascertain hematopoietic restriction (36). Donor-derived T cells primed against these candidate mHAs are undergoing testing in an ongoing phase I/II clinical trial (NCT03091933). As an alternative approach, Lansford et al. predicted 102 novel leukemia-associated mHAs based on the analysis of SNP alleles identified from a cohort of 101 donor-recipient pairs that were only present in recipients with high predicted HLA-binding affinity and expression restricted to hematopoietic tissues (Figure 1B). Altogether, this approach provides a systematic strategy to identify candidate mHAs as targets for personal vaccination or ACT approaches following HSCT (37).

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 broadly 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

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

	Disease	Examples	References
mHAs (A)	MDS, leukemia, MM	HA-1*, HA-2*	27*, 28, 35*
	AML	HEATR, GRK4	37, 176
	CLL	PANE1	30
	CLL, EBV-associated B cell malignancies	HB-1	31
	Hematological malignancies	BCL2A	29
		LB-ARHGDIB-1R	177, 178
		LB-ITGB2-1	179
		HMSD, UTA2-1*	32, 180, 181
		LRH-1*	32*, 182
Somatic mutations (B)	CLL	MGA^{mut}	58
	AML	$NPM1^{mut}$	54, 55
	MPN	$CALR^{mut}$*, $JAK2^{V617F}$	61, 63, 64, 66*
	B-NHL	$MYD88^{L265P}$, $EZH2^{mut}$	73, 74
	FL	$CREBBP^{L145H}$, $MEF2B^{D83A}$	75
	Hematological malignancies	$KRAS^{G12D}$*, $TP53^{R175H}$	138*, 139
Gene fusion (C)	CML and ALL	BCR-ABL*	45, 56, 57*, 87*, 88*, 89*, 90*
	ALL	ETV6-RUNX1	86, 91, 92
	AML	CBFB-MYH11	85
		PML-RARα	84
Posttranslational modifications (D)			
Phosphopeptides	AML and CLL	LSP1, NCOA-1	101
	AML	MLL	101
Glycopeptides	T cell leukemia, CML, MM	Mucin-1	105, 106
	ALL	RNA-binding protein 27	102
Alternative splicing (E)	CML and ALL	BCR-ABL	97
	B cell malignancies	CD20	98
Hypervariable Ig regions (F)	MCL, FL, CLL, and DLBCL	IGHV and IGLV	110–112
	FL, MM	Idiotype*	107*, 108*, 109*, 150*

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.

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).

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). $NPM1^{mut}$ 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 $NPM1^{mut}$ 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 $NPM1^{mut}$-specific T cell responses against these epitopes had superior survival compared with those without (53). Forghieri et al. tracked spontaneous appearance and persistence of $NPM1^{mut}$-specific T cells in 31 AML patients, and 4 of 5 patients without $NPM1^{mut}$-specific T cells relapsed eventually (54). As preclinical studies to develop ACT against $NPM1^{mut}$, van der Lee et al. transduced an HLA-A*02:01 TCR specific for $NPM1^{mut}$ into T cells from healthy donors. These transgenic T cells showed in vitro activity against AML blasts and in a leukemia mouse model (55).

Acute lymphoblastic leukemia. Like patients with CML, patients with Philadelphia chromosome–positive (Ph^+) ALL have detectable T cells with specificity for BCR-ABL. In a long-term follow-up study, Riva et al. highlighted the role of T cell–mediated tumor surveillance by demonstrating an inverse correlation between minimal residual disease levels and T cell activity against BCR-ABL. Patients who relapsed lost BCR-ABL–specific T cell immunity (56).

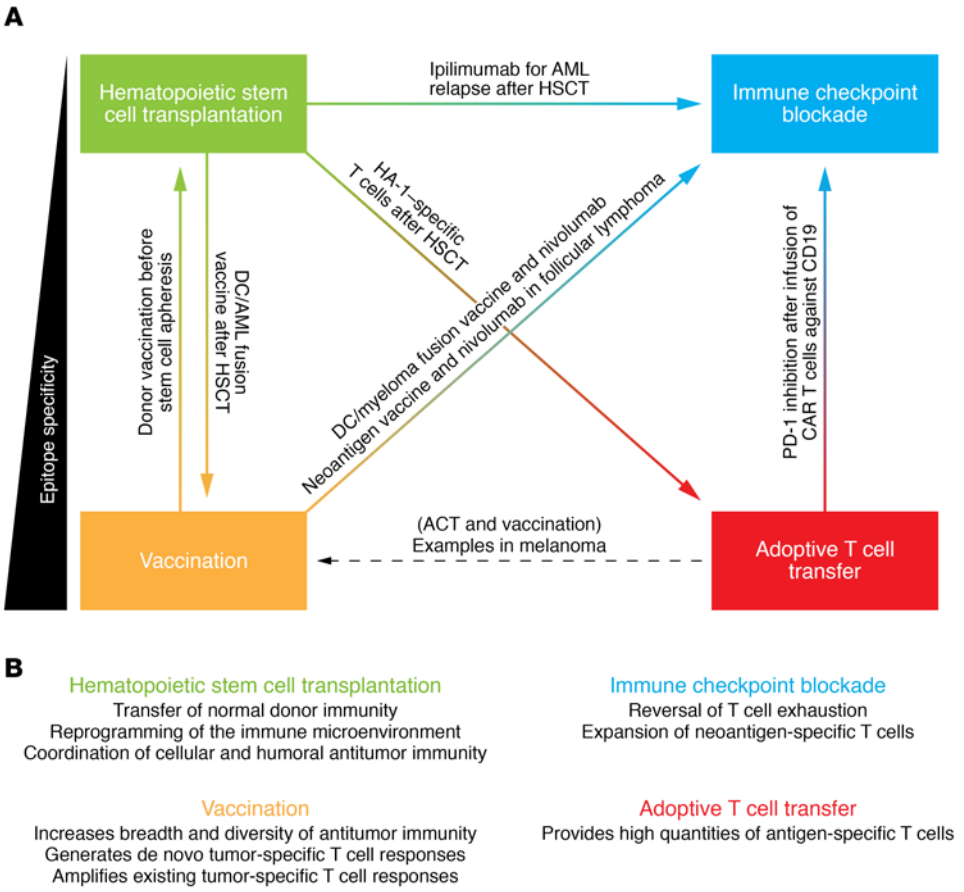

Figure 3. Therapeutic strategies for targeting neoantigens and minor histocompatibility antigens. (**A**) Modalities targeting neoantigens and mHAs can be classified as those with lesser (e.g., allogeneic HSCT or ICB) or higher degrees of epitope specificity (e.g., ACT or vaccination approaches). Examples of combinatorial approaches of these modalities are shown. (**B**) Mechanisms underlying each individual therapeutic modality.

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, JAK2^{V617F}, mutated calreticulin (CALR^{mut}), or MPL$^{W515K/L/A}$. HSCT has been clinically successful in many JAK2^{V617F}-mutated patients (60). CD8$^+$ T cells with higher binding affinity for JAK2^{V617F} than for JAK2^{WT}, which preferentially lyse cells homozygous for JAK2^{V617F}, have been identified from healthy donors (61). Notably, JAK2^{V617F}-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 JAK2^{V617F} in combination with PD-1 blockade (62).

Mutated calreticulin is a driver mutation in 30% of patients with JAK2^{WT} 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). A phase I vaccination trial with CALR^{mut} peptides is ongoing (NCT03566446). Cimen Bozkus et al. demonstrated that CALR^{mut}-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 W515$^{K/L/A}$ 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 MYD88^{L265P} and EZH2^{Y641N} 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 MYD88^{L265P}-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 MYD88^{L265P} in only 1 of 22 patients with MYD88^{L265P}-mutated lymphoma. In contrast, T cells specific for MYD88^{L265P} could be induced in vitro using naive 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-RUNX1*, the most common fusion gene in childhood B-ALL, were stopped because of a lack of endogenous processing (91). Recently, more immunogenic neoepitopes deriving from *ETV6-RUNX1* 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 CD20^{D393} are detectable in patients. In a mouse model, Vauchy et al. could induce CD20^{D393}-specific T cells with a vaccination approach. CD20^{D393} 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 are proteins characterized by β O-linked *N*-acetylglucosamine (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 ICB alone have been largely disappointing, which can be attributed in part to the generally low mutational burdens of these diseases (46). Hodgkin's lymphoma (HL) stands out as a dramatic exception, with overall responses in the relapsed/refractory setting after ASCT of 69% (117). Response to PD-1 inhibition in HL has been linked to its inherent overexpression of PD-L1 due to amplification of the 9p24.1 locus and expression of latent membrane protein 1 in the case of EBV$^+$ HL (118, 119). Other clear examples of response to ICB in the blood malignancy setting have been described in relapsed extramedullary AML following HSCT, wherein administration of CTLA-4 blockade (with ipilimumab) was demonstrated to induce complete remissions (120). In myelodysplastic syndrome (MDS), single ipilimumab or combination of nivolumab with azacytidine generated clinical responses in 2 of 9 and in 6 of 11 patients, respectively (121).

Vaccines. Vaccination approaches have the potential to increase the number and potency and broaden the diversity of T cells with specificity against immunizing antigens, which are presented by the target cells. Given the recent approaches that enable systematic identification of hematopoietically restricted mHAs and tumor neoantigens, vaccination presents an attractive strategy to induce naive antigen-specific T cell responses and generate sustained T cell memory (49, 122). As reviewed below, strategies that enable targeting of these novel antigens can be achieved using whole-tumor, DC, and personal antigen–specific vaccines.

Whole tumor cell vaccines have the advantage of providing a broad repertoire of tumor candidate antigens, including neoantigens, and have been tested over the past two decades. In hematological malignancies, access to large representative tissue samples makes this approach highly feasible. One such example is GVAX (GM-CSF–secreting autologous leukemia cell vaccination), which uses irradiated autologous tumor cells engineered to express GM-CSF or combines irradiated autologous tumor cells with GM-CSF–secreting bystander cells (123). An alternate example, which also provides the advantage of presenting a broad range of tumor antigens along with costimulatory signals, is the autologous DC/tumor cell fusion vaccine. Rosenblatt et al. generated a DC/AML blast fusion vaccine that induced expansion of T cells specific not only to autologous AML but also to cancer/testis antigens and leukemia-associated antigens. Twelve of 17 patients receiving the vaccine remained in remission with a median of 57 months of follow-up (124). Follow-up trials using this platform are ongoing in the nontransplant (NCT03059485) and post-transplant (NCT03679650) settings.

Personal neoantigen vaccines have been demonstrated to induce neoantigen-specific T cell responses using synthetic long peptides (49, 50), RNA-based formulations (125), or peptide-pulsed DCs (122). Because of the unique set of mutations in every patient, the manufacturing process requires sequencing of a representative tumor sample and germline tissue, identification of tumor-specific neoantigens, and prediction of their HLA binding and selection of promising candidates. This has been extensively reviewed elsewhere (126, 127). Personal neoantigen vaccines have been demonstrated to induce strong T cell responses in solid tumors (49, 50, 128, 129). Among hematological malignancies, phase I trials with personal neoantigen vaccines are being conducted in CLL (NCT03219450), ALL (NCT03559413), MM (NCT03631043), and FL (NCT03361852).

Adoptive T cell transfer. ACT directly provides high quantities of functional T cells aimed at eliminating malignant cells. This approach relies on T cells specific for targets expressed selectively on malignant cells. The dramatic successes of CAR T 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. CAR T 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 reinfused after ex vivo coculture with personal neoantigens (NCT03258359). As the manufacture of personal neoantigen–targeting ACT is resource-demanding, the concept of targeting shared neoantigens in this fashion has been actively considered. The identification of HLA-A*02:01 NPM1^{mut}–specific TCRs provides a therapeutic approach targeting a neoantigen frequently found in AML (55). Other examples include TCRs specific for p53^{R175H} and KRAS^{G12D}, mutations shared among blood malignancies (138, 139). As an example of ACT targeting a common mHA, HA-1–specific T cells are currently undergoing testing in a phase I trial in patients with acute leukemia relapse following HSCT (NCT03326921) (28).

Combinatorial genomics-directed therapeutic modalities to overcome resistance mechanisms

While therapeutic efforts directed at targeting neoantigens have shown promising activity, there has been increasing recognition of the negative impact of immune escape mechanisms, which include increased checkpoint expression on mHA-specific T cells (140), loss of HLA class I molecules (138), downregulation of HLA class II molecules (141), and loss of neoantigen or mHA expression (35, 142, 143). Thus, several clinical trials are already under way that combine complementary tumor-directed immune-based treatment strategies to overcome resistance mechanisms (Figure 3A).

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 opportune 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 on-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 TCRαβ 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.

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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.

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