Immunotherapy for hematological malignancies

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Abstract
Tumor immune tolerance remains a major barrier for effective anti-cancer therapy. A growing number of pathways whereby solid tumors escape immune surveillance have been characterized (1). This progress led us to revisit the "hallmarks of cancer" and brought forward many promising immunotherapies. Every growing bodies of research have brought forward many exciting treatment strategies for hematological cancers like chimeric antigen receptor T cells (CAR-T cells) and immune checkpoint inhibitors. Given the distinct characteristics of the different cancers, some benefited profoundly from such therapies while some remain challenging for scientists and physicians. Here, we discuss the unique aspect of hematological malignancies, and briefly review the history, existing and future of immunotherapies for this group of cancer.
Keywords: Cancer, Immunotherapy, CAR-T cells, hematological malignancies.

Background
Tumor microenvironment (TME) contributes to the development, growth and immune invasion of cancer. Immune tolerance, a critical mechanism for cancer immune invasion, remains a major barrier for effective anti-cancer therapy. Although immunotherapy has shown promising activities in a variety of solid tumors, its effects remain variable in hematological malignancies. Given some of the unique features of hematological malignancies, it may provide unique opportunities while present distinct challenges.

Features of hematological malignancies
Several characteristics of hematological malignancies deserve special consideration (1). First of all, many hematological malignancies are systemic disease that involve primary and secondary lymphoid organs. The cell of origin of these cancers are of the immune system (2). For example, chronic lymphocytic leukemia (CLL) or acute lymphoblastic leukemia (ALL) are cancers of the B-cells. Unlike solid tumor where tumor infiltrating lymphocytes are confined in localized tissue, disseminated leukemia involves continuously and systemic contact between the tumor clone and the immune system. Secondly, tumor cells can hijack the niches that belong to normal immune cell development leading to severe immune dysfunction. For example, humoral response where antibodies were produced by B cells in response to foreign antigen requires B-cell–T-cell interactions. In case of B cell malignancies, such interaction maybe be taken over by the accumulating malignant B cells, resulting insufficient antibody production. Thirdly, many cancers of the hematological system can be provided growth stimuli from interactions between the niche and its normal counterparts. As above, such B-cell–T-cell interaction exists as a normal process for antibody production. In the case of B-cell malignancies, this interaction is taken advantage by the malignant B cells for their own survival support. Additionally, some hematological malignancies such as acute leukemia happens at the stage of hematopoietic stem/progenitor cells, resulting in a severe compromised hematopoiesis and immune deficiency. Last but not least, many hematological malignancies have low mutation load therefore less immunogenetic than solid tumors (3). Immunotherapy that revive T cells from exhaustion due to prolonged immune-activation may not be efficacious if T cells were not activated in the first place. While many of the aforementioned features present challenges for immunotherapy, the accessibility of the blood and the existence of precursor states provide an unique opportunity for prospective analysis and an excellent model to examine immune-surveillance. For

example, monoclonal gammopathy of undetermined significance (MGUS) is a precursor to multiple myeloma (MM) (4). While MM is almost always preceded by MGUS, not all MGUS patients progress to MM. Whether immune-surveillance plays a role in the non-progressors remains an active area of research of our group and many others.

Past and present immunotherapy strategies

Earliest "immunotherapy" dates back to 1890, where Emil von Behring and Shibasaburo Kitasato passively immunized people using serum purified from animals containing the antibody (5). In modern medicine, one of the oldest forms of cancer immunotherapy would be allogeneic hematopoietic stem cell transplant (HSCT). The first allogeneic-HSCT was performed in 1957. Since then, it has evolved and expanded throughout the world. Not only this form of therapy is still being practice today, it may be the only curative option for many hematological cancers due to the graft-versus-tumor (GVT) effect (6). However, this approach may be prohibitive due to the high risk of treatment-associated mortality including the development of graft-versus-host disease (GVHD). With supportive care, and the prevention and treatment of transplant associated mortality, there has been a significant improvement in overall survival after allogeneic transplant over the past several decades. However, this strategy still often fails due to the relapse of the malignancy, highlighting the need for developing approaches that better enabling host anti-tumor immunity. There are a few newly approved and experimental immunotherapeutics that have emerged and showed promising activity in a subset of solid tumor and hematological malignancies. We will briefly discuss each of these based on their similar mechanism of action.

Monoclonal antibodies (BITE, ADC)

Monoclonal antibodies (mAb) are one of the most commonly used immunotherapies for cancer. Rituximab, the first mAb approved by the FDA in 1997, is an anti-CD20 antibody designed to treat B-cell malignancies (7). It is a type I antibody that exhibits complement-dependent and antibody-dependent cellular cytotoxicity (CDC & ADCC). Since then, it has become the prototype and backbone for many antibodies that came later. Ofatumumab, approved by the FDA in 2009, is a fully humanized, type I Ab against CD20 developed to recognize a different site than rituximab. This was followed by obinutuzumab, a second-generation anti-CD20 which is a glycoengineered type II antibody that has the ability to induce direct cytotoxicity in addition to ADCC (7) (Table 1).

mAbs are developed based on either lineage-specific antigens (LSAs) or non-lineage-specific antigens (NLSAs) (8). LSA refers to cluster of differentiation (CD) antigens (Ags) that are specific to hematopoietic differentiation. For example, mAb against CD20 (Rituximab), CD19 (Inebilizumab) or CD22 (Epratuzumab) can be used to target B cells. In contrast, NLSAs are molecules not restricted to specific hematopoietic cells but plays critical role in malignant transformation of the cells. These molecules could be glycoproteins and oncogenic receptors, such as CD52 and SLAMF7 for CLL and MM respectively; chemokine receptors CCR4; soluble factors and their associated receptor including BAFF/BAFF-R; adhesion molecules such as CD44 or ICAM-1; and factors for angiogenesis including VEGF.

Bispecific T cell engagers (BiTEs) is a type of antibodies that have two variable fragments that bind to T cell through anti-CD3 fragment and recognize tumor surface antigens through another fragment (8). For example, blinatumomab approved in 2014 for B-ALL, has dual specificity for CD3 and CD19, became the prototype for BiTEs came after. The dual specificity of BiTEs can bring T cells to close proximity of the tumor cells thereby enhancing the immunological synapse formation and anti-tumor cytotoxicity.

Table 1. FDA approved antibody-based therapies

Therapeutic type	Taget	Agents	Hematological malignancies approved for
Monoclonal antibody (LSA)	CD20	Rituximab (Rituxan/Biogen Idec)	B-NHL, CLL
		Ofatumumab (Arzerra/Genmab)	ACLL
		Obinutuzumab (Gazyva/Roche Glycart Biotech)	CLL, FL
Monoclonal antibody (non-LSA)	CD52	Alemtuzumab (Campath/ University of Cambridge)	CLL
	CD38	Daratumumab, JNJ-54767414 (Darzalex/Genmab)	MM
	SLAMF7 (CS1, CD319)	Elotuzumab, HuLuc63, BMS-901608 (Empliciti/PDL BioPharma)	MM
	CCR4	Mogamulizumab, KW-0761 (Poteligeo/Kyowa Hakko Kirin Co.)	ATL, CTCL, PTCL
Bispecific T cell engager	CD19,	BiTE (blinatumomab, Amgen)	ALL
Antibody-drug conjugates (ADC)	CD30	Brentuximab vedotin (Seattle Genetics)	HL

Antibody has also been used to generate antibody-drug conjugates (ADCs) (8). Rather than modulating cellular immunity, these ADCs use antibody as a targeting moiety to delivery cytotoxic agents to specific cell type. For example, brentuximab, a microtubule inhibitor MMAE conjugated with an anti-CD30 antibody has been approved by the FDA for treatment of relapsed/refractory Hodgkin's Lymphoma (HL) in 2011 and for post-autologous transplant consolidation in patients with high risk HL in 2015.

Adoptive cellular therapies and Chimeric antigen receptor T cells (CAR-T)

The idea of adoptive cell therapy derived from the success of infusing donor lymphocytes in recipients of allogeneic stem cell transplant and virus specific T cells in Epstein barr virus-driven lymphomas. Effective T cell immunity requires several components: tumor antigen processing by antigen-presenting cells (APCs); clonal expansion of tumor reactive T cells; recognition of tumor cells by antigen-specific T cells; optimal activation of such tumor-specific T cells. However, these processes are often suppressed by the tumor limiting effective anti-tumor immunity.

In the recent years, Chimeric antigen receptor T (CAR-T) cells therapeutics have emerged and brought incredible promise for hematological cancers. These are autologous T cells engineered to express chimeric antigen receptor against a specific tumor surface antigen, such as CD19 for B-ALL (9). They are antigen specific but HLA independent. This therapy was particularly successful and took the field by storm given several advantages of hematological malignancies, such as clear surface antigen expression allowing CAR-T recognition of tumor cells, easy access to patient samples enabling CAR-T production, and natural homing of T cells to tumor sites in the blood, bone marrow and lymph nodes, facilitating CAR-T-tumor interaction.

Generally, the CAR consists of a single-chain of the antibody variable fragment in the extracellular domain, linked by a hinge and transmembrane domain to an intracellular T cell signaling domain with a costimulatory domain. First generation CAR had only a CD3ζ-derived signaling module, which limited in vivo efficacy and persistence. The second and third generation CAR has one or two costimulatory domains respectively, significantly improving the anti-tumor effects. CAR T cells were first developed to target CD19 for B cell leukemias. Now, they have extended to targeting CD38, CD138, BCMA or SLAM7 for MM, and even tumor antigens like NY-ESO-1.

Checkpoint inhibitors

Immune checkpoint blockade gained the spot light of immunotherapy by winning the Nobel prize of medicine in 2018. This strategy demonstrated impressive efficacy in a wide range of tumor types, evidenced by the success of CTLA-4 and PD-1 pathway blocking antibodies in melanoma, lung cancer renal cell carcinoma and other solid tumors (10). Immune checkpoint molecules are negative regulators of the immune system. They are critical for keeping the immune cells in check to prevent prolonged immune activation and autoimmunity. Cancer cells take advantage of such mechanism as one of the many ways to evade immune surveillance. Inhibiting these checkpoint molecules can re-invigorate T cells leading to tumor regression.

CTLA-4 inhibitors ipilimumab was first approved by the for metastatic melanoma, a proof of concept, leading to more clinical development for solid tumor and hematological malignancies. PD-1 blockade with nivolumab, pembrolizumanb, atezolizumab, duravalumab, has shown activity in multiple solid tumors. Nivolumab and pembrolizumanb are two anti-PD1 Ab at the most advanced stage of clinical trials in hematological malignancies for HL, non-Hodgkin's Lymphoma (nHL), CLL and MM. FDA had approved the use of nivolumab and pembrolizumab in relapsed/refractory HL in 2016 and 2017 respectively (Table 2).

Table 2. FDA approved immune checkpoint inhibitors

Target	Drug	Cancer Type Approved for
CTLA-4	ipilimumab (BMS)	Melanoma
PD-1	pembrolizumab (Merck)	Melanoma, NSCLC. Head and Neck Squamous
PD-1	nivolumab (BMS)	Melanoma, Kidnedy cancer, Hodgkin lymphoma, Head and Neck Squamous

The "success" of PD-1 blockade in HL owing to the high expression of PD-L1 on the tumor cells. Whereas the differential efficacy of PD-1 blockade in NHLs did not directly correlate with PD-L1 expression remains an active area of research. However, despite high PD-L1 expression on MM cells or PD-1 expression on CLL cells, they did not translate into clinical efficacy with PD-1 inhibitor. Additional exhaustion markers like TIM3, LAG-3 that often found co-expressed with PD-1 on exhausted T cells, could be potential target for combination therapies.

Vaccines

While CAR-T is genetically engineering the receptor of T cells, vaccine is educating the T cells through tumor associated antigens to allow its own receptor engineering. The development of cancer vaccine can break down into 1) identifying the best tumor associated antigen(s), 2) culturing blood mononuclear cells (PBMCs) from the patients with recombinant fusion protein based on the antigen, 3) identify the appropriate clinical setting for infusing primed PBMC back to the patients, 4) determining the (combinatory) immunotherapies for optimal T cell activation.

Several tumor-associated antigens have been identified and used for hematological malignancies, including 1) abnormal fusion proteins generated due to the malignancies, such as bcr/abl fusion protein in chronic myeloid leukemia (CML); 2) cancer testis antigens that normally only express during early development, such as NY-ESO, MAGE1, MAGE3, are found in myeloma cells; 3) proteins from aberrantly upregulated oncogenes, such as WT1 and MUC1 in leukemia; 4) lineage specific antigens such as CD138, BCMA, CD38 and CS1 for MM (11). Computational algorithm can predict potential neoantigen peptide epitopes based on whole genome or RNA sequencing of the tumor. This information can be further applied to antigen expressed in the context of HLA-restriction. Despite generally lower mutation burden of hematological malignancies, they often associate with chromosomal translocations that could be used as unique targets.

One vital step for successful vaccination outcome is to determine the best treatment window in clinical practice. One ideal setting is during post-transplant immune reconstitution. Although patients show overall suppressed cellular immunity, preclinical studies suggest that the depletion of regulatory T cells (Tregs) could be beneficial for enhanced vaccine response. In one phase II study, vaccination of post autologous transplant myeloma patients with idiotype-pulsed antigen presenting cells (Mylovenge) significantly improved survival compared to historical controls. To best prevent immune suppression in cancer milieu and enhance vaccine efficiency, combination strategies are often used in effort to counteract with such microenvironment influence. Studies have used hypomethylating agents, checkpoint blockade, CAR-T cells or immunomodulatory agents (IMiD) lenalidomide to enhance overall response to vaccination and prevent the re-establishment of tolerance (11).

Small molecules

Small molecules have several advantages over cellular therapies or antibodies, including low cost, easy to produce, administer and control pharmacokinetics and pharmacodynamics profiles, easy transmembrane transport for intracellular targets, ability to target signaling pathways shared by multiple cell types. Mechanism of these agents includes antagonizing PD-1/PD-L1 interaction, enhancing pattern recognition receptor, targeting STING pathways, and kinase inhibitors skewing immune profile from immune-suppressive to immune-activating (12).

Bruton tyrosine kinase (BTK) inhibitor Ibrutinib or PI3K p110δ inhibitor idelalisib were originally developed for B cell malignancies that depend on their activity for tumor survival, such as CLL or lymphoma mantle cell lymphoma. They were later discovered to have immune activating properties. For example, ibrutinib covalently inhibit both BTK and inducible T cell kinase (ITK), inhibiting which skew T helper (Th) cells to Th2 immunity. Ibrutinib was also shown to increase CD8 T cell numbers in CLL. BTK inhibitors like ibrutinib or acalabrutinib are now being tested in combination with Ab or immune checkpoint inhibitors in clinical trials. Similarly, p110δ inhibition was found to impair Treg expansion thereby improving anti-tumor immunity of the CD8 T cells in CLL (12, 13).

Lenalidomide and pomalidomide, derivatives of thalidomide, were considered to be immunomodulatory drugs (IMiDs) due to their effect on T cell IL-2 productions. Despite the wide clinical use of these IMiDs, their mechanism of action was only recently elucidated. They could bind to cereblon (CRBN), an E3 ubiquitin ligase that regulates the ubiquitination of IKZF1/3. Given that IKZF3 is a transcription repression of the IL2 gene, this mechanism of action explains the IL-2 production induced by IMiDs. Since the IKZF1/3 are also transcription factors critical for B-cell differentiation, it explains the remarkable activity of lenalidomide in certain B cell malignancies like MM (14).

Future perspective

The power of our own immune systems against malignancies has long been appreciated since the curative potential of ASCT. Although hematological malignancies present unique challenges, they also bring unique opportunities for immune-modulation. CAR-T and immune checkpoint blockade have brought extreme excitement to the field. Nevertheless, primary and acquired resistance remain a challenge. Identify robust biomarkers for patient selection is one of the strategies to improve response rate.

Combination therapies are also emerged to enhance therapeutic efficacy. For example, investigators are screening small-molecules to incorporate into CAR-T manufacturing process, attempting to improve the therapeutic properties of the product (15).

While it is crucial for us to apply many of these existing cellular therapies or macromolecules beyond specialized cancer centers and improve clinical care on post treatment management, lots of effort are underway to turn attentions toward small molecule strategies. Needleless to say, these recent development in cancer immunotherapies has completely transformed the way we think about cancer and designing cancer therapies.

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