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TITLE:
Immune checkpoint blockade and CAR-T cell therapy in hematologic malignancies

ABSTRACT:
Harnessing the power of the immune system to recognize and eliminate cancer cells is a longtime exploration. In the past decade, monoclonal antibody (mAb)-based immune checkpoint blockade (ICB) and chimeric antigen receptor T (CAR-T) cell therapy have proven to be safe and effective in hematologic malignancies. Despite the unprecedented success of ICB and CAR-T therapy, only a subset of patients can benefit partially due to immune dysfunction and lack of appropriate targets. Here, we review the preclinical and clinical advances of CTLA-4 and PD-L1/PD-1-based ICB and CD19-specific CAR-T cell therapy in hematologic malignancies. We also discuss the basic research and ongoing clinical trials on emerging immune checkpoints (Galectin-9/Tim-3, CD70/CD27, LAG-3, and LILRBs) and on new targets for CAR-T cell therapy (CD22, CD33, CD123, BCMA, CD38, and CD138) for the treatment of hematologic malignancies.

Introduction:
Our current understanding of hematopoiesis is based on a stem cell model, in which a small pool of multi-potent hematopoietic stem cells (HSCs) self-renew and differentiate into distinct cellular lineages of the blood [1]. This process is tightly regulated to maintain an appropriate number of mature progenies with specific function while not exhausting primitive stem cells [2].
Dysregulation of hematopoiesis results in the development of hematologic malignancy, which is a group of blood cancers arising from cells with reduced capacity to differentiate into mature progeny, leading to the accumulation of immature cells in blood-forming tissues. In 2019, 176,200 new hematologic malignancy cases and 56,770 deaths are projected to occur in the USA according to the data released by the American Cancer Society [3].
Chemotherapy and bone marrow (BM) transplantation are the standard treatments for acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), aggressive Hodgkin's lymphoma (HL), and Non-Hodgkin's lymphoma (NHL) such as diffuse large B cell lymphoma (DLBCL) and Burkitt's lymphoma. Although a temporary remission can be achieved, the risk of relapse remains high because of the existence of chemotherapy-resistant cancer stem cells [4]. Novel methods of immunotherapy, such as immune checkpoint blockade (ICB) and chimeric antigen receptor T (CAR-T) cell therapy have been attracting attention due to their ability to charge the immune system to attack cancer cells.

CTLA-4 ::: Targeting immune checkpoints in hematologic malignancies:
CTLA-4 is expressed on activated T cells, regulatory T cells (Tregs), and AML blasts [11–13]. Anti-CTLA-4 toxin-conjugated mAb treatment induced dramatic apoptosis in AML cells but was only slightly toxic to normal BM precursors [11]. Furthermore, engagement of CTLA-4 by its specific ligands B7-1 and B7-2 induced apoptosis in patient-derived AML cells via a T cell-independent pathway [12]. On the other hand, in murine C1498 myelogenous leukemia model, B7-1+ C1498 cells grew progressively; B7-2+ C1498 cells, however, were rejected spontaneously through a CD8+ T cell-mediated killing. By using anti-CTLA-4 mAb to specifically block the B7-1/CTLA-4 interaction, a significantly higher rate of rejection of B7-1+ C1498 tumor was observed, indicating that B7-1 delivered negative signal to T cell immunity via CTLA-4 [14]. Another group found that in murine DA1-3b AML model, B7-1 and PD-L1 expression were increased in leukemic cells, which were more resistant to host immune responses and thus resulting in worse survival. Blocking PD-L1, B7-1, or CTLA-4 enhanced cytotoxic T cell-mediated lysis and prolonged survival of DA1-3b AML mice [15]. AML patients with the CTLA-4 CT60 AA genotype had increased risk of leukemic relapse after standard chemotherapy and lower overall survival at 3 years. The CTLA-4 CT60 AA genotype has been described to produce a more soluble form of CTLA-4, which is able to suppress proliferation of autoreactive T cells [16].
In HL, TILs were enriched for CTLA-4+ Tregs [17]. T cells from patients with chronic lymphocytic leukemia (CLL) had abnormal upregulation of CTLA-4, which was positively correlated with an increased portion of Tregs and advanced Rai stage [18]. Co-culture of primary T cells with CLL-derived CTLA-4+ Mec1 cells resulted in reduced production of interleukin-2 (IL-2), suggesting that leukemic cells expressing CTLA-4 inhibited T cell co-stimulation [19]. Furthermore, polymorphisms of CTLA-4 were found to be associated with NHL [20]. CTLA-4 has also been reported to upregulate in multiple myeloma (MM) patients [21].

MDS/AML ::: PD-L1/PD-L2/PD-1 ::: Targeting immune checkpoints in hematologic malignancies: PD-L1 expression in murine leukemia cell line C1498 was upregulated in vivo, and blocking PD-L1/PD-1 pathway resulted in decreased AML burden and longer survival time [22]. In myelodysplastic syndromes (MDS) and AML patient samples, PD-L1 was detectable (> 2% PD-L1+ cells) in 100% of patients with common expression on non-tumor hematopoietic cells, while PD-L2 expression was largely absent [23]. PD-L1 expression on AML cells is significantly higher in the relapse setting than at the newly diagnosed stage [24, 25]. In BM aspirates from patients with TP53 mutation, PD-L1 positivity was more frequently noted [25]. Higher PD-L1 expression level was positively correlated with poor risk cytogenetic and molecular abnormalities [25, 26]. In a similar manner to solid tumor, interferon-γ (IFN-γ) induced PD-L1 expression on AML cells protected them from cytotoxic T cell lysis [27]. In BM aspirates from AML patients, T cell subsets, such as CD4+ effector T cells, CD8+ T cells, and Tregs, had significantly higher PD-1 expression in untreated and relapsed AML patients compared with healthy donors [28]. PD-1 expression on CD4+ and CD8+ T cells was upregulated at relapse after allogeneic stem cell transplantation (allo-SCT) [29]. In peripheral blood of patients with chronic myeloid leukemia (CML), PD-1 expression on CD8+ T cells was higher in comparison with healthy donors. In CML mouse model, PD-1 was highly expressed on CML-specific cytotoxic T cells, while PD-L1 expression was higher in blast crisis CML (bcCML) than chronic phase CML (cpCML), indicating that CML cells utilized PD-L1 to avoid immune surveillance. PD-1-deficient mice with bcCML survived significantly longer than wild type mice, suggesting that myeloid leukemia cells impaired host immune responses via PD-L1/PD-1 pathway [30]. Hence, the upregulation of PD-L1 on MDS/AML cells leads to immune escape and supports the potential benefit of using PDL-1/PD-1 inhibitors to treat MDS/AML. Single agent nivolumab (humanized anti-PD-1 IgG4 mAb) as maintenance therapy demonstrated a complete remission (CR) rate of 71% in 14 transplant ineligible patients with high-risk features including adverse cytogenetics, treatment-related AML, and history of prior relapse (Table 1) [33]. Early results of pembrolizumab (humanized anti-PD-1 IgG4 mAb) plus cytarabine yielded CR rate of 35% and minimal residual disease (MRD)-negative remission in 56% of patients (Table 1) [34]. Idarubicin plus cytarabine and nivolumab in newly diagnosed AML reported complete remission or complete remission with incomplete count recovery (CR/CRi) in 34 of 42 patients and MRD-negative remission in 18 patients. Furthermore, the median relapse-free survival for the complete responders was 18.5 months. The risk of graft versus host disease (GvHD) was not significantly elevated in the 18 patients who proceeded to allo-SCT. Interestingly, baseline BM analysis of those who achieved CR/CRi had a higher frequency of CD3+ T cell infiltrate as compared to non-responders who had higher number of CD4+ effector T cells co-expressing markers of an exhausted phenotype (Table 1) [35, 36]. While the use of nivolumab and ipilimumab (humanized anti-CTLA-4 IgG1 mAb) in the post allo-SCT relapse setting in hematologic malignancies has demonstrated potent anti-tumor effects, significant immune-related adverse events (irAE) have also been reported (Table 1) [31, 49, 50]. Ipilimumab use with various hematologic malignancies resulted in significant irAE including 1 death, GvHD leading to drug discontinuation in 4 patients, pneumonitis and colitis [31]. In addition, fatal acute respiratory distress syndrome (ARDS), antiphospholipid syndrome, fatal acute grade 3 GvHD, and worsening of chronic GvHD were reported with nivolumab use in two other clinical trials [49, 50]. These data highlight a need for caution of T cell-mediated GvHD when treating patients with ICB after allo-SCT. Mechanistically, one study has demonstrated that persistent expression of PD-L1 by parenchymal cells reduces the proliferation of donor-derived CD8+ T cells in GvHD target tissues, leading to amelioration of GvHD in a mouse model [51]. Another group has showed similar result that elevated levels of PD-L1 from organ-specific microenvironments (e.g., lymph nodes) dampen cytotoxic T lymphocyte (CTL)-mediated GvHD after allo-SCT [52]. Reduced CTL activity in lymph nodes, however, also contributed to local tumor escape, which could be reversed by anti-PD-1 blockade [52]. It would be important to balance the possible risk of exacerbating GvHD and achieving maximum tumor killing.

Hodgkin's lymphoma ::: PD-L1/PD-L2/PD-1 ::: Targeting immune checkpoints in hematologic malignancies:
PD-L1/PD-L2 expression is increased on HL cell lines and malignant Reed Sternberg (RS) in classical HL (cHL), due to upregulation and amplification of 9p24.1 JAK and MEK/ERK signaling [53, 54]. Although cHL does not have a high mutational burden, a necessary biomarker predicting responses to ICB, high frequency of PD-L1/PD-L2/PD-1/JAK2 genetic alterations in RS cells and high proportion of PD-1+ TILs determine sensitivity to PD-L1/PD-1 inhibitors [55, 56]. Receptor

PD-1 was markedly increased on TILs as well as peripheral T cells of HL patients [55, 57]. Functionally, mAb targeting PD-L1 was able to inhibit tyrosine phosphorylation of SHP-2 and restore the production of IFN-γ by tumor-infiltrating T cells [57]. Within the tumor microenvironment (TME) of cHL, PD-1 and PD-L1 were elevated on natural killer (NK) cells and tumor-associated macrophages (TAMs), respectively. As expected, PD-1 inhibition reactivated both T and NK cells by blocking interactions between PD-1+ T/NK cells and PD-[39]L1+ malignant B cells/TAMs [58]. In addition, expanded numbers of CD4+PD-1− Th1-polarized Tregs and PD-1+ differentiated T effectors were observed within the TME of cHL, where these cells might utilize PD-L1/PD-1 pathway to exert complementary mechanisms to suppress host anti-tumor immune responses [59].
Clinically, both pembrolizumab and nivolumab showed favorable responses and acceptable safety profile in patients with cHL that has relapsed or progressed after autologous stem cell transplantation (auto-SCT) and brentuximab vedotin (BV), leading to their approval in 2016 by US FDA. The phase I clinical trials, KEYNOTE-013 with pembrolizumab and CheckMate 039 with nivolumab, produced overall response rates (ORRs) of 65% (CR 21%) and 87% (CR 17%) in relapsed and refractory (RR) HL, respectively (Table 1) [37, 38, 43]. CheckMate-205, the phase II multi-cohort study of 243 patients with BV naïve-cohort A, BV after auto-SCT cohort B, and BV before and after auto-SCT cohort C, demonstrated ORR of 69% and a median duration of response (DOR) of 16.6 months (Table 1) [41]. Correlative studies of 45 available tumor samples showed concordant alteration of the PD-L1 and PD-L2 loci in the RS cells. Fluorescence in situ hybridization of the RS cells showed 26 cases with copy gain of PD-L1/PD-L2, 12 cases with PD-L1/PD-L2 amplification, and 7 cases with polysomy 9. Furthermore, complete responders had higher PD-L1 than non-responders [42]. Similarly, KEYNOTE-087, the multi-cohort phase II trial with pembrolizumab monotherapy in RR HL patients who progressed after auto-SCT and subsequent BV therapy (cohort 1), salvage chemotherapy and BV (cohort 2), or auto-SCT but no BV (cohort 3), demonstrated ORR of 72% and CR rate of 28% with a median DOR of 11.1 months (Table 1) [45, 46]. Combination therapy of ipilimumab plus nivolumab has also shown efficacy with ORR of 74% in HL (CheckMate 039, Table 1) [40].
Nivolumab plus BV produced ORR of 82% and CR rate of 61% as the first-line salvage therapy (Table 1) [47]. ECOG-ACRIN E4412 study of nivolumab, ipilimumab, and BV demonstrated ORR of 82% (18/22), with a CR rate of 68% (15/22) (Table 1) [48]. Nivolumab followed by treatment with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) for patients at high risk of relapse (NCT03033914) and pembrolizumab for patients unsuitable for ABVD (PLIMATH NCT03331731) are being explored in the first-line setting for HL. Pembrolizumab (NCT02684292) and nivolumab (CheckMate-812 NCT03138499) with or without BV are being evaluated in phase III clinical trials in the relapsed setting as well (Table 1).

Non-Hodgkin's lymphoma ::: PD-L1/PD-L2/PD-1 ::: Targeting immune checkpoints in hematologic malignancies:
In contrast to HL, PD-L1 expression in NHL is markedly heterogeneous. Of two distinct clinical subtypes of DLBCL, PD-L1 expression was rarely detected in germinal center B cell-like (GCB) subtype, while 57% of activated B cell-like DLBCL samples were PD-L1 positive [60]. Other studies showed similar low expression of surface PD-L1 and soluble PD-L1, and the surface PD-L1 expression was positively associated with the number of PD-1+ TILs and inversely correlated with the number of Tregs in GCB-DLBCL [61, 62]. In a small number of follicular lymphoma (FL) patients, PD-L1 expression was high [63]. PD-1 expression on TILs of FL was abundant but with complicated expression patterns: many cell types, including CD4+ Th1 cells, CD8+ cytotoxic T cells, and Tregs, expressed PD-1 [64]. In CLL, histiocytes, not tumor cells, were the main source of PD-L1 expression within the TME [65]. Low numbers of PD-1+ TILs were observed, which had controversial association results among different contexts [56]. One study has shown that PD-1 expression was increased on CD4+ and CD8+ T cells, and the crosstalk between PD-L1 on CLL and PD-1 on CD8+ T cells resulted in decreased IFN-γ production [66].
Although PD-L1 expression is heterogeneous among MM patients, many studies have shown that PD-L1 expression is limited to malignant plasma cells (PCs), and PD-L1 overexpression is associated with increased risk of progression from smoldering multiple myeloma to MM [62, 67]. However, other groups detected very low PD-L1 expression on normal PCs and did not observe significant upregulation of PD-L1 on malignant PCs from MM patients, which could explain why nivolumab monotherapy and in combination with ipilimumab had no significant therapeutic activity in a phase I CheckMate-039 study treating RR MM patients [68]. PD-1 was upregulated on CD4+ T cells, CD8+ T cells, and NK cells within the BM of MM [68–70]. PD-1+ NK and T cells

were less proliferative and cytotoxic, which could be reversed by anti-PD-L1/PD-1 blocking in vitro and in vivo [68, 70]. Furthermore, lenalidomide, an immunomodulatory drug (IMiD), reduced expression of PD-1 on T and NK cells and downregulated PD-L1 expression on PCs and myeloid-derived suppressive cells (MDSCs) [67, 69, 71]. As a result, combined blockade of PD-L1/PD-1 and lenalidomide enhanced granzyme B and IFN-γ production by T and NK cells and inhibited MDSC-mediated MM progression [67, 71].
Pembrolizumab is approved for RR primary mediastinal B cell lymphoma (PMBCL) based on ORRs of 48% (CR 31%) in KEYNOTE-13 and ORRs of 45% in phase II, KEYNOTE-170 studies (Table 1) [44]. CheckMate-039 also evaluated the efficacy of single agent nivolumab in NHL and demonstrated ORRs of 40% in FL, 36% in DLBCL, 15% in mycosis fungoides, and 40% in peripheral T cell lymphoma (PTCL) (Table 1) [39]. Furthermore, the nivolumab plus ipilimumab cohort of CheckMate-039 reported ORRs of 20% in FL/DLBCL and 9% in transplant-naïve T-NHL patients in 65 patients who had a median number of 4 prior therapies [40]. Nivolumab monotherapy in patients with RR DLBCL who were ineligible for auto-SCT and those with post auto-SCT relapse produced ORRs of 3% and 10%, respectively. Furthermore, median progression-free survival (PFS) and OS were 1.9 and 12.2 months in the post auto-SCT relapse cohort and 1.4 months and 5.8 months in the auto-SCT ineligible group, respectively [72]. Similarly, pembrolizumab maintenance in post auto-SCT chemosensitive patients failed to meet its primary end point as well [73]. In RR FL, pembrolizumab plus rituximab (chimeric anti-CD20 IgG1 mAb) showed ORR of 67% (CR 50%, PR 17%) in patients and a median PFS of 11.4 months. Interestingly, PDL-1 expression was not associated with response [74]. Nivolumab plus ibrutinib demonstrated responses 61% in patients with high-risk CLL/small lymphocytic leukemia (SLL), 33% with FL, 36% with DLBCL, and 65% of patients with Richter's transformation [75] Unlike single agent PD-1 blockade that produced minimal responses in RR MM, the combination of PD-1 inhibition with IMiDs was efficacious and produced ORRs of 50–60% [76]. Pembrolizumab monotherapy in patients who did not achieve CR prior to transplant produced a CR rate of 31% and MRD-negative rate of 41% [77]. In addition, pembrolizumab plus lenalidomide and dexamethasone in high-risk patients within 3–6 months of auto-SCT led to stringent CR in 33% patients and 4 patients achieving MRD-negative remission [78]. Despite the obvious preclinical anti-tumor effects of PD-1 blockade and positive results from earlier trials in MM, phase III clinical trials involving combination therapy of PD-1 blockade with IMiDs were placed on hold by the FDA in 2017 due to increased rate of adverse effects observed in KEYNOTE-183 (NCT02576977) and KEYNOTE-185 (NCT02579863) [79]. The pembrolizumab arm in KEYNOTE-183 (pembrolizumab plus pomalidomide and low-dose dexamethasone) experienced myocarditis, hepatitis, Steven Johnson syndrome, hyperthyroidism, pneumonitis, and 2 treatment-related deaths [79]. KEYNOTE-185 (lenalidomide plus pembrolizumab plus low-dose dexamethasone) reported 6 treatment-related deaths, with 4 being due to either cardiac arrest (1), pneumonia (1), myocarditis (1), and cardiac failure [79].
Several ongoing trials are assessing the combination of PD-1 or PD-L1 inhibition with conventional chemotherapy in untreated DLBCL (NCT 03003520) or as consolidation therapy in NHL (NCT03620578) (Table 2). The JAVELIN study (NCT 02951156) is a phase Ib trial assessing efficacy and safety of immunotherapy-based regimens containing avelumab (human anti-PD-L1 IgG1 antibody) in combination with utomilumab (4-1BB agonist), azacytidine (AZA), rituximab, and/or conventional chemotherapy in patients with RR DLBCL (Table 2) [80].

Galectin-9/Tim-3 ::: Targeting immune checkpoints in hematologic malignancies:
T cell immunoglobulin and mucin-domain containing-3 (Tim-3) shares a similar expression pattern as PD-1 on T cells within the TME, where it functions as a co-inhibitory receptor, thus inhibiting T cell proliferation and cytokine production [81]. Galectin-9, one of the ligands of Tim-3, negatively regulates T cell immunity [82]. PD-1highTim-3+ T cell subsets were functionally deficient and were strongly associated with leukemia relapse in AML patients after allo-SCT [83]. The frequency of PD-1+Tim-3+ T cell subsets, including CD8+ T cells, CD4+ effector T cells, and Tregs, was increased in relapsed and new AML in comparison with healthy donors [25]. Interestingly, surface expression of Tim-3 was significantly elevated in CD34+CD38− AML leukemia stem cells (LSCs) and CD34+CD38+ leukemic progenitors, but not in CD34+CD38− normal HSCs or most portion of CD34+CD38+ normal progenitors [84]. Another report showed increased levels of soluble Galectin-9 and Tim-3 in the plasma of AML patients compared with healthy donors [85]. Xenograft experiments demonstrated that Tim-3+ AML cells were able to initiate human AML in NSG mice and anti-Tim-3 mAb treatment dramatically depleted LSCs and leukemic burden in primary and secondary NSG recipients [84]. Of note, it is inferred that Galectin-9/Tim-3 pathway enhances

AML progression via both immune-cell-dependent and immune-cell-independent manners: AML cells take advantage of self-secreted Galectin-9/Tim-3 to attenuate cytotoxic activity of T cells and NK cells; some pathways such as NF-κB, β-catenin, PI3 Kinase/mTOR, and HIF-1 pathways are intrinsically activated with the ligation of Tim-3 by soluble Galectin-9 in human AML cells. As a result, Galectin-9/Tim-3 autocrine loop promotes self-renewal of LSCs [86, 87]. Given that LSCs were considered to be responsible for the relapse of AML after standard therapies, targeting Galectin-9/Tim-3 pathway represents a promising approach in eliminating LSCs. In terms of other hematologic malignancies, Tim-3 was not only detected on tumor cells in DLBCL and HL, but also observed on TILs where it served as a T cell exhaustion marker [88, 89].
Sym023 (NCT03489343), an anti-Tim-3 mAb as single agent or in combination with Sym021, a PD-1 antibody, (NCT03311412) is in phase I clinical trials for both solid and hematologic malignancies (Table 2).

CD70/CD27 ::: Targeting immune checkpoints in hematologic malignancies:
CD27 (also known as TNFRSF7), one of the TNF receptor family members, works as a positive regulator of T cell immunity by CD70 (TNFSF7) engagement [90]. CD27 is constitutively expressed on naïve T cells as well as HSCs. CD27 remains expressed on stem-like memory cells and central-memory-like cells, whereas it is downregulated on effector cells [91]. With regard to hematopoiesis, the CD70/CD27 interaction negatively mediates leukocyte differentiation and decreases myeloid colony-forming capacity of BM progenitor cells [92]. Besides its functions in modulating normal HSC self-renewal and differentiation, CD70/CD27 signaling also promotes LSC growth and disease progression in murine model and leukemia patients [93–95]. In a BCR/ABL-induced CML-like disease murine model, CD27 was expressed by LSCs (defined as Lin−Sac-1−c-Kithigh) and leukemia progenitors, where CD27 signaling enhanced proliferation and cell cycle progression in a Wnt/β-catenin-dependent manner [93]. Furthermore, CD70 was induced in LSCs by upregulating transcription factor specificity protein 1 in tyrosine kinase inhibitor-treated CML patients, triggering CD27 signaling which compensated Wnt pathway and therefore ultimately causing relapse [94]. Combining anti-CD70 mAb blockade with imatinib therapy effectively promoted cell death of human CD34+ CML stem/progenitor cells in vitro, as well as in a patient-derived xenograft model [94]. More recently, both AML stem/progenitor cells and blasts were found to express CD70 and CD27, while normal HSCs and progenitor cells were negative. In primary AML patient samples, CD70/CD27 signaling enhanced symmetric cell divisions and proliferation by activating canonical Wnt pathway via TRAF2 and TNIK [95]. In addition, mAbs against either CD70 or CD27 have been evaluated in hematological malignancies. For example, a human anti-CD27 mAb eliminated CD27-expressing lymphoma and leukemia via multiple mechanisms: antibody-dependent cellular cytotoxicity (ADCC) and enhancing co-stimulation of T cells [96]. Both anti-CD70 mAb and anti-CD70 antibody-drug conjugates (ADCs) have shown significant anti-tumor effects in xenograft models [97]. In B cell NHL, preexisting and TGF-β-induced intratumoral CD70+ effector memory T cells show exhausted phenotype, expressing high levels of PD-1 and Tim-3 [98]. Interestingly, CD27 on malignant B cells triggers CD70 reverse signaling in NK cells, resulting in increased numbers of tumor-infiltrating activated NK cells and prolonged survival of CD27-expressing lymphoma-bearing mice [99].
Based on preclinical data, anti-CD70 therapy is being studied in AML/MDS and T cell lymphomas. ARGX-110, which blocks CD27/CD70 signaling, demonstrated ORR of 23% in heavily pre-treated patients with CD70 expressing advanced cutaneous T cell lymphoma of different subtypes and stages in a phase I/II clinical trial [100]. A phase II clinical trial (NCT03030612) of ARGX-110 with AZA in AML/MDS is also underway. ADCs of CD70 mAb with a small molecule, MED-2460 (MDX-1203; NCT00944905), with pyrrolobenzodiazepine (SGN-70A, NCT02216890) and monomethyl auristatin (SGN-75, NCT01015911), yielded only modest response in NHL and have been limited to phase I due to significant toxicities including pleural effusion, hypersensitivity and facial edema (MDX-1203), grade 3 thrombocytopenia (SGN-70A), and ocular toxicity (SGN-75) (Table 2) [101–103]. Varlilumab (CDX-1127), a human IgG1 anti-CD27 agonist, has produced substantial and durable response in the phase I trial of patients with hematologic malignancies [104].

LAG-3 ::: Targeting immune checkpoints in hematologic malignancies:
Lymphocyte activation gene-3 (LAG-3) is a transmembrane protein mainly expressed on activated CD4+ and CD8+ T cells, as well as Tregs, NK cells, and plasmacytoid dendritic cells [105]. LAG-3 and PD-1 when expressed on CD4+ and CD8+ TILs exhibit an exhausted phenotype [106]. LAG-3 blockade has been shown to synergize with anti-PD-1 blocking, suggesting LAG-3 and PD-1

signaling pathways have non-redundant and synergistic functions in dampening T cell responses within the TME [106]. LAG-3 and PD-1 double positive CD8+ and CD4+ effector T cells were coexpressed more frequently from AML BM aspirates compared with healthy donors [25]. In addition to AML, intertumoral and peripheral blood lymphocytes from HL patients also expressed high levels of LAG-3, and deletion of CD4+LAG-3+ T cells improved lymphoma-specific CD8+ T cell responses [107]. In CLL, both surface and soluble LAG-3 were upregulated, which were associated with a more aggressive clinical course and poor prognostic features [108]. Blocking LAG-3, but not PD-L1/PD-1 pathway, enhanced T cell activation in patients with CLL, making LAG-3 a potential target to treat CLL [108]. LAG-3 also defined the exhaustion of tumor-infiltrating PD-1+ T cells in B cell NHL [88, 89]. Notably, the interaction between LAG-3 and its canonical ligand, MHC-II, was unable to fully explain its suppressive functions to CD8+ T cells and NK cells [106]. Most recently, fibrinogen-like protein 1 (FGL1) was identified to be a major functional ligand of LAG-3 [109]. Elevated FGL1 was found in the plasma of cancer patients, and high FGL-1 level was correlated with poor prognosis and resistance to anti-PD-1 therapy [109]. It would be interesting to investigate whether FGL1/LAG-3 pathway plays a role in hematologic malignancies. Several phase I/II clinical trials of LAG-3 antibodies as single agent (NCT03489369) or in combination with PD-1 inhibitor (NCT03005782, NCT02061761) are ongoing (Table 2). In addition, MGD013, a dual-affinity re-targeting antibody specific to both PD-1 and LAG-3 is being studied in hematologic malignancies (NCT03219268) (Table 2). As of March 2019, there are close to 27 clinical trials targeting LAG-3.

LILRBs ::: Targeting immune checkpoints in hematologic malignancies:
The leukocyte immunoglobulin-like receptors subfamily B (LILRBs) are transmembrane glycoproteins with intracellular immunoreceptor tyrosine-based inhibitory motifs [110]. LILRB contains five members (LILRB1-5) in humans and primates, but has only two orthologs in mouse, paired immunoglobulin-like receptor B (PirB) and gp49B1, making the xenograft murine model more suitable for LILRB-related preclinical research. LILRBs are expressed on cancer cells as well as a wide range of immune cells, including NK cells, T cells, B cells, macrophages, and monocytes [110]. LILRB1 (also known as CD85J, ILT2, LIR1, and MIR7) and LILRB3 (CD85A, ILT5, LIR3, and HL9) are widely expressed on malignant cells of hematologic malignancies, such as AML, B cell leukemia/lymphoma, and T cell leukemia, where they intrinsically promote tumor progression [111]. LILRB2 (CD85D, ILT4, LIR3, and MIR10) expression was observed on human HSCs, and the binding of angiopoietin-like proteins (ANGPTLs) to LILRB2 supports ex vivo expansion of HSCs. In a transplantation AML mouse model, expression of PirB (the mouse ortholog of human LILRB2 and LILRB3) on MLL-AF9-induced AML cells was able to suppress differentiation and enhance self-renewal of LSCs [112]. It was later demonstrated that ANGPTL2/LILRB2 binding was more potent than another ligand, HLA-G [113]. LILRB4 (CD85K, ILT3, LIR5, and HM8) was restrictively expressed on monocytes and monocytic AML cells [114]. LILRB4 expression on leukemia cells suppress T-cell proliferation, as well as promote AML cell migration and infiltration. Apolipoprotein E (APOE) was identified as an extracellular binding ligand of LILRB4. APOE was able to activate LILRB4 on human monocytic AML cells, where SHP-2 was phosphorylated and NF-kB pathway was subsequently activated, resulting in the upregulation of urokinase receptor (uPAR) and arginase-1 (ARG1). As a result, ARG1 inhibited T cell proliferation, which could be augmented by uPAR signaling [114]. In addition, considering that LILRB4 was a monocytic AML-specific antigen, LILRB4-CAR-T was developed and showed efficient effector function in vitro and in vivo against LILRB4+ AML cells, but no toxicity to normal CD34+ cells [114]. As for LILRB5, its role in hematologic malignancies remains unclear [110]. Currently, there is no ongoing clinical trial evaluating LILRBs in hematologic malignancies.

Combination of ICB with bispecific T cell engager ::: Combination of ICB with other therapies ::: Targeting immune checkpoints in hematologic malignancies:
Currently, bispecific antibodies, which recruit patient's T cells or NK cells against cancer cells expressing tumor-associated antigens, have been attracting attention for treating hematologic malignancies. A typical example is CD33/CD3 bispecific T cell engager (BiTE). Given that CD33 is overexpressed in AML blasts, a BiTE antibody against both CD3 and CD33 has been developed to recruit T cells to kill CD33+ AML cells [115]. Similarly, bispecific antibody targeting both CD3 and CD123 has been designed as CD123 is overexpressed in a wide range of hematologic malignancies, particularly on LSCs [116, 117]. However, ongoing clinical trials have showed that only a small fraction of patients could benefit from bispecific antibody treatment. A major mechanism limiting the therapeutic efficacy is due to T cell anergy and exhaustion driven by

inhibitory immune checkpoint pathways, such as PD-L1/PD-1 axis [118]. For example, T cells recruited to CD33-positive cells showed impaired cytotoxicity due to high expression of PD-L1 on AML cells, which was induced by CD33/CD3 BiTE antibody treatment. Inspired by the inhibitory role of PD-L1/PD-1 pathway in AML, combining PD-L1/PD-1 blockade with CD33/CD3 BiTE antibody showed enhanced T cell proliferation and IFN-γ production [119].

Combination of ICB with hypomethylating agents ::: Combination of ICB with other therapies ::: Targeting immune checkpoints in hematologic malignancies:
The expression of PD-L1, PD-L2, PD-1, and CTLA-4 was upregulated in a cohort of MDS, CMML, and AML patients treated with epigenetic therapy, suggesting inhibitory immune checkpoint signaling pathways might be involved in hypomethylating agent (HMA) resistance [13]. HMAs triggered demethylation of the PD-1 promoter leading to increased expression of PD-1 on T cells, which promoted exhaustion of tumor-specific T cells and therefore resulting in immune escape [32]. Therapeutically, number of ongoing clinical trials have been designed to combine HMAs with ICB (Tables 1 and 2). Notably, AZA plus nivolumab showed better OS (16.1 months vs 4.1 months) and better ORR (33% vs 20%) in heavily treated RR AML patients compared to a historical cohort with AZA-based salvage therapy. A second cohort in this trial treated with nivolumab and ipilimumab plus AZA led to 6 of 14 patients achieving CR/CRi [32]. Responders had a progressive increase of CD4+ and CD8+ TILs in the BM, demonstrating that AML patients could benefit from PD-1 blocking therapy. Furthermore, CTLA-4+CD8+ cell numbers were increased in both responders and non-responders, indicating a dual combination of PD-1 blockade and CTLA-4 blockade with AZA might be able to further improve response rates [32].

Combination of ICB with cytokine therapy ::: Combination of ICB with other therapies ::: Targeting immune checkpoints in hematologic malignancies:
Cytokines like IFN-α was approved for the treatment of hairy cell leukemia in 1986 and IL-2 for the treatment of metastatic renal cell carcinoma (1992) and advanced melanoma (1998) [120]. Although being one of the first forays in immunotherapy, nowadays, cytokine therapy is mainly used in combination with other anti-tumor treatments. For example, recently bempegaldesleukin (NKTR-214), an IL2Rβ (CD122)-biased agonist, has shown capabilities of enhancing the proliferation and activation of CD8+ T cells and NK cells without increasing the number of Tregs [121]. Results of PIVOT-02 trial, combination of NKTR-214 and nivolumab, has shown that this combination is safe and efficacious (ORR 48% in 23 patients) in metastatic urothelial carcinoma [122]. Aside from IL-2, IL-15 has also been evaluated in stimulating NK cells and T cells. Combination therapy with IL-15 and blocking antibodies against PD-1 and CTLA-4 has been shown to synergistically activate T cells and prolong the survival of tumor-bearing mice [123]. In addition, a recent study has demonstrated that DC-derived IL-12 is necessary for successful anti-PD-1 cancer therapy, suggesting that IL-12 and PD-1 blockade could be rationally combined [124]. In an earlier study, synergistic effects were observed when tumor-bearing mice were treated with Semliki Forest virus-based vector encoding IL-12 and anti-PD-L1 mAb [125]. Currently, there are limited pre-clinical and clinical trials based on the combination of ICB and cytokine therapy in hematologic malignancies although much more trails are ongoing in solid tumors.

CD22 ::: CAR-T cell immunotherapy for hematologic malignancies:
Although the CD19 CAR-T therapy has yielded potent antileukemic effects in children and adults with RR B-ALL, acquisition of CD19-negative cells and selection of alternatively spliced CD19 isoforms with the compromised epitope were recognized as mechanisms for tumor escape [132, 133]. Similar to CD19, CD22 (also known as Siglec-2) is also expressed on most B-ALL cells, but has a limited expression in normal tissues except B cell lineage [134, 135]. CD22 is therefore proposed as an alternative target for CAR design to treat patients with CD22-expressing B-ALL and CD19dim or CD19– relapse following CA19 CAR-T therapy [136]. Although CD22 CAR-T therapy demonstrated robust antileukemic activity with CR in 11 of the 15 patients and similar safety profile as CA19 CAR-T, relapse still occurred due to the loss of CD22 surface expression [136]. Importantly, a bispecific CAR targeting both CD19 and CD22 was reported to be able to overcome the resistance arising from loss of either CD19 or CD22 expression [136]. Currently, there are 17 ongoing CAR-T clinical trials targeting CD22. One particular dual specificity CD19 and CD22 CAR-T encodes truncated epidermal growth factor receptor (EGFRt) and truncated human epidermal growth factor receptor 2 (HER2t) safety switch, allowing for detection of the CAR-T cells and ADCC-directed elimination of the CAR-T cell (NCT03330691) (Table 3).

CD33 ::: CAR-T cell immunotherapy for hematologic malignancies:
CD33 (Siglec-3) is well known as a marker of myeloid progenitor cells and expressed on all normal myeloid cells [135]. Like CD22, CD33 has long been identified as a diagnostic marker and a therapeutic target for B cell lymphomas and myeloid leukemias [134]. Gemtuzumab ozogamicin (GO), a CD33-specific ADC to calicheamicin, was approved again in 2017 after being withdrawn from the market in 2010 due to safety concerns, for combination therapy with daunorubicin and cytarabine in newly diagnosed CD33+ AML after it doubled the event-free survival from 9.5 to 17.3 months [137]. GO is also approved as a single agent in the RR setting. Meanwhile, SGN-CD33A, another CD33 targeting ADC, was demonstrated to be more potent than GO in vitro and in a xenograft model, but the FDA called a halt to all clinical testing of SGN-CD33A after failure in a phase III trial [138]. Alternatively, CD33-specific CAR-Ts in AML are in preclinical and clinical development [139–141]. For example, CD33-CAR-T therapy exhibited potent antileukemic activities in vitro and in vivo and hematopoietic toxicity [140]. In one patient with RR CD33+ AML, CD33 CAR-T cell infusion led to rapid degradation of blasts in the BM within 2 weeks of infusion; however, the disease relapsed after 9 weeks as CD33+ blasts gradually increased. Even though the clinical toxicities observed in the patient were controllable, more patient data is needed to further validate the safety and efficacy profile of CD33 CAR-T therapy [141]. Most recently, in order to avoid potential serious adverse events caused by CD33 CAR-T therapy, a group came up with an idea to combine allogeneic transplantation of CD33 knockout (KO) HSPCs with CD33 CAR-T therapy [142]. To support this assumption, they engrafted human and rhesus macaques CD33 KO HSPCs into NSG mice and rhesus macaques model, respectively, and found that CD33 was not essential for human myeloid cell functions and rhesus macaques neutrophil functions [142]. Importantly, they demonstrated that human myeloid cells lacking expression of CD33 were resistance to CD33 CAR-T therapy in NSG mice [142]. Therapeutically, a 6-year old heavily pre-treated AML patient achieved MRD-negative remission 19 days post infusion of compound CAR (cCAR) comprising of anti-CLL1 CAR linked to anti-CD33 CAR via a self-cleaving P2A peptide [143]. Some of other ongoing CD33 CAR-T clinical trials include NCT02958397 and NCT03126864 (Table 3)

CD123 ::: CAR-T cell immunotherapy for hematologic malignancies:
CD123 (IL-3Rα) is normally expressed on a fraction of myeloid progenitors and a wide range of hematologic malignancies, including blastic plasmacytoid dendritic cell neoplasm (BPDCN), hairy cell leukemia, B-ALL, MDS, and AML [116, 117, 144]. Antibody-based therapies targeting CD123 have been effective in eliminating AML blasts [145]. CD123 CAR-T cells have also shown activity against CD123+ AML cell lines and primary patient samples in vitro and in vivo [146]. Furthermore, CD123-specific CAR cytokine-induced killer (CIK) cells had limited toxicity on normal BM HSPCs compared to CD33-specific CAR CIK cells, suggesting that CD123 CIK has a better safety profile [139]. Another group, however, raised safety concerns for the use of CD123 CAR-T due to its effect on hematopoiesis [147]. They later dementated that ablation of CAR-T cells with optimal timing after AML eradication might enable durable leukemia remission, controllable hematologic toxicity, and subsequent HSC transplantation [148]. Notably, CD123 CAR-T therapy showed remissions of AML and BPDCN, as well as acceptable feasibility and safety in the first-in-human clinical trial [149]. CD123 CAR-T therapy also exhibited specific killing activity against BPDCN and high-risk MDS in preclinical models [144, 149]. Some CD123 CAR-T trials are ongoing (Table 3). Furthermore, a dual CAR targeting both CD19 and CD123 showed highly anti-leukemia activity against B-ALL in vivo and was able to eradicate CD19− leukemic cells at relapse after CD19 CAR-T administration [150]. Treatment of 3 post allo-SCT relapse B-ALL patients with donor-derived double 4SCART19/4SCAR123 T cells helped achieve MRD-negative remission within 1 month after CAR-T infusion, without evidence of severe CRS or GvHD [151]. The pilot trial of a fourth-generation apoptosis inducible CAR targeting CD123 (CD123-scFv/CD28/CD137/CD27/CD3ζ-iCasp9) decreased disease burden from 60 to 45% in a 47-year-old patient with AML post-allo-SCT relapse [152]. CD123-CLL1 cCAR phase I clinical trial is also ongoing (Table 3).

BCMA ::: CAR-T cell immunotherapy for hematologic malignancies:
B cell maturation antigen (BCMA; CD269), a member of the TNF receptor superfamily, is predominantly expressed on plasma cells and a small subset of normal B cells [153]. In patients with MM, BCMA is expressed uniformly on the surface of malignant plasma cells [154]. A novel ADC targeting BCMA has demonstrated to specifically kill MM cells without causing serious side effect, suggesting BCMA was a suitable and safe candidate for MM treatment [153]. BCMA-specific CAR-T cells have shown effective depletion of MM cells both in vitro and in vivo [155].

Clinical data over the past 2 years with BCMA-specific CAR-T cells has produced MRD-negative remission in heavily pre-treated MM patients [156–159]. NCI published the first-in-humans clinical trial and reported ORR of 81% and a very good partial response (VGPR) of 63% in RR MM patients with median number of 10 prior therapies [156]. The bb2121 CAR-T (Bluebird Bio) produced ORR of 85%, median DOR of 10.9 months, and median PFS of 11.8 months in 33 heavily pretreated (median number of 7 prior therapies; range 3-23) in the phase I, CRB-401 clinical trial [159]. Further, 45% achieved CR (n = 15), 9% achieved stringer CR, and 27% achieved VGPR. Sixteen patients achieved MRD negative remission and the median time to at least a PR was 1 month [159]. The LCAR-B38M CAR-T (LEGEND) uses a new antigen-binding domain that binds to two different antigen epitopes and reported ORR of 88% in 57 patients and MRD-negative remission in 39 of 42 patients in complete remission [158]. Two other abstracts presented by the Memorial Sloan Kettering group at American Society of Hematology annual meeting (ASH 2018) reported ORRs of 64% and 82% with the MCARH171 and JCAR125 CAR-T cells, respectively. The MCARH171 CAR-T encodes for the truncated epidermal growth factor receptor safety system [160]. The University of Pennsylvania CART-BCMA demonstrated ORR of 62% in patients with high-risk cytogenetics including 67% with TP53 or del17p mutation. In vivo CAR-T expansion was higher with the use of cyclophosphamide conditioning and a trend towards benefit was observed with higher peak CAR-T levels although this was not statistically significant [157]. BCMA-targeted CAR-Ts have produced impressive results thus far. However, the durability of the responses remains to be explored.

CD38 ::: CAR-T cell immunotherapy for hematologic malignancies:
CD38 is a type II transmembrane glycoprotein associated with cell-surface receptors in lipid rafts and is able to induce cell growth signal in myeloid leukemia [161]. CD38 is highly and consistently expressed on MM cells and is absent on normal myeloid and lymphoid cells, as well as other nonhematopoietic tissues [161, 162]. Several modified anti-CD38 mAbs, such as daratumumab, isatuximab, and MOR202, have been developed to treat CD38+ RR MM via mechanisms of action including Fc-dependent immune-effector manner and immunomodulatory effects [161, 163, 164]. Of note, daratumumab was approved by the FDA in 2015 to treat MM patients who had received at least three prior lines of therapy. In the presence of rituximab, combining anti-CD19 and anti-CD38 CARs showed synergistic cytotoxicity against B-NHL in vitro and in xenograft mice, providing a powerful rationale for clinical evaluation of CD38 CAR and/or CD19 CAR in the treatment of patients with relapsed B-NHLs after rituximab therapy [165]. However, with high-affinity CD38 CAR-T, off-target toxicities were also observed in addition to expected anti-MM effects. To address the safety concerns, a CAR with lower affinity anti-CD38 scFv was designed. It exhibited better discriminative capacity between MM cells and normal cells without significant loss of expansion, persistence, and cytotoxic potential [166]. Another attempt of CD38 CAR-T optimization utilized “light-chain exchange” technology, which generates new antibodies with up to 1000-fold lower affinities to CD38. By incorporation of scFv with different affinities, high-affinity and low-affinity CD38 CAR-Ts were made. As predicted, low-affinity CD38 CAR-T cells had similar effects as high-affinity CD38 CAR-T cells in eradicating MM cell line UM9, while showed no obvious effect on normal HSPCs in vivo [166].
Clinical trials with CD38 CAR-T in RR MM (NCT03464916) and RR B-ALL (NCT03754764) are underway. In addition, dual specificity CD38/BCMA CAR-T (NCT03767751) is also being explored (Table 3).

CD138 ::: CAR-T cell immunotherapy for hematologic malignancies:
CD138 (Syndecan-1) is a membrane glycoprotein expressed on malignant and healthy differentiated plasma cells, as well as in normal and neoplastic epithelial tissues [167]. CD138 is one of the most specific primary diagnostic markers of MM [162]. A phase I/IIa study in MM patients showed that CD138-specific ADC was well tolerated, suggesting CD138 was a targetable MM-specific antigen [168]. Importantly, in a pilot clinical trial evaluating CD138-directed CAR-T therapy, 4 out of 5 patients diagnosed with chemotherapy-refractory MM experienced myeloma regression and had stable disease longer than 3 months. The study suggests that CD138 CAR-T is safe and tolerable [169]. Dual CD138 and BCMA as well as multi-target CAR-T trials NCT03672318, NCT03196414, NCT03778346 are ongoing (Table 3).

Combination of CAR-T and ICB in hematologic malignancies ::: CAR-T cell immunotherapy for hematologic malignancies:

Despite the encouraging outcomes of CD19 CAR-T therapy in B cell malignancies, poor T cell expansion and short-term T cell persistence remain one of the main causes for lack of response and relapse following CAR-T therapy. Development of T cell exhaustion induced by co-inhibitory pathways has been suspected to contribute to poor persistence and dysfunctions of CAR-T cells [170]. In order to understand why only 26% of CLL patients benefited from CD19 CAR-T therapy while over 90% of CD19-positive B-ALL experienced CR, a detailed transcriptomic analysis was performed to compare T cells from CLL responders and non-responders post CD19 CAR-T therapy. It revealed that CAR-T cells from non-responders showed upregulated pathways involved in exhaustion and apoptosis [130, 171]. The expression level of T cell co-inhibitory receptors, such as PD-1, Tim-3, and LAG-3, were upregulated on CAR-T cells, suggesting possible inhibitory effects induced by these molecules [172, 173]. The PD-L1/PD-1 pathway was able to directly inactivate CD28 signaling in CAR-T using CD28 as co-stimulatory domain and therefore inhibiting CAR-T cell function [173, 174]. Furthermore, PD-1 or LAG-3-deficient CAR-T cells showed improved anti-tumor efficacy in vitro and in vivo [175]. The addition of PD-1 blockade to CD19 CAR-T therapy in 14 children (13 with pembrolizumab and 1 with nivolumab) with heavily pre-treated B-ALL including allo-SCT who initially had poor response to CD19 CAR-T therapy had improved persistence of CAR-T cells, thus resulting in better outcomes in this small, single-center study at Children's Hospital of Pennsylvania (CHOP). Seven of the 14 patients maintained either PR or CR. Three of 6 patients treated with PD-1 inhibitor re-established B cell aplasia suggesting ongoing CAR-T function [176].

Conclusion:
ICB with PD-1/PDL-/CTLA4 inhibitors and CAR-T therapy targeting CD19+ leukemia/lymphoma have forever changed the landscape of cancer therapeutics. The identification of novel immune checkpoints will fill in the gap in which our current therapeutics do not work or after disease relapse. CAR-T therapy has expanded beyond CD19+ with newer targets, and the engineering has become safer and sophisticated with the introduction of cytokines or safety switches. Dual specificity CAR-Ts combat disease relapse due to antigen loss, and the combination of ICB and CAR-T also has shown enhanced therapeutic efficacy. Much remains to be investigated about the optimal method of administrating the new CAR-Ts, their safety, and durability of response. However, as we garner a better understanding of the interplay between these targets and their mechanism of action, the field of immune therapy has the potential to reach more patients and transform cancer care.