Published in final edited form as:

Trends Immunol. 2017 July ; 38(7): 513–525. doi:10.1016/j.it.2017.04.004.

Mechanisms of Immune Tolerance in Leukemia and Lymphoma

Emily K. Curran^{1,2,3}, James Godfrey¹, and Justin Kline^{1,3,4,*}

¹Department of Medicine, Section of Hematology, University of Chicago, Chicago, IL

²Committee on Clinical Pharmacology and Pharmacogenomics, University of Chicago, Chicago, IL

³University of Chicago Comprehensive Cancer Center, University of Chicago, Chicago, IL

⁴Committee on Immunology, University of Chicago, Chicago, IL

Abstract

The mechanisms through which immune responses are generated against solid cancers are well-characterized, and knowledge of the immune evasion pathways exploited by these malignancies has grown considerably. However, for hematological cancers, which develop and disseminate quite differently than solid tumors, the pathways which regulate immune activation or tolerance are less clear. Growing evidence suggests that, while a number of immune escape pathways are shared between hematological and solid malignancies, there are several unique pathways exploited by leukemia and lymphoma. Below, we discuss immune evasion mechanisms in leukemia and lymphoma, highlighting key differences from solid tumors. A more complete characterization of the mechanisms of immune tolerance in hematological malignancies is critical to inform the development of future immunotherapy approaches.

Cancer immune escape — implications for immunotherapy

Tumor immune responses are raised spontaneously in a subset of human solid cancers. However, by the time of clinical diagnosis, cancer cells and their stromal network, sensing the presence of activated immune cells and effector cytokines, have triggered immune suppressive pathways that cooperate to enforce the dysfunction of tumor antigen-specific T cells, ultimately leading to a profound immune tolerant state [1]. Significant advances in the identification of immune evasion mechanisms employed by solid tumors have catalyzed the development of immunotherapeutic strategies to reverse immune tolerance in cancer, for example through blockade of immune checkpoints, most notably programmed death — 1/programmed death — ligand 1 (PD-1/PD-L1) interactions.

*Corresponding author: Justin Kline, M.D., 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, jkline@medicine.bsd.uchicago.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The success of immunotherapy for solid cancers [2–4] has generated enthusiasm for its use in hematological malignancies, and the excellent clinical results of PD-1 blockade therapy in classical Hodgkin lymphoma (cHL) [5, 6] are extremely encouraging. However, immune checkpoint blockade therapy has been less impressive in many other hematological malignancies [8–10] highlighting the need for better understanding of immune evasion in these cancers.

Hematologic malignancies develop and disseminate differently than solid tumors. Likewise, the pathways that control immune activation or tolerance in these diseases may be markedly different than what has been described for solid cancers. Although some immune escape pathways are shared between solid and hematological cancers [11–15], unique tolerance mechanisms are operational in the latter [16] (Figure 1). It is essential to have a more complete understanding of immune evasion in hematological malignancies in order to facilitate data-driven testing of immunotherapeutic approaches for these cancers. Below, we discuss immune tolerance mechanisms employed by leukemia and lymphoma, including those that are common to cancer in general, and those unique to hematological cancers.

Evidence for unique regulation of immune tolerance in leukemia and lymphoma

Hematological cancers originate and progress in primary or secondary lymphoid organs where immune cells develop and reside, and in which anti-tumor immune responses are typically initiated. This suggests that most are either poorly immunogenic, and fail to alert innate or adaptive immune sensing mechanisms (immunological ignorance), or that they are adept at suppressing immune responses when they do occur (immune evasion). cHL is a prototypical hematological cancer that employs distinctive mechanisms to achieve immune subversion. Analysis of lymph nodes involved by cHL reveals a remarkable accumulation of innate and adaptive immune cells, suggesting that immunological ignorance is not an issue in this disease. T cells can be readily identified surrounding malignant Hodgkin Reed-Sternberg (HRS) cells [17–21], indicative that a spontaneous anti-lymphoma response has occurred. Rather than eliminating HRS cells, the function of lymphoma-resident immune cells is redirected to promote HRS cell survival and to generate an immune suppressive microenvironment [22–25]. HRS cells further escape host immune surveillance through defective antigen presentation on major histocompatibility complex (MHC) class I molecules [26], and blunt effector T cell function through their near universal expression of PD-L1 [27, 28]. PD-L1 expression on HRS cells results from amplification of the *PD-L1* locus on chromosome 9p24.1 [28] — a phenomenon that occurs rarely in solid tumors [29]. Thus, unique drivers of immune checkpoint ligand expression are active in cHL.

Acute leukemias disseminate rapidly after inception, which may negatively impact the initiation and execution of anti-leukemia immunity. This notion is supported by recent observations in a murine AML model [16]. While localized implantation of AML cells (to mimic solid tumor development) induced a robust leukemia antigen-specific CD8⁺ T cell response in the tumor-draining lymph node, systemic introduction of AML cells induced a potent T cell tolerant state, characterized by the abortive proliferation and deletion of

leukemia-specific CD8⁺ T cells. The tolerant CD8⁺ T cell phenotype could be prevented following administration of an agonistic anti-CD40 antibody, implicating host antigen-presenting cells (APCs), and perhaps a lack of APC activation, as drivers of the phenomenon [16]. APCs were also essential for the induction of T cell tolerance in animals with systemic lymphoma [30, 31]. Here, lymphoma-specific CD4⁺ T cells were anergized in animals harboring systemic A20 lymphomas through a mechanism requiring antigen presentation by bone marrow-derived cells. These data indicate that the regulation of T cell tolerance in systemic hematological cancers occurs at the level of APCs. A paradigm begins to emerge regarding the disparate fates of cancer-specific T cells in solid versus systemic hematological cancers. In the former, tumor-specific T cells are primed, but become functionally impaired in the tumor environment. In the later, leukemia (or lymphoma) — specific T cells are never properly activated, but rather are deleted or anergized upon initial antigen encounter. This paradox has potential ramifications for the efficacy of immune therapies aimed at restoring T cell function (for example through checkpoint blockade therapy) in these unique disease settings.

Subversion of immune surveillance

Recognition and elimination of malignant cells by T cells requires antigen processing and presentation in the context of MHC molecules. Acquired defects in these processes are a well-established mechanism of immune evasion in solid and hematological cancers [32–34]. For example, whole exome sequencing of HRS cells demonstrated recurrent loss-of-function mutations in β_2 -microglobulin (β_2 M), a key component of MHC class I [26]. Similarly, MHC class I expression was absent in more than 50% of follicular lymphomas, again often resulting from mutations in β_2 M [35]. MHC class II expression is also frequently downregulated in lymphomas due to mutations and epigenetic silencing of transcription factors and adapters required for MHC class II gene expression [26, 36]. Furthermore, deletion of the MHC class II locus on chromosome 6 is a recurrent finding in testicular and CNS DLBCL [37], and the activated B-cell (ABC) subtype of DLBCL has been shown to lose MHC class II expression due to upregulation of the transcription factor forkhead box P1 (FOXP1) [38]. Recurrent MHC class II loss or downregulation in B cell lymphomas is interesting as B cells can present antigens in the context of MHC class II molecules (unlike solid cancer cells). These observations suggest that antigen presentation by lymphoma B cells to CD4⁺ T cells may promote immune surveillance, and that downregulation or deletion of MHC class II may be important for induction of T cell tolerance in B cell lymphomas. In contrast, loss of MHC expression is rare in newly-diagnosed acute leukemia, but may occur more commonly upon disease relapse [39]. Loss of mismatched human leukocyte antigen (HLA) has also been described in patients with relapsed AML following haploidentical stem cell transplantation, and is caused by loss of heterozygosity for the shared HLA haplotype [40–42].

Cancers can also avoid immune recognition through selective outgrowth of cells that have lost expression of immunogenic antigens (antigen loss variants). This phenomenon has been described in solid tumors [43], but its role in hematologic malignancies has not been evaluated. Furthermore, next generation sequencing has revealed relatively low numbers of somatic mutations in leukemias and lymphomas compared to many solid tumors [44].

Somatic mutations in cancer cells generate altered proteins (neo-antigens) that can be “sensed” as foreign by the adaptive immune system and thus, the “load” of somatic mutations in a given tumor may affect its immunogenicity [29]. It is possible that the low mutational burden of leukemias and lymphomas results in a paucity of neo-antigens, which may limit host immunosurveillance. One notable exception, however, is cHL. Data in several solid tumor subtypes (melanoma, lung cancer and mismatch repair-deficient cancers) has revealed a strong correlation between mutational burden and responsiveness to immune checkpoint blockade therapy [45–47]. Whole exome sequencing of HRS cells from cHL samples revealed a median of only 244 somatic mutations per case [26] — a much lower number compared to that found in the above-mentioned solid tumors [44]. Nevertheless, PD-1 blockade therapy is extraordinarily effective in cHL [5, 6, 48], suggesting, at least in this disease, that antigenic quality is as important as quantity. It is noteworthy that a significant proportion of cHL samples harbor evidence of EBV infection. Thus, in some cases, the “high quality” HRS-specific antigens may be derived from immunogenic EBV antigens [49], and PD-1 blockade therapy may elicit clinical activity by restoring cytotoxic T cell responses against virally-encoded antigens. Regardless, the effectiveness of PD-1 blockade in cHL remains somewhat surprising given the high incidence of MHC class I downregulation on HRS cells [26]. Additional studies are needed to clarify the nature of immunogenic antigens (viral- or neo-antigens) in cHL, and to investigate the role of CD4⁺ T cells in the response to anti-PD-1 antibody therapy. Lastly, it will be important to determine how MHC class I antigens are recognized by CD8⁺ T cells in the setting of diminished or absent MHC class I expression on HRS cells.

Evasion of innate immune sensing

Innate immune sensing of cancer is a critical first step in the generation of adaptive anti-tumor immune responses. “Immunogenic cancer cell death” results in the exposure or release of danger-associated molecular patterns (DAMPs), including calreticulin [50], high motility group nucleosome binding domain 1 (HMGB1) [51] and nucleic acids [52, 53]. Conserved pattern recognition receptors (PRRs) mediate DAMP recognition by DCs and macrophages, resulting in enhanced antigen presentation and expression of co-stimulatory ligands and cytokines that culminate in anti-tumor T cell cross-priming [54]. In solid cancer models, type I interferon (IFN) is critical to link innate and adaptive anti-tumor immune responses, largely through its effect on basic leucine zipper transcription factor ATF-like3 (Batf3) dependent DCs [55–57]. Tumor DNA is a key mediator of type I IFN production in DCs by triggering a cytosolic sensor called Stimulator of Interferon Genes (STING) [53]. Thus, tumor DNA and the STING pathway link innate immune sensing of cancer with the subsequent activation of anti-tumor T cell immune responses. A type I IFN gene signature was also identified in human melanomas densely infiltrated by CD8⁺ T cells [56] — an observation that linked the importance of type I IFN and adaptive anti-tumor immunity in cancer patients. It was subsequently shown that these so-called “inflamed” tumors were more responsive to cancer immunotherapies compared to “non-inflamed” cancers that lacked the type I IFN gene signature.

Conversely, pre-clinical evidence suggests that hematologic malignancies, such as acute leukemia, may harbor a “non-inflamed” phenotype, due to their inability to trigger innate

immune activation [16, 58]. The precise cause remains unclear, but the disseminated nature of these diseases may prevent sufficient DAMP accumulation necessary for the maturation of leukemia antigen cross-presenting DCs, resulting in T cell tolerance and disease progression. This notion is supported by data demonstrating that systemic activation of host APCs via agonistic anti-CD40 antibodies or STING agonist compounds is sufficient to overcome DC-mediated T cell tolerance in murine leukemia models [16, 58]. Below, we discuss two innate immune pathways that may be particularly relevant to immune escape by hematological cancers.

Type I interferon

In solid cancer models, type I IFN production by DCs in the tumor-draining lymph node is a critical early event required for spontaneous cross-priming of tumor-specific CD8⁺ T cells [56]. Mice lacking the IFN- α/β receptor (*Ifnar*^{-/-}) in DCs fail to control immunogenic solid tumors due to defective priming of tumor-specific CD8⁺ T cells [55, 56]. Conversely, we have shown that leukemia-bearing mice fail to generate a type I IFN response, and that the inability of the host to sense type I IFN does not substantially decrease survival [58]. These observations suggest that the T cell tolerant phenotype in mice with leukemia may be due to the failure of AML cells to trigger type I IFN production by DCs, and provide rationale for the development of immunotherapies that activate type I IFN in hosts with systemic hematological cancers. Tumor-derived DNA is a potent inducer of type I IFN in DCs through activation of the STING pathway [53], and it is possible that the failure of leukemia to induce type I IFN may stem from diminished activation or negative regulation of the STING pathway. Interestingly, mutations in the DEAD/H-box helicase gene *Ddx41* have been demonstrated in familial leukemias [59, 60], and DDX41 has been proposed to function as a critical component of the STING pathway [61, 62]. Whether predisposition to leukemia in these families results from impaired immune surveillance via STING has not been elucidated. Regardless, the role of type I IFN in leukemia appears to differ considerably from solid malignancies and further study of these mechanisms will be critical for understanding the host immune response to leukemia.

CD47

Phagocytosis of host cells, including cancer cells, by macrophages and DCs is regulated by a balance of “eat me” and “do not eat me” signals. Phosphatidylserine (PS) and calreticulin are dominant pro-phagocytic receptors on apoptotic cells [63]. The CD47 (integrin associated protein; IAP) receptor, on the other hand, inhibits phagocytosis upon ligation with signal regulatory protein α (SIRP α) on macrophages and DCs [64, 65]. CD47/SIRP α binding results in phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIM) and recruitment of Src homology phosphatase 1 and 2 (SHP-1 and SHP-2), which inhibit accumulation of myosin-IIA at the phagocytic synapse [65–67]. Under homeostatic conditions, CD47 regulates normal cell turnover [68]. However, increased CD47 expression has been observed on a variety of malignant cells, including AML [69], ALL [70], leukemia stem cells (LSC) [69], and B cell NHL [71]. In fact, high CD47 expression levels were an independent predictor of poor survival in cohorts of AML and NHL patients [71, 72]. Thus, increased CD47 expression on leukemia/lymphoma cells promotes innate immune subversion through evasion of phagocytosis. Human CD47-blocking antibodies have

demonstrated efficacy in xenograft AML and ALL models [69, 71, 72], and CD47 blockade synergized with anti-CD20 antibody therapy to enhance the phagocytosis of lymphoma cells in a xenograft model [71]. High-affinity SIRP α variants also promote enhanced cancer cell phagocytosis when combined with tumor-specific mAbs [73], representing another approach to interrupt CD47-SIRP α interactions.

The efficacy of CD47 blockade in xenograft systems was thought to derive exclusively from enhanced uptake and elimination of cancer cells by phagocytes. However, in syngeneic tumor models, including A20 lymphoma, CD47 blockade resulted in enhanced cross-priming of tumor-specific T cells by DCs in a type I IFN and STING-dependent manner [74], indicating that the mechanisms underlying the activity of CD47 blockade therapy involve adaptive immunity, and are more complex than initially proposed. Moreover, it was recently demonstrated in a MYC-driven mouse T cell ALL model that CD47 mRNA and cell-surface protein expression was directly regulated by MYC, which directly bound the promoter region of *Cd47* [75]. Inhibition of MYC resulted in decreased *Cd47* expression [75]. As MYC is commonly dysregulated in Burkitt lymphoma and DLBCL, these findings may inform the identification of lymphomas that are more likely to be sensitive to blockade of the CD47/SIRP α axis. Increased CD47 expression has also been demonstrated in solid tumors, and in some cases, higher CD47 mRNA expression correlated with decreased progression-free and overall survival. Furthermore, CD47 blockade promoted in vitro phagocytosis of human breast, bladder and hepatocellular cancer cells by macrophages, and inhibited growth of solid tumors in xenograft and syngeneic models [74, 76]. Taken together, these observations indicate that targeting CD47 or SIRP α may be an effective immunotherapeutic strategy for hematological cancers (and solid cancers as well), and early-phase clinical trials testing the safety and activity of CD47 blocking antibodies are underway (NCT02678338, NCT02641002, NCT02663518, NCT02367196).

Negative regulatory receptors and ligands

The induced expression of negative regulatory immune receptors, or immune “checkpoints,” on activated T cells represents a natural adaptive response aimed at mitigating tissue damage mediated by release of cytokines and lytic molecules [77, 78]. In solid cancers, expression of negative regulatory receptors, such as PD-1, cytotoxic T lymphocyte antigen — 4 (CTLA-4), and lymphocyte activation gene — 3 (LAG-3), on tumor-infiltrating T cells, serves as a marker of both tumor antigen specificity and a dysfunctional state [77–79]. Expression of ligands for negative regulatory receptors by tumor cells or APCs represents a highly effective mechanism through which cancers suppress immune responses directed against them [80]. Furthermore, the finding of upregulated expression of negative regulatory receptors on T cells or their ligands on malignant cells indicates the presence of a spontaneous anti-cancer adaptive immune response. These observations have culminated in the development of checkpoint blockade therapy, which has revolutionized the treatment of various malignancies by restoring functional anti-tumor T cell responses. Below, we discuss evidence supporting the involvement of key negative costimulatory receptors and their ligands in the regulation of immune responses against hematologic malignancies.

PD-1/PD-L1

Engagement of PD-1 with its ligand, PD-L1, leads to downregulation of T cell effector function through SHP-2-mediated inhibition of proximal TCR signaling events [81, 82]. Similar to solid tumors, the PD-1/PD-L1 axis also promotes immune escape in some hematologic malignancies. For instance, increased expression of PD-L1 was observed on leukemic blasts and peripheral blood mononuclear cells from patients with myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia [83]. Pre-clinical leukemia models have also demonstrated enhanced PD-L1 expression on AML cells following transplantation into immunocompetent hosts. Furthermore, leukemia-specific CD8⁺ T cell responses and survival were significantly augmented in leukemia-bearing *Pdl*^{-/-} mice, and in those treated with an anti-PD-L1 antibody [11, 84].

Additionally, a unique mechanism of PD-L1 expression has been uncovered in cHL and PMBL — lymphomas that share morphologic and genetic similarities. Through gene copy number analysis, cHL and PMBL cell lines and primary samples were shown to harbor recurrent copy gains of chromosome 9p24.1, which contains the loci encoding PD-L1, PD-L2 and Janus kinase 2 (Jak2) [28]. The fact that chromosome 9p24.1 copy number alterations (CNA) are found in nearly all cases of cHL [27], suggests this genetic aberration is crucial to early tumorigenesis, and the finding that the degree of 9p24.1 amplification correlates with disease stage in cHL further illustrates the importance of this pathway in promoting disease progression [27]. Although JAK2 is amplified in conjunction with PD-L1, and could be the primary driver of tumorigenesis, strong evidence refutes this notion. For instance, JAK2 inhibition demonstrates quite modest clinical activity in cHL compared to PD-1 blockade [6, 85]. Furthermore, the degree of 9p24.1 amplification correlates with best overall response and progression-free survival following treatment with nivolumab in cHL, which suggests it is the impairment of anti-lymphoma immune responses by PD-1/PD-L1 interactions, and not enhanced JAK2 activity that promotes lymphoma progression in the setting of 9p24.1 amplification [86]. In addition to cHL and PMBL, 9p24.1 CNA have also been recurrently observed in primary CNS and testicular lymphomas [87], and sporadically in DLBCLs [88]. In contrast, 9p24.1 amplification is an uncommon driver of PD-L1 upregulation in solid cancers [29].

Collectively, these findings have led to clinical trials evaluating therapeutic PD-1 blockade in hematologic malignancies. While anti-PD-1 therapy has been effective in selected lymphomas [6], other hematologic cancers appear less responsive. Numerous trials are now underway testing anti-PD-1 or anti-PD-L1 antibodies in combination with other immune checkpoint modulating therapies, CAR T cell therapy, hypomethylating agents, radiation, and stem cell transplantation in attempts to enhance their efficacy (NCT02677155, NCT02362997, NCT02926833, NCT02961101, NCT03005782, NCT02981914).

CTLA-4

CTLA-4 is a negative regulatory receptor with structural similarity to the T cell costimulatory molecule, CD28 [89]. Like PD-1, the cell-surface expression of CTLA-4 is rapidly induced in conventional T cells upon activation [90]. While both CTLA-4 and CD28 bind the B7 family members CD80 and CD86 on APCs, CTLA-4 has 10–100 times greater

affinity [91, 92], and competitive binding of CTLA-4 to B7 molecules promotes the inhibition of T cell effector function through a mechanism different from PD-1 [82]. CTLA-4 is also constitutively expressed on Treg cells [93], and is critical for their suppressive function [94]. CTLA-4 was the first negative regulatory receptor discovered, and the first to be manipulated to modulate anti-tumor T cell responses [95]. In hematological malignancies, overexpression of CTLA-4 on lymphoma-resident regulatory T cells and PBMCs has been reported in cHL when compared to healthy donor PBMCs, and treatment with CTLA-4 blocking antibodies abrogated regulatory T cell mediated suppression of effector T cells in vitro [96]. In a murine AML model, CTLA-4 blockade had no effect on disease progression [97], although it enhanced anti-lymphoma immunity when combined with anti-CD20 antibody therapy in the A20 lymphoma model [98]. Moreover, CTLA-4 expression on donor T cells can occur following allogeneic stem cell transplantation, where certain polymorphisms in CTLA-4 have been associated with the risk of AML relapse [99], suggesting that CTLA-4 may be important in modulating allo-immune responses. Clinical trials of anti-CTLA-4 antibodies in hematological cancers [10], including in the context of allogeneic stem cell transplantation [100, 101], have revealed modest efficacy, but a small number of patients have achieved durable responses [10]. Overall, the impact of CTLA-4 on the regulation of anti-cancer immunity in hematological malignancies appears low, and CTLA-4 blockade therapy has demonstrated limited efficacy in these diseases.

LAG-3

LAG-3 is another coinhibitory receptor expressed on activated T cells. LAG-3 shares structural similarity to CD4, and binds MHC class II with higher affinity [102]. Unlike *Pd1*^{-/-} and *CTLA-4*^{-/-} mice, *LAG-3*^{-/-} mice do not develop spontaneous autoimmunity [103]. However, LAG-3 is clearly a negative regulator of T cell function [104]. LAG-3 also mediates the suppressive function of Treg cells [105]. Expression of LAG-3 concurrently with other negative regulatory receptors on dysfunctional T cells in the solid tumor microenvironment also suggests that it contributes to the generation of the tolerant T cell phenotype [106, 107]. The mechanism through which LAG-3/MHC class II interactions inhibit T cell effector function remains unclear, and while LAG-3 blockade alone has not been effective in pre-clinical cancer models, combined PD-1 and LAG-3 blockade synergized to enhance anti-tumor immunity [106]. Emerging evidence also supports a role for LAG-3 in promoting T cell dysfunction in hematological malignancies. For example, in a murine AML model, LAG-3 was upregulated on tolerant leukemia-specific CD8⁺ T cells, and the addition of an anti-LAG-3 antibody to combination CTLA-4 and PD-1 blockade resulted in enhanced expansion and effector function of AML-specific CD8⁺ T cells, which was associated with prolonged survival [108]. LAG-3 also appears to play an important role in the regulation of EBV-specific immune responses in cHL [109]. Clinical trials investigating LAG-3 blockade alone or in combination with anti-PD-1 antibodies are ongoing in patients with selected hematological malignancies (NCT02061761 and NCT03005782).

Recruitment and expansion of immunosuppressive cells

Several immunosuppressive cell types are known to be important in promoting tumor immune evasion, including Tregs, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) [110]. In the cancer context, the expansion of immunosuppressive cells results from both skewing or conversion of pre-existing immune cells to harbor immune suppressive phenotypes (“re-education”), as well as enhanced recruitment through secretion of chemokines and cytokines in the tumor niche [111]. Interestingly, the degree of dependence on these immune suppressive cell populations varies widely among hematologic malignancies. For instance, cHL represents an extreme example, where the normal lymph node architecture is completely replaced by an inflammatory milieu rich in immune suppressive cells [17, 18]. This is in contrast to lymphomas such as Burkitt lymphoma, where there is a relative paucity of immune suppressive cells, and the normal nodal tissue is almost completely replaced by malignant cells [111]. Thus, immunosuppressive cells have a heterogeneous role in hematologic malignancies.

Regulatory T cells

Tregs are a population of CD4⁺ T cells with naturally immunosuppressive functions that rely on constitutive expression of the FOXP3 transcription factor [112], and are essential for the maintenance of peripheral tolerance to self-antigens [113, 114]. In solid malignancies, the Treg cell population expands, and Treg cell frequency in the tumor environment has often correlated with tumor stage and poorer prognosis [115, 116]. In animal cancer models, Treg cell depletion or functional modulation has resulted in significantly enhanced anti-tumor T cell responses, and in some cases, tumor rejection [117]. Tregs also expand in mice with AML, and Treg cell depletion, achieved through an IL-2 — diphtheria toxin (DT), led to enhanced anti-leukemia immunity, particularly when combined with PD-L1 blockade [13, 84]. Patients with AML harbor increased frequencies of peripheral blood Tregs when compared to healthy controls [118]. Furthermore, among AML patients, an increase in Tregs correlates with reduced remission rates following standard chemotherapy [118]. Given their prominent role in facilitating cancer immune escape, there has been great interest in developing strategies to deplete Tregs or to reduce their suppressive function. Although this has been accomplished time and again in pre-clinical experiments, similar approaches in humans have not been successful to date [119].

Interestingly, in contrast to solid tumors and AML, there is evidence that a higher Treg frequency may instead be associated with improved prognosis in certain lymphomas [120]. For example, in cHL, FOXP3 expression by immunohistochemistry in biopsy specimens is correlated with improved overall survival [121, 122]. Although the underlying mechanisms are unclear, it is possible that Treg cells may directly inhibit the proliferation of HRS cells [123]. Alternatively, as has been shown in solid tumors [124], the increased frequency of Tregs in the tumor environment may occur in response to the activation of an anti-tumor immune response.

Tumor-associated macrophages (TAMs)

“Tumor-associated macrophages” (TAMs) are abundant in many solid tumors, are highly immunosuppressive, and inhibit anti-tumor immune responses through various mechanisms, including expression of inhibitory ligands, triggering of caspase-dependent T cell apoptosis, recruitment and induction of regulatory T cells, and arginase-mediated suppression of T cell activity [125]. In cHL, gene expression profiling revealed a “TAM gene signature” that was associated with a higher risk for primary treatment failure, and an increased number of CD68⁺ macrophages correlated with a decreased progression-free and disease-specific survival [126]. The role of TAMs in other lymphomas is less clear. A study of patients with DLBCL demonstrated that higher numbers of CD68⁺ macrophages were associated with significantly worse progression-free and overall survival when treated with chemotherapy alone, but the inverse association was demonstrated when rituximab, an anti-CD20 antibody, was added to treatment [127]. In murine sarcoma, breast, colon, and cervical cancer models, colony-stimulating factor 1 receptor (CSF1R) blockade reduced TAMs, enhanced T cell infiltration and inhibited tumor growth [128, 129]. A small trial of an anti-CSF1-receptor antibody in humans demonstrated partial clinical responses in 5 of 7 patients with diffuse-type giant cell tumor [129], and clinical trials are underway in advanced solid tumors (NCT01494688). Whether anti-CSF1R blocking antibodies will be effective in leukemia and lymphoma is unknown.

Myeloid-derived suppressor cells

MDSCs are a heterogeneous population of immature myeloid cells [130]. While present at low frequencies in the blood of healthy individuals, MDSCs accumulate in multiple cancers and contribute to immune evasion through production of immunosuppressive cytokines and reactive oxygen species, induction of Tregs, depletion of key amino acids required for T cell proliferation, and inhibition of T cell tumor infiltration [130]. Interestingly, MDSCs develop and accumulate in the same compartments as do leukemias and lymphomas (bone marrow and peripheral lymphoid organs), and recent evidence suggests that tumor-resident MDSCs possess more potent antigen-independent suppression compared to those in peripheral lymphoid organs [130]. Whether this observation has implications for the behavior of MDSCs in lymphoma has not been evaluated.

MDSCs are increased in the bone marrow of patients with AML, and elevated numbers of MDSCs following chemotherapy correlate with residual disease [131]. Similarly, increased MDSC frequencies in the peripheral blood of cHL patients predicted for poor clinical outcomes [132]. Strategies to deplete MDSCs or modulate their suppressive function have been thoroughly investigated in solid tumor models [134]. However, in a murine lymphoma model, administration of MDSC-targeted peptide-Fc fusion proteins resulted in depletion of MDSCs and impaired tumor growth [133]. Overall, the potential efficacy of MDSC-targeted immunotherapeutic approaches in leukemia and lymphoma has been under-explored.

Conclusions and future perspectives

Immune escape is a major obstacle to the delivery of effective cancer immunotherapy. A thorough characterization of immune tolerance mechanisms in solid cancers has been critical

for the development of the immune-based therapies available in the clinic today, many of which are aimed at reversal of tumor-induced immune suppression. Our understanding of immune evasion pathways orchestrated by hematological malignancies is growing but insufficient. Although many pathways are shared between solid and hematological cancers, several are unique to leukemia and lymphoma. One essential but under-explored question is the degree to which hematological cancers are immunogenic, given their low mutational load. Also, the rapid kinetics with which many hematological cancers develop, their disseminated nature, and the chemotherapies used in treatment protocols may negatively impact not only spontaneously-generated host immune responses, but also those induced following immunotherapy. Pre-clinical AML and lymphoma models have suggested that due to lack of functional T cell priming in these disease settings, strategies aimed at restoring T cell function with checkpoint blockade therapies may be relatively ineffective. Rather, immune-based strategies targeting activation of APCs, for example through type I IFN, could lead to improved results. Furthermore, the interrogation of immune surveillance and evasion mechanisms in hematological cancers could be aided by development of genetically engineered leukemia/lymphoma models that more accurately recapitulate human disease (see Outstanding Questions).

Glossary

Batf3

Basic leucine zipper transcription factor ATF-like 3. A transcription factor critical for CD8 α dendritic cell development

Beta-2 Microglobulin

the invariant soluble component of MHC class I molecules. It is crucial to provide stability to MHC class I structure

BET Inhibitor

Bromodomain extra-terminal inhibitor. Prevents protein-protein interactions between BET proteins and oncogenic transcriptional programs such as MYC

CD28

Cluster of differentiation 28. Costimulatory protein expressed on T cells. When ligated by CD80 and CD86 costimulatory ligands on antigen-presenting cells, CD28 transmits signals required for naïve T cell activation

CD40

Cluster of differentiation 40. TNF receptor superfamily member expressed on antigen-presenting cells. Engagement of this CD40 activates antigen presenting cells to promote T cell activation

CD80/86

Cluster of differentiation 80 and 86. Cell surface proteins expressed by antigen-presenting cells that provide co-stimulatory signals necessary for T cell activation

Cereblon

Critical protein component of an ubiquitin-ligase complex that is necessary to target senescent proteins for degradation by the proteasome

DAMPs

Danger-associated molecular patterns. Various biomolecules that initiate or perpetuate an inflammatory response upon binding of pattern recognition receptors expressed by antigen-presenting cells

Granzyme A

Protease contained in the granules released by cytotoxic T cells and NK cells to induce programmed cell death in target cells

Haploidentical

Sharing half of the genetic information for a specified allele with another individual

HLA

Human leukocyte antigen. Gene complex that encodes the major histocompatibility proteins

JAK2

Janus kinase 2. Non-receptor tyrosine kinase critical in mediating signal transduction from cell surface receptors

Major Histocompatibility Complex

Cell surface proteins that present antigens to the adaptive immune system

MYC

Oncogene that functions to promote cell growth and cell cycle progression

Perforin

Pore-forming cytolytic protein found in the granules of cytotoxic T cells and NK cells that promotes target cell death

PRRs

Pattern recognition receptors. Protein receptors expressed by cells of the innate immune system that recognize and bind danger-associated molecular pattern molecules to activate pro-inflammatory signaling pathways

SHP

Src homology phosphatase. Phospho-tyrosine binding domains that are important mediators of signal transduction for a variety of cytokines and growth factors

STING

Stimulator of interferon genes. Intracellular receptor which is critical in activating transcription of type I interferon in response to cytosolic tumor DNA and is required for anti-tumor immunity

Uniparental Disomy

A situation where the two copies of a specific chromosome are both from the same parent

References

1. Dunn GP, et al. The three Es of cancer immunoediting. *Annu Rev Immunol.* 2004; 22:329–360. [PubMed: 15032581]
2. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010; 363:711–723. [PubMed: 20525992]
3. Robert C, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet.* 2014; 384:1109–1117. [PubMed: 25034862]
4. Topalian SL, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012; 366:2443–2454. [PubMed: 22658127]
5. Younes A, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol.* 2016; 17:1283–1294. [PubMed: 27451390]
6. Ansell SM, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med.* 2015; 372:311–319. [PubMed: 25482239]
7. Zinzani PL, et al. Phase 1b Study of Pembrolizumab in Patients with Relapsed/Refractory Primary Mediastinal Large B-Cell Lymphoma: Results from the Ongoing Keynote-013 Trial. *Blood.* 2016; 128:619–619.
8. Daver N, et al. Phase IB/II Study of Nivolumab in Combination with Azacytidine (AZA) in Patients (pts) with Relapsed Acute Myeloid Leukemia (AML). *Blood.* 2016; 128:763–763. [PubMed: 27354720]
9. Lesokhin AM, et al. Nivolumab in Patients With Relapsed or Refractory Hematologic Malignancy: Preliminary Results of a Phase Ib Study. *J Clin Oncol.* 2016; 34:2698–2704. [PubMed: 27269947]
10. Ansell SM, et al. Phase I study of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with relapsed and refractory B-cell non-Hodgkin lymphoma. *Clin Cancer Res.* 2009; 15:6446–6453. [PubMed: 19808874]
11. Zhang L, et al. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. *Blood.* 2009; 114:1545–1552. [PubMed: 19417208]
12. Zhou Q, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood.* 2011; 117:4501–4510. [PubMed: 21385853]
13. Zhou Q, et al. Depletion of endogenous tumor-associated regulatory T cells improves the efficacy of adoptive cytotoxic T-cell immunotherapy in murine acute myeloid leukemia. *Blood.* 2009; 114:3793–3802. [PubMed: 19724059]
14. Wang X, et al. Increased population of CD4(+)CD25(high), regulatory T cells with their higher apoptotic and proliferating status in peripheral blood of acute myeloid leukemia patients. *Eur J Haematol.* 2005; 75:468–476. [PubMed: 16313258]
15. Mussai F, et al. Acute myeloid leukemia creates an arginase-dependent immunosuppressive microenvironment. *Blood.* 2013; 122:749–758. [PubMed: 23733335]
16. Zhang L, et al. CD40 ligation reverses T cell tolerance in acute myeloid leukemia. *J Clin Invest.* 2013; 123:1999–2010. [PubMed: 23619361]
17. Vardhana S, Younes A. The immune microenvironment in Hodgkin lymphoma: T cells, B cells, and immune checkpoints. *Haematologica.* 2016; 101:794–802. [PubMed: 27365459]
18. Aldinucci D, et al. The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape. *J Pathol.* 2010; 221:248–263. [PubMed: 20527019]
19. Kuppers R. New insights in the biology of Hodgkin lymphoma. *Hematology Am Soc Hematol Educ Program.* 2012; 2012:328–334. [PubMed: 23233600]
20. Steidl C, et al. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. *J Clin Oncol.* 2011; 29:1812–1826. [PubMed: 21483001]
21. Liu Y, et al. The microenvironment in classical Hodgkin lymphoma: an actively shaped and essential tumor component. *Semin Cancer Biol.* 2014; 24:15–22. [PubMed: 23867303]

22. Niens M, et al. Serum chemokine levels in Hodgkin lymphoma patients: highly increased levels of CCL17 and CCL22. *Br J Haematol.* 2008; 140:527–536. [PubMed: 18275430]
23. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res.* 2016; 27:109–118. [PubMed: 27995907]
24. Yaddanapudi K, et al. Control of tumor-associated macrophage alternative activation by macrophage migration inhibitory factor. *J Immunol.* 2013; 190:2984–2993. [PubMed: 23390297]
25. Juszczynski P, et al. The AP1-dependent secretion of galectin-1 by Reed Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. *Proc Natl Acad Sci U S A.* 2007; 104:13134–13139. [PubMed: 17670934]
26. Reichel J, et al. Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells. *Blood.* 2015; 125:1061–1072. [PubMed: 25488972]
27. Roemer MG, et al. PD-L1 and PD-L2 Genetic Alterations Define Classical Hodgkin Lymphoma and Predict Outcome. *J Clin Oncol.* 2016; 34:2690–2697. [PubMed: 27069084]
28. Green MR, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood.* 2010; 116:3268–3277. [PubMed: 20628145]
29. Rooney MS, et al. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell.* 2015; 160:48–61. [PubMed: 25594174]
30. Sotomayor EM, et al. Cross-presentation of tumor antigens by bone marrow-derived antigen-presenting cells is the dominant mechanism in the induction of T-cell tolerance during B-cell lymphoma progression. *Blood.* 2001; 98:1070–1077. [PubMed: 11493453]
31. Staveley-O'Carroll K, et al. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc Natl Acad Sci U S A.* 1998; 95:1178–1183. [PubMed: 9448305]
32. Mottok A, et al. Genomic Alterations in CIITA Are Frequent in Primary Mediastinal Large B Cell Lymphoma and Are Associated with Diminished MHC Class II Expression. *Cell Rep.* 2015; 13:1418–1431. [PubMed: 26549456]
33. Leone P, et al. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. *J Natl Cancer Inst.* 2013; 105:1172–1187. [PubMed: 23852952]
34. Restifo NP, et al. Identification of human cancers deficient in antigen processing. *J Exp Med.* 1993; 177:265–272. [PubMed: 8426105]
35. Morin RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature.* 2011; 476:298–303. [PubMed: 21796119]
36. Green MR, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *Proc Natl Acad Sci U S A.* 2015; 112:E1116–1125. [PubMed: 25713363]
37. Twa DD, et al. Recurrent genomic rearrangements in primary testicular lymphoma. *J Pathol.* 2015; 236:136–141. [PubMed: 25712539]
38. Brown PJ, et al. FOXP1 suppresses immune response signatures and MHC class II expression in activated B-cell-like diffuse large B-cell lymphomas. *Leukemia.* 2016; 30:605–616. [PubMed: 26500140]
39. Masuda K, et al. Loss or down-regulation of HLA class I expression at the allelic level in freshly isolated leukemic blasts. *Cancer Sci.* 2007; 98:102–108. [PubMed: 17083564]
40. Vago L, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med.* 2009; 361:478–488. [PubMed: 19641204]
41. Stolzel F, et al. Clonal evolution including partial loss of human leukocyte antigen genes favoring extramedullary acute myeloid leukemia relapse after matched related allogeneic hematopoietic stem cell transplantation. *Transplantation.* 2012; 93:744–749. [PubMed: 22314337]
42. McCurdy SR, et al. Loss of the mismatched human leukocyte antigen haplotype in two acute myelogenous leukemia relapses after haploidentical bone marrow transplantation with post-transplantation cyclophosphamide. *Leukemia.* 2016; 30:2102–2106. [PubMed: 27211264]
43. Matsushita H, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature.* 2012; 482:400–404. [PubMed: 22318521]

44. Alexandrov LB, et al. Signatures of mutational processes in human cancer. *Nature*. 2013; 500:415–421. [PubMed: 23945592]
45. Rizvi NA, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015; 348:124–128. [PubMed: 25765070]
46. Snyder A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014; 371:2189–2199. [PubMed: 25409260]
47. Le DT, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 2015; 372:2509–2520. [PubMed: 26028255]
48. Armand P, et al. Programmed Death-1 Blockade With Pembrolizumab in Patients With Classical Hodgkin Lymphoma After Brentuximab Vedotin Failure. *J Clin Oncol*. 2016; 34:3733–3739. [PubMed: 27354476]
49. Flavell KJ, Murray PG. Hodgkin's disease and the Epstein-Barr virus. *Mol Pathol*. 2000; 53:262–269. [PubMed: 11091850]
50. Obeid M, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med*. 2007; 13:54–61. [PubMed: 17187072]
51. Apetoh L, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*. 2007; 13:1050–1059. [PubMed: 17704786]
52. Ghiringhelli F, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nat Med*. 2009; 15:1170–1178. [PubMed: 19767732]
53. Woo SR, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity*. 2014; 41:830–842. [PubMed: 25517615]
54. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013; 39:1–10. [PubMed: 23890059]
55. Diamond MS, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med*. 2011; 208:1989–2003. [PubMed: 21930769]
56. Fuertes MB, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. *J Exp Med*. 2011; 208:2005–2016. [PubMed: 21930765]
57. Hildner K, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science*. 2008; 322:1097–1100. [PubMed: 19008445]
58. Curran E, et al. STING Pathway Activation Stimulates Potent Immunity against Acute Myeloid Leukemia. *Cell Rep*. 2016; 15:2357–2366. [PubMed: 27264175]
59. Lewinsohn M, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. *Blood*. 2016; 127:1017–1023. [PubMed: 26712909]
60. Polprasert C, et al. Inherited and Somatic Defects in DDX41 in Myeloid Neoplasms. *Cancer Cell*. 2015; 27:658–670. [PubMed: 25920683]
61. Parvatiyar K, et al. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat Immunol*. 2012; 13:1155–1161. [PubMed: 23142775]
62. Zhang Z, et al. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol*. 2011; 12:959–965. [PubMed: 21892174]
63. Poon IK, et al. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol*. 2014; 14:166–180. [PubMed: 24481336]
64. Blazar BR, et al. CD47 (integrin-associated protein) engagement of dendritic cell and macrophage counterreceptors is required to prevent the clearance of donor lymphohematopoietic cells. *J Exp Med*. 2001; 194:541–549. [PubMed: 11514609]
65. Tsai RK, et al. Self inhibition of phagocytosis: the affinity of 'marker of self' CD47 for SIRPalpha dictates potency of inhibition but only at low expression levels. *Blood Cells Mol Dis*. 2010; 45:67–74. [PubMed: 20299253]
66. Oldenborg PA, et al. Role of CD47 as a marker of self on red blood cells. *Science*. 2000; 288:2051–2054. [PubMed: 10856220]

67. Tsai RK, Discher DE. Inhibition of “self” engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. *J Cell Biol.* 2008; 180:989–1003. [PubMed: 18332220]
68. Gardai SJ, et al. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell.* 2005; 123:321–334. [PubMed: 16239148]
69. Jaiswal S, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell.* 2009; 138:271–285. [PubMed: 19632178]
70. Chao MP, et al. Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia. *Cancer Res.* 2011; 71:1374–1384. [PubMed: 21177380]
71. Chao MP, et al. Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. *Cell.* 2010; 142:699–713. [PubMed: 20813259]
72. Majeti R, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell.* 2009; 138:286–299. [PubMed: 19632179]
73. Weiskopf K, et al. Engineered SIRPalpha variants as immunotherapeutic adjuvants to anticancer antibodies. *Science.* 2013; 341:88–91. [PubMed: 23722425]
74. Liu X, et al. CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. *Nat Med.* 2015; 21:1209–1215. [PubMed: 26322579]
75. Casey SC, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science.* 2016; 352:227–231. [PubMed: 26966191]
76. Krampitz GW, et al. Identification of tumorigenic cells and therapeutic targets in pancreatic neuroendocrine tumors. *Proc Natl Acad Sci U S A.* 2016; 113:4464–4469. [PubMed: 27035983]
77. Driessens G, et al. Costimulatory and coinhibitory receptors in anti-tumor immunity. *Immunol Rev.* 2009; 229:126–144. [PubMed: 19426219]
78. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012; 12:252–264. [PubMed: 22437870]
79. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol.* 2015; 36:265–276. [PubMed: 25797516]
80. Topalian SL, et al. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell.* 2015; 27:450–461. [PubMed: 25858804]
81. Chemnitz JM, et al. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol.* 2004; 173:945–954. [PubMed: 15240681]
82. Parry RV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol.* 2005; 25:9543–9553. [PubMed: 16227604]
83. Yang H, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia.* 2014; 28:1280–1288. [PubMed: 24270737]
84. Zhou Q, et al. Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia. *Blood.* 2010; 116:2484–2493. [PubMed: 20570856]
85. Younes A, et al. Phase I study of a novel oral Janus kinase 2 inhibitor, SB1518, in patients with relapsed lymphoma: evidence of clinical and biologic activity in multiple lymphoma subtypes. *J Clin Oncol.* 2012; 30:4161–4167. [PubMed: 22965964]
86. Roemer MGM, et al. Chromosome 9p24.1/PD-L1/PD-L2 Alterations and PD-L1 Expression and Treatment Outcomes in Patients with Classical Hodgkin Lymphoma Treated with Nivolumab (PD-1 Blockade). *Blood.* 2016; 128:2923.
87. Chapuy B, et al. Targetable genetic features of primary testicular and primary central nervous system lymphomas. *Blood.* 2016; 127:869–881. [PubMed: 26702065]
88. Georgiou K, et al. Genetic basis of PD-L1 overexpression in diffuse large B-cell lymphomas. *Blood.* 2016; 127:3026–3034. [PubMed: 27030389]
89. Brunet JF, et al. A new member of the immunoglobulin superfamily—CTLA-4. *Nature.* 1987; 328:267–270. [PubMed: 3496540]
90. Linsley PS, et al. Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. *J Exp Med.* 1992; 176:1595–1604. [PubMed: 1334116]

91. Collins AV, et al. The interaction properties of costimulatory molecules revisited. *Immunity*. 2002; 17:201–210. [PubMed: 12196291]
92. Peach RJ, et al. Complementarity determining region 1 (CDR1)-and CDR3-analogous regions in CTLA-4 and CD28 determine the binding to B7-1. *J Exp Med*. 1994; 180:2049–2058. [PubMed: 7964482]
93. Takahashi T, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med*. 2000; 192:303–310. [PubMed: 10899917]
94. Wing K, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008; 322:271–275. [PubMed: 18845758]
95. Leach DR, et al. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996; 271:1734–1736. [PubMed: 8596936]
96. Marshall NA, et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood*. 2004; 103:1755–1762. [PubMed: 14604957]
97. LaBelle JL, et al. Negative effect of CTLA-4 on induction of T-cell immunity in vivo to B7-1+, but not B7-2+, murine myelogenous leukemia. *Blood*. 2002; 99:2146–2153. [PubMed: 11877291]
98. Ren Z, et al. CTLA-4 Limits Anti-CD20-Mediated Tumor Regression. *Clin Cancer Res*. 2017; 23:193–203. [PubMed: 27354469]
99. Perez-Garcia A, et al. CTLA-4 genotype and relapse incidence in patients with acute myeloid leukemia in first complete remission after induction chemotherapy. *Leukemia*. 2009; 23:486–491. [PubMed: 19092854]
100. Bashey A, et al. CTLA4 blockade with ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. *Blood*. 2009; 113:1581–1588. [PubMed: 18974373]
101. Davids MS, et al. Ipilimumab for Patients with Relapse after Allogeneic Transplantation. *N Engl J Med*. 2016; 375:143–153. [PubMed: 27410923]
102. Huard B, et al. CD4/major histocompatibility complex class II interaction analyzed with CD4-and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. *Eur J Immunol*. 1995; 25:2718–2721. [PubMed: 7589152]
103. Miyazaki T, et al. Independent modes of natural killing distinguished in mice lacking Lag3. *Science*. 1996; 272:405–408. [PubMed: 8602528]
104. Workman CJ, et al. Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. *J Immunol*. 2004; 172:5450–5455. [PubMed: 15100286]
105. Workman CJ, Vignali DA. Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223). *J Immunol*. 2005; 174:688–695. [PubMed: 15634887]
106. Woo SR, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012; 72:917–927. [PubMed: 22186141]
107. Matsuzaki J, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci U S A*. 2010; 107:7875–7880. [PubMed: 20385810]
108. Berrien-Elliott MM, et al. Durable adoptive immunotherapy for leukemia produced by manipulation of multiple regulatory pathways of CD8+ T-cell tolerance. *Cancer Res*. 2013; 73:605–616. [PubMed: 23188506]
109. Gandhi MK, et al. Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. *Blood*. 2006; 108:2280–2289. [PubMed: 16757686]
110. Gajewski TF, et al. Innate and adaptive immune cells in the tumor microenvironment. *Nature Immunology*. 2013; 14:1014–1022. [PubMed: 24048123]
111. Scott DW, Gascoyne RD. The tumour microenvironment in B cell lymphomas. *Nat Rev Cancer*. 2014; 14:517–534. [PubMed: 25008267]
112. Fontenot JD, et al. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity*. 2005; 22:329–341. [PubMed: 15780990]

113. Sakaguchi S, et al. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol.* 1995; 155:1151–1164. [PubMed: 7636184]
114. Sakaguchi S, et al. Regulatory T cells and immune tolerance. *Cell.* 2008; 133:775–787. [PubMed: 18510923]
115. Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol.* 2006; 6:295–307. [PubMed: 16557261]
116. deLeeuw RJ, et al. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. *Clin Cancer Res.* 2012; 18:3022–3029. [PubMed: 22510350]
117. Turk MJ, et al. Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *J Exp Med.* 2004; 200:771–782. [PubMed: 15381730]
118. Szczepanski MJ, et al. Increased frequency and suppression by regulatory T cells in patients with acute myelogenous leukemia. *Clin Cancer Res.* 2009; 15:3325–3332. [PubMed: 19417016]
119. Attia P, et al. Inability of a fusion protein of IL-2 and diphtheria toxin (Denileukin Diftitox, DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. *J Immunother.* 2005; 28:582–592. [PubMed: 16224276]
120. Tzankov A, et al. Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. *Haematologica.* 2008; 93:193–200. [PubMed: 18223287]
121. Alvaro T, et al. Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. *Clin Cancer Res.* 2005; 11:1467–1473. [PubMed: 15746048]
122. Greaves P, et al. Expression of FOXP3, CD68, and CD20 at diagnosis in the microenvironment of classical Hodgkin lymphoma is predictive of outcome. *J Clin Oncol.* 2013; 31:256–262. [PubMed: 23045593]
123. Fowler NH, et al. Role of the tumor microenvironment in mature B-cell lymphoid malignancies. *Haematologica.* 2016; 101:531–540. [PubMed: 27132279]
124. Spranger S, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci Transl Med.* 2013; 5:200ra116.
125. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity.* 2014; 41:49–61. [PubMed: 25035953]
126. Steidl C, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med.* 2010; 362:875–885. [PubMed: 20220182]
127. Riihijarvi S, et al. Prognostic influence of macrophages in patients with diffuse large B-cell lymphoma: a correlative study from a Nordic phase II trial. *Haematologica.* 2015; 100:238–245. [PubMed: 25381134]
128. Strachan DC, et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. *Oncoimmunology.* 2013; 2:e26968. [PubMed: 24498562]
129. Ries CH, et al. Targeting tumor-associated macrophages with anti-CSF-1 R antibody reveals a strategy for cancer therapy. *Cancer Cell.* 2014; 25:846–859. [PubMed: 24898549]
130. Kumar V, et al. The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Trends Immunol.* 2016; 37:208–220. [PubMed: 26858199]
131. Sun H, et al. Increase in myeloid-derived suppressor cells (MDSCs) associated with minimal residual disease (MRD) detection in adult acute myeloid leukemia. *Int J Hematol.* 2015; 102:579–586. [PubMed: 26358057]
132. Romano A, et al. Circulating myeloid-derived suppressor cells correlate with clinical outcome in Hodgkin Lymphoma patients treated up-front with a risk-adapted strategy. *Br J Haematol.* 2015; 168:689–700. [PubMed: 25376846]
133. Qin H, et al. Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice. *Nat Med.* 2014; 20:676–681. [PubMed: 24859530]
134. Draghiciu O, et al. Myeloid derived suppressor cells-An overview of combat strategies to increase immunotherapy efficacy. *Oncoimmunology.* 2015; 4:e954829. [PubMed: 25949858]

Trends Box

- Leukemia and lymphoma develop and progress in the same compartments in which immune responses are typically generated, suggesting that these diseases fail to trigger immune sensing mechanisms (immunologic ignorance), or effectively impair anti-tumor immune responses when they do occur (immune evasion).
- A number of immune evasion mechanisms are shared between solid and hematologic cancers, but leukemia and lymphoma employ several “exclusive” mechanisms of immune escape.
- Classical Hodgkin lymphoma is the prototypical example of an immunogenic hematologic cancer that promotes immune evasion through a unique genetic mechanism of PD-L1 upregulation.
- T cell priming often occurs against solid cancer antigens, but is followed by functional impairment in the tumor environment. Conversely, T cell tolerance in disseminated leukemia and lymphoma is regulated at the level of “tolerogenic” antigen-presenting cells, leading to a defective priming upon initial antigen encounter.

Outstanding Questions Box

What is the role of innate immune sensing in leukemia and lymphoma? Innate immunity is critical to generate adaptive immune responses against solid tumors. However, early evidence suggests that acute leukemias may evade innate immune sensing mechanisms in the host. If this is the case, then what are the underlying mechanisms? Is this related to their disseminated nature? Or do these malignancies possess other mechanisms to avoid or dampen the innate immune response?

Leukemia and lymphoma have a relatively low mutational burden compared to solid tumors and, as a result, likely harbor fewer neo-antigens. Does this paucity of antigens lead to decreased immunogenicity? And, if so, does immunosurveillance occur to any meaningful degree in leukemia and lymphoma? Or, is the “quality” of the neo-antigens in these cancers sufficient to invoke antitumor immune response?

Checkpoint blockade therapy has demonstrated impressive results in cHL. However, preliminary evidence suggests that it may not be as effective in other hematologic malignancies, such as AML. Like other hematologic malignancies, cHL has a relatively low mutational burden, so the decreased efficacy is unlikely to be related to antigenic load, as has been reported in solid malignancies. Better understanding of the mechanisms of these disparate responses may lead to important insights into additional important (and perhaps unique) mechanisms of immune evasion in leukemia and lymphoma.

Our current animal models of leukemia and lymphoma may not accurately recapitulate the biology of these diseases in patients. Will emerging genetically-engineered leukemia and lymphoma models prove more useful in understanding how host immune activation and tolerance are regulated?

Figure 1. Unique and shared mechanisms of immune evasion in leukemia and lymphoma
 Leukemias and lymphomas utilize many of the same mechanisms of immune evasion as solid tumors (top). These include induction of programmed death-ligand 1 (PD-L1) by interferon (IFN)- γ , downregulation of major histocompatibility complex class I (MHC I), inhibition of phagocytosis, and recruitment or induction of immunosuppressive cells such as tumor-associated macrophages (TAM), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). However, several immune evasion mechanisms are unique to leukemia and lymphoma. In lymphoma (bottom left), genetic changes such as amplification of the PD-L1 locus on chromosome 9p24.1, or mutations, deletions or epigenetic silencing of the MHC II locus on chromosome 6 results in increased PD-L1 expression or loss of MHC class II (MHC II), respectively. In leukemia (bottom right), a relatively low mutational burden may result in fewer neoantigens available for recognition by host T cells. Further, danger-associated molecular patterns (DAMPs) may not be present in concentrations

sufficient to mediate dendritic cell (DC) maturation, and leukemia antigen presentation by immature DCs results in T cell tolerance. Teff, effector T cell; PD-1, programmed death-1.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript