NIH Public Access

Author Manuscript

Cancer J. Author manuscript; available in PMC 2012 September 1.

Published in final edited form as:

Cancer J. 2011 September ; 17(5): 309–324. doi:10.1097/PPO.0b013e3182341fde.

Evaluation of Current Cancer Immunotherapy: Haemato-Oncology

Christopher Hourigan and **Hyam I. Levitsky**

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA 21231-1000

Abstract

Hematological malignancies were the first diseases in clinical oncology for which the potential of harnessing the immune system as targeted therapy was unequivocally demonstrated. Unfortunately the use of this highly efficacious modality has been limited to only a subset of patients and diseases due to immune-mediated toxicities resulting from incomplete specificity, and disease-specific determinants of sensitivity versus resistance to immune effector mechanisms. Recent studies however, have begun to elucidate the molecular basis of the observed clinical effects allowing the rational development of next generation of immunotherapeutic combinations. We discuss here cancer antigen targets in hematological malignancies and the specific approaches to induce immunity being pursued, the importance of modulating the host immunoregulatory environment, and the special features of immunological monitoring in clinical investigation. The hematological malignancies represent an ideal setting for the development of immunotherapy due to logistical, clinical monitoring and disease biology factors and may represent an exemplar for immune based treatment in other cancer types.

Keywords

Immunotherapy; Cancer Vaccines/Immunology; Cancer Vaccines/Therapeutic Use; Human

Evidence for Efficacious Anti-cancer Immunity in the Hematological Malignancies

The field of Malignant Hematology has been at the forefront of adopting cancer immunotherapy into the standard of care in clinical practice. This is exemplified by the development of successful hematopoietic stem cell transplantation in the late 1960s and 1970s (1)(2), the development of monoclonal antibody therapy in the 1980s and 1990s (3)(4), and the demonstrated efficacy of defined antigen-specific adoptive T cell therapy in the past decade (5)(6). The use of the immune system as a targeted therapeutic agent for hematological malignancies has thus been recognized as an attractive approach that has been used clinically for over 40 years. Of particular interest is the ability to specifically target the malignant clone thereby avoiding the side effects seen with most conventional therapies while also providing long-term prevention against disease relapse. While cancer immunotherapy in the form of allogeneic bone marrow transplantation (Allo-BMT) can already be considered standard of care for some patients with leukemia and lymphoma, immune responses targeting "allo-antigens" shared by both malignant and non-malignant

Please address correspondence to: Hyam I Levitsky MD, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, CRB 1, Room 4M51, Baltimore, Maryland, USA 21231-1000, Phone: 410-614-0552, Fax: 410-614-9705, hy@jhmi.edu.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

cells of the host fall well short of this desired specificity, with an accompanying risk of morbidity and mortality that has historically limited the use of this therapy to those with greatest risk of relapse following conventional chemotherapy. Nevertheless, recent advances in transplant conditioning and immune modulation have circumvented some of these barriers, allowing the possibility of integrating more targeted immunotherapeutic strategies using the BMT platform as discussed below. As such, the path of cancer immunotherapy is coming full circle in the treatment of hematologic malignancies.

Perhaps the strongest evidence that immune responses can be effective in controlling hematological malignancies is the example of donor lymphocyte infusion (DLI) therapy in patients who relapse after allo-BMT. Re-induction of clinical remission using DLI can typically be achieved in around 75% of chronic phase CML patients and 15–20% of AML patients (7). While response to DLI is usually associated with clinical graft versus host disease (GVHD), a distinct graft versus leukemia (GVL) effect alone can be observed, with complete remissions of relapsed chronic phase CML being seen in patients without clinical GVHD (8). The "graft vs. leukemia" effect of transplantation itself was noted early with the observation that those receiving allo-BMT and experiencing moderate to severe GVHD had a dramatically lower leukemic relapse rate compared to those having little or no GVHD or compared to those receiving transplants from identical-twins (syngeneic BMT)(9). The major contribution of T lymphocytes to the prevention of leukemia relapse following successful BMT was later demonstrated by historical comparisons showing better outcomes from unmanipulated versus T-cell depleted bone marrow grafts for patients with chronic myeloid leukemia (10).

Finally, analogous to the situation seen with immune escape of chronic viral infections, loss of mismatched HLA has been observed in mutated leukemic blasts from patients with relapse following haplo-identical BMT. Acquired uniparental disomy of chromosome 6p lead to a loss of recognition by donor T cells which could previously recognize and kill the original unmutated leukemic cells (11). This escape mutation most likely results from selective pressure from the T cell allo-response to leukemia analogous to the phenomena of epitope mutations already observed in HIV (12), HBV (13) and HCV (14) viral infections.

While much of the evidence above speaks to the potency of an allogenic response, it has been known for over 40 years that humans can mount endogenous immune responses to leukemia as well (15, 16). It has only recently become possible however to dissect the immunological specifics underlying this "host versus leukemia" immunity. Whether induced by responses to allo-MHC, minor histocompatibility antigens, or leukemia associated antigens (LAAs) however, durable remissions are not-infrequently obtained following immunotherapy of hematologic maligancies (e.g. with BMT/DLI, interferons, or monoclonal antibodies), providing an opportunity to characterize and correlate the specific features of the anti-tumor immune response with the hard clinical endpoints of remission versus disease progression.

Cancer Antigens in the Hematological Malignancies

The most compelling rationale for the use of the immune response as a therapy for cancer is the ability to specifically target the malignant clone while sparing the normal host tissue. This however relies on the ability of the immune system to recognize "non-self" characteristics of a malignancy in the context of appropriately pro-effector environmental cues. Extensive efforts have been made to define antigenic targets that can confer specificity of the immune response against hematological malignancies. The prediction would be that antigens arising from genes essential for cellular transformation and/or conferring a growth advantage (such as "driver" mutations) would represent better antigens than "passenger"

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mutations (17) due to obligate expression; indeed it has been noted that the majority of the LAAs identified so far are linked to the cell cycle or proliferation (18). The last 2–3 years has seen an explosion of information regarding somatic mutations in cytogenetically normal hematological malignancies during first presentation and relapse; we expect this will result in the identification of multiple new antigens within this category.

Cancer-associated antigens can be divided into three main classes. Firstly, true "neo antigens", defined as molecules uniquely expressed by cancer cells with no normal tissue expression, can be created by somatic genetic events within the leukemia itself. These range from products of chromosomal fusions (eg; BCR:ABL in CML), to atypically spliced isoforms (19, 20), to single nucleotide variants (SNV) only discoverable in the first instance via whole genome sequencing (21). One member of this category of antigens unique to hematological malignancies is the idiotypic determinant of a B cell or T cell receptor expressed by the clonally expanded cells comprising most lymphoid malignancies. In this setting, DJ and VDJ recombination of B and T cell receptor gene segments together with additional genetic mechanisms for generating antigen receptor diversity result in genes encoding amino acid sequences that are unique to the clonal lymphocyte population (22, 23). Experimental therapy with monoclonal antibodies raised against such antigens have led to durable remissions in lymphoma patients (24), and active immunization with patient-specific idiotypic sequences has been shown to raise host antibody and T cell responses against idiotypic epitopes which correlate with clinical endpoints such as relapse free survival (25). Unfortunately however, these promising results have proven challenging to reproduce in the phase III trial setting (26). It remains to be seen if in the appropriate clinical setting, such biological responses can translate into clinically meaningful outcomes.

The second class of cancer-associated antigens in the hematological malignancies are those antigens with germline (unmutated) sequence, whose expression is limited to the leukocyte subset from which the hematological malignancy is derived. Examples include CD20 expression in most B-cell lymphomas (4), or CD52 expression in chronic lymphocytic leukemia (CLL) and some lymphomas (3). Given that members of this class of antigens are also expressed on normal hematopoietic cell lineages, they have been most useful as targets for passive immunotherapy with monoclonal antibodies, either alone or as an adjunct to cytotoxic chemotherapy, rather than as a target for the induction of long-lived immunity via vaccination where self-tolerance limits the host response. B cell lineage surface antigens have also been the target of adoptive T cell immunotherapy in the case of CLL, via the adoptive transfer of autologous T cells transduced with a chimeric antigen receptor (CAR) specific for CD19 (27, 28). This strategy utilizes an engineered transmembrane receptor that exploits the antigen-binding properties of a monoclonal antibody (extracellular domain) specific for the cell surface antigen CD19, fused to an intracellular domain consisting of the zeta chain of the T cell receptor signal transduction complex along with a co-stimulatory motif from CD137. Such systems enable a self-sustaining, amplifiable cytotoxic effector response with T cells that can migrate to tumor compartments not easily accessed by naked antibodies. As for the rationale for using a target that does not distinguish the malignancy from its normal cellular counterpart, even the complete elimination of normal CD19+ cells and the resulting impairment of humoral immunity may be a manageable and acceptable outcome in some clinical settings. Furthermore, future strategies may seek to only transiently target CD19 expressing cells (e.g. with T cells co-transduced with a suicide gene together with the CAR, or by using mRNA rather than viral vectors for CAR transduction). Since CD19 is not expressed on normal hematopoietic stem cells, normal B cell lymphopoiesis would be expected to recover once the transduced T cells are eliminated. Finally, the potency of linking antibody mediated tumor targeting with T cell activation may be achievable with a more easily exportable approach, i.e. via so-called bi-specific antibodies. As a result of significant progress in antibody engineering, antibodies having

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

multiple valences and two (or more) specificities are now routinely generated (29, 30). Recently, such a chimeric antibody was tested in patients with CD19+ Acute Lymphoblastic Leukemia (ALL) who had persistence or relapse of molecularly measureable disease following intensive induction and consolidation chemotherapy (31). The experimental antibody, Blinatumomab, a bispecific single-chain antibody for both CD19 (the leukemia target) and CD3 (the T cell target leading to T cell activation) was well tolerated, resulted in clearance of detectable minimal residual disease (MRD) in 16 of 21 patients treated, and was associated with a relapse-free survival of 78% at a median follow-up of 405 days. Of note 12 of the 16 responders in this study had been molecularly refractory to prior chemotherapy.

The third, and final, category is the one most extensively studied so far; those proteins of wild-type sequence that are over-expressed within the tumor target tissue compared to normal host tissue. This class includes the Wilms tumor protein WT-1 (32, 33), PR1 derived from azurophil granule proteases proteinase-3 and neutrophil elastase (34) and the cancer testis antigens (eg: PRAME, MAGE, Cyclin A1, CALR-3) (35). Given their import in current leukemia vaccination efforts WT1, PR1 and PRAME antigens are all discussed individually below.

The Wilms tumor 1 (WT-1) gene encodes a transcription factor that has an essential role in the normal development of the urogenital, nervous and hematopoietic systems and mesothelium. Its normal expression in post-natal life is thought to be limited to highly differentiated glomerular podocytes in the mature kidney (36) and CD34+CD38− hematopoietic stem cells (37). It undergoes a loss of function mutation in a subset of patients with Wilm’s tumors, where it functions as a tumor-suppressor gene (38). Paradoxically, the unmutated gene is expressed by the vast majority of human acute leukemias (39–41) where it can function as an oncogene. The amount of WT1 expression in normal CD34+ cells compared to leukemic blasts is controversial, with most studies limited by the analysis of bulk populations rather than defined subsets. WT-1 expression is found in a high percentage of leukemic blasts, whereas its expression is limited to a small subset (1–2%) of normal CD34+ cells (typically in more primitive progenitors) (42). Nevertheless, analysis at the single cell level did not reveal significant differences in the level of WT-1 mRNA as measured by qRT-PCR. In contrast, other studies comparing phenotypically defined ($CD34^{+}CD38^{-}ALDH^{hi}$) hematopoietic stem cells (HSCs) and leukemic stem cells (LSCs) have reported 2–5 fold levels of over-expression by LSCs (37). Differences in protein expression and turnover have not been fully explored, although the demonstration that human WT-1 specific T cells can distinguish LSCs vs normal HSCs (43) highlights the potential therapeutic window. In spite of low-level self-antigen expression, WT-1 has been found to be an immunogenic cancer antigen in the mouse (44) and the human with both T-lymphocyte (45)(46) and humoral responses being seen (47)(48). Given the observations it is overexpressed in myeloid leukemia stem cells (49) and that human CTLs specific for this antigen were able to eliminate engraftment of leukemia initiating, but not normal CD34+, stem cells in a mouse model (43), much effort has focused on inducing protective immunity to this antigen. These observations are strengthened by the observations that WT1 (but not PR1 or PRAME) specific CTL could frequently be detected after Allo-HSCT (50), that an apparent graft-versus-leukemia effect was associated with detectable Wilms tumor-1 specific T lymphocytes after allo-BMT for ALL (51) and that detectable WT1 expression following allo-BMT is an independent prognostic factor for leukemia relapse (52). One note of caution about WT1 as a therapeutic target however, comes from the recent report that WT1 is one of the antigens that triggers T cell-mediated myelosuppression in myelodysplastic syndrome (53). Whether this represents T cell targeting (but not eradication) of early transformed cells that are over-expressing WT-1 versus collateral damage to normal hematopoietic stem cells is currently an important unknown. Clinical trials of vaccination with this promising LAA are currently underway at a variety of medical

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

centers including MSKCC, Moffitt, NCI, NHLBI and the University of Southampton (detailed in Table 1).

Proteinase 3 and neutrophil elastase are expressed at high concentrations in the primary granules of myeloid leukemia blasts while normal expression is primarily confined to the early promyelocytic/myelocytic stage of bone marrow development (54)(55). PR1 is a HLA-A*0201 restricted T cell epitope within these proteins against which CTL lines and clones have been expanded in vitro. Such cells demonstrated highly specific recognition of AML and CML blasts isolated directly ex vivo (34). This work was extended by the finding that T cell responses to PR1 were strongly correlated with clinical responses in patients with CML treated with IFN-alpha and allogeneic HSCT (56). Furthermore, in patients with CML treated with allo-BMT, donor-derived PR1 specific T cells demonstrated an effector memory phenotype early post-BMT and expansion of this population was followed by complete remission in a patient with CML (57). The recent development of an anti-PR1/HLA-A2 TCR-like antibody capable of mediating complement-dependant cytotoxicity of AML progenitor cells is an interesting spin-off from these studies with potential therapeutic utility (58).

PRAME was identified in 1997 by Ikeda *et. al.*, from a human melanoma cell line derived from a late recurrence metastasis (59). A CTL clone capable of lysing the melanoma line was generated from autologous lymphocytes and used to screen pools from a cDNA library derived from the line. The single positive pool contained a clone with an open reading frame encoding a 509 amino acid protein nearly identical to sequences expressed by myeloid leukemia cell line K562 and promyelocytic leukemia cells HL-60 (60) and 97% homologous to a 332 bp cDNA from human testis (61). The authors named the gene PRAME for “preferentially expressed antigen of melanoma”. It was later shown that this antigen is also over-expressed in at least 25% of acute leukemia cells and almost all of those with the 8:21 translocation (62). PRAME, which is up-regulated by BCR-ABL in CML, is thought to act via inhibition of retinoic acid signaling to both block myeloid differentiation (63) and to increase resistance to apoptosis by down-regulating the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (64). As a member of the cancer testis family, expression in post-natal life is thought to be limited to germ cells. Within the normal hematopoietic compartment, PRAME expression is below the limits of detection by sensitive techniques. One intriguing observation is that PRAME expression is induced or increased by leukemias treated with DNA methyltransferase inhibitors and histone deacetylase inibitors, both of which are being actively used as “differentiating agents” in the treatment of leukemia (65). As such, immunotherapies targeting PRAME, either through vaccination or adoptive T cell transfer may benefit from systemic modulation of the target antigen expression, if such maneuvers prove to be selective.

The cancer antigens identified to date in the hematological malignancies have been discovered in one of three ways. “Reverse Immunology” identifies candidate antigens based on gene expression profiling. Proteins that are uniquely or preferentially expressed by cancer cells relative to normal cells are evaluated for amino acid sequences that are predicted by MHC binding algorithms to be potential epitopes. Candidate peptides are synthesized, confirmed to bind to the relevant MHC, and then pulsed onto dendritic cells (DCs), which are used to stimulate polyclonal T cells to generate lines. These are then tested to see if they recognize tumor targets that endogenously express and process this antigen. WT1 and PR1 are examples of antigens discovered using this method (45)(34). This technique has the advantage that it can limit the search for novel cancer antigens to those proteins known to be overexpressed by cancer cells compared to normal cell types, and can even focus the search to those antigens found in self-renewing cancer stem cells, which are relatively well-defined in hematological malignancies (37). However the approach is relative inefficient, and as

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

relates to vaccine candidates, it assumes that the ability to generate T cell lines *in vitro* (used to screen the candidate) predicts how immunogenic the antigen will be *in vivo*.

The second approach, "Forward Immunology" seeks to identify the antigenic targets of an endogenous (or experimentally induced) host response. In the case of antigens recognized by T cells, tumor-reactive T cell lines or clones are first generated against a primary leukemia or cell line. Once the MHC restriction is determined, the specific antigen and presented peptide epitope can be identified either biochemically (by fractionating and sequencing peptides stripped from tumor MHC that sensitize targets for T cell recognition) or genetically (by transfection of targets sharing the restricting MHC with pools from tumor derived cDNA libraries and screening for T cell recognition). PRAME is an example of an antigen discovered via this methodology (66). A significant bias of this approach arises from uncontrolled factors that influence which T cell specificities emerge from the in vitro T cell expansion; a process that is notoriously inefficient and does not necessarily reflect the full spectrum (or even the hierarchy) of the host T cell response present in vivo.

The third approach, which we refer to as "Fast-Forward Immunology" is the direct characterization of the antigen specificity of an actual immune response from a cancer patient without the bias introduced by in vitro T cell expansion. The classic example of this is the combined use of "SEREX" (serological analysis of recombinant cDNA expression libraries of human tumors with autologous serum) (67, 68) to define the specificity of antibodies present in cancer patients, followed by evaluation of T cell responses to the serologically defined candidates. The antigen RHAMM was identified using this technique by screening patient sera against a cDNA library derived from the CML blast phase cell line K562 expressed by phage display, and comparing "hits" to those found in healthy volunteers and autoimmune patients (69). T cell responses to the antibody targets were then examined with standard techniques. Starting with the humoral response provides a more comprehensive picture of the host response than T cell cloning approaches and avoids some of the educated guesswork and inefficiency embedded in the reverse immunology approach. It can be highly focused by creating the cDNA library from highly purified autologous tumor or subsets such as the stem cell fraction. It additionally offers the opportunity to study patients at defined clinical timepoints, for example, before and after response to immunotherapy. Investigators from the Dana-Farber Cancer Institute have elegantly exploited this feature to study the antigenic targets associated with a clinical remission induced by DLI in a CML patient (70). The antigen identified, CML66, is expressed in CML blast crisis, AML and on normal myeloid progenitor cells but not other normal tissues (71) and is the target of both B and T cell immunity after CD4+ donor lymphocyte infusion in CML (72). Strikingly, CML66 specific immunity was first detectable after DLI, and just prior to the decline of Bcr-Abl positive cells in the index patient, peaking when a complete molecular remission was first documented, and persisting for over a year.

While many individual human LAAs have now been described it is unfortunately not yet possible to integrate this information into a complete systems-level understanding of the character of the immune response in those patients whose leukemia is well controlled. Given that the immune system can be effective in controlling leukemia, and that the T cell appears to be an important component of this response, it is reasonable to hypothesize that the functional T lymphocyte repertoire against leukemia antigens would differ for patients having different clinical outcomes (e.g. differences should be able to be observed in remission versus relapsed disease settings) and it is on this basis that clinical trials of vaccination to induce leukemia-specific immunity have proceeded.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Clinical Monitoring: Considerations for the Development of Immunotherapy for Hematologic Malignancies

A major challenge for virtually all forms of cancer immunotherapy is the development of immunologic biomarkers or upstream surrogate endpoints that reflect the relevant biological mechanism of action of the therapy. Such assays should ideally be able to quantitatively distinguish key features of robust versus weak immune responses, enabling meaningful comparisons between groups, such as subjects given the same vaccine with different adjuvants, or on a different schedule or dose. However, because such responses may be necessary, but not always sufficient for tumor rejection, these measurements may have more negative than positive predictive value for clinical responses unless examined across large numbers of patients. In any event, the vetting of a candidate biomarker still begins with clinical associations and therefore requires reasonable numbers of patients deemed to be "clinical responders", a limitation that has plagued many immunotherapy studies in solid tumor patients.

As described above, immune mediated clinical responses are not uncommon in hematologic malignancies. In addition, it is possible to molecularly quantify systemic changes in tumor burden at a limit of detection approaching one in a million cells for most cases of leukemia, lymphomas, and myeloma. As such, clinical responses to immunotherapy can be made in real-time in the setting of MRD. In spite of these opportunities however, the correlation between clinical responses and immune responses to the multitude of LAA that have been identified has not been rigorously studied. The next phase of leukemia immunology research will need to integrate observations regarding the size, composition and character of human immune responses against a wide range of LAA in patients from both longitudinal observational studies as well as clinical vaccination trials with hard clinical endpoints.

The challenge of assessing the human immune response to optimize vaccination has been discussed elsewhere (73–75) and while a universally agreed upon set of parameters to comprehensively document and compare immune responses between individuals has not yet been established, initial efforts at standardization have been made (75–77).

Measurable parameters range from the descriptive phenotyping of whole lymphocyte populations (78) to more sophisticated monitoring of individual antigen-specific responses in patients versus controls (79), in patients over time as a function of antigen expression and disease burden (51, 57) and the correlation of specific induced responses with defined clinical circumstances; for example following DLI in CML (71, 72) and CLL ((80). Whereas there is obvious scientific appeal for the more precise measures of the frequency and function of T cells of a defined specificity, it must be acknowledged that seemingly crude readouts such as delayed type hypersensitivity reactions to irradiated leukemia cells often correlate more closely with clinical benefit, perhaps because they reflect the summation of the global response rather than any single component. Furthermore, because chemotherapy for hematologic malignancies profoundly alters normal lymphocyte homeostasis, measures of the absolute and relative numbers of lymphocyte subsets (irrespective of specificity) is a key parameter of all immune assessments in this patient population. Finally, with relevance to measuring systemic immunomodulation and changes to the tumor microenvironment, serial gene expression profiling of the bone marrow (a site routinely sampled in standard of care) has the potential to reveal global patterns of the effector response most likely to impact on disease control or progression.

At the level of quantifying individual antigen specific responses, there are important differences in the methodology typically used for analysis of cytotoxic/cellular immunity compared to humeral responses. Antibody responses can be assayed by titering based on

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

binding to antigen alone (e.g. ELISA) whereas conventional immunological assays for T and NK function typically depend on functional capacity (e.g. assays of proliferation, cytokine production, ELISPOT (81), intracellular cytokine production, cytotoxic killing). However the immune response to cancer antigens in patients with hematological malignancies is by definition suboptimal (82, 83), and defects in the functional capacity of potentially responsive cells is likely to be underestimated by such methods. To accurately assess the full repertoire of potential T cell responses to leukemia antigens, including those that have been rendered functionally unresponsive by peripheral tolerance, anergy or exhaustion, it may be necessary to utilize assays more analogous to the methods used to detect antibody responses. For example MHC tetramer staining (84) and/or TCR whole repertoire sequencing technology (85) are likely to offer insights that would be missed by conventional functional T cell assays alone. Again, comparison of both the frequency and function of such populations between the blood and the marrow are likely to reveal important differences. For example it has been shown that CD8+ T cells against LAA in patients with myeloid leukemia can be found in the bone marrow, but not the peripheral blood (86).

Current Immunotherapy Approaches: Induction of Effector Immunity

Investigational immunotherapy approaches for the hematological malignancies can broadly be broken into two major, complementary and overlapping, classes; direct induction and/or amplification of effector immunity versus immunomodulation of an ongoing response, potentially within a tolerant host milieu. Any successful immunotherapeutic strategy is likely to include elements from both classes.

Direct induction of effector immunity is perhaps the most familiar conceptually, given the similarity to traditional clinical vaccination approaches commonly used in the prevention of infectious diseases. Nevertheless, the immunobiology of priming an immune response to persisting antigens present long before vaccination, and to which some degree of endogenous immune recognition is ongoing, is quite distinct from prophylactic vaccination of a naïve host (87) (88).

Many recent and ongoing attempts have been made to directly induce effector immunity by vaccination with known cancer antigens as described in the previous section. Peptide specific approaches have largely been limited to epitopes within those proteins restricted by common HLA alleles such as HLA-A*0201 or HLA-A*2402. Typically these trials have been conducted using subcutaneous injections of peptide in an adjuvant preparation (e.g. Montanide (89)) and often with concurrent GM-CSF (Table 1). This general approach showed some promising early success using WT-1 and PR-1 epitopes (90)(91) but unfortunately follow-up reports have shown this to be insufficient when used alone to maintain sustained high avidity LAA-specific responses (92).

Attempts have been made to vaccinate using the potential neo-antigen created by the unique protein sequence of the Bcr-Abl fusion product of the Philadelphia chromosome (seen in almost all cases of CML and approximately 25% of adult cases of ALL). A phase II study in CML patients using vaccination with a panel of six Bcr-Abl breakpoint junction derived peptide epitopes restricted by HLA-A2, A3, A11 and HLA class II demonstrated induction of antigen-specific CD4+ T cell responses in 11 of 14 patients but CD8+ responses in only 4 of 14. Assessments of vaccine clinical efficacy were not possible as patients concurrently received a variety of other therapies (including interferon, imatinib mesylate, DLI) (93). Attempts were made to improve the immunogenicity of these peptide vaccines in respect to class I restricted responses by generating synthetic immunogenic analog peptides ("heteroclitic peptides") with improved binding to MHC Class I (94). Unfortunately in a subsequent phase II trial of both naïve and heteroclitic peptides performed in CML patients

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

on stable dose imatinib mesylate only 3 of 10 patients had a 1 log reduction in Bcr-Abl transcript level (95). An alternative strategy using these naïve peptides, both alone and also fused with the 15-mer PADRE (pan DR epitope) sequence for adjuvant effect, was more successful in Phase I/II trial of adults with CML on stable imatinib (96). 14 of 19 patients developed T cell responses to Bcr-Abl peptides, and 13 of the 14 patients in a major cytogenetic response at baseline developed at least a 1 log decrease in Bcr-Abl transcript level. Unfortunately the induced T cell responses were only transient, peaking at 64–92 days and largely absent after six months. Similar kinetics have since been observed in healthy volunteers vaccinated with these peptides again calling into question the immunogenicity of Bcr-Abl as a cancer antigen (97).

In an attempt to overcome the limitations of peptide vaccination (i.e. the need to have identified the specific antigen/epitope, the necessity for selecting patients expressing a given HLA allele, and the unknown hierarchy of antigenic dominance within the spectrum of LAA), clinical trials using whole cell vaccination have been performed; either with an irradiated genetically modified allogeneic leukemia cell line, autologous tumor cells, or a combination of the two. For example, the K562 cell line, derived from blast crisis phase of CML, has been transfected with a plasmid vector encoding human GM-CSF, a potent immunomodulatory cytokine that promotes DC maturation (98)(99) and "cross-presentation" of antigens captured by DCs that are released from the irradiated cell line (100). Such "cancer cell-based" vaccines ("GVAX") have been used in a number of vaccine formulations (101)(102). As a "stand alone" allogeneic tumor cell vaccine for myeloid leukemias, K562/GM-CSF cells express high levels of several known LAA candidates including WT-1, PRAME, RHAMM, survivin, and the Bcr/Abl p210 fusion protein, and as such, might be considered a "polyvalent vaccine" when used as an allogeneic source of tumor antigen. In a pilot study of K562/GM-CSF vaccination in CML patients who had persistent measurable disease despite a year or more (median 37 months) on imatinib mesylate (Gleevec), 13 of 19 vaccinated patients had a progressive decline in disease burden, 8 of whom had increasing disease burden before vaccination (103). A total of 7 patients became PCR undetectable following the initiation of immunotherapy at a median of 24 (range 6–82) weeks from the start of vaccination. Five patients achieving a complete molecular response (CMR) continue to show evidence of response @ 6, 6, 18, 24, and 28 months while 2 others had short-lived CMR of 3 and 5 months. 5 of the 19 subjects can be categorized as vaccine responders having achieved a lasting CMR with a clear "inflection" in their measured disease burden bracketing the vaccination period. A follow up study is ongoing to see if patients who achieve a sustained CMR following vaccination can discontinue Gleevec.

Hematologic malignancies, especially leukemia, represent the most feasible setting in which autologous tumor cell vaccine approaches might be developed. Leukemia cell collection (typically by blood draw or apheresis), irradiation, and cryopreservation are within the technical capabilities of most centers that treat leukemia. As an example, an autologous leukemia cell-based vaccine trial was conducted for newly diagnosed AML patients <60 years old, in which the vaccine formulation consisted of a defined mixture of irradiated autologous leukemic blasts with K562-GM-CSF cells. This "bystander GVAX" platform for AML maintains the advantages associated with using autologous tumor (i.e. not assuming "shared antigens") without the need for in vitro manipulation or individualized *ex vivo* gene transfer (104). In support of the feasibility of this approach, of 54 subjects enrolled in this multi-institutional clinical trial, sufficient leukemia cell numbers (median 5.6×10^9 cells collected) for > 9 vaccine cycles were obtained from all but one patient.

A critical element of any novel therapy being developed for hematologic malignancies is how to best integrate the treatment with existing standards of care. In the AML vaccine trial

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

noted above, vaccination was integrated into the platform of autologous hematopoietic stem cell transplantation (HSCT). This work was based on mouse models that revealed two key opportunities in this setting (105). First, vaccination of syngeneic donor mice followed by the transfer of vaccine primed T cells into conditioned tumor-bearing transplant recipients exerted a potent anti-tumor response that was accompanied by dramatic expansion of tumor specific T cells in the recipient. Second, the response to recipient vaccination given in the post-transplant period during a defined period of immune reconstitution far exceeded vaccine responses generated during the immune steady state. This latter finding was subsequently shown to be at least partially attributable to effector cells "outcompeting" regulatory T cells (Tregs) during lymphopenia and homeostatic expansion, resulting in an increase in the effector: Treg ratio (106) (107).

Based on these pre-clinical findings, AML patients who achieved a complete remission with cytotoxic chemotherapy were first vaccinated after full recovery from consolidation chemotherapy and autologous stem cell collection. Two weeks after this single vaccination, aphersis was performed to collect "vaccine-primed lymphocytes". Following myeloablative conditioning, adoptive transfer of "vaccine primed T cells" accompanied the stem cell infusion. Post-transplant booster vaccinations were initiated 6 weeks post autologous HSCT and given every 3 weeks × 8 vaccinations. For the 46 patients who achieved complete remission, the relapse-free survival (RFS) at 3 years was 47.4% and overall survival (OS) was 57.4%. 28 patients received at least one vaccine and the relapse-free and overall survival at 3 years was 61.8% and 73.4% in this group, respectively. Delayed-type hypersensitivity (DTH) reactions to autologous tumor were present in 2 subjects at baseline and were induced post vaccination in 7 other subjects. Conversion from DTH− to DTH+ was associated with a longer relapse-free survival (RFS) at 3 years (100% vs. 48%, p = 0.029). Of the 16 pts with molecularly detectable MRD on the day of the pre-transplant vaccine 11 (68.8%) showed a 1-log or greater decline in tumor burden two weeks later (at the time of the vaccine primed lymphocyte apheresis). Intriguingly, patients who met this criterion had longer RFS than those without a vaccine associated fall in molecular MRD (median, 37.0 vs 9.0 months, P .029; 3-year RFS 60.6% vs 0%) (108).

The integration of vaccines is also being pursued in the context of Allo-BMT. Soiffer and colleagues immunized high-risk AML or MDS patients with irradiated, autologous, GM-CSF-secreting tumor cells early after allogeneic, nonmyeloablative HSCT. Despite the administration of a calcineurin inhibitor as prophylaxis against GVHD, vaccination elicited local and systemic reactions that were qualitatively similar to those previously observed in nontransplanted, immunized solid-tumor patients. While the frequencies of acute and chronic GVHD were not increased, 9 of 10 subjects who completed vaccination achieved durable complete remissions, with a median follow-up of 26 months (range 12–43 months) (109). Whereas the ability to prime systemic vaccine responses in Allo-BMT patients on pharmacologic immunosuppression is a welcome, if not surprising finding, one major advantage of the Allo-BMT setting is the availability of the healthy donor's immune system, with a presumably fully functional T cell repertoire. In this regard, strategies for immunizing the donor as a means to augment adoptive transfer of primed lymphocytes have obvious theoretical appeal. In animal models, priming allogeneic donor mice with recipient tumor cell-based vaccines exacerbated lethal GVHD (110), presumably by priming donor T cells against recipient minor histocompatibility antigens, whereas recipient vaccination was well tolerated once tolerance to host antigens is already established (111). Importantly, while immunization of donors to recipient minor antigens has been shown to exacerbate GVHD in mouse models, in clinical trials, donor immunization to defined antigens such as hepatitis B (112), pneumococcal antigens (113), tetanus–diphtheria, Haemophilus influenzae type b and inactivated poliovirus (114) did not increase the incidence or severity of GVHD, and evidence for the transfer of donor vaccine primed immunity was observed in transplant

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

recipients. Indeed, there is evidence that vaccine-primed tumor immunity can be transferred from donor to recipient in the context of either allo or syngeneic BMT. Kwak and colleagues immunized 5 HLA matched donor-recipient pairs undergoing BMT for the treatment of multiple myeloma with patient idiotype (Id) protein conjugated to keyhole limpet hemocyanin (KLH) (115,116). Although all donors responded to pre-BMT vaccination, the patients did not. After BMT however, donor derived T cell responses and antibody to Id and KLH were seen in the three patients who survived past transplant day 30. Immune responses increased after recipient vaccination, and persisted for 18 months in association with a conversion from partial to complete clinical remission. At last follow-up, two patients were alive and disease-free 7 and 8 years after transplantation, while one patient died of renal failure after 5.5 years while in complete remission from myeloma. This small proof of concept study established the ability to transfer donor vaccine primed T cell and antibody responses, and suggests that donors might be able to be safely immunized against other fully "non-self" defined LAA as a means to improve the outcome of allo-BMT.

Two other cell-based vaccination approaches are worthy of mention. Firstly, infusion of activated DCs either after pulsing with tumor lysate, a peptide of interest, or after transfection with mRNA encoding the target protein antigen such as WT-1 (117) is being studied in an attempt to harness the migratory capacities, superior antigen presentation and co-stimulation of this cell type. The optimal DC isolation, culture, and maturation conditions remain to be defined, and variables such as the best route of administration and distribution are still being evaluated, but clear evidence of biological activity has been reported in small pilot clinical trials, including increased levels of WT1 specific T cells and conversion of partial to complete remissions. Efforts are currently ongoing in larger trials that have both immunological and clinical endpoints. Secondly, DCs can be successfully generated from AML blasts in vitro (118, 119). Such cells have been shown to retain the expression of some relevant antigens and be capable of stimulating defined T cell populations in vitro. Nevertheless, the in vitro protocols for generating the cells involve classic cellular differentiation that by definition alters the gene expression profile relative to the transformed leukemic cells against which immunity is sought.

Finally, as noted above, adoptive cellular therapies truly had their origin in the treatment of hematologic malignancies/bone marrow transplantation, and it is remarkable to observe that many of the most promising immunotherapeutic treatments for metastatic solid cancers such as melanoma now are built on fairly classic myeloablative BMT platforms (120). Since adoptive cellular therapies for have been extensively reviewed elsewhere (121–123) a thorough discussion will not be given here, although it should be noted that with relevance to hematologic malignancies, strategies being pursued include; the infusion of EBV specific T cells in the treatment of EBV associated lymphoproliferative disease (5), ex-vivo expanded CTLs against minor histocompatibility antigens (124), polyclonal ex vivo expansion and infusion of marrow infiltrating lymphocytes (MILs) (125), "KIR-mismatched" NK cells following a T-cell depleted haplo-identical HSCT (126), and transfer of antigen specificity by transduction of a T-cell receptor (TCR) or chimeric antigen receptor (CAR) by gene-transfer in autologous cells (27, 28, 127–131). Whereas each of these platforms has shown exciting signs of clinical efficacy, issues pertaining to persistence of the progeny of the infused cells and pathways of immune escape remain areas of active investigation likely to vastly improve the outcome of these approaches.

Current Immunotherapy Approaches: Immunomodulation Strategies

It has been recognized for at least a decade now that the interactions between the immune system and cancer are bi-directional with reciprocal editing, evolution and prolonged phases of dynamic equilibrium (132–134). Changes in the antigenicity of tumors due to immune

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

selective pressure have been well described (11, 133) as has suppression of immune effectors by the tumor microenvironment by CD11b+ GR1+ myeloid-derived suppressor cells (MDSCs), production of Indoleamine 2,3-dioxygenase (IDO), CD4+CD25+ FOXP3+ Tregs or by secretion of immune-suppressive cytokines (eg: IL-10, TGF- and VEGF) from tumor stroma or the cancer cell itself (135). There is both preclinical (136) and clinical (92) evidence that any attempt to induce cancer specific effector immunity by, for example, peptide vaccination strategies alone may be counterproductive and in fact increase tolerance and Tregs and/or delete the high-affinity antigen-specific portion of the T-cell repertoire.

While there are likely to be disease specific differences between the dominant resistance mechanism(s) to immune effector cell generation, maintenance, and execution of effector function, each of the pathways described below have been reported to varying degrees in patients with hematologic malignancies. As strategies for circumventing these barriers are tested, it will be of paramount importance to identify which of the many (but finite) resistance pathways is being exploited by the cancer to evade immune elimination.

Tregs

In contrast to the nearly uniformly negative prognostic value that Treg frequency has for most solid tumors, correlations between the abundance and localization of Tregs with clinical outcomes is quite variable in hematological malignancies (137). In the case of AML, the frequency of circulating Tregs at presentation has been reported to be higher than normal controls (138–140). Patients with persistent disease after induction therapy had higher Treg frequencies at clinical presentation than those who achieved complete remission to chemotherapy (138). During the period of early lymphocyte recovery after intensive AML induction chemotherapy, recovering T lymphocytes were reported to be predominantly $CD4^{+}$ and included a greatly expanded population of $CD3^{+}CD4^{+}CD25^{+}Foxp3^{+}$ T cells (78). Recovering Tregs were phenotypically activated and showed suppressive activity in vitro. Furthermore, these cells showed marked oligoclonal skewing, suggesting that their peripheral expansion was antigen-driven. In spite of these data, there are no published studies in AML evaluating the correlation between Treg frequencies (and/or effector/Treg ratios) in blood or marrow, either at presentation or remission, with clinical outcomes such as remission duration or overall survival.

In the case of follicular B cell lymphoma (FL), high numbers of infiltrating Tregs in the FL microenvironment have been correlated with a *favorable* prognosis (141–144). When evaluating clinical parameters, FL patients who present with low numbers of tumor infiltrating Tregs are more likely to have refractory disease. Similar findings have been reported for subsets of diffuse large B cell lymphoma (DLBCL). As these are entirely descriptive studies, these immunologically counterintuitive findings lack a clear mechanistic explanation, but the findings have been consistent across many studies, prompting some to speculate that the Tregs may exert a degree of regulation on the B lymphoma cells directly.

Indoleamine 2,3-dioxygenase (IDO)

IDO degrades the essential amino acid tryptophan (Trp) and produces kynurenine (Kyn), resulting in immune tolerance by reducing the local concentration of Trp required by T cells, and by directly inhibiting T and NK cells through Kyn and its derivatives (145). First identified as being essential during pregnancy for preventing immune mediated rejection of the fetus, IDO has since been shown to be produced in the microenvironment of several cancers, as well as in draining lymph nodes. A positive feedback loop between IDO production, induction of Tregs, and tolerogenic DCs has also been established. IDO production has been shown to correlate with Treg frequency in patients with AML at diagnosis (138), and serum Kyn/Trp ratios correlated with reduced survival (146). IDO was

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

also reported to be abundantly expressed in 32% of 119 cases of DLBCL in both the lymphoma cells themselves and the tumor resident DCs. Complete remission rates to chemotherapy plus rituximab in patients with IDO-positive DLBCL and IDO-negative DLBCL were 55.3% and 79.0% (p=0.008), while 3-year overall survival rates were 49.8% and 78.8%, respectively (p=0.0003) (147) suggesting this resistance pathway may be important in a subset of patients with this disease.

B7-H1 (PD-L1)

Programmed death-1 (PD-1) is expressed on activated T cells, B cells and monocytes and delivers an inhibitory (and/or proapoptotic) signal to T cells crucial for regulating peripheral tolerance (148). In the face of persistent antigen exposure (e.g. chronic viral infection, HIV (149), Hepatitis (150), "exhausted" T cells upregulate PD-1 and display impaired effector function that can be reversed by PD-1 blockade (151). Two known ligands (PD-L1 and PD-L2) have distinct patterns of expression, but PD-L1 has been demonstrated to be expressed by a significant fraction of solid tumors, and its blockade (via antibodies) unmasks endogenous anti-tumor immunity (152). The data pertaining to PD-L1 expression in AML is controversial, with some studies reporting a low frequency of expression (153) and others reporting an expression rate of 57% and 66% in M4 vs. M5 AML respectively (154). Some of this discrepancy may relate to PD-L1 expression being inducible by cytokines such as γ-interferon. In the case of B cell lymphoma, PD-L1 was reported to be expressed in nearly all anaplastic large cell lymphoma cell lines and in 24% of primary DLBCL. In vitro antibody blockade of lymphoma cell: T cell co-cultures enhanced the secretion of several inflammatory cytokines, again suggesting a role for this resistance pathway in a subset of patients.

Interestingly, both classical Hodgkin lymphoma (cHL) and mediastinal large B cell lymphoma (MLBCL) are diseases that share a number of molecular features and can be characterized as often having minority of malignant cells within a predominant inflammatory infiltrate, suggesting these tumors require a local immune resistance mechanism to persist. Shipp and colleagues utilized transcriptional profiles together with high-resolution copy number data from micro-dissected cHL and MLBCL samples, and identified a disease-specific structural alteration (chromosome 9p24.1 amplification) that increases the gene dosage of PD-1 ligands in both cHL and MLBCL (155). These findings suggest that these tumors may be particularly responsive to PD-1 ligand/PD-1 receptor blockade.

Soluble NKG2D ligands

NK cells and subsets of CD8+ T cells express an activating receptor, NKG2D that triggers cytolytic activity (and in the case of T cells, co-stimulation). Ligands for NKG2D include MICA, MICB, ULBP1-4, and RAET1, which although distinct, have in common their induced expression on cells undergoing genotoxic stress, including most cancers (156). This pathway plays a key role in cancer immune surveillance. Cancers can escape this immunologic selective pressure by cleavage of NKG2D ligands from the surface of transformed cells via the enzyme protein disulfide isomerase, resulting in readily detectable "soluble" NKG2D ligands in the serum that down regulates NKG2D expression on T cells and NK cells, inhibiting their lytic capacity (157). Low level expression of NKG2D ligands has been reported on AML blasts, which could be upregulated with differentiating agents in association with increased sensitivity to NK cell-mediated lysis (158). Whereas estimates of the frequency of soluble ligands in AML patients vary (159), in the aforementioned AML vaccine trial conducted in the setting of Allo-BMT, 13/15 immunized subjects had high levels of soluble MICA and MICB at study entry (160). Similar to earlier reports from this group in solid tumor patients, 6 of 7 long-term responding subjects with detectable shed

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NKG2D ligands showed significant decreases in response to post-transplant vaccination, and in 2 long term responders, these decreases were associated with restored NKG2D expression on NK cells. Interestingly, in the case of CML, one of the factors influencing MICA expression is the Bcr/Abl fusion gene, and NKG2D ligand expression by cells in the CML lineage is blocked by imatinib (161), making these cells less recognizable by NK cells.

NKG2D ligand regulation has also been shown to play a role in the progression of plasma cell dyscrasias, from the precursor known as monoclonal gammopathy of undetermined significance (MGUS) to overt multiple myeloma. Whereas plasma cells from MGUS patients were shown to express MICA as compared to normal donors, in the evolution to myeloma, plasma cells shed MICA, triggering the down-regulation of NKG2D and impairing lymphocyte cytotoxicity. Remarkably, some MGUS patients were found to have endogenous anti-MICA antibodies that antagonized the suppressive effects of sMICA.

Based on the above, a variety of approaches targeting each of these immune resistance pathways are under development, seeking to tip the balance of cancer-specific responses from tolerance/resistance to effector function. Most of these are in early phase clinical trials, and only a few have been tested in significant numbers of patients with hematologic malignancies. These targets provide a wealth of opportunity for the astute clinical investigator who has a detailed appreciation for the specific diseases and the underlying immunobiology that is ripe for exploiting.

Discussion: Future Directions

In patients with clinically active maligancies, the immune response to cancer is by definition suboptimal. This review has focused on the current approaches to both define the potential for the immune system to recognize and control the hematological malignancies and investigate how such responses can be augmented. The challenge remains to correlate clinical outcomes with observed variations in human immunity. It is increasing clear that the interplay between the immune system and cancer is dynamic with clear bi-directional immunoediting over the lifespan of the cancer and the host (134). Attempts to co-opt the immune system as a therapeutic agent for these diseases therefore must be sophisticated and rationally designed based on an understanding of the un-manipulated natural history of immune responses to these malignancies. An example of the potential for harm was recently highlighted by the report that repeated vaccination and boosting with PR1 and WT1 peptide in patients with myeloid malignancies, while resulting in transient anti-leukemia immunity, ultimately led to selective deletion of high-affinity peptide specific CD8+ T-cells while maintaining low-affinity responses (92).

Careful analysis of clinical material from patients during all stages their disease, from cancer diagnosis to remission, relapse and survivorship is needed, along with information on the unedited potential immune repertoire against hematological malignancies from patients without cancer, to build a complete and coherent foundation upon which subsequent clinical trial efforts can be built. This is unlikely to come from systematic large-scale investigation but rather from integration of carefully performed immune correlate data collected during clinical trials and following standard-of-care therapies (e.g. chemotherapy, transplantation, donor lymphocyte infusions).

In addition to the use of the immune system as therapeutic tool, significant opportunities exist to use observed responses as a marker of MRD or as a sensitive biomarker for relapse risk. Efficacious therapeutic use, by contrast, will likely require induction of specific immunity via vaccination and/or adoptive transfer in the context of immunomodulation such as immune checkpoint inhibition in the appropriate patient, disease, and phase of treatment.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Clinician scientists trained in the "fourth arm" of cancer therapy have an unprecedented opportunity to exploit the interface between disease specific cancer biology and the host response to tame the beast within.

References

1. Thomas E, Storb R, Clift RA, et al. Bone-marrow transplantation (first of two parts). N Engl J Med. 1975; 292:832–843. [PubMed: 234595]
2. Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation (second of two parts). N Engl J Med. 1975; 292:895–902. [PubMed: 235092]
3. Hale G, Bright S, Chumbley G, et al. Removal of T cells from bone marrow for transplantation: A monoclonal antilymphocyte antibody that fixes human complement. Blood. 1983; 62:873–882. [PubMed: 6349718]
4. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-hodgkin's lymphoma. Blood. 1997; 90:2188–2195. [PubMed: 9310469]
5. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010; 115:925–935. [PubMed: 19880495]
6. O'Reilly RJ, Dao T, Koehne G, et al. Adoptive transfer of unselected or leukemia-reactive T-cells in the treatment of relapse following allogeneic hematopoietic cell transplantation. Semin Immunol. 2010; 22:162–172. [PubMed: 20537908]
7. Deol A, Lum LG. Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. Cancer Treat Rev. 2010; 36:528–538. [PubMed: 20381970]
8. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol. 1997; 15:433–444. [PubMed: 9053463]
9. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med. 1979; 300:1068–1073. [PubMed: 34792]
10. Apperley JF, Mauro FR, Goldman JM, et al. Bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: Importance of a graft-versus-leukaemia effect. Br J Haematol. 1988; 69:239–245. [PubMed: 3291930]
11. Vago L, Perna SK, Zanussi M, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. N Engl J Med. 2009; 361:478–488. [PubMed: 19641204]
12. Phillips RE, Rowland-Jones S, Nixon DF, et al. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature. 1991; 354:453–459. [PubMed: 1721107]
13. Bertoletti A, Costanzo A, Chisari FV, et al. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. J Exp Med. 1994; 180:933–943. [PubMed: 7520476]
14. Wang H, Eckels DD. Mutations in immunodominant T cell epitopes derived from the nonstructural 3 protein of hepatitis C virus have the potential for generating escape variants that may have important consequences for T cell recognition. J Immunol. 1999; 162:4177–4183. [PubMed: 10201944]
15. Santos GW, Anderson PN, Klein DL, et al. Immunological studies in acute leukemia. Trans Am Clin Climatol Assoc. 1977; 88:95–107. [PubMed: 143105]
16. Dore JF, Motta R, Marholev L, et al. New antigens in human leukaemic cells and antibody in the serum of leukaemic patients. Lancet. 1967; 2:1396–1398. [PubMed: 4170060]
17. Bozic I, Antal T, Ohtsuki H, et al. Accumulation of driver and passenger mutations during tumor progression. Proc Natl Acad Sci U S A. 2010; 107:18545–18550. [PubMed: 20876136]
18. Greiner J, Bullinger L, Guinn BA, et al. Leukemia-associated antigens are critical for the proliferation of acute myeloid leukemia cells. Clin Cancer Res. 2008; 14:7161–7166. [PubMed: 19010831]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

19. Zhang PY, Zhang WG, He AL, et al. Identification and functional characterization of the novel acute monocytic leukemia associated antigen MLAA-34. Cancer Immunol Immunother. 2009; 58:281–290. [PubMed: 18592235]
20. de Rijke B, van Horssen-Zoetbrood A, Beekman JM, et al. A frameshift polymorphism in P2X5 elicits an allogeneic cytotoxic T lymphocyte response associated with remission of chronic myeloid leukemia. J Clin Invest. 2005; 115:3506–3516. [PubMed: 16322791]
21. Ley TJ, Mardis ER, Ding L, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature. 2008; 456:66–72. [PubMed: 18987736]
22. Meeker TC, Lowder J, Maloney DG, et al. A clinical trial of anti-idiotype therapy for B cell malignancy. Blood. 1985; 65:1349–1363. [PubMed: 3888313]
23. Burdette S, Schwartz RS. Current concepts: Immunology. idiotypes and idiotypic networks. N Engl J Med. 1987; 317:219–224. [PubMed: 3299091]
24. Miller RA, Maloney DG, Warnke R, et al. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med. 1982; 306:517–522. [PubMed: 6173751]
25. Kwak LW, Campbell MJ, Czerwinski DK, et al. Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. N Engl J Med. 1992; 327:1209–1215. [PubMed: 1406793]
26. Bendandi M. Idiotype vaccines for lymphoma: Proof-of-principles and clinical trial failures. Nat Rev Cancer. 2009; 9:675–681. [PubMed: 19701243]
27. Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med. 2011; 3:95ra73.
28. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011
29. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 2009; 69:4941–4944. [PubMed: 19509221]
30. Beck A, Wurch T, Bailly C, et al. Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol. 2010; 10:345–352. [PubMed: 20414207]
31. Topp MS, Kufer P, Gokbuget N, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011; 29:2493–2498. [PubMed: 21576633]
32. Call KM, Glaser T, Ito CY, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 wilms' tumor locus. Cell. 1990; 60:509–520. [PubMed: 2154335]
33. Rose EA, Glaser T, Jones C, et al. Complete physical map of the WAGR region of 11p13 localizes a candidate wilms' tumor gene. Cell. 1990; 60:495–508. [PubMed: 2154334]
34. Molldrem J, Dermime S, Parker K, et al. Targeted T-cell therapy for human leukemia: Cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. Blood. 1996; 88:2450–2457. [PubMed: 8839835]
35. Luetkens T, Schafhausen P, Uhlich F, et al. Expression, epigenetic regulation, and humoral immunogenicity of cancer-testis antigens in chronic myeloid leukemia. Leuk Res. 2010; 34:1647–1655. [PubMed: 20409582]
36. Hohenstein P, Hastie ND. The many facets of the wilms' tumour gene, WT1. Hum Mol Genet. 2006; 15(Spec No 2):R196–201. [PubMed: 16987884]
37. Gerber JM, Qin L, Kowalski J, et al. Characterization of chronic myeloid leukemia stem cells. Am J Hematol. 2011; 86:31–37. [PubMed: 21132730]
38. Huff V. Wilms' tumours: About tumour suppressor genes, an oncogene and a chameleon gene. Nat Rev Cancer. 2011; 11:111–121. [PubMed: 21248786]
39. Inoue K, Ogawa H, Sonoda Y, et al. Aberrant overexpression of the wilms tumor gene (WT1) in human leukemia. Blood. 1997; 89:1405–1412. [PubMed: 9028964]
40. Miwa H, Beran M, Saunders GF. Expression of the wilms' tumor gene (WT1) in human leukemias. Leukemia. 1992; 6:405–409. [PubMed: 1317488]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

41. Qin Y, Zhu H, Jiang B, et al. Expression patterns of WT1 and PRAME in acute myeloid leukemia patients and their usefulness for monitoring minimal residual disease. Leuk Res. 2009; 33:384–390. [PubMed: 18950857]
42. Hosen N, Sonoda Y, Oji Y, et al. Very low frequencies of human normal CD34+ haematopoietic progenitor cells express the wilms' tumour gene WT1 at levels similar to those in leukaemia cells. Br J Haematol. 2002; 116:409–420. [PubMed: 11841446]
43. Gao L, Xue SA, Hasserjian R, et al. Human cytotoxic T lymphocytes specific for wilms' tumor antigen-1 inhibit engraftment of leukemia-initiating stem cells in non-obese diabetic-severe combined immunodeficient recipients. Transplantation. 2003; 75:1429–1436. [PubMed: 12792492]
44. Oka Y, Udaka K, Tsuboi A, et al. Cancer immunotherapy targeting wilms' tumor gene WT1 product. J Immunol. 2000; 164:1873–1880. [PubMed: 10657636]
45. Oka Y, Elisseeva OA, Tsuboi A, et al. Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type wilms' tumor gene (WT1) product. Immunogenetics. 2000; 51:99–107. [PubMed: 10663572]
46. Azuma T, Makita M, Ninomiya K, et al. Identification of a novel WT1-derived peptide which induces human leucocyte antigen-A24-restricted anti-leukaemia cytotoxic T lymphocytes. Br J Haematol. 2002; 116:601–603. [PubMed: 11849218]
47. Elisseeva OA, Oka Y, Tsuboi A, et al. Humoral immune responses against wilms tumor gene WT1 product in patients with hematopoietic malignancies. Blood. 2002; 99:3272–3279. [PubMed: 11964293]
48. Gaiger A, Carter L, Greinix H, et al. WT1-specific serum antibodies in patients with leukemia. Clin Cancer Res. 2001; 7:761s–765s. [PubMed: 11300470]
49. Gerber JM, Qin L, Kowalski J, et al. Characterization of chronic myeloid leukemia stem cells. Am J Hematol. 2011; 86:31–37. [PubMed: 21132730]
50. Morita Y, Heike Y, Kawakami M, et al. Monitoring of WT1-specific cytotoxic T lymphocytes after allogeneic hematopoietic stem cell transplantation. Int J Cancer. 2006; 119:1360–1367. [PubMed: 16596644]
51. Rezvani K, Yong AS, Savani BN, et al. Graft-versus-leukemia effects associated with detectable wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. Blood. 2007; 110:1924–1932. [PubMed: 17505014]
52. Zhao XS, Jin S, Zhu HH, et al. Wilms' tumor gene 1 expression: An independent acute leukemia prognostic indicator following allogeneic hematopoietic SCT. Bone Marrow Transplant. 2011
53. Sloand EM, Melenhorst JJ, Tucker ZC, et al. T-cell immune responses to wilms tumor 1 protein in myelodysplasia responsive to immunosuppressive therapy. Blood. 2011; 117:2691–2699. [PubMed: 21097671]
54. Sturrock AB, Franklin KF, Rao G, et al. Structure, chromosomal assignment, and expression of the gene for proteinase-3. the wegener's granulomatosis autoantigen. J Biol Chem. 1992; 267:21193–21199. [PubMed: 1400430]
55. Alatrash G, Molldrem JJ. Vaccines as consolidation therapy for myeloid leukemia. Expert Rev Hematol. 2011; 4:37–50. [PubMed: 21322777]
56. Molldrem JJ, Lee PP, Wang C, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med. 2000; 6:1018–1023. [PubMed: 10973322]
57. Rezvani K, Price DA, Brenchley JM, et al. Transfer of PR1-specific T-cell clones from donor to recipient by stem cell transplantation and association with GvL activity. Cytotherapy. 2007; 9:245–251. [PubMed: 17464756]
58. Sergeeva A, Alatrash G, He H, et al. An anti-PR1/HLA-A2 T-cell receptor-like antibody mediates complement-dependent cytotoxicity against acute myeloid leukemia progenitor cells. Blood. 2011; 117:4262–4272. [PubMed: 21296998]
59. Ikeda H, Lethe B, Lehmann F, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity. 1997; 6:199–208. [PubMed: 9047241]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

60. Murakawa K, Matsubara K, Fukushima A, et al. Chromosomal assignments of 3′-directed partial cDNA sequences representing novel genes expressed in granulocytoid cells. Genomics. 1994; 23:379–389. [PubMed: 7835887]
61. Pawlak A, Toussaint C, Levy I, et al. Characterization of a large population of mRNAs from human testis. Genomics. 1995; 26:151–158. [PubMed: 7782076]
62. van Baren N, Chambost H, Ferrant A, et al. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. Br J Haematol. 1998; 102:1376–1379. [PubMed: 9753074]
63. Oehler VG, Guthrie KA, Cummings CL, et al. The preferentially expressed antigen in melanoma (PRAME) inhibits myeloid differentiation in normal hematopoietic and leukemic progenitor cells. Blood. 2009; 114:3299–3308. [PubMed: 19625708]
64. De Carvalho DD, Binato R, Pereira WO, et al. BCR-ABL-mediated upregulation of PRAME is responsible for knocking down TRAIL in CML patients. Oncogene. 2011; 30:223–233. [PubMed: 20838376]
65. Luetkens T, Schafhausen P, Uhlich F, et al. Expression, epigenetic regulation, and humoral immunogenicity of cancer-testis antigens in chronic myeloid leukemia. Leuk Res. 2010; 34:1647–1655. [PubMed: 20409582]
66. Ikeda H, Lethe B, Lehmann F, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity. 1997; 6:199–208. [PubMed: 9047241]
67. Sahin U, Tureci O, Pfreundschuh M. Serological identification of human tumor antigens. Curr Opin Immunol. 1997; 9:709–716. [PubMed: 9368781]
68. Tureci O, Sahin U, Pfreundschuh M. Serological analysis of human tumor antigens: Molecular definition and implications. Mol Med Today. 1997; 3:342–349. [PubMed: 9269687]
69. Greiner J, Ringhoffer M, Taniguchi M, et al. Receptor for hyaluronan acid-mediated motility (RHAMM) is a new immunogenic leukemia-associated antigen in acute and chronic myeloid leukemia. Exp Hematol. 2002; 30:1029–1035. [PubMed: 12225794]
70. Yang XF, Wu CJ, McLaughlin S, et al. CML66, a broadly immunogenic tumor antigen, elicits a humoral immune response associated with remission of chronic myelogenous leukemia. Proc Natl Acad Sci U S A. 2001; 98:7492–7497. [PubMed: 11416219]
71. Wu CJ, Biernacki M, Kutok JL, et al. Graft-versus-leukemia target antigens in chronic myelogenous leukemia are expressed on myeloid progenitor cells. Clin Cancer Res. 2005; 11:4504–4511. [PubMed: 15958636]
72. Zhang W, Choi J, Zeng W, et al. Graft-versus-leukemia antigen CML66 elicits coordinated B-cell and T-cell immunity after donor lymphocyte infusion. Clin Cancer Res. 2010; 16:2729–2739. [PubMed: 20460482]
73. Germain RN. Vaccines and the future of human immunology. Immunity. 2010; 33:441–450. [PubMed: 21029956]
74. Davis MM. A prescription for human immunology. Immunity. 2008; 29:835–838. [PubMed: 19100694]
75. Nussenblatt RB, Bielekova B, Childs R, et al. National institutes of health center for human immunology conference, september 2009. Ann N Y Acad Sci. 2010; 1200 (Suppl 1):E1–23. [PubMed: 20633145]
76. Britten CM, Janetzki S, van der Burg SH, et al. Minimal information about T cell assays: The process of reaching the community of T cell immunologists in cancer and beyond. Cancer Immunol Immunother. 2011; 60:15–22. [PubMed: 21080166]
77. Janetzki S, Britten CM, Kalos M, et al. "MIATA"-minimal information about T cell assays. Immunity. 2009; 31:527–528. [PubMed: 19833080]
78. Kanakry CG, Hess AD, Gocke CD, et al. Early lymphocyte recovery after intensive timed sequential chemotherapy for acute myelogenous leukemia: Peripheral oligoclonal expansion of regulatory T cells. Blood. 2011; 117:608–617. [PubMed: 20935254]
79. Rezvani K, Yong AS, Tawab A, et al. Ex vivo characterization of polyclonal memory CD8+ T-cell responses to PRAME-specific peptides in patients with acute lymphoblastic leukemia and acute and chronic myeloid leukemia. Blood. 2009; 113:2245–2255. [PubMed: 18988867]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

80. Marina O, Hainz U, Biernacki MA, et al. Serologic markers of effective tumor immunity against chronic lymphocytic leukemia include nonmutated B-cell antigens. Cancer Res. 2010; 70:1344–1355. [PubMed: 20124481]
81. Czerkinsky CC, Nilsson LA, Nygren H, et al. A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. J Immunol Methods. 1983; 65:109–121. [PubMed: 6361139]
82. Frey AB, Monu N. Signaling defects in anti-tumor T cells. Immunol Rev (Denmark). 2008; 222:192–205.
83. Beatty GL, Smith JS, Reshef R, et al. Functional unresponsiveness and replicative senescence of myeloid leukemia antigen-specific CD8+ T cells after allogeneic stem cell transplantation. Clin Cancer Res. 2009; 15:4944–4953. [PubMed: 19602548]
84. Altman JD, Moss PA, Goulder PJ, et al. Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996; 274:94–96. [PubMed: 8810254]
85. Quigley MF, Greenaway HY, Venturi V, et al. Convergent recombination shapes the clonotypic landscape of the naive T-cell repertoire. Proc Natl Acad Sci U S A. 2010; 107:19414–19419. [PubMed: 20974936]
86. Melenhorst JJ, Scheinberg P, Chattopadhyay PK, et al. High avidity myeloid leukemia-associated antigen-specific CD8+ T cells preferentially reside in the bone marrow. Blood. 2009; 113:2238–2244. [PubMed: 18997173]
87. Levitsky HI. Augmentation of host immune responses to cancer: Overcoming the barrier of tumor antigen-specific T-cell tolerance. Cancer J. 2000; 6 (Suppl 3):S281–90. [PubMed: 10874499]
88. Zhou G, Drake CG, Levitsky HI. Amplification of tumor-specific regulatory T cells following therapeutic cancer vaccines. Blood. 2006; 107:628–636. [PubMed: 16179369]
89. Lawrence GW, Saul A, Giddy AJ, et al. Phase I trial in humans of an oil-based adjuvant SEPPIC MONTANIDE ISA 720. Vaccine. 1997; 15:176–178. [PubMed: 9066035]
90. Rezvani K, Yong AS, Mielke S, et al. Leukemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. Blood. 2008; 111:236–242. [PubMed: 17875804]
91. Keilholz U, Letsch A, Busse A, et al. A clinical and immunologic phase 2 trial of wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. Blood. 2009; 113:6541–6548. [PubMed: 19389880]
92. Rezvani K, Yong AS, Mielke S, et al. Repeated PR1 and WT1 peptide vaccination in montanide-adjuvant fails to induce sustained high-avidity, epitope-specific CD8+ T cells in myeloid malignancies. Haematologica (Italy). 2011; 96:432–440.
93. Cathcart K, Pinilla-Ibarz J, Korontsvit T, et al. A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia. Blood. 2004; 103:1037–1042. [PubMed: 14504104]
94. Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, et al. Synthetic peptide analogs derived from bcr/abl fusion proteins and the induction of heteroclitic human T-cell responses. Haematologica (Italy). 2005; 90:1324–1332.
95. Jain N, Reuben JM, Kantarjian H, et al. Synthetic tumor-specific breakpoint peptide vaccine in patients with chronic myeloid leukemia and minimal residual disease: A phase 2 trial. Cancer. 2009; 115:3924–3934. [PubMed: 19536894]
96. Rojas JM, Knight K, Wang L, et al. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: Results of the EPIC study. Leukemia. 2007; 21:2287–2295. [PubMed: 17637811]
97. Rojas JM, Knight K, Watmough S, et al. BCR-ABL peptide vaccination in healthy subjects: Immunological responses are equivalent to those in chronic myeloid leukaemia patients. Leuk Res. 2011; 35:369–372. [PubMed: 20573399]
98. Witmer-Pack MD, Olivier W, Valinsky J, et al. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal langerhans cells. J Exp Med. 1987; 166:1484–1498. [PubMed: 2445889]
99. Caux C, Dezutter-Dambuyant C, Schmitt D, et al. GM-CSF and TNF-alpha cooperate in the generation of dendritic langerhans cells. Nature. 1992; 360:258–261. [PubMed: 1279441]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

100. Huang AY, Golumbek P, Ahmadzadeh M, et al. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. Science. 1994; 264:961–965. [PubMed: 7513904]
101. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci U S A. 1993; 90:3539–3543. [PubMed: 8097319]
102. Dummer R. GVAX (cell genesys). Curr Opin Investig Drugs. 2001; 2:844–848.
103. Smith BD, Kasamon YL, Kowalski J, et al. K562/GM-CSF immunotherapy reduces tumor burden in chronic myeloid leukemia patients with residual disease on imatinib mesylate. Clin Cancer Res. 2010; 16:338–347. [PubMed: 20048335]
104. Borrello I, Sotomayor EM, Cooke S, et al. A universal granulocyte-macrophage colony-stimulating factor-producing bystander cell line for use in the formulation of autologous tumor cell-based vaccines. Hum Gene Ther. 1999; 10:1983–1991. [PubMed: 10466632]
105. Borrello I, Sotomayor EM, Rattis FM, et al. Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. Blood. 2000; 95:3011–3019. [PubMed: 10807763]
106. Mirmonsef P, Tan G, Zhou G, et al. Escape from suppression: Tumor-specific effector cells outcompete regulatory T cells following stem-cell transplantation. Blood. 2008; 111:2112–2121. [PubMed: 18063750]
107. Brody JD, Goldstein MJ, Czerwinski DK, et al. Immunotransplantation preferentially expands T-effector cells over T-regulatory cells and cures large lymphoma tumors. Blood. 2009; 113:85–94. [PubMed: 18812472]
108. Borrello IM, Levitsky HI, Stock W, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cellular immunotherapy in combination with autologous stem cell transplantation (ASCT) as postremission therapy for acute myeloid leukemia (AML). Blood. 2009; 114:1736–1745. [PubMed: 19556425]
109. Ho VT, Vanneman M, Kim H, et al. Biologic activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. Proc Natl Acad Sci U S A. 2009; 106:15825–15830. [PubMed: 19717467]
110. Anderson LD Jr, Petropoulos D, Everse LA, et al. Enhancement of graft-versus-tumor activity and graft-versus-host disease by pretransplant immunization of allogeneic bone marrow donors with a recipient-derived tumor cell vaccine. Cancer Res. 1999; 59:1525–1530. [PubMed: 10197624]
111. Anderson LD Jr, Savary CA, Mullen CA. Immunization of allogeneic bone marrow transplant recipients with tumor cell vaccines enhances graft-versus-tumor activity without exacerbating graft-versus-host disease. Blood. 2000; 95:2426–2433. [PubMed: 10733517]
112. Ilan Y, Nagler A, Zeira E, et al. Maintenance of immune memory to the hepatitis B envelope protein following adoptive transfer of immunity in bone marrow transplant recipients. Bone Marrow Transplant. 2000; 26:633–638. [PubMed: 11035369]
113. Molrine DC, Antin JH, Guinan EC, et al. Donor immunization with pneumococcal conjugate vaccine and early protective antibody responses following allogeneic hematopoietic cell transplantation. Blood. 2003; 101:831–836. [PubMed: 12393732]
114. Parkkali T, Kayhty H, Hovi T, et al. A randomized study on donor immunization with tetanus-diphtheria, haemophilus influenzae type b and inactivated poliovirus vaccines to improve the recipient responses to the same vaccines after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2007; 39:179–188. [PubMed: 17211432]
115. Kwak LW, Taub DD, Duffey PL, et al. Transfer of myeloma idiotype-specific immunity from an actively immunised marrow donor. Lancet. 1995; 345:1016–1020. [PubMed: 7723498]
116. Neelapu SS, Munshi NC, Jagannath S, et al. Tumor antigen immunization of sibling stem cell transplant donors in multiple myeloma. Bone Marrow Transplant. 2005; 36:315–323. [PubMed: 15968284]
117. Van Tendeloo VF, Van de Velde A, Van Driessche A, et al. Induction of complete and molecular remissions in acute myeloid leukemia by wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci U S A. 2010; 107:13824–13829. [PubMed: 20631300]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

118. Mohty M, Olive D, Gaugler B. Leukemic dendritic cells: Potential for therapy and insights towards immune escape by leukemic blasts. Leukemia. 2002; 16:2197–2204. [PubMed: 12399962]

119. Kremser A, Dressig J, Grabrucker C, et al. Dendritic cells (DCs) can be successfully generated from leukemic blasts in individual patients with AML or MDS: An evaluation of different methods. J Immunother. 2010; 33:185–199. [PubMed: 20139775]

120. Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008; 26:5233–5239. [PubMed: 18809613]

121. Okur FV, Brenner MK. Cellular immunotherapy of cancer. Methods Mol Biol. 2010; 651:319–345. [PubMed: 20686975]

122. Grupp SA, June CH. Adoptive cellular therapy. Curr Top Microbiol Immunol. 2011; 344:149–172. [PubMed: 20700700]

123. Hudecek M, Anderson LD Jr, Nishida T, et al. Adoptive T-cell therapy for B-cell malignancies. Expert Rev Hematol. 2009; 2:517–532. [PubMed: 21083018]

124. Warren EH, Fujii N, Akatsuka Y, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. Blood. 2010; 115:3869–3878. [PubMed: 20071660]

125. Noonan K, Matsui W, Serafini P, et al. Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. Cancer Res. 2005; 65:2026–2034. [PubMed: 15753403]

126. Gill S, Olson JA, Negrin RS. Natural killer cells in allogeneic transplantation: Effect on engraftment, graft- versus-tumor, and graft-versus-host responses. Biol Blood Marrow Transplant. 2009; 15:765–776. [PubMed: 19539207]

127. Ochi T, Fujiwara H, Okamoto S, et al. Novel adoptive T-cell immunotherapy using a WT1-specific TCR vector encoding silencers for endogenous TCRs shows marked anti-leukemia reactivity and safety. Blood. 2011

128. Giordano Attianese GM, Marin V, Hoyos V, et al. In vitro and in vivo model of a novel immunotherapy approach for chronic lymphocytic leukemia by anti-CD23 chimeric antigen receptor. Blood. 2011; 117:4736–4745. [PubMed: 21406718]

129. Hudecek M, Schmitt TM, Baskar S, et al. The B-cell tumor-associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. Blood. 2010; 116:4532–4541. [PubMed: 20702778]

130. Marin V, Pizzitola I, Agostoni V, et al. Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: Improvement of their immune activity by expression of CD33-specific chimeric receptors. Haematologica (Italy). 2010; 95:2144–2152.

131. James SE, Orgun NN, Tedder TF, et al. Antibody-mediated B-cell depletion before adoptive immunotherapy with T cells expressing CD20-specific chimeric T-cell receptors facilitates eradication of leukemia in immunocompetent mice. Blood. 2009; 114:5454–5463. [PubMed: 19880489]

132. Shankaran V, Ikeda H, Bruce AT, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001; 410:1107–1111. [PubMed: 11323675]

133. Zhou G, Lu Z, McCadden JD, et al. Reciprocal changes in tumor antigenicity and antigen-specific T cell function during tumor progression. J Exp Med. 2004; 200:1581–1592. [PubMed: 15596524]

134. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–1570. [PubMed: 21436444]

135. Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol. 2009; 27:83–117. [PubMed: 19007331]

136. Zhou G, Drake CG, Levitsky HI. Amplification of tumor-specific regulatory T cells following therapeutic cancer vaccines. Blood. 2006; 107:628–636. [PubMed: 16179369]

137. Kelley TW, Parker CJ. CD4 (+)CD25 (+)Foxp3 (+) regulatory T cells and hematologic malignancies. Front Biosci (Schol Ed). 2010; 2:980–992. [PubMed: 20515837]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

138. Szczepanski MJ, Szajnik M, Czystowska M, 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]
139. Wang X, Zheng J, Liu J, 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 (Denmark). 2005; 75:468–476.
140. Ersvaer E, Liseth K, Skavland J, et al. Intensive chemotherapy for acute myeloid leukemia differentially affects circulating TC1, TH1, TH17 and TREG cells. BMC Immunol. 2010; 11:38. [PubMed: 20618967]
141. Carreras J, Lopez-Guillermo A, Roncador G, et al. High numbers of tumor-infiltrating programmed cell death 1-positive regulatory lymphocytes are associated with improved overall survival in follicular lymphoma. J Clin Oncol. 2009; 27:1470–1476. [PubMed: 19224853]
142. Carreras J, Lopez-Guillermo A, Fox BC, et al. High numbers of tumor-infiltrating FOXP3−positive regulatory T cells are associated with improved overall survival in follicular lymphoma. Blood. 2006; 108:2957–2964. [PubMed: 16825494]
143. Tzankov A, Meier C, Hirschmann P, 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 (Italy). 2008; 93:193–200.
144. Lee AM, Clear AJ, Calaminici M, et al. Number of CD4+ cells and location of forkhead box protein P3-positive cells in diagnostic follicular lymphoma tissue microarrays correlates with outcome. J Clin Oncol. 2006; 24:5052–5059. [PubMed: 17033038]
145. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J Clin Invest. 2007; 117:1147–1154. [PubMed: 17476344]
146. Corm S, Berthon C, Imbenotte M, et al. Indoleamine 2,3-dioxygenase activity of acute myeloid leukemia cells can be measured from patients' sera by HPLC and is inducible by IFN-gamma. Leuk Res. 2009; 33:490–494. [PubMed: 18639339]
147. Ninomiya S, Hara T, Tsurumi H, et al. Indoleamine 2,3-dioxygenase in tumor tissue indicates prognosis in patients with diffuse large B-cell lymphoma treated with R-CHOP. Ann Hematol. 2011; 90:409–416. [PubMed: 20938662]
148. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008; 26:677–704. [PubMed: 18173375]
149. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006; 443:350–354. [PubMed: 16921384]
150. Isogawa M, Furuichi Y, Chisari FV. Oscillating CD8(+) T cell effector functions after antigen recognition in the liver. Immunity. 2005; 23:53–63. [PubMed: 16039579]
151. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006; 439:682–687. [PubMed: 16382236]
152. Curran MA, Montalvo W, Yagita H, et al. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A. 2010; 107:4275–4280. [PubMed: 20160101]
153. Tamura H, Dan K, Tamada K, et al. Expression of functional B7-H2 and B7. 2 costimulatory molecules and their prognostic implications in de novo acute myeloid leukemia. Clin Cancer Res. 2005; 11:5708–5717. [PubMed: 16115907]
154. Chen X, Liu S, Wang L, et al. Clinical significance of B7-H1 (PD-L1) expression in human acute leukemia. Cancer Biol Ther. 2008; 7:622–627. [PubMed: 18756622]
155. Green MR, Monti S, Rodig SJ, 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]
156. Gonzalez S, Groh V, Spies T. Immunobiology of human NKG2D and its ligands. Curr Top Microbiol Immunol. 2006; 298:121–138. [PubMed: 16329186]

157. Groh V, Wu J, Yee C, et al. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002; 419:734–738. [PubMed: 12384702]

158. Nowbakht P, Ionescu MC, Rohner A, et al. Ligands for natural killer cell-activating receptors are expressed upon the maturation of normal myelomonocytic cells but at low levels in acute myeloid leukemias. Blood. 2005; 105:3615–3622. [PubMed: 15657183]

159. Salih HR, Antropius H, Gieseke F, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood. 2003; 102:1389–1396. [PubMed: 12714493]

160. Ho VT, Vanneman M, Kim H, et al. Biologic activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. Proc Natl Acad Sci U S A. 2009; 106:15825–15830. [PubMed: 19717467]

161. Terme M, Borg C, Guilhot F, et al. BCR/ABL promotes dendritic cell-mediated natural killer cell activation. Cancer Res. 2005; 65:6409–6417. [PubMed: 16024645]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Recruiting, Active and Recently Completed Vaccine Clinical Trials in Acute Myeloid Leukemia

Agent	Sponsor	Details	Patients	Primary Endpoints	Secondary Endpoints	Status	NCT ID
WT-1 Peptide Vaccine	Memorial Sloan-Kettering Cancer Center	Peptides/Montanide and GM-CSF injected SC in weeks 0, 4, 6, 8, 10, and 12. If immune response and no disease progression up to 6 additional monthly doses.	AML (in CR, with no plans for additional chemotherapy) or MDS (≥Int-2, not candidate for chemotherapy) with documented pretreatment WT-1 positive disease (by protein on bone marrow biopsy or RQ-PCR).	Safety Immune *(D, Ie, P, T)*	Tumor response	**Completed** Phase I	NCT00398138
WT-1 peptide Vaccine	Memorial Sloan-Kettering Cancer Center	6x SC vaccinations with WT1 peptide over ten week period.	AML in first CR, completed post-remission therapy, not eligible for allo-BMT, with WT-1 positive PCR detectable MRD on bone marrow within 4wks of 1st vaccine.	Safety OS (3yr)	DFS (3yr) Immune *(T, P, $I_{f/e}$)* MRD *(WT-1+ by RT-PCR)*	Recruiting Phase II Opened Dec 2010	NCT01266083
WT-1 derived Peptides	Duke University	WT-1 derived epitopes restricted by HLA-A2, A24, DR15 and DRw53 injected ID and SC in Montanide and GM-CSF.	Following autologous or allogeneic stem cell transplantation for AML, CML, ALL, B cell malignancies or MDS. Patient must be HLA A2, A24, DR15 or DRw53.	Safety Feasibility	Immune Clinical response	Recruiting Phase I Opened June 2007	NCT00672152
WT-1 Peptide	H. Lee Moffitt Cancer Center	6 bi-weekly vaccinations of peptide/montanide and GM-CSF over 10 weeks. Patients with immunologic response and not disease progression may continue with 6 extra monthly vaccinations.	AML (in CR1 or 2, with no plans for additional chemotherapy) or MDS (≥Int-2, not candidate for chemotherapy) with documented pretreatment WT-1 positive disease (by protein on bone marrow biopsy or RQ-PCR).	Safety Toxicity Immune (D, In, P, T)	Tumor Response (*WT-1+ by IHC and RQ-PCR, Multiparameter Flow Cytometry*)	Recruiting Opened June2008	NCT00665002
WT-1 peptide vaccine with lymphodepletion	National Heart, Lung, and Blood Institute (NHLBI)	Peptide/Montanide and GM-CSF	MDS, CML (relapsed or	Efficacy *(change in frequencies of*	Hematological (marrow blasts, blood	**Completed** Phase II	NCT00433745

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Agent	Sponsor	Details	Patients	Primary Endpoints	Secondary Endpoints	Status	NCT ID
and lymphocyte infusion.		injections weekly for 9 wks.	refractory, with accelerated or blast phase), AML (secondary, relapsed or refractory) ALL (relapsed, refractory or in CR), CMML. HLA-A0201+	*circulating WT-1 specific T cells)* Toxicity	counts, transfusion dependence) TTP OS		
WT-1 epitopes DNA vaccine	Southampton University Hospitals NHS Trust	If HLA A2+ will be vaccinated with two DNA vaccines given IM followed by electroporation, 6 times at 4 weekly intervals followed by every 3 month vaccinations (for max of 24 mo) in responders. HLA A2-ve patients monitored with molecular testing only.	CML: Philadelphia chromosome positive CML in chronic phase, maintained in complete cytogenetic response (CCyR) for ≥ 24months but with detectable BCR-ABL transcripts. AML patients: AML: WT1+ AML in CR or morphologic CR with incomplete blood count recovery (CRi).	Molecular Response	CML: TTP TNT 2yr Survival AML: 2yr Survival OS	Recruiting Phase II Opened Feb 2010	NCT01334060
WT-1 Peptide Fusion Protein	GlaxoSmith-Kline	Recombinant fusion protein WT1-A10 combined with the adjuvant ASO1B GSK2130579A	Post-induction therapy in adults with WT1-positive AML and a suboptimal clinical response (PR or CRi) to induction chemotherapy	Severe Toxicity Clinical Activity	Immune Safety Clinical Activity	Recruiting Phase 1	NCT0151063
WT-1 Peptide Fusion Protein	GlaxoSmith-Kline	Recombinant fusion protein WT1-A10 combined with the adjuvant ASO1B GSK2130579A	Post-consolidation therapy in adults with WT1-positive AML in first complete remission	Severe Toxicity Immune (H/S)	Adverse Events	Recruiting Phase 1	NCT00725283
WT-1 Peptide-Pulsed Dendritic Cells	National Cancer Institute (NCI)	Donor-derived dendritic cell/WT-1 vaccine and DLI given every 14 days for 6 doses.	HLA-A2 plus patients aged 1 to 75 with relapsed or residual WT1 expressing hematologic malignancy following (at least 5/6 match) allogeneic SCT	Safety Toxicity Feasibility	Immune Clinical response MRD *(WT-1+ by RT-PCR)*	Recruiting Phase I/II Opened Jan 2008	NCT00923910

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Agent	Sponsor	Details	Patients	Primary Endpoints	Secondary Endpoints	Status	NCT ID
			Donor also HLA-A2 plus.				
WT1 and PR1 Peptide Vaccine	National Heart, Lung, and Blood Institute (NHLBI)	Single dose of PR1:169–177 and WT-1:126–134 peptide (in Montanide) concomitantly with GM-CSF	HLA-A*0201 positive. AML in CR (w/in 5yrs of treatment), CP CML, MDS, Between 6–36 months from allo-BMT for MDS, CML, AML with 100% engraftment and <5% blasts in marrow.	Safety	N/A	**Completed** Phase I	NCT00270452
WT-1 and PR-1 Peptide Vaccines	National Heart, Lung, and Blood Institute (NHLBI)	Six doses of a combination of WT-1: 126–134 and PR-l: 169–177 peptide vaccines in Montanide and GM-CSF	HLA-A*0201 positive. MDS, AML in CR or CP CML and unsuitable for stem cell transplantation or at 6–36 mos after Allo-BMT with <5% blasts in marrow, 100% engraftment	Efficacy *(change in frequencies of circulating PR-1 and WT-1 specific T cells)* Toxicity	Hematological (marrow blasts, blood counts, transfusion dependence) TTP OS Boost Response	**Completed** Phase II Ref: (92)	NCT00488592
PR-1 Peptide Vaccine	M.D. Anderson Cancer Center	PR1 peptide SC with Montanide and GM-CSF every 3 weeks for total of 6 vaccinations.	HLA-A*0201 positive patients with high risk myeloid leukemias.	Immune *(3wk after last vaccine)* Clinical *(3wk after last vaccine)*	EFS OS	Ongoing, but not recruiting Phase I/II Opened Dec 1999	NCT00004918
Dendritic Cell/AML Vaccine and either PD-1 blockade or GM-CSF	Beth Israel Deaconess Medical Center/NIH/ CureTech Ltd	CT-011 (anti PD-1 mAb) or GM-CSF 4–8wks after chemotherapy. Followed by 3 doses of DC/AML vaccine given at 4wk intervals.	AML (initial diagnosis or relapse) in chemotherapy induced CR.	Toxicity	Immune (D) TTP (2yrs)	Recruiting Phase II Opened May 2010	NCT01096602
GM-CSF/K562 with irradiated autologous leukemia vaccine.	Dana-Farber Cancer Institute	K562 expressing GM-CSF mixed with irradiated autologous leukemia. Total of 6 doses over 9 wks.	Advanced Myelodysplastic Syndrome (MDS) or Acute Myeloid Leukemia (AML) after Allogeneic Hematopoetic Stem Cell Transplantation	Safety	Immune (D) Disease Response DFS OS	Recruiting Phase I Opened Nov 2008	NCT00809250
Lentivirus Transduced AML Blasts to express B7.1 (CD80) & IL-2	King's College Hospital NHS Trust, London.	RFUSIN2-AML1 (AML Blasts transduced to	New or relapsed poor prognosis AML with no CR or persistent	Safety and Toxicity	Relapse LFS OS	Recruiting Phase I Opened May 2008	NCT00718250

Agent	Sponsor	Details	Patients	Primary Endpoints	Secondary Endpoints	Status	NCT ID
		express B7.1 and IL-2) and/or DLI.	cytogenetic abnormality >100 days post BMT.				
GM-CSF/K562 with irradiated autologous leukemia vaccine, before and after Auto-PBSC and primed DLI.	Cell Genesys	Autologous leukemia admixed with GM-CSF secreting K562 (“GVAX”) given once pre-and every 3wks for 8 doses starting ≥ 6wks post-auto-BMT. Primed autologous DLI with PBSC.	De novo, untreated, AML in patients 18–60 with 18 and 60 years of age.	Safety Feasibility	GM-CSF PKData Immune (A, D, H, I_f) MRD*(WT-1+ by RT-PCR)* RFS OS	**Completed** Phase II Ref: (108)	NCT00116467
WT-1 mRNA-transfected Autologous Dendritic Cell Vaccination	University Hospital, Antwerp	Intradermal injection of WT1-RNA-transfected autologous dendritic cells.	Adults with AML or MDS, with over-expression of WT-1, in PR, CR or smoldering course and high risk of relapse.	Toxicity Feasibility	Immune	**Completed** Phase I/II	NCT00834002
BMT followed by autologous cancer vaccine and donor DLI.	SKCCC at Johns Hopkins	HLA matched related sibling nonmyeloablative hematopoietic stem cell transplantation followed by autologous tumor cell vaccine +/− DLI from donor.	AML: *Relapsed, from MDS, Primary Refractory or De Novo with High Risk Features.* ALL: *Ph chromosome +, t(4,11) or relapsed.* Multiple Myeloma: *In need of treatment.*	TTP	Safety and Tolerability Determine MTD of DLI	Completed	NCT00469820
Autologous Mature Dendritic Cells transfected with hTERT mRNA	Geron Corporation	Vaccinated weekly for 6 weeks, “rest” for 4 weeks, then 6 boost injections over 12 weeks.	AML in first CR or in CR2 with CR1 >/ = 6 months Has completed at least one cycle of consolidation chemotherapy within past 6 months.	Feasibility	Immune (D, S) EFS	Active, Not Recruiting Phase II Opened July 2007	NCT00510133
Therapeutic autologous dendritic cells.	Institut Paoli-Calmettes	Increasing doses of blastic cells transformed in vitro by autologous dendritic cells (1/3 subcutaneously and 2/3 IV) every 3 weeks for up to 5 doses.	HLA-A*0201 positive. AML, M4 or M5, in CR2 or later following CR1 lasting <12months. Not Eligible for BMT.	Tolerability	Immune Clinical Response	Active, Not Recruiting Phase I	NCT00963521

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Agent	Sponsor	Details	Patients	Primary Endpoints	Secondary Endpoints	Status	NCT ID
BMT followed by irradiated autologous myeloblastic leukemia engineered to secrete GM-CSF	Dana-Farber Cancer Institute	Allogeneic reduced intensity stem cell transplant followed 30–45 days later by vaccination SC and ID 6 times over a period of 9 weeks.	AML, MDS-RAEB or RAEB-T, CML, myeloid blast crisis not in remission or CML accelerated phase. Subjects must have > 5% blasts in bone marrow or peripheral blood prior to admission for transplant. HLA 6/6 matched related or unrelated donor available	Feasibility	Safety Biological activity DFS OS	Active, Not Recruiting Opened June 2004	NCT00426205
Irradiated autologous myeloblastic leukemia engineered to secrete GM-CSF	Dana-Farber Cancer Institute	Vaccinations will be given weekly for three weeks, followed by every other week, for a min of 6 doses.	MDS, AML (relapsed, refractory, not candidate for myelosuppressive chemotherapy).	Feasibility	Safety Biological Activity	Completed Phase I	NCT00136422

Key

AML = Acute Myeloid Leukemia

DC = Dendritic Cell

DLI = Donor Lymphocyte Infusion

DFS = Disease Free Survival

EFS= Event Free Survival

ID = Intradermally

IM = Intramuscular

LFS = Leukemia Free Survival

MRD = minimal residual disease

OS = Overall Survival

PD-1 = Programmed death-1 receptor

RFS = Relapse Free Survival

SC = Subcutaneous

TNT = Time to next treatment

TTP = Time to Progression

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

WT-1 = Wilms Tumor Antigen

Immune Monitoring

A = Skin Test Anergy Panel

D = Delayed Type Hypersensitivity Testing (DTH)

H= Humeral/Antibody Response

If = IFN-Y Cytokine Assay - Flow

Ie = IFN-Y Cytokine Assay – Elispot

In = IFN-Y Cytokine – Assay Not Specificed

P = CD4 Proliferation Assay

S= Measurement of Antigen Specific T cells – Assay not specificied.

T = MHC Tetramer Assay