Published in final edited form as:

Expert Rev Hematol. 2011 February ; 4(1): 37–50. doi:10.1586/ehm.10.80.

Vaccines as consolidation therapy for myeloid leukemia

Gheath Alatrash¹ and Jeffrey J Molldrem^{†,1}

¹Department of Stem Cell Transplantation and Cellular Therapy, University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 900, Houston, TX 77030, USA

Abstract

Immunotherapy for myeloid leukemias remains a cornerstone in the management of this highly aggressive group of malignancies. Allogeneic (allo) stem cell transplantation (SCT), which can be curative in acute and chronic myeloid leukemias, exemplifies the success of immunotherapy for cancer management. However, because of its nonspecific immune response against normal tissue, allo-SCT is associated with high rates of morbidity and mortality, secondary to graft-versus-host disease, which can occur in up to 50% of allo-SCT recipients. Targeted immunotherapy using leukemia vaccines has been heavily investigated, as these vaccines elicit specific immune responses against leukemia cells while sparing normal tissue. Peptide and cellular vaccines have been developed against tumor-specific and leukemia-associated self-antigens. Although not yet considered the standard of care, leukemia vaccines continue to show promising results in the management of the myeloid leukemias.

Keywords

acute myeloid leukemia; allogeneic stem cell transplantation; cell-based vaccines; chronic myeloid leukemia; dendritic cell vaccines; leukemia vaccines; peptide vaccines; targeted immunotherapy

Myeloid leukemia accounts for approximately 50% of the newly diagnosed leukemias. Acute myeloid leukemia (AML) has an aggressive course with an overall 5-year survival rate of only 24%. Chronic myeloid leukemia (CML) has a more indolent course during its chronic phase but can be as similarly aggressive as AML in its accelerated and blast phases. Currently, cytarabine-based chemotherapy is the mainstay treatment for AML, and the tyrosine kinase inhibitors, imatinib, dasatinib and nilotinib, provide long-term responses for patients in chronic-phase CML[1-4]. Despite overall advances in the treatment of myeloid leukemia, the ideal management for aggressive forms of AML and for CML in accelerated or blast phases is less well defined, thus providing the impetus to discover novel therapeutic approaches for these aggressive leukemias. Since AML and CML often present with a large burden of disease in the bone marrow and sometimes in the peripheral blood, thereby necessitating prompt therapy that is so far limited to chemotherapy, vaccines for myeloid leukemia would most probably be beneficial to eradicate minimal residual disease in the setting of consolidation therapy or following stem-cell transplantation (SCT).

© 2011 Expert Reviews Ltd

[†]Author for correspondence: Tel.: +1 713 563 3334, Fax: +1 713 563 3364, jmolldre@mdanderson.org.

Financial & competing interests disclosure The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

The success of allogeneic (allo)-SCT, which can lead to long-term cures in AML and CML, highlights the importance of immunotherapy in myeloid leukemia. However, the curative graft-versus-leukemia (GvL) immune response following allo-SCT is often nonspecific and is counterbalanced by the allograft attacking normal tissues, leading to graft-versus-host disease (GvHD). GvHD can occur in up to 50% of allo-SCT recipients, causes significant morbidity and mortality, and limits the benefit that can be attained with allo-SCT. Numerous patient, donor, graft and disease characteristics have been identified to aid the selection of the most appropriate candidates for allo-SCT; nevertheless, the toxicity of allo-SCT is often unacceptable and, in some cases, precludes the use of this therapeutic option. The direct toxicity of allo-SCT is further amplified by the older median age of patients with AML (70 years) and CML (65 years) [1,201] and by the fact that many patients undergo allo-SCT after having previously received numerous cycles of highly toxic chemotherapy [1].

Targeted immunotherapy using leukemia vaccines has been heavily investigated, as these vaccines elicit specific immune responses against leukemia targets while sparing normal tissue. Target antigens for GvL are believed to be either minor histocompatibility antigens [5] or leukemia-associated antigens (LAAs) [6]; the latter are preferentially expressed by leukemia but are also expressed by normal tissue, albeit to a lesser degree. It is the expression of these antigens by normal tissue that is believed to be, in part, the culprit for GvHD. The optimal target antigens for immunotherapy would be leukemia-specific antigens; however, such antigens that are expressed exclusively by leukemia and are absent in normal tissues, and that also elicit potent immune responses, are rare. The benefit of leukemia vaccines is that they elicit immunity against one or a few known antigens, thereby providing specific immunity against myeloid leukemia cells and fewer nonspecific immune responses against normal tissues. Peptide/antigen vaccines prime an antigen-specific immune response through their interactions with antigen-presenting cells (APCs) and subsequently cytotoxic T lymphocytes (CTLs), which ultimately kill antigen-expressing leukemia targets (Figure 1). These interactions, which require functional APCs that can provide adequate costimulation to T cells, in addition to antigen presentation, are complex and are the subject of a large body of investigations.

Tumor vaccines can be divided into two broad categories – peptide-based vaccines and cell-based vaccines – as determined by the immunogen used in the vaccine (peptide vs cells). Peptide vaccines are HLA restricted and therefore are applicable to distinct patient populations expressing the HLA type, require prior knowledge and characterization of the antigenic epitope of the target protein, and elicit a narrow immune response against a single antigen. Peptide vaccines primarily focus on enhancing the CTL immune response, although a few peptide vaccines have elicited immune responses by helper CD4⁺ T cells (see later). Conversely, cell-based vaccines are not HLA-restricted and elicit an immune response against a broad spectrum of antigens that are expressed by the target cells, although often these vaccines elicit only a narrow immune response against very few epitopes, a phenomenon known as epitope dominance [7,8]. In addition, cell-based vaccines can enhance not only a CTL-mediated immune response but also antibody and CD4⁺ T-cell-mediated immunity.

Vaccines have been developed against tumor-specific and leukemia-associated self antigens, including peptide- and cellular-based vaccines, and have demonstrated efficacy, not only in preclinical but also in clinical trials, mainly Phase I and II studies. This article will focus on these two vaccine categories in the context of the myeloid leukemias.

Peptide-based vaccines

The discovery of tumor-associated and -specific antigens has paved the way for engineering peptide vaccines to elicit specific anti-tumor immune responses. Peptide vaccines have been used in solid and hematologic malignancies with various success rates. The low toxicity and ease of administration of peptide vaccines has made them very attractive for the treatment of malignancies, especially compared with the highly toxic chemotherapeutic agents and stem cell transplantation that are currently the standard of care. Peptide vaccines are HLA restricted and are usually developed for HLA-A2, since HLA-A2 is the most frequently occurring HLA serotype found in up to 50% of Caucasians [9] (of interest, HLA-A24 is the most common HLA serotype in Japan). However, despite their favorable toxicity profile, peptide vaccines have, to date, been primarily used in clinical trials in the adjuvant setting following standard therapy, and these vaccines have not yet become the standard of care. Nevertheless, numerous reports have already documented long disease remissions and cures following the use of peptide vaccines, and these results support the use of these vaccines in clinical trials in specific high-risk populations [10,11].

BCR-ABL

The Philadelphia chromosomal translocation t(9;22)(q34;q11), although not unique to CML, is essential to its pathogenesis and is found in approximately 95% of patients with CML. This translocation also can be found in 25–30% of adults with acute lymphoblastic leukemia and occasionally in patients with AML [12,13]. The result of this translocation is the BCR-ABL fusion protein, which is associated with increased ABL tyrosine kinase activity that is directly associated with leukemo-genesis [14]. The absence of BCR-ABL protein in normal cells makes this protein an ideal target for leukemia immunotherapy.

Two different proteins result from the BCR-ABL fusion: b2a2 and b3a2. The breakpoint in BCR usually occurs where either BCR exons 13 (b2) or 14 (b3) are fused to ABL exon 2 (a2), termed b2a2 and b3a2 fusion transcripts. The b3a2 rearrangement is found in approximately 60% of CML patients [15]. Although immunogenic peptides were previously identified from both fusion proteins [16–18], most studies have focused on the b3a2 fusion protein. Bocchia *et al.* were the first to identify HLA class I peptides from the BCR-ABL fusion protein derived from the b3a2 exon junction [19]. In that study, four overlapping peptides were shown to bind with intermediate (≤ 500 nmol/l) or high affinity (≤ 50 nmol/l) to the HLA class I molecules HLA-A3 (KQSSKALQR), -A11 (ATGFKQSSK), -B8 (GFKQSSKAL) or both -A3 and -A11 (HSATGFKQSSK). Although cell-surface HLA binding of these peptides was demonstrated, a subsequent study showed that the HLA-A3 and HLA-A3/A11 binding peptides elicited the most potent CTLs that lysed targets that were loaded with these respective peptides [16]. In a Phase I dose-escalation trial of a BCR-ABL peptide vaccine (that included a mix of the four previously mentioned b3a2 fusion protein peptides, in addition to a 25 amino acid peptide that included all four overlapping sequences) in patients with CML, increasing doses of the peptide vaccine (50, 150, 500 and 1500 μ g per vaccine dose) were all well tolerated without any major adverse events [20]. In addition, T-cell proliferative response and delayed-type hypersensitivity reactions were detected in patients who had received the two highest doses of the vaccine, but no peptide-specific CTLs were identified. Because clinical immunity was identified in the absence of peptide-specific CTLs, the authors suggested that the vaccine might have generated a CD4⁺ T-cell response as was demonstrated by prior peptide vaccines [21,22].

The promising data from preclinical and Phase I studies with BCR-ABL led to a Phase II trial of multivalent BCR-ABL fusion peptide vaccine in 14 patients with CML [23]. In this trial, in addition to the previously mentioned four HLA class I peptides, the investigators also used HLA-A0201 peptides (SSKALQRPV) and HLA class II DR1, DR4 and DR11

binding peptide (IVHSATGFKQSSKALQRPVASFEP) as part of the vaccine. Although peptide-specific CD8⁺ T-cell responses were seen in four patients, the majority of patients (n = 11) demonstrated a CD4⁺ immunologic response. Clinical responses were seen in seven of the 14 vaccinated patients and included molecular and cytogenetic responses. However, many of the patients in this trial were receiving concurrent CML therapy that included IFN- α , imatinib or donor lymphocyte infusion. Using a similar vaccine strategy, Bocchia *et al.* also documented clinical efficacy following vaccination with BCR-ABL multi-peptide vaccine in CML patients receiving either imatinib (n = 10) or IFN- α (n = 6) [24].

Since the CD8⁺ T-cell responses to BCR-ABL vaccines were variably detected in the aforementioned trials, and since CD4⁺ T-cell responses were more consistently detected following BCR-ABL vaccine, Rojas *et al.* [25] linked some of the BCR-ABL peptides to the pan DR epitope peptides [26], which induce CD4⁺ T-cell responses and have been used as adjuvants in studies of vaccines for cancer and infectious diseases [27,28]. In the Phase I/II study, 14 of 19 imatinib-treated patients who received the vaccination demonstrated T-cell responses, and a drop in BCR-ABL transcripts was seen in 13 patients [25]. CD8⁺ T-cell responses to the HLA class I 9-nonamer peptides and CD4⁺ T-cell responses to the 18-nonamer peptide that contains the sequences for numerous 9-nonamer peptides were reported in six and 13 patients, respectively. Molecular response was demonstrated in those patients who had shown a major cytogenetic response at entry into the study. No major toxicities were reported.

Another strategy to improve the immunogenicity of BCR-ABL peptide vaccines employed synthetic peptide analogs derived from the BCR-ABL b3a2 and b2a2 fusion protein [29]. These peptides, termed heteroclitic peptides, were designed with single or double amino acid substitutions at key HLA-0201 binding positions that would increase peptide-HLA-0201 binding. In one study, 13 patients received a multi-peptide vaccine that included native and heteroclitic HLA-A2-binding peptides; immunologic and clinical responses were seen and the vaccine was well tolerated [29]. Using the same synthetic and native peptide combinations derived from the b3a2 and b2a2 breakpoints, Jain *et al.* demonstrated, in a Phase II trial with ten patients, that vaccination was associated with a 1-log reduction in BCR-ABL transcripts and with a major molecular response in three and five of those patients, respectively [30]. All ten patients entered into that trial had been in complete cytogenetic response at study entry and had been taking imatinib concurrently with vaccination. However, many of the responses were transient. Because of this observation, Rojas *et al.*, in another study, vaccinated five healthy individuals with the BCR-ABL junctional peptides discussed previously in an attempt to investigate whether CML was inhibiting potent immune responses to the BCR-ABL vaccine [31]. However, their results demonstrated comparable moderate and transient immune responses in healthy donors, similar to those noted in CML patients. As a result, a question has been raised regarding the immunogenicity and potency of junctional BCR-ABL peptides and their use in vaccine therapy. Nevertheless, to date, junctional BCR-ABL peptides remain the main components of the BCR-ABL vaccine.

Wilm's tumor gene

Wilm's tumor (*WT1*) gene is a zinc finger transcription factor that is involved in cell proliferation, differentiation and apoptosis [32-34]. WT1 is overexpressed in AML and CML [35-37] in addition to solid tumors [38-41]. WT1 was initially identified as an immunogenic tumor-associated antigen in animal models using WT1-derived nonamer peptides Db126, Db221 and Db235 [42]. CTLs were generated targeting WT1-peptides in mice and were shown to eliminate WT1-expressing target cells. In addition, mice that were immunized with Db126 peptide rejected challenges by WT1-expressing tumors. Using nonobese diabetic severe combined immunodeficiency mice, Gao *et al.* demonstrated that

human WT1-CTLs were able to eliminate CD34⁺ CML cells while sparing normal CD34⁺ stem cells [43]. Moreover, WT1-CTLs were generated from normal donors against numerous WT1 epitopes that bind to different HLA-A molecules, including HLA-A01 [44], HLA-A0206 [45] and HLA-A2402 [46], and demonstrated specific target killing of WT1-expressing leukemia [47,48].

Although numerous HLA class I and II WT1 peptides have been identified [44-46,49-53], most clinical trials have focused on three HLA class I 9-nonamer peptides: the HLA-A0201-restricted peptide RMFPNAPYL and the HLA-A2402-restricted peptides CMTWNQMNL and its modified version CYTWNQMNL. Clinical trials have been conducted and are currently ongoing to further determine the efficacy of WT1 in solid [54,55] and hematologic malignancies. The low toxicity of WT1 HLA-A2402-restricted peptide vaccine was initially established following Phase I studies in patients with lung and breast cancers, AML and myelodysplastic syndrome (MDS); in these studies, local erythema at the vaccine injection site was the primary toxicity, and severe leukopenia was noted in the vaccinated MDS patients [54,56]. A reduction in blast number was reported in the leukemia patients who received this vaccine.

The WT1 HLA-A0201 peptide was also demonstrated to be safe and efficacious when administered to patients with AML [10,57]. In view of their mild toxicity and promising efficacy, WT1 peptide vaccines were subsequently studied in Phase II clinical trials. In a Phase II trial by Keilholz *et al.*, AML patients were administered the HLA-A0201-restricted WT1 peptide along with keyhole limpet hemocyanin (KLH) and granulocyte-macrophage colony-stimulating factor as adjuvants [58]. Of 17 AML patients enrolled on this trial, 12 had stable diseases and one had a complete remission. Correlative data showed a reduction in WT1 mRNA levels, a tumor marker in AML, compared with baseline in 35% of the patients, and an increase in blood and bone marrow WT1-tetramer⁺ CTLs in 44 and 47% of patients, respectively. Rezvani *et al.* conducted a clinical trial investigating HLA-A0201-WT1 peptide vaccine administered in conjunction with PR1 vaccine (discussed in the following section) to patients with AML (n = 5), CML (n = 1) and MDS, and observed an increase in functional WT1-CTLs following vaccination in five of eight patients. Furthermore, disease regression, as measured by WT1 expression, was correlated with an increase in WT1 and PR-1 CTLs [10].

PR1

The HLA-A2-restricted nonameric peptide PR1 (VLQELNVTV) is derived from the azurophil granule proteases proteinase-3 (P3) and neutrophil elastase (NE), both of which are aberrantly expressed by myeloid leukemia [59]. In addition to leukemia, NE and P3 are also expressed by normal neutrophils and hematopoietic myeloid cells and their expression is primarily limited to the early stages of hematopoietic development [60]. Since NE and P3 expression is somewhat limited to early immature myeloid cells, which is often the phenotype of myeloid leukemia blasts, targeting PR1-expressing cells would therefore selectively eliminate leukemia while sparing normal hematopoietic cells.

Immunity to PR1 was first demonstrated by expanding PR1-CTLs from healthy donor blood and showing the ability of PR1-CTLs to lyse PR1-pulsed targets and primary CML blasts, with minimal killing of normal bone marrow cells [59,61]. This killing of myeloid targets by PR1-specific CTLs was HLA-A2 restricted and correlated with P3 expression by target leukemia. Furthermore, a correlation between the detection of PR1-specific CTLs and clinical responses to IFN- α 2b or allo-SCT was demonstrated in CML patients [62].

The toxicity and efficacy of PR1 peptide vaccination in patients with AML, CML and MDS was demonstrated following a Phase I/II trial conducted at the MD Anderson Cancer Center

(Houston, TX, USA) [63,64]. In that study, 13 of the 66 patients treated with PR1-peptide vaccine achieved complete remission, and toxicity was primarily limited to injection-site reactions (Figure 2). In addition, an immunologic response (defined as at least a twofold increase in PR1-CTL) was observed in 25 of 53 (47%) patients with measurable disease; this response was associated with a longer event-free survival (8.7 vs 2.4 months; $p = 0.03$), and nine of the 25 immune responders demonstrated clinical responses. Moreover, following the administration of PR1 peptide and WT1 peptide vaccines in combination, Rezvani *et al.* showed a positive correlation between emergence of PR1-CTL and clinical response in three of six evaluable patients with myeloid leukemia or MDS [10]. Further studies are ongoing with PR1 peptide vaccination in patients following standard leukemia therapy and in healthy donors prior to stem cell harvest to enhance the GvL effect provided by PR1-specific CTLs.

RHAMM (CD168)

The receptor for hyaluronic acid-mediated motility (RHAMM) was shown to be expressed in hematologic malignancies, including 80% of cases of AML or MDS, as well as CML [65]. RHAMM is normally a cytoplasmic protein that can be exported to the cell surface as a result of redistribution of intracellular protein pools under specific stimuli or malignant transformation [66,67]. RHAMM has been demonstrated to play a role in oncogenesis. In conjunction with CD44 and hyaluronic acid, RHAMM activates ERK1/2 signaling, which promotes motility and invasion [68]. RHAMM expression is low in normal human tissue and it is upregulated in some tissues in response to stress, including wound repair and hypoxia [69]. In addition, high expression of RHAMM has been detected in many cancers, including solid tumors [65].

RHAMM was demonstrated to be immunogenic, eliciting humoral and cell-mediated immune responses [65,70]. Numerous immunogenic HLA-A2-binding peptides originating from RHAMM protein were discovered and shown to elicit a cellular immune response. Using HLA-A2 binding algorithms, Greiner *et al.* identified ten potential peptides derived from RHAMM [70]. Two of these ten peptides, R3 (ILSLELMKL) and R5 (SLEENIVIL), were shown to elicit an immune response, as demonstrated by the detection of peptide-tetramer⁺ CTLs in the blood of leukemia patients and by lysis of peptide-expressing leukemia targets by peptide-primed CTLs. Vaccination with R3 peptide was subsequently carried out in patients with AML, MDS and multiple myeloma (MM). In a Phase I clinical trial, Schmitt *et al.* vaccinated ten patients (six with AML/MDS and four with MM) with R3 peptide and reported clinical and immunologic responses following vaccination [71]. Specifically, three of the six patients with AML/MDS demonstrated a decrease in blast count or a hematologic improvement following vaccination. Four of the six patients with AML/MDS demonstrated an increase in tetramer⁺ R3-specific CTLs, and all three patients with immunologic responses also demonstrated clinical responses. Furthermore, R3 CTLs that were generated *in vivo* following vaccination were shown to have specific lysis of R3-expressing targets. In view of the low toxicity reported with the RHAMM peptide vaccine in the Phase I study, Greiner *et al.* administered a higher dose of R3 peptide vaccine (1000 μg vs 300 μg /vaccination) to nine patients (six with AML/MDS and three with MM) and demonstrated similarly low toxicity and equivalent clinical and immunologic responses compared with the higher dose. Notably, no improvement in either clinical or immunologic responses was recorded with the higher dose, compared with the lower R3 peptide vaccine dose [72]. Further investigation of the RHAMM peptide vaccine is warranted to conclusively determine its use in the treatment of myeloid leukemia.

hTERT

The human telomerase reverse transcriptase (hTERT) is a catalytic subunit of the enzyme telomerase, which is a ribonucleoprotein that plays a role in stabilizing and maintaining

chromosomal length by adding DNA bases (telomers) to the ends of eukaryotic chromosomes that physiologically shorten with every cell division [73]. Telomerase is expressed in normal dividing cells (i.e., progenitor cells), is repressed in somatic cells and is expressed by more than 85% of tumors, including myeloid leukemia [74-76]. Although it does not directly cause malignant transformation, hTERT overexpression provides for the unlimited replication of cancerous cells. hTERT, although not unique to tumors, is a good target antigen since it is primarily expressed by malignant tissue.

Human telomerase reverse transcriptase is immunogenic, as it has been shown to elicit CD8⁺ and CD4⁺ T-cell immune responses. Minev *et al.* demonstrated the presence of precursor CTLs to two HLA-A2 hTERT peptides – ILAKFLHWL and RLVDFFLLV – in normal individuals and prostate cancer patients [77]. These CTLs were expanded and demonstrated lysis of hTERT-expressing cells. Also in that study, vaccination with these hTERT peptides elicited an hTERT-specific immune response in mice [77]. Moreover, two HLA-A24 peptides, VYAETKHFL and VYGFVRACL, have also been shown to elicit hTERT-specific immune responses against HLA-A24 leukemia cell lines [78]. Since HLA-A24, the most common allele found in the Japanese population, is also frequently found in individuals of European decent, these peptides may prove useful for patients with myeloid leukemia. Furthermore, Schroers *et al.* identified HLA class II epitopes derived from hTERT that were able to induce CD4⁺ T-cell responses in the context of several HLA-DR alleles [79]. Mouse vaccinations with one of these HLA class II peptides elicited peptide-specific immune responses that were demonstrated *in vitro* against the acute promyelocytic leukemia cell line HL-60.

Although vaccination approaches targeting hTERT have been primarily been used in solid malignancies [80-82], hTERT peptide-specific CTLs have been detected in CML patients irrespective of prior treatment, which included IFN- α , SCT and imatinib therapies [83]. Furthermore, these hTERT peptide-specific CTLs were successfully reactivated and demonstrated IFN- γ production following stimulation with HLA-matched leukemia cells. Despite these promising data, hTERT peptide vaccines have yet to be clinically tested in patients with myeloid leukemia [84].

G250/carbolic anhydrase IX

The tumor-specific antigen G250/CA IX, or carbolic anhydrase IX (CA IX), is a membrane-associated CA and a marker of hypoxia that is regulated by hypoxia-inducible factor- α [85,86]. G250/CA IX is expressed in many cancers, including AML, and is absent in normal tissues and hematopoietic stem cells, thus making it an ideal target for immunotherapy [84,87-89]. In addition, high expression of G250/CA IX mRNA has been correlated with a longer overall survival in patients with AML, implicating a role for the immune system in eliminating G250/CA IX-expressing leukemia cells [89]. Immunogenicity of the G250/CA IX HLA-A2-restricted peptide HLSTAFARV was first demonstrated by Vissers *et al.*, who demonstrated that CTLs targeting this peptide were elicited *in vivo* from mice and *in vitro* from human peripheral blood mononuclear cells [84]. In addition, these CTLs lysed G250/CA IX-pulsed cells or cells that endogenously expressed G250/CA IX.

Despite the promising potential of G250/CA IX in myeloid leukemia, no clinical studies have yet investigated its role in this disease. Most *in vivo* studies with G250-targeting immunotherapy have been performed in renal cell carcinoma (RCC). Using a preclinical animal model, Herbert *et al.* demonstrated that vaccinating BALB/c mice with G250 peptide-loaded dendritic cells (DCs) was sufficient to cause rejection of RCC tumors [90]. A Phase I clinical trial determined that vaccinating RCC patients with RCC RNA-transfected DCs was safe and elicited peptide-specific immune responses, including a CTL response to G250/CA IX [91]. Combining these *in vivo* data with those of prior studies showing G250

activity in myeloid leukemia has set the stage for future investigations using G250-targeting vaccines in patients with myeloid leukemia.

PRAME

Cancer testis antigens are non-mutated genes expressed at high levels in germinal tissues and tumors; in nongermlinal normal tissues, these antigens are absent or have low expression. Preferentially expressed antigen in melanoma (PRAME) is a cancer testis antigen that is expressed by solid tumors and hematologic malignancies [89,91-98]. PRAME was also demonstrated to be expressed by normal ovarian, endometrial, placental and adrenal tissues [99]. Similar to findings for RHAMM and G250/CA IX, increased levels of PRAME mRNA have been correlated with improved outcomes in patients with AML [89,95,100]. In CML, however, an elevated PRAME level was correlated with disease progression and blast crisis and was shown to inhibit myeloid differentiation [101,102].

Immunity to PRAME was first reported by Kessler *et al.*, who identified four HLA-A2-binding epitopes derived from PRAME – VLDGLDVLL (PRA100), SLYSFPEPEA (PRA142), ALYVDSLFFL (PRA300) and SLLQHLIGL (PRA425) – using proteasome cleavage pattern analysis [103]. In that study, Kessler *et al.* also demonstrated the expansion of peptide-specific CTLs from healthy donors that lysed PRAME-expressing RCC, melanoma, lung cancer and mammary cell lines. Griffioen *et al.* demonstrated PRA100-specific CTLs in four of 11 melanoma patients who primarily had a naive phenotype (CD45 RA⁺) that was similar to the phenotype of the PRAME-specific CTLs observed in four of 14 healthy donors [104].

Anti-PRAME immunity was first demonstrated in leukemia by Morita *et al.*, who demonstrated the presence of low-frequency PRA300-specific CTLs in samples from AML and MDS patients following SCT [105]. Subsequent to these findings, Quintarelli *et al.* expanded PRAME CTLs from healthy donors and CML patients and demonstrated that these CTLs were functional and killed PRAME-expressing tumor cell lines and primary CML cells [106]. Furthermore, in that study, a higher frequency of CTLs was elicited against the PRA300 and PRA100, thus leading the authors to conclude that these peptides are more dominant than other PRAME-derived epitopes, although immunity was also elicited against some of the other PRAME peptides. Rezvani *et al.* corroborated and extended these findings by showing PRAME-specific CTLs in acute lymphoblastic leukemia, AML and CML [107]. Immunity was again most frequently detected against PRA300. Furthermore, in patients with relatively high expression of PRAME, PRAME-specific CTLs were primarily low-avidity CTLs, in contrast to findings in patients with low PRAME expression, who demonstrated the presence of high-avidity PRAME-specific CTLs [107]. Although PRAME peptide vaccines are yet to be tested in leukemia patients, the availability of multiple immunogenic epitopes that have elicited immune responses make this protein a good potential immunotherapeutic option for the management of myeloid leukemia.

Other peptides in early development for myeloid leukemia immunotherapy

A list of the most clinically well-developed leukemia peptide vaccines are provided in Table 1. Although an exhaustive list of antigens that are targets for leukemia immunotherapy is beyond the scope of this article, some antigens have been extensively studied in solid tumors and lymphoma. Since leukemia and other tumor types often share common features, studies are currently underway to determine the role of some of these antigens in leukemia. Two such antigens will be briefly reviewed in this section: MUC1 and NY-ESO-1.

MUC1 is a transmembrane type I glycoprotein that is overexpressed and distinctly glycosylated in many types of malignancies, including AML [108-110]. Immunogenicity of MUC1 peptides was initially demonstrated against solid tumor cell lines [109] and subsequently in AML, including primary AML cells [108,111]. In clinical studies, many formulations of MUC1 peptide have been used to treat solid tumors and have demonstrated low toxicities and some efficacy [112-115]. At this point, there are no published MUC1 vaccine trials in AML; however, Kapp *et al.* showed MUC1-specific CTLs in the peripheral blood of patients with AML and MM following allo-SCT and correlated LAA-specific CTLs (MUC1, PR1 and WT1) with clinical remission [116], thereby providing the impetus for further studies with MUC1 in myeloid leukemia.

Similar to PRAME, NY-ESO-1 is a cancer testis antigen that is expressed in a variety of solid tumors; expression of NY-ESO-1 in normal tissues is limited to the testis [117]. NY-ESO-1 is considered to be highly immunogenic, as it elicits a humoral immune response as well as CD8⁺ and CD4⁺ T-cell responses [118,119]. Clinical trials with NY-ESO-1 peptide vaccines have been undertaken in solid tumors and have shown promising results [120-123]. However, despite the availability and proven safety of NY-ESO-1 peptide vaccines, the role of NY-ESO-1 in myeloid leukemia remains unknown, primarily owing to the conflicting evidence regarding the expression of NY-ESO-1 in leukemia. In one report, Adams *et al.* found NY-ESO-1 expression in one of 26 primary AML samples but a lack of expression in CML and AML cell lines [124]. By contrast, Niemeyer found absence of NY-ESO-1 and other cancer testis antigens in 19 AML samples [125]. Schmitt *et al.* further corroborated these findings in CML as they reported absence of NY-ESO-1 in 34 CML samples from patients examined in their study [126]. Whether NY-ESO-1 will play a role in future therapy for myeloid leukemia remains to be determined and will depend primarily on the results of further studies that explore subtypes of AML and phases of CML disease during which NY-ESO-1 expression might be significant enough to warrant using NY-ESO-1-targeting immunotherapy.

Cell-based vaccines

The use of APCs, specifically DCs, to directly elicit anti-tumor immunity has shown promise in the management of malignant diseases. Since DCs are the link between innate and adaptive immune responses, they have been the main focus of cell-based vaccines. Normal and leukemia-derived DCs have been used successfully in leukemia vaccines, but their use remains primarily in the preclinical stages of development. Although using peptide-loaded DCs to expand peptide-specific CTLs is common practice to demonstrate anti-leukemia immunity against a specific LAA *in vitro*, the following discussion will focus on the use of DCs to directly elicit anti-leukemia immunity *in vivo*.

The main function of DCs is to present antigens to B cells as well as CD4⁺ and CD8⁺ T-cells, which, in turn will generate an immune response against the antigen that is presented by the DC. Since the leukemia microenvironment is thought to be immunosuppressive, causing quantitative and functional defects in immune cells including DCs [127-129], enhancing leukemia immunity by augmenting DC function provides an attractive means of leukemia therapy. DC vaccines can be divided into two broad categories based on the ability of DCs to present exogenously pulsed/transfected antigen versus the presentation of endogenous antigens that are similarly expressed by myeloid leukemia. In the first category, DCs can be loaded with exogenous antigens, including peptides, apoptotic cells and whole cell lysates, or transfected with DNA/mRNA nucleotides that are either directly antigenic or transcribed/translated by DCs to produce protein antigens. In the second category, since DCs and leukemia cells are both derived from common hematopoietic cells and thus often share common antigens, increasing the antigen presentation machinery of leukemia cells by

differentiating them into DCs or DC-like cells can increase antigen presentation by the leukemia-derived DCs, thereby generating an immune response against leukemia-associated/specific antigens.

Peptide-specific DC vaccines

The use of peptides to directly pulse DCs *in vitro* prior to administration offers some advantages. For example, peptides can be loaded onto DCs and peptide loading can subsequently be directly confirmed. Since DCs can be dysfunctional in leukemia, this approach allows for the administration of competent DCs to present the antigen against which an immune response is expected [127-129]. One of the disadvantages of using this approach, which is also limiting to peptide vaccines, is that peptide-pulsed DCs elicit immunity against a single epitope or a predetermined group of epitopes whose expression by the leukemia cell can change over time, a process known as antigenic drift. Another disadvantage is HLA restriction that is also critical to peptide-loaded DC vaccines, thereby limiting these vaccines to a group of patients with specific HLA subtypes. Therapy with peptide-pulsed DCs has been attempted in CML by Takahashi *et al.*, who compared three different methodologies of DC vaccinations using BCR-ABL peptide-pulsed DCs [130]. In the first method, DCs were isolated from patient blood, loaded with peptide and administered intravenously to patients. In the other two methods, DCs were generated from patient blood using standard techniques and immature DCs were pulsed with peptide and administered to patients intradermally or were matured with TNF- α and then administered intradermally. Immunologic responses were detected using the three methodologies, but no clinical responses were noted. Furthermore, the immunologic responses were most effective following the administration of mature peptide-pulsed DCs. Although promising, much work still needs to be completed to further define the role of peptide-pulsed DC vaccines in leukemia therapy.

DC vaccines against undefined epitopes

Many approaches have been undertaken to avoid some of the limitations of direct peptide pulsing of DCs. One way to broaden immunity against a known antigen is to transfect DCs with known antigen mRNA and then allow the DC to naturally process and present antigenic epitopes. Although still in its nascent phases of development, this method will allow for the unmanipulated presentation of the most dominant epitopes derived from a known antigen and is not restricted by the patient's HLA type. However, the immune response elicited by the transfected DCs is unpredictable and can lead to either autoimmunity or anergy. The safety of this approach was demonstrated by Van Driessche *et al.*, who administered increasing doses of WT1-transfected DCs to patients with AML in a Phase I clinical trial [131]. In that study, WT1 mRNA was electroporated into DCs and subsequently administered to patients. No major side effects were reported. In another study, Van Tendeloo *et al.* showed the efficacy of WT1 mRNA-electroporated DCs in preventing relapse in AML patients [132]. In that Phase I/II clinical trial, five of ten AML patients had clinical responses to the vaccine, and in three patients the clinical response lasted for at least 3 years. A significant correlation was seen between clinical response to the vaccine and detection of WT1-specific CTLs.

Since transfection of DCs and subsequent stable expression of antigen can be limiting and since DCs are the most efficient cross-presenting cell type [133,134], DCs have been pulsed with apoptotic and necrotic leukemia blasts and subsequently used in vaccine studies to elicit anti-leukemia immune responses. Cross-presentation is a process whereby exogenous antigens are taken up and presented on HLA class I molecules [135]. The ability of leukemia-derived DCs and nonleukemic DCs pulsed with leukemia blasts to elicit anti-leukemia immunity has been well documented in animal models and *in vitro* studies

[136-140]. However, little has been done using these approaches to elicit anti-leukemia immunity in clinical trials. In one study, autologous monocyte derived-DCs were generated from two AML patients, matured and pulsed with leukemic lysates and the immunomodulant KLH, and then administered to the patients. An immunological response was detected in both patients following vaccination, but blast count increased within 2–5 months, indicating disease progression [141].

Moreover, because some DC subsets are derived from myeloid progenitors that also give rise to myeloid leukemia, these DC subsets often express an antigen profile similar to that of leukemia cells and can be used to elicit anti-leukemia immune responses. Generating leukemia-derived DCs was shown to be feasible, yielding fully functional DCs that have activation markers and functional activities similar to those of normal DCs [138,142,143]. Vaccination with DCs from leukemia patients or leukemia cell-derived DCs was demonstrated to be an option in the treatment of patients with AML and CML. In a Phase I/II clinical trial, Westermann *et al.* generated mature DCs from ten CML patients using a standard methodology [144]. The mean percentage of BCR-ABL⁺ DCs was 33%. After maturation with TNF- α , cells were pulsed with KLH and then administered to patients. The vaccine was well tolerated, and T-cell responses were detected, not only against BCR-ABL but also against PR1. Although clinical responses were seen following vaccination, conclusions regarding the efficacy of the vaccine and a correlation between the emergence of immune and clinical responses could not be determined.

Tumor cell-based vaccines

Since the expression of target peptides and antigens by the malignant cell is an essential criterion in the development of tumor vaccines, using actual tumor cells/lysates in vaccine preparations has been studied in many tumor types. This type of vaccination provides similar advantages to some of the DC cell-based vaccinations, in that it bypasses HLA restriction and provides a broad range of epitopes against which an immune response can be elicited. One of the approaches to this type of immunotherapy employs the GVAX[®] platform for vaccine development. Using this methodology, granulocyte-macrophage colony-stimulating factor (GM-CSF), which has been demonstrated to elicit anti-tumor responses [145,146], can be transduced into tumor cells and subsequently administered to patients as part of the vaccine. Numerous clinical trials have been conducted using vaccinations with GM-CSF-secreting tumor cells in solid malignancies and have demonstrated safety and efficacy of this approach [147,148].

Although most studies with the GVAX platform have been in the setting of solid malignancies, success with immunizations using GM-CSF-secreting tumor cells was also demonstrated in hematologic malignancies. Borrello *et al.* demonstrated in a mouse model that immunization with irradiated GM-CSF-transduced B-cell lymphoma tumor cells in the early post-transplant period decreased the incidence of tumor relapse [149]. In a Phase II trial in patients with AML, Borrello *et al.* showed that the administration of a mixed GVAX platform (autologous leukemia cells and GM-CSF-secreting K562 cells) along with autologous SCT and adoptive immunotherapy of vaccine-primed lymphocytes led to anti-tumor T-cell and antibody responses, as well as a reduction in minimal residual disease in some patients [150]. Although the published data on the GVAX platform in myeloid leukemia are scarce, there are currently ongoing trials to further study the therapeutic role of this approach in myeloid leukemia [147,148,151-153].

Expert commentary

Vaccine therapies have clearly shown promising results in the treatment of AML and CML. Although their efficacy remains suboptimal, the low toxicity associated with tumor vaccines

highlights their potential in the management of myeloid leukemia patients, especially for those patients who are unable to tolerate further chemotherapy or allo-SCT. The timing of vaccine administration in the course of the leukemia and in relation to SCT is yet to be determined. The consolidation or postconsolidation settings may be the ideal time periods to administer leukemia vaccines to eradicate minimal residual disease that chemotherapy has failed to eliminate. Owing to the evolving nature of the underlying myeloid leukemia and antileukemia immune responses in individual patients, the optimal immunotherapy may ultimately require the targeting of multiple different antigens/epitopes and the tailoring of vaccines to address the distinct patterns of antigen expression by individual patients. Irrespectively of how studies are approached, the shortcomings of standard chemotherapy and allo-SCT, which are the cornerstones in the management of AML and advanced-stage CML, highlight the urgent need for further investigations of leukemia vaccines in order to discover novel curative treatments for myeloid leukemia.

Five-year view

In 5 years time, consolidation therapy for myeloid leukemia will be more immune based. Novel approaches to antigen discovery will allow for the development of vaccines targeting peptides that are highly specific to myeloid leukemia cells, thereby sparing normal progenitor and hematopoietic cells. Such discoveries will also revolutionize allo-SCT, as novel antigen discoveries and immune modulators that stimulate anti-leukemia immunity will allow for the specific targeting of leukemia cells, thereby harnessing the benefits of GvL while minimizing GvHD. Furthermore, as we further understand the processes involved in immune evasion and tolerance, we will be able to more accurately determine the optimal timing for the application of vaccine therapies, to elicit the most potent, yet highly leukemia-specific, immune responses. With a plethora of laboratory and animal data supporting leukemia vaccines, studies will be moving rapidly into the clinical setting. Future research will focus on clinical trials applying leukemia vaccines at the earlier stages of disease, specifically following first complete remission for AML and in chronic phase or early stages of CML, to consolidate remissions following standard therapy.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Estey E, Dohner H. Acute myeloid leukaemia. *Lancet*. 2006; 368(9550):1894–1907. [PubMed: 17126723]
2. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2010; 362(24):2260–2270. [PubMed: 20525995]
3. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2010; 362(24):2251–2259. [PubMed: 20525993]
4. Marin D. Current status of imatinib as frontline therapy for chronic myeloid leukemia. *Semin Hematol*. 2010; 47(4):312–318. [PubMed: 20875547]
5. Falkenburg JH, van de Corput L, Marijt EW, Willemze R. Minor histocompatibility antigens in human stem cell transplantation. *Exp Hematol*. 2003; 31(9):743–751. [PubMed: 12962719]
6. Greiner J, Dohner H, Schmitt M. Cancer vaccines for patients with acute myeloid leukemia – definition of leukemia-associated antigens and current clinical protocols targeting these antigens. *Haematologica*. 2006; 91(12):1653–1661. [PubMed: 17145602]
7. Chen W, Norbury CC, Cho Y, Yewdell JW, Bennink JR. Immunoproteasomes shape immunodominance hierarchies of antiviral CD8⁺ T cells at the levels of T cell repertoire and presentation of viral antigens. *J Exp Med*. 2001; 193(11):1319–1326. [PubMed: 11390439]

8. Yewdell JW. Confronting complexity: real-world immunodominance in antiviral CD8⁺ T cell responses. *Immunity*. 2006; 25(4):533–543. [PubMed: 17046682]
9. Ellis JM, Henson V, Slack R, Ng J, Hartzman RJ, Katovich Hurley C. Frequencies of HLA-A2 alleles in five U.S population groups. Predominance of A*02011 and identification of HLA-A*0231. *Hum Immunol*. 2000; 61(3):334–340. [PubMed: 10689125]
10. 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(1):236–242. [PubMed: 17875804] . •• Shows clinical applicability and immunologic response to the combined PR1 and Wilm's tumor (WT1) peptide vaccines.
11. Oji Y, Oka Y, Nishida S, et al. WT1 peptide vaccine induces reduction in minimal residual disease in an imatinib-treated CML patient. *Eur J Haematol*. 2010; 85(4):358–360. [PubMed: 20633041]
12. Moorman AV, Chilton L, Wilkinson J, Ensor HM, Bown N, Proctor SJ. A population-based cytogenetic study of adults with acute lymphoblastic leukemia. *Blood*. 2010; 115(2):206–214. [PubMed: 19897583]
13. Soupir CP, Vergilio JA, Dal Cin P, et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. *Am J Clin Pathol*. 2007; 127(4):642–650. [PubMed: 17369142]
14. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the *P210bcr/abl* gene of the Philadelphia chromosome. *Science*. 1990; 247(4944):824–830. [PubMed: 2406902]
15. Shepherd P, Suffolk R, Halsey J, Allan N. Analysis of molecular breakpoint and m-RNA transcripts in a prospective randomized trial of interferon in chronic myeloid leukaemia: no correlation with clinical features, cytogenetic response, duration of chronic phase, or survival. *Br J Haematol*. 1995; 89(3):546–554. [PubMed: 7734353]
16. Bocchia M, Korontsvit T, Xu Q, et al. Specific human cellular immunity to *bcr-abl* oncogene-derived peptides. *Blood*. 1996; 87(9):3587–3592. [PubMed: 8611681] . • Reports clinical results of BCR-ABL-derived peptides that elicit immunity.
17. Buzyn A, Ostankovitch M, Zerbib A, et al. Peptides derived from the whole sequence of BCR-ABL bind to several class I molecules allowing specific induction of human cytotoxic T lymphocytes. *Eur J Immunol*. 1997; 27(8):2066–2072. [PubMed: 9295046]
18. Greco G, Fruci D, Accapezzato D, et al. Two bcr-abl junction peptides bind HLA-A3 molecules and allow specific induction of human cytotoxic T lymphocytes. *Leukemia*. 1996; 10(4):693–699. [PubMed: 8618449]
19. Bocchia M, Wentworth PA, Southwood S, et al. Specific binding of leukemia oncogene fusion protein peptides to HLA class I molecules. *Blood*. 1995; 85(10):2680–2684. [PubMed: 7742526]
20. Pinilla-Ibarz J, Cathcart K, Korontsvit T, et al. Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses. *Blood*. 2000; 95(5):1781–1787. [PubMed: 10688838]
21. Mannering SI, McKenzie JL, Fearnley DB, Hart DN. HLA-DR1-restricted bcr-abl (b3a2)-specific CD4⁺ T lymphocytes respond to dendritic cells pulsed with b3a2 peptide and antigen-presenting cells exposed to b3a2 containing cell lysates. *Blood*. 1997; 90(1):290–297. [PubMed: 9207464]
22. Pawelec G, Max H, Halder T, et al. BCR/ABL leukemia oncogene fusion peptides selectively bind to certain HLA-DR alleles and can be recognized by T cells found at low frequency in the repertoire of normal donors. *Blood*. 1996; 88(6):2118–2124. [PubMed: 8822931]
23. 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(3):1037–1042. [PubMed: 14504104]
24. Bocchia M, Gentili S, Abruzzese E, et al. Effect of a p210 multi-peptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet*. 2005; 365(9460):657–662. [PubMed: 15721470]
25. Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. *Leukemia*. 2007; 21(11):2287–2295. [PubMed: 17637811]

26. Alexander J, Sidney J, Southwood S, et al. Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides. *Immunity*. 1994; 1(9):751–761. [PubMed: 7895164]
27. Rosa DS, Tzelepis F, Cunha MG, Soares IS, Rodrigues MM. The pan HLA DR-binding epitope improves adjuvant-assisted immunization with a recombinant protein containing a malaria vaccine candidate. *Immunol Lett*. 2004; 92(3):259–268. [PubMed: 15081621]
28. Rensing ME, van Driel WJ, Brandt RM, et al. Detection of T helper responses, but not of human papillomavirus-specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma. *J Immunother*. 2000; 23(2):255–266. [PubMed: 10746552]
29. Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Roberts W, Scheinberg DA. Synthetic peptide analogs derived from bcr/abl fusion proteins and the induction of heteroclitic human T-cell responses. *Haematologica*. 2005; 90(10):1324–1332. [PubMed: 16219568]
30. 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(17):3924–3934. [PubMed: 19536894] . •• Reports clinical results of a BCR-ABL peptide vaccine for patients with chronic myeloid leukemia.
31. 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*. 2010 Epub ahead of print. 10.1016/j.leukres.2010.05.028
32. Rauscher FJ 3rd, Morris JF, Tournay OE, Cook DM, Curran T. Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. *Science*. 1990; 250(4985):1259–1262. [PubMed: 2244209]
33. Englert C, Hou X, Maheswaran S, et al. WT1 suppresses synthesis of the epidermal growth factor receptor and induces apoptosis. *EMBO J*. 1995; 14(19):4662–4675. [PubMed: 7588596]
34. Harrington MA, Konicek B, Song A, Xia XL, Fredericks WJ, Rauscher FJ 3rd. Inhibition of colony-stimulating factor-1 promoter activity by the product of the Wilms' tumor locus. *J Biol Chem*. 1993; 268(28):21271–21275. [PubMed: 8407965]
35. Hossain A, Nixon M, Kuo MT, Saunders GF. N-terminally truncated WT1 protein with oncogenic properties overexpressed in leukemia. *J Biol Chem*. 2006; 281(38):28122–28130. [PubMed: 16698800]
36. Inoue K, Ogawa H, Sonoda Y, et al. Aberrant overexpression of the Wilms tumor gene (*WT1*) in human leukemia. *Blood*. 1997; 89(4):1405–1412. [PubMed: 9028964]
37. Miwa H, Beran M, Saunders GF. Expression of the Wilms' tumor gene (*WT1*) in human leukemias. *Leukemia*. 1992; 6(5):405–409. [PubMed: 1317488]
38. 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(3):509–520. [PubMed: 2154335]
39. Oji Y, Miyoshi S, Maeda H, et al. Overexpression of the Wilms' tumor gene *WT1* in *de novo* lung cancers. *Int J Cancer*. 2002; 100(3):297–303. [PubMed: 12115544]
40. Dohi S, Ohno S, Ohno Y, Soma G, Kyo S, Inoue M. Correlation between WT1 expression and cell proliferation in endometrial cancer. *Anticancer Res*. 2009; 29(11):4887–4891. [PubMed: 20032452]
41. Oji Y, Yamamoto H, Nomura M, et al. Overexpression of the Wilms' tumor gene *WT1* in colorectal adenocarcinoma. *Cancer Sci*. 2003; 94(8):712–717. [PubMed: 12901797]
42. Oka Y, Udaka K, Tsuboi A, et al. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. *J Immunol*. 2000; 164(4):1873–1880. [PubMed: 10657636]
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(9):1429–1436. [PubMed: 12792492]
44. Asemussen AM, Keilholz U, Tenzer S, et al. Identification of a highly immunogenic HLA-A*01-binding T cell epitope of WT1. *Clin Cancer Res*. 2006; 12(24):7476–7482. [PubMed: 17189421]
45. Li Z, Oka Y, Tsuboi A, et al. Identification of a WT1 protein-derived peptide, WT1, as a HLA-A 0206-restricted, WT1-specific CTL epitope. *Microbiol Immunol*. 2008; 52(11):551–558. [PubMed: 19090835]

46. Ohminami H, Yasukawa M, Fujita S. HLA class I-restricted lysis of leukemia cells by a CD8⁺ cytotoxic T-lymphocyte clone specific for WT1 peptide. *Blood*. 2000; 95(1):286–293. [PubMed: 10607714]
47. Gao L, Bellantuono I, Elsasser A, et al. Selective elimination of leukemic CD34⁺ progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood*. 2000; 95(7):2198–2203. [PubMed: 10733485]
48. 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(2):99–107. [PubMed: 10663572]
49. Fujiki F, Oka Y, Kawakatsu M, et al. A WT1 protein-derived, naturally processed 16-mer peptide, WT1(332), is a promiscuous helper peptide for induction of WT1-specific Th1-type CD4⁺ T cells. *Microbiol Immunol*. 2008; 52(12):591–600. [PubMed: 19120973]
50. Knights AJ, Zaniou A, Rees RC, Pawelec G, Muller L. Prediction of an HLA-DR-binding peptide derived from Wilms' tumour 1 protein and demonstration of *in vitro* immunogenicity of WT1(124–138)-pulsed dendritic cells generated according to an optimised protocol. *Cancer Immunol Immunother*. 2002; 51(5):271–281. [PubMed: 12070714]
51. Kobayashi H, Nagato T, Aoki N, et al. Defining MHC class II T helper epitopes for WT1 tumor antigen. *Cancer Immunol Immunother*. 2006; 55(7):850–860. [PubMed: 16220325]
52. Guo Y, Niiya H, Azuma T, et al. Direct recognition and lysis of leukemia cells by WT1-specific CD4⁺ T lymphocytes in an HLA class II-restricted manner. *Blood*. 2005; 106(4):1415–1418. [PubMed: 15845894]
53. Tsuboi A, Oka Y, Uda K, et al. Enhanced induction of human WT1-specific cytotoxic T lymphocytes with a 9-mer WT1 peptide modified at HLA-A*2402-binding residues. *Cancer Immunol Immunother*. 2002; 51(11–12):614–620. [PubMed: 12439606]
54. Oka Y, Tsuboi A, Taguchi T, et al. Induction of *WT1* (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci USA*. 2004; 101(38):13885–13890. [PubMed: 15365188]
55. Tsuboi A, Oka Y, Osaki T, et al. WT1 peptide-based immunotherapy for patients with lung cancer: report of two cases. *Microbiol Immunol*. 2004; 48(3):175–184. [PubMed: 15031530]
56. Oka Y, Tsuboi A, Murakami M, et al. Wilms tumor gene peptide-based immunotherapy for patients with overt leukemia from myelodysplastic syndrome (MDS) or MDS with myelofibrosis. *Int J Hematol*. 2003; 78(1):56–61. [PubMed: 12894852]
57. Mailander V, Scheibenbogen C, Thiel E, Letsch A, Blau IW, Keilholz U. Complete remission in a patient with recurrent acute myeloid leukemia induced by vaccination with WT1 peptide in the absence of hematological or renal toxicity. *Leukemia*. 2004; 18(1):165–166. [PubMed: 14603333]
58. 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(26):6541–6548. [PubMed: 19389880] . •• Reports clinical results of WT1 peptide vaccine in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome.
59. 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(7):2450–2457. [PubMed: 8839835]
60. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood*. 1997; 89(10):3503–3521. [PubMed: 9160655]
61. Molldrem JJ, Clave E, Jiang YZ, et al. Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. *Blood*. 1997; 90(7):2529–2534. [PubMed: 9326217]
62. 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(9):1018–1023. [PubMed: 10973322]
63. Qazilbash M, Wieder E, Rios R, et al. Vaccination with the PR1 leukemia-associated antigen can induce complete remission in patients with myeloid leukaemia. *Blood*. 2004; 104:77a. Abstract 259.

64. Qazilbash MH, Thall PF, Wang X, et al. PR1 peptide vaccination for patients with myeloid leukemias. *J Clin Oncol (ASCO Annual Meeting Proceedings)*. 2007; 25:18S.
65. 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(9):1029–1035. [PubMed: 12225794]
66. Maxwell CA, McCarthy J, Turley E. Cell-surface and mitotic-spindle RHAMM: moonlighting or dual oncogenic functions? *J Cell Sci*. 2008; 121(Pt 7):925–932. [PubMed: 18354082]
67. Hamilton SR, Fard SF, Paiwand FF, et al. The hyaluronan receptors CD44 and Rhamm (CD168) form complexes with ERK1,2 that sustain high basal motility in breast cancer cells. *J Biol Chem*. 2007; 282(22):16667–16680. [PubMed: 17392272]
68. Tolg C, Hamilton SR, Nakrieko KA, et al. Rhamm^{-/-} fibroblasts are defective in CD44-mediated ERK1,2 mitogenic signaling, leading to defective skin wound repair. *J Cell Biol*. 2006; 175(6): 1017–1028. [PubMed: 17158951]
69. Samuel SK, Hurta RA, Spearman MA, Wright JA, Turley EA, Greenberg AH. TGF- β 1 stimulation of cell locomotion utilizes the hyaluronan receptor RHAMM and hyaluronan. *J Cell Biol*. 1993; 123(3):749–758. [PubMed: 7693717]
70. Greiner J, Li L, Ringhoffer M, et al. Identification and characterization of epitopes of the receptor for hyaluronic acid-mediated motility (RHAMM/CD168) recognized by CD8⁺ T cells of HLA-A2-positive patients with acute myeloid leukemia. *Blood*. 2005; 106(3):938–945. [PubMed: 15827130]
71. Schmitt M, Schmitt A, Rojewski MT, et al. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. *Blood*. 2008; 111(3):1357–1365. [PubMed: 17978170]
72. Greiner J, Schmitt A, Giannopoulos K, et al. High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma. *Haematologica*. 2010; 95(7):1191–1197. [PubMed: 20081055] . • Reports the clinical and immunologic results of receptor for hyaluronic acid-mediated motility (RHAMM)-R3 peptide vaccine in patients with AML, myelodysplastic syndrome and multiple myeloma.
73. Blackburn EH. Structure and function of telomeres. *Nature*. 1991; 350(6319):569–573. [PubMed: 1708110]
74. Hiyama E, Hiyama K. Telomerase as tumor marker. *Cancer Lett*. 2003; 194(2):221–233. [PubMed: 12757980]
75. Hartmann U, Brummendorf TH, Balabanov S, Thiede C, Illme T, Schaich M. Telomere length and hTERT expression in patients with acute myeloid leukemia correlates with chromosomal abnormalities. *Haematologica*. 2005; 90(3):307–316. [PubMed: 15749662]
76. Warner JK, Wang JC, Takenaka K, et al. Direct evidence for cooperating genetic events in the leukemic transformation of normal human hematopoietic cells. *Leukemia*. 2005; 19(10):1794–1805. [PubMed: 16094415]
77. Minev B, Hipp J, Firat H, Schmidt JD, Langlade-Demoyen P, Zanetti M. Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. *Proc Natl Acad Sci USA*. 2000; 97(9):4796–4801. [PubMed: 10759561]
78. Arai J, Yasukawa M, Ohminami H, Kakimoto M, Hasegawa A, Fujita S. Identification of human telomerase reverse transcriptase-derived peptides that induce HLA-A24-restricted antileukemia cytotoxic T lymphocytes. *Blood*. 2001; 97(9):2903–2907. [PubMed: 11313288]
79. Schroers R, Shen L, Rollins L, et al. Human telomerase reverse transcriptase-specific T-helper responses induced by promiscuous major histocompatibility complex class II-restricted epitopes. *Clin Cancer Res*. 2003; 9(13):4743–4755. [PubMed: 14581345]
80. Bernhardt SL, Gjertsen MK, Trachsel S, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating Phase I/II study. *Br J Cancer*. 2006; 95(11): 1474–1482. [PubMed: 17060934]
81. Brunsvig PF, Aamdal S, Gjertsen MK, et al. Telomerase peptide vaccination: a Phase I/II study in patients with non-small cell lung cancer. *Cancer Immunol Immunother*. 2006; 55(12):1553–1564. [PubMed: 16491401]

82. Mavroudis D, Bolonakis I, Cornet S, et al. A Phase I study of the optimized cryptic peptide TERT(572y) in patients with advanced malignancies. *Oncology*. 2006; 70(4):306–314. [PubMed: 17047402]
83. Gannage M, Abel M, Michallet AS, et al. *Ex vivo* characterization of multiepitopic tumor-specific CD8 T cells in patients with chronic myeloid leukemia: implications for vaccine development and adoptive cellular immunotherapy. *J Immunol*. 2005; 174(12):8210–8218. [PubMed: 15944330]
84. Vissers JL, De Vries IJ, Schreurs MW, et al. The renal cell carcinoma-associated antigen G250 encodes a human leukocyte antigen (HLA)-A2.1-restricted epitope recognized by cytotoxic T lymphocytes. *Cancer Res*. 1999; 59(21):5554–5559. [PubMed: 10554034]
85. Lancaster JA, Harris AL, Davidson SE, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. *Cancer Res*. 2001; 61(17):6394–6399. [PubMed: 11522632]
86. Grabmaier K, A de Weijert MC, Verhaegh GW, Schalken JA, Oosterwijk E. Strict regulation of CAIX(G250/MN) by HIF-1 α in clear cell renal cell carcinoma. *Oncogene*. 2004; 23(33):5624–5631. [PubMed: 15184875]
87. Le QT, Chen E, Salim A, et al. An evaluation of tumor oxygenation and gene expression in patients with early stage non-small cell lung cancers. *Clin Cancer Res*. 2006; 12(5):1507–1514. [PubMed: 16533775]
88. Troost EG, Bussink J, Kaanders JH, et al. Comparison of different methods of CAIX quantification in relation to hypoxia in three human head and neck tumor lines. *Radiother Oncol*. 2005; 76(2):194–199. [PubMed: 16024110]
89. Greiner J, Schmitt M, Li L, et al. Expression of tumor-associated antigens in acute myeloid leukemia: implications for specific immunotherapeutic approaches. *Blood*. 2006; 108(13):4109–4117. [PubMed: 16931630]
90. Herbert N, Haferkamp A, Schmitz-Winnenthal HF, Zoller M. Concomitant tumor and autoantigen vaccination supports renal cell carcinoma rejection. *J Immunol*. 2010; 185(2):902–916. [PubMed: 20548033]
91. Su Z, Dannull J, Heiser A, et al. Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res*. 2003; 63(9):2127–2133. [PubMed: 12727829]
92. Doolan P, Clynes M, Kennedy S, Mehta JP, Crown J, O'Driscoll L. Prevalence and prognostic and predictive relevance of PRAME in breast cancer. *Breast Cancer Res Treat*. 2008; 109(2):359–365. [PubMed: 17624586]
93. Figueiredo DL, Mamede RC, Proto-Siqueira R, Neder L, Silva WA Jr, Zago MA. Expression of cancer testis antigens in head and neck squamous cell carcinomas. *Head Neck*. 2006; 28(7):614–619. [PubMed: 16475205]
94. Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high-stage neuroblastoma and associated with poor outcome. *Clin Cancer Res*. 2004; 10(13):4307–4313. [PubMed: 15240516]
95. Steinbach D, Hermann J, Viehmann S, Zintl F, Gruhn B. Clinical implications of *PRAME* gene expression in childhood acute myeloid leukemia. *Cancer Genet Cytogenet*. 2002; 133(2):118–123. [PubMed: 11943337]
96. Steinbach D, Viehmann S, Zintl F, Gruhn B. *PRAME* gene expression in childhood acute lymphoblastic leukemia. *Cancer Genet Cytogenet*. 2002; 138(1):89–91. [PubMed: 12419593]
97. 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*. 2010; 30(2):223–233. [PubMed: 20838376]
98. 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(5):1376–1379. [PubMed: 9753074]
99. 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(2):199–208. [PubMed: 9047241]

100. Santamaria CM, Chillon MC, Garcia-Sanz R, et al. Molecular stratification model for prognosis in cytogenetically normal acute myeloid leukemia. *Blood*. 2009; 114(1):148–152. [PubMed: 19398719]
101. 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(15):3299–3308. [PubMed: 19625708]
102. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. *Proc Natl Acad Sci USA*. 2006; 103(8):2794–2799. [PubMed: 16477019]
103. Kessler JH, Beekman NJ, Bres-Vloemans SA, et al. Efficient identification of novel HLA-A(*0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J Exp Med*. 2001; 193(1):73–88. [PubMed: 11136822]
104. Griffioen M, Kessler JH, Borghi M, et al. Detection and functional analysis of CD8⁺ T cells specific for PRAME: a target for T-cell therapy. *Clin Cancer Res*. 2006; 12(10):3130–3136. [PubMed: 16707612]
105. 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(6):1360–1367. [PubMed: 16596644]
106. Quintarelli C, Dotti G, De Angelis B, et al. Cytotoxic T lymphocytes directed to the preferentially expressed antigen of melanoma (PRAME) target chronic myeloid leukemia. *Blood*. 2008; 112(5):1876–1885. [PubMed: 18591381]
107. 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(10):2245–2255. [PubMed: 18988867]
108. Brossart P, Schneider A, Dill P, et al. The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T-lymphocytes. *Cancer Res*. 2001; 61(18):6846–6850. [PubMed: 11559560]
109. Brossart P, Heinrich KS, Stuhler G, et al. Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. *Blood*. 1999; 93(12):4309–4317. [PubMed: 10361129]
110. Girling A, Bartkova J, Burchell J, Gendler S, Gillett C, Taylor-Papadimitriou J. A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary carcinomas. *Int J Cancer*. 1989; 43(6):1072–1076. [PubMed: 2471698]
111. Brugger W, Schneider A, Schammann T, et al. Dendritic cell-based vaccines in patients with hematological malignancies. *Ann NY Acad Sci*. 2001; 938:359–363. [PubMed: 11458523]
112. Ramanathan RK, Lee KM, McKolanis J, et al. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol Immunother*. 2005; 54(3):254–264. [PubMed: 15372205]
113. Palmer M, Parker J, Modi S, et al. Phase I study of the BLP25 (MUC1 peptide) liposomal vaccine for active specific immunotherapy in stage IIIB/IV non-small-cell lung cancer. *Clin Lung Cancer*. 2001; 3(1):49–57. discussion 58. [PubMed: 14656392]
114. North SA, Graham K, Bodnar D, Venner P. A pilot study of the liposomal MUC1 vaccine BLP25 in prostate specific antigen failures after radical prostatectomy. *J Urol*. 2006; 176(1):91–95. [PubMed: 16753376]
115. Butts C, Murray N, Maksymiuk A, et al. Randomized Phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J Clin Oncol*. 2005; 23(27):6674–6681. [PubMed: 16170175]
116. Kapp M, Stevanovic S, Fick K, et al. CD8⁺ T-cell responses to tumor-associated antigens correlate with superior relapse-free survival after allo-SCT. *Bone Marrow Transplant*. 2009; 43(5):399–410. [PubMed: 19139738]

117. Chen YT, Scanlan MJ, Sahin U, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci USA*. 1997; 94(5):1914–1918. [PubMed: 9050879]
118. Stockert E, Jager E, Chen YT, et al. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med*. 1998; 187(8):1349–1354. [PubMed: 9547346]
119. Jager E, Chen YT, Drijfhout JW, et al. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J Exp Med*. 1998; 187(2):265–270. [PubMed: 9432985]
120. Odunsi K, Qian F, Matsuzaki J, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. *Proc Natl Acad Sci USA*. 2007; 104(31):12837–12842. [PubMed: 17652518]
121. Khong HT, Yang JC, Topalian SL, et al. Immunization of *HLA-A*0201* and/or *HLA-DPβ1*04* patients with metastatic melanoma using epitopes from the NY-ESO-1 antigen. *J Immunother*. 2004; 27(6):472–477. [PubMed: 15534491]
122. Fourcade J, Kudela P, Andrade Filho PA, et al. Immunization with analog peptide in combination with CpG and montanide expands tumor antigen-specific CD8⁺ T cells in melanoma patients. *J Immunother*. 2008; 31(8):781–791. [PubMed: 18779741]
123. Bioley G, Guillaume P, Luescher I, et al. Vaccination with a recombinant protein encoding the tumor-specific antigen NY-ESO-1 elicits an A2/157–165-specific CTL repertoire structurally distinct and of reduced tumor reactivity than that elicited by spontaneous immune responses to NY-ESO-1-expressing tumors. *J Immunother*. 2009; 32(2):161–168. [PubMed: 19238015]
124. Adams SP, Sahota SS, Mijovic A, et al. Frequent expression of HAGE in presentation chronic myeloid leukaemias. *Leukemia*. 2002; 16(11):2238–2242. [PubMed: 12399967]
125. Niemeyer P, Tureci O, Eberle T, Graf N, Pfreundschuh M, Sahin U. Expression of serologically identified tumor antigens in acute leukemias. *Leuk Res*. 2003; 27(7):655–660. [PubMed: 12681366]
126. Schmitt M, Li L, Giannopoulos K, et al. Chronic myeloid leukemia cells express tumor-associated antigens eliciting specific CD8⁺ T-cell responses and are lacking costimulatory molecules. *Exp Hematol*. 2006; 34(12):1709–1719. [PubMed: 17157168]
127. Mohty M, Jarrossay D, Lafage-Pochitaloff M, et al. Circulating blood dendritic cells from myeloid leukemia patients display quantitative and cytogenetic abnormalities as well as functional impairment. *Blood*. 2001; 98(13):3750–3756. [PubMed: 11739182]
128. Vuckovic S, Fearnley DB, Gunningham S, Spearing RL, Patton WN, Hart DN. Dendritic cells in chronic myelomonocytic leukaemia. *Br J Haematol*. 1999; 105(4):974–985. [PubMed: 10554809]
129. Eisendle K, Wolf D, Gastl G, Kircher-Eibl B. Dendritic cells from patients with chronic myeloid leukemia: functional and phenotypic features. *Leuk Lymphoma*. 2005; 46(5):663–670. [PubMed: 16019503]
130. Takahashi T, Tanaka Y, Nieda M, et al. Dendritic cell vaccination for patients with chronic myelogenous leukemia. *Leuk Res*. 2003; 27(9):795–802. [PubMed: 12804637]
131. Van Driessche A, Van de Velde AL, Nijs G, et al. Clinical-grade manufacturing of autologous mature mRNA-electroporated dendritic cells and safety testing in acute myeloid leukemia patients in a Phase I dose-escalation clinical trial. *Cytotherapy*. 2009; 11(5):653–668. [PubMed: 19530029]
132. 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 USA*. 2010; 107(31):13824–13829. [PubMed: 20631300]. •• Results of a study reporting promising clinical and immunologic responses to WT1 antigen-targeted dendritic cell vaccine in AML patients.
133. Matheoud D, Perie L, Hoeffel G, et al. Cross-presentation by dendritic cells from live cells induces protective immune responses *in vivo*. *Blood*. 2010; 115(22):4412–4420. [PubMed: 20308597]

134. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. *Cell*. 2001; 106(3):255–258. [PubMed: 11509172]
135. Ackerman AL, Cresswell P. Cellular mechanisms governing cross-presentation of exogenous antigens. *Nat Immunol*. 2004; 5(7):678–684. [PubMed: 15224093]
136. Zeng Y, Graner MW, Thompson S, Marron M, Katsanis E. Induction of BCR-ABL-specific immunity following vaccination with chaperone-rich cell lysates derived from BCR-ABL⁺ tumor cells. *Blood*. 2005; 105(5):2016–2022. [PubMed: 15374884]
137. Weigel BJ, Panoskaltsis-Mortari A, Diers M, et al. Dendritic cells pulsed or fused with AML cellular antigen provide comparable *in vivo* antitumor protective responses. *Exp Hematol*. 2006; 34(10):1403–1412. [PubMed: 16982333]
138. Spisek R, Chevallier P, Morineau N, et al. Induction of leukemia-specific cytotoxic response by cross-presentation of late-apoptotic leukemic blasts by autologous dendritic cells of nonleukemic origin. *Cancer Res*. 2002; 62(10):2861–2868. [PubMed: 12019165]
139. Fujii S, Fujimoto K, Osato M, Matsui K, Takatsuki K, Kawakita M. Induction of antitumor cytotoxic activity using CD34⁺ cord blood cell-derived and irradiated tumor cell-primed dendritic cells. *Int J Hematol*. 1998; 68(2):169–182. [PubMed: 9803675]
140. Jarnjak-Jankovic S, Pettersen RD, Saeboe-Larssen S, Wesenberg F, Gaudernack G. Evaluation of dendritic cells loaded with apoptotic cancer cells or expressing tumour mRNA as potential cancer vaccines against leukemia. *BMC Cancer*. 2005; 5:20. [PubMed: 15720715]
141. Lee JJ, Kook H, Park MS, et al. Immunotherapy using autologous monocyte-derived dendritic cells pulsed with leukemic cell lysates for acute myeloid leukemia relapse after autologous peripheral blood stem cell transplantation. *J Clin Apher*. 2004; 19(2):66–70. [PubMed: 15274198]
142. 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(2):185–199. [PubMed: 20139775]
143. Li L, Schmitt A, Reinhardt P, et al. Reconstitution of CD40 and CD80 in dendritic cells generated from blasts of patients with acute myeloid leukemia. *Cancer Immun*. 2003; 3:8. [PubMed: 12862419]
144. Westermann J, Kopp J, van Lessen A, et al. Vaccination with autologous non-irradiated dendritic cells in patients with bcr/abl⁺ chronic myeloid leukaemia. *Br J Haematol*. 2007; 137(4):297–306. [PubMed: 17408402]
145. 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 USA*. 1993; 90(8):3539–3543. [PubMed: 8097319]
146. Schwaab T, Tretter CP, Gibson JJ, et al. Tumor-related immunity in prostate cancer patients treated with human recombinant granulocyte monocyte-colony stimulating factor (GM-CSF). *Prostate*. 2006; 66(6):667–674. [PubMed: 16425182]
147. Soiffer R, Hodi FS, Haluska F, et al. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J Clin Oncol*. 2003; 21(17):3343–3350. [PubMed: 12947071]
148. Nemunaitis J. Granulocyte-macrophage colony-stimulating factor gene-transfected autologous tumor cell vaccine: focus[correction to fcous] on non-small-cell lung cancer. *Clin Lung Cancer*. 2003; 5(3):148–157. [PubMed: 14667270]
149. Borrello I, Sotomayor EM, Rattis FM, Cooke SK, Gu L, Levitsky HI. Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood*. 2000; 95(10):3011–3019. [PubMed: 10807763]
150. 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(9):1736–1745. [PubMed: 19556425]

151. Simons JW, Sacks N. Granulocyte-macrophage colony-stimulating factor-transduced allogeneic cancer cellular immunotherapy: the GVAX vaccine for prostate cancer. *Urol Oncol.* 2006; 24(5): 419–424. [PubMed: 16962494]
152. Small EJ, Sacks N, Nemunaitis J, et al. Granulocyte macrophage colony-stimulating factor – secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. *Clin Cancer Res.* 2007; 13(13):3883–3891. [PubMed: 17606721]
153. Le DT, Pardoll DM, Jaffee EM. Cellular vaccine approaches. *Cancer J.* 2010; 16(4):304–310. [PubMed: 20693840]

Website

201. SEER: Surveillance, Epidemiology and End Results. <http://seer.cancer.gov>

Figure 1. T-cell activation and killing of the target cell

In order for CD8⁺ T cells to be activated into antigen-specific cytotoxic T lymphocytes (CTLs), they must recognize the target antigen on APC and also receive signals from costimulatory molecules (e.g., CD80 and CD86). This causes CTL activation, which upon an encounter with the appropriate antigen on the target cell leads to target cell death. By contrast, antigen encounter without costimulation leads to T-cell anergy.

APC: Antigen-presenting cell; MHC: Major histocompatibility complex; TCR: T-cell receptor.

Figure 2. Local skin reaction at the site of peptide vaccination

(A) Left thigh of a leukemia patient showing local painful erythema that developed at the injection site 2 weeks following vaccination with 1.0 mg PR1. (B) A small area of firm induration at a site of previous PR1 peptide vaccination on the anterior right thigh. This photograph was taken 1 year after vaccination with 0.5 mg PR1 peptide. (C) Skin biopsy from the local injection site shown in (A) demonstrating perivascular lymphocytic infiltrates (hematoxylin and eosin staining, 400 $\times$). A total of 39% of the lymphocytes were CD8⁺ T cells, 65% of which were specific for the PR1 peptide.

Table 1

Leukemia peptide vaccines.

Antigen	HLA type	Type of immune response	Clinical trial in myeloid leukemia
BCR-ABL	Multiple HLA class I and II	CD4 ⁺ /CD8 ⁺	Phase II
WT1	A0201/A2402/DR.B1	CD4 ⁺ /CD8 ⁺	Phase II
PR1	A0201	CD8 ⁺	Phase II
RHAMM	A0201	CD8 ⁺	Phase II
hTERT	A0201/A2402/multiple DR	CD4 ⁺ /CD8 ⁺	None
G250/CA IX	A0201 and DR	CD4 ⁺ /CD8 ⁺	None
PRAME	A0201	CD8 ⁺	None

CA IX: Carbonic anhydrase IX; hTERT: Human telomerase reverse transcriptase; PRAME: Preferentially expressed antigen in melanoma; RHAMM: Receptor for hyaluronic acid-mediated motility; WT1: Wilm's tumor.