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Adoptive Transfer of Unselected or Leukemia-Reactive T-cells in the Treatment of Relapse Following Allogeneic Hematopoietic Cell Transplantation

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Abstract

Adoptive transfer of in vivo generated antigen-specific donor-derived T-cells is increasingly recognized as an effective approach for the treatment or prevention of EBV lymphomas and cytomegalovirus infections complicating allogeneic hematopoietic cell transplants. This review examines evidence from preclinical experiments and initial clinical trials to critically assess both the potential and current limitations of adoptive transfer of donor T-cells sensitized to selected minor alloantigens of the host or to peptide epitopes of proteins, differentially expressed by clonogenic leukemia cells, such as the Wilms tumor protein, WT-1, as a strategy to treat or prevent recurrence of leukemia in the post transplant period.

Keywords

Allogeneic; Leukemia-targeted; T-cell; Immunotherapy

Introduction

Treatments employing tumor-selective monoclonal antibodies or T-cells have recently emerged as a promising approach for treating hematopoietic malignancies and particularly for eradicating recurrent or minimal residual disease following allogeneic hematopoietic cell transplants (HSCT). Several reviews have documented the increasingly important role played by antibodies such as Rituxan in the treatment of B cell lymphomas [1, 2] and CD33 specific monoclonal antibodies as adjuncts to the therapy of myeloid malignancies [3, 4]. In this review, we will examine the current status of adoptive T-cell therapies as adjuncts to allogeneic HSCT for the treatment of hematologic malignancies. Particular attention will be focused on preclinical and clinical experience with the use of T-cells specific for minor alloantigens and antigens, such as WT-1, that are differentially expressed by host leukemic

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cells. We will also examine some of the cellular interactions which may limit the effectiveness of adoptive T-cell therapies, and the strategies now being explored to overcome them.

Allogeneic Marrow Transplants as a Platform for Adoptive T-cell Therapies

Allogeneic hematopoietic stem cell transplants (HSCT) derived from HLA-matched related donors are now recognized as a potentially curative treatment of choice for patients afflicted with acute leukemia relapsing after initial remission, high risk forms of ALL and AML in 1° CR, MDS and advanced stages of NHL [5, 6, 7]. Furthermore, as a result of the improvements in the precision of HLA typing provided by DNA typing and the efficiency of donor identification and recruitment from the National Marrow Donor Program and an integrated international network of donor registries [8, 9], the results of transplants from matched unrelated donors for these diseases have also markedly improved [10, 11]. However, despite advances in donor matching and the introduction of more effective combinations of immunosuppressive drugs for its prevention and treatment, Graft. vs. host disease (GVHD), its treatment and its associated infectious complications remain significant obstacles to the success of these transplants and to the application of transplantation to the majority of individuals who lack an HLA-matched related or unrelated donor [12, 13].

In 1980, our group introduced an efficient method employing lectin agglutination and E-rosette depletion for depleting T-cells from a marrow graft [14] and demonstrated that in man, as in mouse [15, 16], T-cell depletion can prevent Graft vs. host disease both in HLA matched and in HLA haplotype disparate transplant recipients [14, 17, 18]. Thereafter, a variety of techniques were introduced [19–24] which employed T-cell specific monoclonal antibodies varying significantly in their potential to deplete T-cells [25]. As a result, consistent reductions in the incidence and severity of acute and chronic GVHD were regularly observed only when those grafts were administered together with post transplant immunosuppression. In contrast, marrow grafts depleted by lectin agglutination and E-rosette depletion [17, 18, 26] or more recently, GCSF mobilized peripheral blood stem cell transplants depleted of T-cells by positive selection of $CD34^+$ hematopoietic progenitors [27–29] may be administered without post transplant prophylaxis. Current protocols employing such transplants achieve consistent engraftment with low incidences of both acute and chronic GVHD [30, 31, 32]. For example, at our center over 70% of adults (median age 45) transplanted for high risk forms of AML in 1° remission are surviving disease-free at 5–10 years of follow-up [30, 31]. These results have recently been confirmed in a multicenter trial conducted by the BMT Clinical Trials Network [33].

Early studies of adoptive T-cell therapy for the treatment of CMV infections following unmodified allogeneic HSCT showed that the growth, persistence and therapeutic potential of transferred T-cells was compromised by the immunosuppressive drugs coadministered to treat or prevent GVHD [35]. In contrast, techniques that deplete alloresponsive T-cells sufficiently to prevent acute and chronic GVHD without prophylaxis with immunosuppressive agents circumvent this limitation and thus provide a unique platform for exploring adoptive T-cell therapies. Indeed, clinical trials conducted in recipients of allogeneic HSCT have already indicated the potential of adoptive transfer of donor –derived

in vitro generated pathogen-specific T-cells to treat or prevent EBV-induced lymphomas [34–37], and infections caused by CMV [38–40], adenovirus [41–43] and Aspergillus [44]. These studies have shown that transferred virus-specific T-cells proliferate extensively in the transplant host, accumulate in affected tissues and persist for extended periods of time after transfer [36, 45, 46]. These findings have thus provided useful benchmarks for assessment of the growth and survival of leukemia-targeted T-cells. An added advantage of the use of donor T-cells in this allogeneic HSCT setting as opposed to autologous, patient-derived T-cells is that the donors of the T-cells are healthy donors rather than tumor-bearing hosts whose potential for effective immune responses may already be blunted by their tumor burden and/or the treatments used to control it. Furthermore, in the treatment of acute leukemia, these cells may amplify the resistance to relapse already conferred by an allogeneic unmodified or T-cell depleted graft.

The Cellular Basis of the Allogeneic Graft Vs. Leukemia Response

In early clinical trials of allogeneic HSCT, the anti-leukemic effects of the treatment were ascribed to the leukemia-ablating activity of supralethal doses of total body irradiation and cyclophosphamide [47]. However, subsequent studies by Fefer et al [48], confirmed by others [49] demonstrated that for patients transplanted in early remission, the incidence of relapse following a graft from a genotypically identical twin was almost twice that recorded for a transplant from an HLA-matched sibling. Subsequently, Weiden et al [50] demonstrated that patients who developed acute and/or chronic GVHD following an HLA-matched marrow graft had a lower incidence of relapse than those who did not. These studies in HLA-matched, presumptively minor alloantigen disparate human hosts were consistent with prior experiments demonstrating a reduced incidence of disease recurrence in lethally irradiated leukemic mice following H-2 disparate vs. H-2 compatible marrow grafts [51, 52]. Based on these results, Weiden et al [50] hypothesized that minor-alloreactive T-cells inducing GVHD were the principal effectors of the transplants anti-leukemic activity.

In 1990, Kolb et al [53] reported three patients with CML that had relapsed following an allogeneic marrow graft who were induced into a complete and durable cytogenetic and molecular remission following infusion of a large dose of unselected lymphocytes from the marrow transplant donor. This study provided the first direct evidence that adoptive transfer of human lymphocytes could induce remission of a hematologic malignancy, and that the enhanced resistance to leukemia acquired following a marrow allograft was mediated by donor cells.

Cumulative experience now indicates that up to 80% of patients relapsing with CML can be induced into remission by this approach [54, 55]. The fact that over 75% of the patients responding to these high doses of donor leukocytes also developed GvHD was initially taken as confirmatory evidence of the anti-leukemic effects of GvHD. However, in a trial examining the effects of escalating doses of donor PBMC on CML relapses developing after T-cell depleted marrow grafts, our group showed that T-cell doses as low as $0.3–1.0\times10^7$ $CD3^+$ cells/kg, could induce durable molecular remissions of disease in over 60% of individuals treated for histologic or cytogenetic relapse and that if these T-cell doses were

administered > 9 months after transplant, fewer than 10% develop acute or chronic GvHD [56]. Our findings, which were soon confirmed by Dazzi et al [57], suggest that, at this time post transplant, T-cell doses approximating the 1–3 × 10^7/kg dose given in an unmodified HLA-matched graft that, would be expected to induce grade II-IV GVHD in 30–40% of cases despite drug prophylaxis against GVHD are limited in their potential to initiate GVHD. We hypothesize that this is due to the replacement of host-type dendritic cells and other professional APCs that present minor alloantigens with donor APC populations. As shown by Streilein et al [58], and more recently by Schlomcik et al [59] and others [60] these host-derived APCs are essential instigators of GVHD in minor alloantigen disparate hosts.

The hypothesis that alloreactive donor T-cells contribute to the adoptively transferred resistance to CML is also supported by the increased incidence of CML relapse observed following transplants of T-cell depleted HSCT [61, 62, 63]. Furthermore, when donor leukocytes have been infused, remissions of CML have been correlated with elimination of both leukemic Ph^+ myeloid cells and presumably normal Ph^- T-cells of host origin [56]. Such infusions have resulted in significant increases in the frequencies of donor-derived helper $CD4^+$ and $CD8^+$ T-cells reactive against both Ph^- host B-cells [64] and host-type clonogenic Ph^+CD34^+ CML precursors [65]. Indeed, Falkenburg et al [66] have recently used $CD4^+$ T-cell clones selected on the basis of their capacity to inhibit the growth of Ph^+CD34^+ host CML precursors to treat a patient with CML who relapsed in accelerated phase post transplant and had failed to respond to donor leukocyte infusions. Three infusions providing a total of 3.2×10^9 T-cells from these lines induced molecular remission and reestablished complete donor chimerism. That the donor T-cell lines were reactive against an alloantigen expressed on the HLA matched host's CML cells was inferred from the fact that these lines had no effect on normal clonogenic donor $CD34^+$ cells. Strikingly, however, GvHD was not exacerbated post infusion. Thus, this experience raised the possibility that T-cells reactive against alloantigens or other determinants differentially expressed on clonogenic Ph^+ CML precursors and other hematopoietic cells of the host induced the remission observed.

For patients with AML and ALL, a lower incidence of relapse has also been observed following an allogeneic HSCT when compared to a syngeneic HSCT [62]. However, the contribution of alloreactive T-cells participating in GVHD to enhanced resistance is, at best, unclear. In contrast to the situation in CML, T-cell depleted HSCT administered without post transplant immunosuppression to patients with AML or ALL have not been associated with an increase in the incidence of relapse. This has been demonstrated both in large single center series and in multicenter randomized trials [30, 31, 32, 33, 63]. Conversely, for patients relapsing with AML or ALL, infusions of unselected donor lymphocytes have been largely ineffective. Cumulative experience in patients with AML or MDS who relapse post transplant indicates that, only 20–30% will respond to DLI, even at doses exceeding 10^9 $CD3^+$ T cells/kg [55]. Responses in patients relapsing with ALL have been rare [54, 55]. Furthermore, donor leukocyte infusions intentionally administered with a marrow graft to increase GVHD and thereby reduce risk of ALL relapse have indeed increased the incidence and severity of GVHD without affecting the risk of relapse [67].

At least two features of AML and ALL cells may contribute to these treatment failures. First, the rate of growth of ALL and AML populations at relapse is much more rapid than that observed following recurrence of CML. In contrast, the expansion of leukemia-reactive T-cells following donor leukocyte infusions is slow and limited. As a consequence, the size of the leukemic burden may eclipse the number of potential effector T-cells transferred. This hypothesis is consistent with the fact that in CML, responses to DLI are rarely observed before 8–12 weeks post infusion, at which time donor T-cells reactive against host leukemic precursors are first detected [64, 66, 68]. That the magnitude of the leukemic burden affects response is also consistent with studies of the effects of DLI on CML at different levels of disease. For example, doses of T-cells required to induce remissions in patients treated for molecular relapse of CML ($0.3–1.0\times10^7$ T –cells/kg) are an order of magnitude lower than the $1.0–5.0 \times 10^8$ T-cells/kg required to induce remission in patients with hematologic recurrence of chronic phase CML [56]. These higher doses are, in turn, inadequate to induce remission in over 60% of patients treated for accelerated phase disease or blast crisis in. However, the fact that reducing tumor burden with chemotherapy provides only a limited additional antileukemic effect to DLI in AML and ALL, and that GVH is often induced without eliciting a demonstrateable anti-leukemic effect [55, 69] suggests that other features of the acute leukemias may either limit the capacity of these cells to stimulate a clinically significant donor T-cell response or reduce their sensitivity to the ongoing cytotoxic and/or cytoinhibitory activities of allospecific and/or leukemia-reactive T-cells transferred in the donor leukocyte infusions.

A second feature of human AMLs and ALLs, which likely limits their capacity to elicit an immune response, is their failure to express the costimulatory molecules B7.1 and B7.2. In our own series of primary human AML and ALL cells, fewer than 15% express these molecules. As shown by Cardoso et al [70], and confirmed by us and others [71, 72, 73], AML and ALL cells lacking these costimulatory molecules are incapable of inducing a proliferative or cytotoxic T-cell response in mixed leukocyte cultures with HLA matched or fully allogeneic T-cells. In fact, T-cells sensitized with these $B7.1^{1-}$ leukemic blasts are anergized and are relatively refractory to restimulation.

Given that T-cells participating in GVHD are relatively ineffective in controlling AML or ALL, are there other mechanisms that could explain the enhanced resistance provided by an allogeneic relative to a syngeneic graft? For patients with AML, natural killer cells likely play a significant role. In 2002, Ruggieri et al [74] reported that among patients receiving HLA haplotype disparate T-cell depleted grafts for AML, those whose normal and leukemic cells expressed HLA-C or B alleles inherited on the unshared haplotype that would not engage inhibitory killer immunoglobulin-like receptors (KIRs) expressed on donor NK cells and would therefore be susceptible to NK-mediated cytolysis, were at minimal risk of leukemic relapse post transplant. Indeed, relapses in AML patients were only observed when all inhibitory KIRs expressed by donor NK cells were engaged. Subsequent studies have confirmed these findings [75]. Furthermore, KIR genotypes, which are inherited in man as a constellation of activating and inhibitory KIRs encoded by a complex of genes on chromosome 19 [76], have also been found to influence the risk of AML in HLA-matched recipients. Early after transplant, NK cells express the full panoply of inherited KIR genes, which, in over 30% of individuals, include inhibitory KIRs not satisfied by either the

donor's or matched recipient's HLA genotype [77, 78]. As a consequence, these NK cells can engage and kill residual leukemic cells in the host.

In contrast, KIR disparities and NK cell activity have not been associated with enhanced resistance in patients transplanted for ALL. However, donor T-cells previously sensitized to minor alloantigens and oncofetal proteins differentially expressed by ALL cells may contribute to enhanced resistance. For example, Riddell et al [79] have documented a significantly lower risk of recurrence of ALL, AML and CML in male recipients of transplants from parous female donors, suggesting that transplants containing pre-sensitized T-cells specific for minor alloantigens encoded by genes on the Y chromosome may contribute to leukemic resistance. Case reports have also recorded the isolation of T-cells specific for other minor alloantigens, such as HA-1, that exhibit cytotoxic activity against ALL cells, in patients following HSCT [80]. In addition, recent studies have correlated clinical evidence of the anti-tumor activity of donor lymphocyte infusions with the emergence of donor $CD8^+$ T-cells reactive against oncofetal proteins, such as WT-1, that are differentially expressed by ALL blasts [68, 81].

Minor Alloantigens As Targets for Adoptive Immunotherapy of Acute Leukemias

To date, only a limited number of antigens differentially expressed by host leukemia cells and sufficiently immunogenic to elicit T cell responses have been identified. These include a group of minor alloantigens which are differentially expressed on normal and malignant host hematopoietic cells and a limited set of peptide antigens encoded by leukemia-specific fusion genes or proteins, such as WT-1, protease 3, hTERT and others that are differentially expressed at high concentrations in most leukemias.

The effectiveness of a T-cell mediated adoptive immunotherapy in an allogeneic HSCT recipient critically depends upon the selection of the antigen to be targeted. Several characteristics are now recognized to be important. First, the antigen must be sufficiently immunogenic to elicit a specific response by T-cells derived from the normal transplant donor. Second, the antigen must be selectively or differentially expressed by clonogenic host leukemic cells if the T-cells generated are to be effective in eradicating disease and preventing recurrence. Thirdly, the antigen should not be expressed by normal tissues of the host, other than, possibly, host hematopoietic and lymphoid cells, so as to avoid severe GVHD. Ideally, the antigenic protein should also be essential to the growth and/or survival of the leukemic cell, such that failure to express the protein would result in growth arrest or apoptosis. This attribute reduces the possibility of the emergence of antigen negative clones that can evade T cell recognition.

The array of minor alloantigens that can stimulate donor T-cells to induce GVHD or provoke an allospecific response against residual host leukemic cells is large, complex and still poorly understood Goulmy et al. [82] were the first to isolate and characterize minor alloantigen reactive T cell clones generated from recipients of HLA-matched marrow who had developed GVHD. This led to the identification of the inheritance, allelic frequency, tissue distribution and presenting HLAs of a set of minor alloantigens, termed HA-1 to 5

[83]. By HPLC analysis of peptides eluted from cells presenting these alloantigens, she was thereafter able to identify the alloantigenic peptide of HA-1 [84]. Since then, a large number of minor alloantigens has been identified [85–93]. The gene origins and allelic frequencies of many of these minor alloantigens have now been characterized and mapped, their immunogenic peptide epitopes sequenced and each epitope's presenting HLA allele determined Reviewed in [94]. Most of these minor alloantigens are broadly expressed on normal tissues and could therefore serve as targets of GVHD. However, a subset of antigens has been identified which are selectively expressed by normal or malignant hematopoietic and lymphoid cells [94]. Because of this limited distribution, it has been hypothesized that T-cells specific for such minor alloantigens might exhibit anti-leukemic activity without inducing GVHD [85. 94]. In fact, T-cells generated against several of these minor alloantigens exhibit striking cytotoxic activity against leukemic cells in vitro. Furthermore, T-cells reactive against certain minor alloantigens can prevent outgrowth of Ag^+ leukemias in NOD/SCID mice, indicating their activity against clonogenic leukemia stem cells [95–97]. However, the allelic frequencies of most of these minor alloantigen polymorphisms are such that, with the notable exception of Y chromosome encoded minor alloantigens, disparities between a transplant donor and host that also share the minor alloantigen's presenting HLA allele occur at low frequency [86, 98, 99].As a consequence, clinical trials of T-cells specific for minor alloantigens such as HA-1, HA-2, PANE 1 or LRH-1 that are expressed selectively on host leukemic cells and normal hematopoietic progenitors have proceeded slowly [100, 101, 102].

Proteins Differentially Expressed by Leukemic Cells as Targets for Adoptive T-cell Therapy

Other immunogenic proteins differentially expressed by leukemias of different lineages that have been proposed as targets for immunotherapy include: the Wilms Tumor protein, WT-1 which is overexpressed in a high proportion of ALLs, AMLs, blastic CMLs and advanced MDS, [103– 107] the serine proteases, proteinase 3 and neutrophil elastase which are overexpressed in AML and CML [108], human telomerase reverse transcriptase, hTERT, [109] and the apoptosis inhibitor, survivin [110] which are overexpressed in a wide range of malignancies, as well as the immature laminin receptor protein [OFA-I LRP] [111] and the receptor for hyaluronic acid-mediated motility (RHAMM/CD168) [112] which are aberrantly expressed in myeloid leukemias. Each of these proteins is immunogenic and can elicit responses in vitro by T-cells from normal individuals. T-cell responses against several of these proteins have also been detected at significant frequencies in the blood of tumor – bearing patients. That the expression of certain of these proteins may be important to the survival and growth of leukemic cells is suggested by experiments demonstrating, on the one hand, that SiRNA mediated inhibition of expression of the protein induces apoptosis in leukemic cell population [113–115] and, on the other, that T-cells specific for antigenic peptide epitopes of these proteins can suppress the clonogenicity of leukemias in vitro and their growth in vivo in xenografted NOD SCID mice [116].

The Wilms Tumor Protein, WT-1 As A Model Antigen for Adoptive T-cell Therapy

Experimental work examining the potential of T-cells specific for epitopes of the Wilms tumor protein, WT-1, to inhibit clonogenic leukemic cells in vitro and in vivo in preclinical models is particularly advanced and provides a useful illustration of both the potential and the current limitations of antigen-specific T-cell therapies applied to the treatment of acute leukemias.

WT1 was first identified in a mutated form in the childhood neoplasm, Wilms' tumor [117]. This gene, located at 11p13, comprises 10 exons [118] (Fig 1). Exons 7–10 on the carboxyl terminus of the gene encode a series of four zinc finger domains which mediate binding to DNA. At the N-terminus, exon 1 encodes a repression domain while exons 2–5 comprise an activating domain. Alternative splicing of transcribed mRNA contributes to the generation of a series of different isoforms of WT-1which, in normal tissues, are expressed in defined ratios [119]. The principal isoforms of WT-1 result from splice variants that do or do not include a 17 amino acid sequence in exon 5 or a three amino acid sequence (KTS) between zinc fingers 3 and 4. Isoforms with or without the exon 5 sequence differ in their patterns of WT-1 mediated gene transcription [120], while the presence or absence of KTS alters the spectrum of promoters bound by WT-1 and the genes that it activates or represses [121]. Recently, an isoform termed SWT-1 containing a truncated N terminal sequence of exon 1 has also been described [122]. This isoform, which is predominant in a high proportion of primary leukemias and leukemia cell lines, lacks the repressor domain of exon 1.

Non-mutated WT-1 has been categorized as a tumor suppressor gene [117, 123, 124]. The WT-1 protein is a transcription factor which binds and either activates or represses a spectrum of early growth factor gene promoters, including platelet-deriver growth factor A chain, colony stimulating factor-1, transforming growth factor-β1, and insulin-like growth factor II [123]. Unlike the tumor suppressor genes Rb and p53, which are ubiquitously expressed, the expression of WT1 is restricted to a limited number of normal tissues including: fetal kidney, ovary, testis, spleen, hematopoietic precursors, and the mesothelial cell lining of visceral organs [124]. In normal hematopoietic progenitor cells, WT-1 is expressed at low levels. Its expression in primitive $CD34^+$ progenitor cells inhibits proliferation, inducing a sustained quiescence in G_o [125, 126] .Expression in later stage progenitors, on the other hand, induces terminal differentiation [126]. Thus, in these normal progenitor cells, WT-1's functions are also consistent with those of a tumor suppressor gene.

In contrast to its limited expression in normal hematopoietic cells, WT1 is expressed at high levels in up to 70 % of adult and childhood AMLs, ALLs, CMLs [127] and high risk myelodysplastic syndromes as well as in several solid tumors including: ovarian cancer, mesothelioma, desmoplastic small round cell tumor, Wilms' tumor, breast cancer, renal cell carcinoma, and non-small cell lung cancer [128–134]. Among patients with acute myelogenous leukemia, high expression of WT-1 by the patient's leukemic blasts is associated with a poor response to chemotherapy, a higher risk of leukemic relapse and a significantly lower probability of extended disease-free survival [135]. Similarly, among patients with different stages of myelodysplasia, patients with refractory anemia tend to have

low numbers of marrow cells expressing WT-1, while patients with RAEB, RAEB and overt AML have incrementally higher levels of WT-1 [136]. Accordingly, WT-1 expression is now being used by several groups to estimate prognosis [137, 138]. Furthermore, pcr-based methods quantitating WT-1 RNA in the blood are now being used to follow disease response and to monitor minimal residual disease in patients who achieve remission [139, 140].

The function of WT-1 in leukemic cells is still poorly understood. However, in leukemic cells, expression of WT-1 is associated with enhanced proliferation of blasts [141]. Introduction of anti-sense oligomers consistently induces apoptosis in these leukemic blasts [142]. Similarly, SiRNA-induced inhibition of WT-1 expression in primary leukemia blasts and leukemic cell lines inhibits their proliferation and enhances apoptosis [113, 115]. Alterations in the balance of WT-1 isoforms may also be critical to leukemic cell growth. Thus, while introduction of an SiRNA inhibiting wild type WT-1 into K562 leukemic cells in which the SWT-1 isoform is dominant, only moderately reduces their growth, SiRNA that knocks down the S-WT-1 isoform profoundly inhibits proliferation [122]. The contribution of the S-WT-1 isoform rather than wild type WT-1 to oncogenesis is further suggested by the differentially enhanced capacity of the SWT-1 to induce 3T3 cell transformation when cotransfected with H-ras^{V12} [122]

Several studies [144–147]; have shown that peptides derived from the WT-1 protein are immunogenic in man. Ohminami et al [144] and Oka et al [145] were the first to identify peptides of WT-1 which, when presented by HLA A2402 or HLA A0201, could elicit WT-1 peptide specific T cell clones with *in vitro* leukomocidal activity. These included: 1)$_{126–134}$RMFPNAPYL and 2) $_{187–195}$SLGEQQYSV presented by HLA A0201, and HLA A2402. $_{235–243}$CMTWVQMNL and $_{417–425}$RWPSCQKKF. The identification of these epitopes catalyzed studies of the anti-tumor activity of WT-1 specific T-cells. However, until recently, only a limited number of WT-1 epitopes, presented by prevalent class I and class II HLA alleles have been identified. These epitopes are listed in Table 1; their distribution within the WT-1 protein is mapped in Figure 1.

In our own studies [147], two WT-1 peptides presented by HLA A0201 elicited IFNγ^+ cytotoxic WT-1 specific HLA A0201 restricted CD8$^+$ T cells in each of 16 normal HLA A0201$^+$ normal donors tested. Furthermore, the T cells generated consistently exhibited cytotoxic activity specific for WT-1$^+$ HLA A0201$^+$ leukemias, including AML, ALL and CML blasts *in vitro*, but had no cytotoxic or colony inhibiting activity CD34$^+$ cells from normal adult marrow or cord blood [169]. Studies of Gao et al 116, [146] have also shown that a T cell clone specific for RMF bound to HLA A0201 was capable of selectively inhibiting the clonogenic activity of Ph$^+$ CD34$^+$ CML blasts *in vitro* but did not affect normal Ph$^-$ CD34 cells.

A proportion of patients with leukemia are also able to generate T cell responses against these WT-1 peptides. Scheibenbogen et al [148] detected T cell responses against the $_{126–134}$RMF WT-1 peptide presented by HLA A0201 in up to 30 % of patients with AML in remission who share this HLA allele. Rezvani et al [68] also documented the emergence of WT-1 peptide specific T cells in patients transplanted for CML who had

received donor lymphocytes as treatment for recurrence of disease. Strikingly, in these patients, the emergence of WT-1 specific T cells was temporally associated with leukemic response, suggesting that the WT-1 peptide specific T cells participated in the inductions of molecular remission observed.

The immunogenicity of specific peptides of WT-1 presented by HLAA0201 and HLA A2402 has also been tested in patients with leukemia as a vaccine. In the initial trial reported by Oka et al [149], HLAA2402$^+$ patients with hematologic malignancies, including 12 with de novo AML in complete remission, 1 with 2° AML evolving from MDS and 1 with MDS and myelofibrosis, received biweekly injections of the HLA A2402 presented WT-1 epitope CMT WNQMNL or a modification of this peptide CYT WNQMNL. Of these patients, 9/13 tested developed tetramer$^+$ T-cells; 6/11 tested generated peptide-specific IFNγ^+ T-cells. The two patients with prior MDS developed severe leukopenia, a potentially reflecting the activity of induced WT-1 specific T-cells against malignant stem cells. WT-1 transcripts were also decreased in the blood after vaccination. Among the 12 patients with AML in remission, no hematopoietic toxicities were observed. However, WT-1 transcript levels fell in 5 patients with elevated WT-1 transcripts in the blood. Furthermore, only 2 of the 12 patients developed disease progression over the 3–4 months in which the vaccinations were administered. Subsequently Rezvani et al [150] also vaccinated 8 HLA A0201$^+$ patients with AML, MDS or CML in remission who had molecular evidence of minimal residual disease. The vaccine consisted of immunogenic peptides derived from WT-1 (RMFPNAPYL) and protease 3 (VLQELNVTV) that are presented by HLA A0201. These epitopes emulsified in the adjuvant Montamide. Two 100ug doses of GM CSF were also administered in adjacent areas. Again, no suppression of normal white cell, platelet or red cell counts was observed. Of the 8 patients, 5 patients had significant increases in the number of RMF/HLAA0201 tetramer$^+$ T cells and IFNγ^+ T-cells in the circulation; in 3/6 evaluable responders, a ≥2.0 $\log_{10}$ reduction in circulating WT-1 transcripts was also observed.

More recently, Keilholz et al [151] used a combination of the HLA A0201 presented WT-1 peptide RMF and KLH, together with GMCSF to vaccinate 19 patients with AML (N=17) or MDS with RAEB (N=2) who had failed to achieve remission (N=10), or could not receive chemotherapy because of comorbidities or advanced age (N=9). Vaccinations were administered biweekly × 4 and then monthly. All but 4 received at least 6 vaccinations over 4 months without additional intervention. Of the 17 patients with AML, 1 patient achieved a complete remission and 13 had stabilization of disease for a median interval of 155 days [101–571 days]. In four of these patients, there was a reduction in marrow blasts of 50% or more.

Employing a different approach, Scheinberg et al [152, 153] have been conducting trials of a polyvalent WT-1 peptide vaccine designed to induce both CD8 and CD4 T-cells and, thereby, to enhance both the amplitude and persistence of T-cell responses. This vaccine contains a heteroclitic analog of the RMFPNAPYL [126–134] peptide, YMFPNAPYL designed to enhance binding to the HLA A0201 allele. The vaccine also includes three epitopes, either 22aa or 19aa in length, known to be presented by HLA DRB1 of which one is also presented by DRB, 0405, DRB1501 and 1502. One of these 19aa peptides also includes within its sequence the HLA-A0201 presented epitope RMFPNAPYL [145]. The

peptides have been administered as an emulsion with the adjuvant, Montanide, biweekly over 12 weeks, together with GMCSF. Preliminary evidence suggests this approach can induce CD4 and/or CD8 T-cell responses to the natural peptides of WT-1 in a high proportion of patients inheriting the HLA alleles that present these epitopes. Duration of responses and the effects of these responses on minimal residual disease are being evaluated [152].

Preclinical and Clinical Studies of Adoptive Transfer of WT-1 Specific T-cells

The continuing success of adoptive therapy with donor-derived pathogen-specific T-cells in the treatment and prevention of EBV lymphomas and CMV infections developing following allogeneic HSCT [34–40], and the demonstrated capacity of the transferred virus-specific T-cells to expand and persist in the immunoablated transplant recipient [46], coupled with the finding that T-cells specific for "self antigens" like WT-1 that are differentially expressed by clonogenic leukemic cells can be generated from the blood of normal healthy donors [145, 147] has spawned considerable interest in the potential of adoptive transfer of such T-cells, generated in vitro, to control or eradicate residual disease in allogenic HSCT recipients.

In preclinical studies, Ohminami et al [144] was the first to demonstrate that a human HLA $A2402^+$ T cell clone (TAK-1) specific for the CMT peptide of WT-1 could lyse WT-1+ leukemic cells in vitro. Thereafter, MAKITA et al [154] showed that this clone could induce regressions of HLA $A2402^+$ $WT\text{-}1^+$ lung cancer xenografts in NOD SCID mice. Subsequently, Gao et al [146] demonstrated that T-cells generated from an HLA A0201-donor that were specific for the RMF peptide of WT-1 together with HLA A0201 could lyse HLA $A0201^+$ $WT\text{-}1^+$ $CD34^+$ myeloid leukemia blasts in vitro and could deplete clonogenic cells capable of transferring leukemia into NOD/SCID mice [116]. More recently Xue et al [143, 155] from the same group, have shown that T-cells from HLA $A0201^+$ leukemic patients modified to express a TCR specific for the WT-1/HLA A0201 complex can also prevent outgrowths of autologous leukemic cells in this model. Our group has recently also shown that co-infusion of HLA A0201 restricted T-cells specific for the RMF peptide can durably prevent outgrowth of $WT\text{-}1^+$ pre B ALL cells bearing this allele [156]. We also demonstrated that T-cells specific for the RMF and SLG peptides of WT-1 presented by adoptive transfer of this HLA A0201 allele into NOD/SCID mice bearing 4 established human tumor xenografts differing in their expression of WT-1 and the HLA A0201 allele, led to selective accumulations of the T-cells in subcutaneous tumor xenografts expressing both WT-1 and HLA A0201 [147]. Strikingly, these T cells also did not accumulate in $WT\text{-}1^+$ tumors that were genotypically $HLAA0201^+$ but failed to express HLA A0201 on their surface. Because the transferred T-cells had been transduced to express HSV thymide kinase we could also sequentially quantitate these accumulations by position emission tomography (PET) following intravenous infusion of I^{124} F1AU. Targeted accumulations of these HLA-restricted WT-1 specific T-cells in $WT\text{-}1^+$ HLA $A0201^+$ tumor xenografts could be demonstrated within 4 hours of their transfer. T-cells continued to selectively accumulate in the $WT\text{-}1^+$, HLA $A0201^+$ tumors for up to 12 days post transfer, resulting in tumor regressions. In contrast, HLA A0201 restricted T-cells specific for EBV did not migrate to

or alter the growth of the leukemic xenografts. Thus, these experiments provided evidence that, following adoptive transfer in vivo, WT-1 peptide specific T-cells selectively accumulate in and can induce regressions of leukemic xenografts but only if they co express the WT-1 epitope and its presenting HLA allele.

Based on these studies, we and others have recently initiated Phase I and II trials of donor-derived WT-1 peptide specific T-cells for the treatment of persistent minimal residual disease or recurrence of WT-1^+ AML, ALL, or MDS following allogeneic HSCT. In our studies, we have focused efforts on the development of techniques whereby transplant donor derived WT-1 specific T-cells can be produced for any transplant recipient, irrespective of their HLA genotype. Accordingly, we have generated WT-1 peptide specific T-cells by repeated sensitizations with autologous dendritic cells loaded with a pool of synthetic pentadecapeptides that each overlap the sequence of the next by 11 amino acids and collectively span the sequence of WT-1. Using this approach, we have been able to elicit both $CD8^+$ and $CD4^+$ T-cell responses specific for WT-1 peptides in the pool from over 80% of normal donors, irrespective of their HLA genotype [157]. We have subsequently mapped and identified the epitopes eliciting responses and thereafter, the HLA alleles presenting each epitope. A striking feature of this approach is the fact that the T cells generated from these normal donors have consistently exhibited cytocidal and/or cytoinhibitory activity against WT-1^+ leukemic cell lines bearing their restricting HLA allele [157]. Thus far, infusions of these T-cells at the lower doses in this phase I dose escalation trial have been well tolerated without renal, hematopoietic or other toxicities and without GVHD unpublished observations. These T-cells can also at least transiently reduce or eliminate WT-1^+ cells from the circulation. However, it is too early to assess whether or to what degree these T-cells can substantively affect recurrent or residual disease

An alternative strategy also being explored is the use of autologous T-cells transduced to express high affinity T cell receptors specific for a WT-1 epitope presented by a prevalent HLA allele such as HLA A0201 [143]. Initial trials with T-cells transduced with retroviral vectors encoding α and β chains of TCRs specific for the melanoma antigens MART-1 and tyrosinase have demonstrated their safety and, in a proportion of patients treated, has led to significant regressions of disease [158]. However, the sustained production of functional T-cell's in vivo was limited by mispairing of transduced α and β chains with the α and β chains of each T-cells endogenous receptor. As a result, in vivo expansion and persistence of T cells bearing a functional receptor was short-lived. More recently, several approaches have been developed to reduce mispairing. A particularly effective strategy has been to introduce cysteine residues into the constant regions of the transduced α and β chains of the tumor-epitope specific TCR to promote preferential pairing of the transduced α and β chains by disulfide linkages [159]. Using this approach, Xue et al [155] have been able to generate large populations of T-cells effectively transduced to express a high affinity WT-1 specific TCR and have also shown that adoptive transfer of these T-cells into NOD/SCID mice that had received an infusion of autologous CML blasts, prevented the outgrowth of this leukemia. Based on these studies, a clinical trial of autologous T-cells transduced to express a TCR specific for the RMF peptide of WT-1 presented by HLA A0201 is being conducted in leukemia patients bearing this allele.

Approaches to Enhance the Anti-Leukemic Activity and Persistence of Transferred T-cells

In early trials of adoptive therapy using in vitro generated autologous T-cell clones specific for antigenic epitopes of Mart-1, tyrosinase or GP100, the persistence of the induced or transferred antigen-specific T-cells in vivo was short-lived [160]. Initially, the limited survival of these T-cells was ascribed to their limited potential for replication after extended growth in vitro [161]. However, the striking expansion and long-term persistence of adoptively transferred EBV-specific T-cells in marrow allograft recipients suggested that other mechanisms were also operative. Accordingly, Dudley et al [162] introduced preparatory immunoablative chemotherapy of the patient prior to adoptive transfer of T-cells to test the possibility that the expansion of transferred T-cells was limited either by inadequate space or by active inhibition by host cells regulating reactivity against "self" antigens. This approach radically enhanced the potential of the transferred T-cell clones to expand and persist post infusion and is now increasingly incorporated into trials evaluating adoptive transfer of tumor-reactive autologous T-cells in patients with solid tumors. Further studies have also provided evidence underscoring the importance of depleting regulatory cells in the host to ensure the persistence and sustained growth of the transferred T-cells [163]. This finding, in turn, raises the possibility that treatment of the host with other agents selectively targeting regulatory cells such as anti-CTLA-4 [164] or anti-PD-1, [165] rather than broadly immunoablative drugs like cyclophosphamide or fludarabine might potentiate the growth and persistence of adoptively transferred T-cells while avoiding the systemic toxicities of these chemotherapeutic agents.

Recent evidence also indicates that the types of T cells administered affect the magnitude and persistence of the T cell response. Walter et al [135] provided early evidence of the contribution of $CD4^+$ helper T-cells to the persistence of adoptively transferred CMV specific T-cells. Recent studies of Hunder et al [166] also indicate that tumor antigen-specific $CD4^+$ T-cells can persist and exact significant anti-tumor effects without prior lymphoablation or concominant administration of cytokines. More recently, Berger et al [167] also demonstrated that $CD8^+$ T cells originating from central memory T-cells [T_{CM}] can survive and propagate in vivo for extended periods post transfer while $CD8^+$ effector memory cells T_{EM} are short-lived. These studies have focused efforts on the development of techniques for early selection and propagation of tumor antigen-specific T_{CM} in vitro and stimulation of their growth in vivo. One particularly promising approach is to sensitize and expand T-cells in vitro in the presence of IL-15 [168] or on antigen presenting cells coexpressing IL-15 and IL-15Rα [169] which stimulate the growth of both T_{CM} and T_{EM} without stimulating Fox $P3^+$ regulatory T cells. Recent studies of Berger et al [170] also suggest that IL-15 can stimulate the growth and persistence of both T_{CM} and T_{EM} in vivo. In the non-human primate, M. nemestrina, intermittent subcutaneous doses of 2.5–10ug/kg human recombinant IL-15 were well tolerated and resulted in significant increases in circulating populations of CMV-specific T_{CM} and T_{EM} cells following adoptive transfer which persisted for periods up to 4 weeks post cessation of IL-15 treatment. Unlike what has been observed in primates and humans treated with IL-2 [171], IL-15 only marginally increased the number of $CD4^+$ Fox $P3^+$ CD25 high regulatory T-cell. Thus, IL-15 and

possibly other cytokines such as IL-7 [172] or IL-21 may foster expansion of memory T-cells, and particularly T_{CM} to enhance the persistence of adoptively transferred T-cells and sustain their anti-tumor effects.

Introduction of a tumor-specific TCR into central memory T-cells has also been proposed as a strategy to enhance their persistence in vivo [173]. However, in the setting of an allogeneic HSCT, the specificities of the endogenous receptors expressed by the T_{CM} must also be considered, since unselected T_{CM} populations derived from a transplant donor would be expected to also contain alloreactive T cells capable of inducing GVHD. To circumvent this limitation, Heemskerk et al [174] have proposed and examined the feasibility of selectively transducing T-cells specific for the latent virus CMV to express a second TCR specific for the minor alloantigen, HA-1. Their initial studies demonstrated the HLA-restricted dual specificity of these transduced T-cells. Subsequent studies have also shown that these cells, after extended propagation in vitro, preserve their dual specificity [175]. These T-cells are soon to be introduced in clinical trials. Pule et al [176] have also recently documented that EBV-specific T-cells transduced to express a chimeric antigen receptor [CAR] specific for GD2 persisted for significantly longer periods than non specifically activated T-cells expressing this CAR after adoptive transfer into patients with neuroblastoma. Their data support the hypothesis that such bispecific T-cells may persist and exert more sustained antitumor effects as a result of ongoing stimulation of the T-cells through their endogenous TCR in response to the small populations of EBV^+ B cells that are maintained in the circulation.

In summary, adoptive transfer of unselected T-cells in allogeneic HSCT recipients provided the first clear evidence of the potential of T-cells to control or eradicate residual leukemia in man. Furthermore, T-cell depleted allogeneic HSCT, which can prevent GVHD without administering immunosuppressive drugs post transplant provide a unique platform for the evaluation of cell therapies applied to the treatment of human hematologic malignancies. Preclinical studies have now clearly demonstrated the potential of donor-derived T-cells specific for minor alloantigens expressed by a patient's hematopoietic cells or antigens differentially expressed by the patient's leukemic cells to eradicate clonogenic human leukemic cells in immunodeficient mice. Early results of vaccine trials employing immunogenic peptides of differentially expressed proteins such as WT-1 suggest that induction of T-cell responses to such antigens can lead to significant reductions or eradication of leukemic cell populations. Trials of adoptive transfer of leukemia-reactive T-cells generated from normal transplant donors have recently been initiated. The results of preclinical and early clinical studies thus provide considerable evidence in support of the potential of tumor-reactive T-cells to control or eradicate leukemia both in animals and in man and suggest that adoptive T-cell-based immune therapies will emerge as an important and increasingly effective targeted therapy for the treatment of hematologic malignancies.

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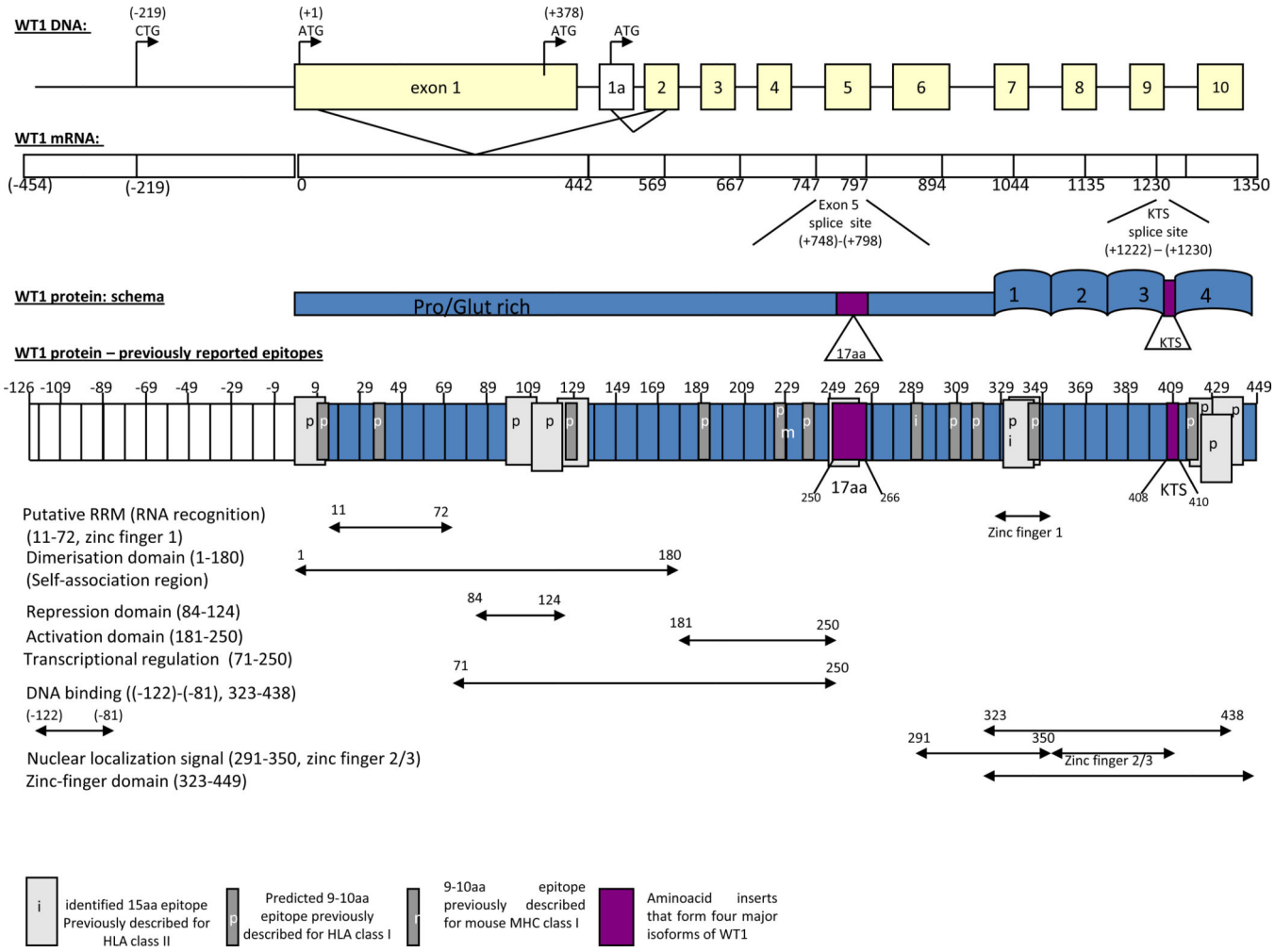

Figure 1.

The structure of the WT-1 gene and the WT-1 protein, with mapping of reported peptide epitopes presented by class I or II HLA alleles. The figure also defines domains within the WT-1 protein that may be altered in their function in different isoforms of WT-1 created by splicing within exons 1, 5 or 9.

Table

Summary of the WT1 epitopes described.

Epitope	HLA allele	Anti-leukemia cytotoxicity	Authors
$_{124–138}$QARMFPNAPYLPSCL	HLA-DR53	+	177
$_{247–261}$LKGVAAGSSSSVKWT	HLA-DR	+	177
$_{235–243}$CMTWNQMNL	HLA-A2402	+	144
$_{332–347}$KRYFKLSHLQMHSRKH	DRB$_1$0405	−	178
$_{331–345}$NKRYFKLSHLQMHSR	DRB$_1$0401	−	179
$_{(-3)–(-12)}$PQQMGSDVRDLNALL	DRB$_1$0401	−	180
$_{417–425}$RWPSCQKKF	A2402	+	190
$_{187–195}$SLGEQQYSV	HLA-A0201	+	145
$_{235–243}$CMTWNQMNL	HLA-A0201	+	182
$_{126–134}$RMFPNAPYL	HLA-A0201	+	146
$_{37–45}$VLDFAPPGA	HLA-A0201		191
$_{337–347}$LSHLQMHSRKH	DP5	+	184
$_{317–327}$ TSEKRPFMCAY	HLA-A0101	+	186
$_{286–294}$ YRIHTHGVF	HLA-Cw07	−	187
$_{10–19}$ALLPAVPSL	HLA-A0201	−	188
$_{303–311-}$VPGVAPTLV	HLA-A0201 HLA-B5101	−	189
$_{97–111}$ SGQFTGTAGACRYGP	HLA-A6801 DRB$_1$1501	−	189
$_{109–124}$YGPFGPPPPSQASGQ	MHC class II	−	189
$_{422–436}$QKKFARSDELVRHHN	MHC class II	−	189
$_{418–432}$QKKFARSDELVRHHN	MHC class II	−	189