Adoptive T-Cell Transfer as a Clinical Antitumor Strategy for Hematologic Malignancies

Aaron P. Rapoport and Nadia Ijaz

Abstract Allogeneic stem cell transplantation remains the only widely accepted and effective form of T-cell immunotherapy for blood cancers including lymphoma, myeloma, and leukemia. However this therapy carries substantial risks and is available to only a minority of patients who have suitable donors. The goal of harnessing autologous (patient derived) T-cells to treat blood cancers has been elusive. Nonetheless, new insights into T-cell biology and advances in vaccine and T-cell culture technology have provided a foundation for the development and clinical application of autologous T-cell immunotherapy. Two major but intersecting strategies have been used to stimulate antitumor immunity in patients: therapeutic or "active" immunization using putative cancer-based vaccines and "passive" immunization chiefly referring to the transfer of autologous (or allogeneic) T-cells into tumor-bearing hosts. This chapter briefly reviews the early studies that formed the basis for adoptive T-cell immunotherapy and then focuses on the growing clinical experience of using adoptive T-cell transfer therapy for immune reconstitution and treatment of hematological malignancies. Historically, most of this experience involves the transfer of cultured, poly-specific T-cells obtained from tumor-bearing tissues or peripheral blood. However, advances in the efficiency and safety of gene-transfer technology are driving efforts to generate T-cells with predetermined specificity for known tumor antigens and enhanced functional properties as well. Recent clinical success using adoptive transfer of genetically altered T-cells in the setting of chronic lymphocytic leukemia and pediatric acute lymphoblastic leukemia, although limited to a small number of patients, has generated increasing interest and has validated the therapeutic potential inherent in T-cell transfer strategies.

Keywords Adoptive T-cell Transfers • Autologous T-cell immunotherapy • Cellular Immunotherapy • Gene-modified T-cells • Chimeric Antigen Receptors • CART cells

A.P. Rapoport, M.D. (✉) • N. Ijaz, M.D.
Department of Medicine, University of Maryland School of Medicine,
Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD 21201, USA
e-mail: Arapoport@umm.edu

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1 Background

Despite impressive advances in the treatment of nearly all types of hematological malignancies, cures remain uncommon for the majority of patients with myeloma and relapsed or refractory lymphoma and leukemia. Dose-intensive chemotherapy/radiotherapy followed by autologous (patient derived) stem cell transplantation leads to complete remissions and extended (~5 years) disease-free survival in about 20–40 % of myeloma patients, but the 10-year disease-free survival is < 20 % and the likelihood of cure is < 10 % [1–4]. Autologous stem cell transplants induce cures in about 40 % of patients with relapsed lymphoma [5]. Allogeneic (donor derived) stem cell transplants induce cures in about 20–60 % of patients with acute leukemia depending on remission status and may increase the likelihood of cure for patients with myeloma and high-risk aggressive lymphoma largely through a T-cell-mediated graft-vs.-tumor effect [6–11]. However, the benefit of the graft-vs.-tumor effect is offset to a significant extent by increased treatment-related morbidity and mortality from graft-vs.-host disease (GVHD) wherein donor-derived T-cells attack certain healthy cells and tissues in the patient (e.g., skin, intestinal tract, and liver in the acute phase). Furthermore, immune depletion after all forms of high-dose chemotherapy may be long-lasting and increases the risk for serious bacterial and viral infections [12–14].

Enhanced immune cell number and/or function may be associated with better outcomes after the treatment of a variety of hematologic malignancies. For example, higher lymphocyte counts at diagnosis and after transplantation predicted better disease-free and overall survival for patients with myeloma [15, 16]. Higher lymphocyte counts at diagnosis and relapse have also been associated with improved progression-free and/or overall survivals for patients with lymphoma and myelodysplastic syndromes [17–21], while a few studies have not demonstrated an association between lymphocyte recovery and outcome [22]. In the case of myeloma, tumor-reactive T-cells have been detected at low frequencies in the marrow or the blood of untreated patients [23, 24]. Furthermore, CD4+ and CD8+ T-cells directed against epitopes from the mutated region of NPM1 can be detected in about 30–40 % of patients with NPM1-mutated AML, and in vitro studies reveal that these cells can elicit specific lysis of leukemic blasts [25]. These lines of indirect evidence suggest that tumor-reactive T-cells may be found in a significant proportion of patients with a variety of hematologic malignancies and also provide a justification for the notion that forced increases in the number and function of these tumor-reactive T-cells may contribute to better tumor control. Adoptive transfer of functionally enhanced T-cells which are either poly specific or preferably tumor specific may help repair the immunodepletion that inevitably follows the treatment of hematologic malignancies with standard-dose or high-dose chemotherapy and may exert antitumor effects. While infusion of allogeneic T-cells is a widely accepted and effective if potentially toxic form of adoptive cellular therapy, recent clinical experience suggests that autologous T-cell transfers may also exhibit clinical benefits. These benefits include accelerated immune recovery, protection from infections, enhanced

responses to microbial and putative cancer vaccines, and possibly antitumor effects as well. There is growing hope that further developments will allow autologous adoptive T-cell immunotherapy to become a new and highly effective therapeutic branch of transfusion medicine.

2 Allogeneic (Donor) Lymphocyte Infusions

Weiden et al. were among the first to recognize the therapeutic impact of passenger lymphocytes in marrow/stem cell products when they discovered a significantly lower rate of leukemia relapse among recipients of allogeneic marrow grafts vs. recipients of marrow grafts from syngeneic (identical twin) donors [26]. A logical extension of this donor lymphocyte-mediated "graft-vs.-leukemia" effect has been the development of therapeutic donor lymphocyte infusions (DLI). DLI involves the transfer of lymphocytes from the original stem cell donor in an effort to treat relapse of the hematologic malignancy after prior allogeneic stem cell transplantation. DLI can be given alone or following chemotherapy, monoclonal antibody therapy, or other form of cytoreductive treatment to achieve a lower burden of disease prior to DLI. DLI induces durable complete remissions in the majority of patients with chronic myelogenous leukemia (CML) in early-stage relapse. However DLI induces remission in less than 30 % of patients with relapsed acute leukemia, myelodysplastic syndrome, and multiple myeloma, and the majority of these patients eventually relapse again.

Preemptive DLI has also been used for patients with hematologic malignancies who have not formally relapsed with the intention of generating full donor chimerism (complete donor stem cell engraftment in the recipient's blood and bone marrow) and potentiating the graft-vs.-tumor effect.

However the success of DLI comes at the price of GVHD and sometimes marrow suppression with frequencies of 50–60 and 20–40 %, respectively. Several groups have studied different strategies to overcome these complications including combining DLI with chemotherapy, using lymphocyte subset selection, or genetically modifying T-cells to express suicide genes which can be activated in the event of serious GHVD.

2.1 Efficacy of DLI in Various Hematologic Malignancies

2.1.1 Chronic Myeloid Leukemia

The most favorable outcomes after DLI occur in patients with relapsed chronic-phase CML. A large multicenter study showed complete cytogenetic remission in 60 % of relapsed CML patients without pre-DLI cytoreduction [27]. The best response to DLI was evident in chronic-phase CML patients with molecular and/or

cytogenetic relapse only. Nearly all of these patients had a complete cytogenetic response, defined as complete absence of the Philadelphia chromosome on standard cytogenetic testing. Patients with cytogenetic relapses or chronic-phase CML at the time of relapse had a complete cytogenetic response rate of 75.7 vs. 33.3 and 16.7 % in patients with accelerated or blast-phase relapses, respectively. Complete responses when achieved were durable with a projected probability of 89 % for remaining in complete remission at 2 years of follow-up. By multivariate analysis, predictors of complete response to DLI included chronic GVHD after the original transplant, chronic-phase disease at relapse, and a time interval of 2 years or less between transplant and DLI. Furthermore, the development of acute and chronic GVHD post DLI also correlated significantly with disease response ($P < 0.00001$).

2.1.2 Acute Myeloid Leukemia

Table 1 summarizes the response rates to DLI for a variety of hematological malignancies including CML, AML, acute lymphoblastic leukemia (ALL), lymphoma, and myeloma. From this table it is evident that patients with other hematologic malignancies respond less frequently and durably to DLI. For example, the complete remission rate is about 21 % for patients with relapsed AML, and the long-term survival for AML patients treated with DLI is less than 20 %.

DLI in AML from unrelated donors is associated with a higher response rate when compared to related donors, with one series reporting that 42 % of patients achieved a complete remission after unrelated DLI [29]. A retrospective analysis of DLI in AML performed by the European Group for Blood and Marrow Transplantation showed an estimated survival of 21 % among 171 patients who received DLI vs. a 9 % 2-year overall survival for 228 patients who did not receive DLI for post-transplant relapse [33] After adjustment for all the pertinent clinical variables in the two groups, DLI administration appeared to be associated with improved outcome in younger patients and in those patients who relapsed more than 5 months after transplantation. Bone marrow blast count at the time of relapse, female gender, favorable cytogenetics, and disease remission at the time of DLI were predictive of survival by multivariate analysis. For patients who received DLI in remission and had favorable cytogenetics, the 2-year overall survival was estimated at 56 vs. 9–20 % for those who received DLI with active leukemia. The reasons for reduced DLI efficacy in acute myeloid leukemia as compared to CML may be due to rapid growth kinetics of the acute leukemia cells. Other potential mechanisms for decreased efficacy of DLI in AML include lack of surface expression of costimulatory molecules, defective tumor antigen presentation, involvement of immunologically privileged sites, or down regulation of HLA molecules and especially patient-specific major or minor histocompatibility antigens. In this regard, a study of 43 patients who received haploidentical bone marrow transplants and donor T-cell infusions for acute myeloid leukemia or high-risk myelodysplastic syndrome reported that 5 of the 17 patients (29 %) who relapsed had developed resistance to donor lymphocytes due to genomic loss of the mismatched patient-specific HLA haplotype in the leukemic cells [43].

Table 1 Studies of DLI for relapses after SCT

Disease	Ref.	Patients	Other treatments	DLI dose (per kg)	Responses	Survival
CML	[28]	84	None	3×10^8	Cyto relapse: 82 % Hematological:78 % Accel/blast: 12.5 %	67 % at 2 years
	[27]	56	None	$1–8.2\times10^8$	Cyto relapse: 100 % Hematologic: 73.5 % Accel/blast: 33 %	60 % at 2 years
	[29]	25	None	0.85×10^8	CR 46 %	53 % for early phase, 12 % for late phase
	[30]	66	None	1.5×10^8	CR 68 %	91 % at 2 years in responders
	[31]	23	Five patients received IFN alpha	3×10^8	91 % in chronic phase	82 % in chronic phase 16 % in active chase
AML	[28]	23	Eight patients received chemotherapy	2.4 (0.25–12.3) $\times10^8$	CR 29 %	15 % at 2 years
	[27]	44	Seven patients received chemotherapy	$1–8.2\times10^8$	15.4 % CR in patients without chemotherapy	17 % at 2 years
	[29]	23	Four patients were in remission prior to receiving DLI	1.34×10^8	42 %	21 % at 1.4 years
	[31]	21	None	2.3×10^8	CR in 38 %	7 % at 2 years
	[32]	16	All patients received pre DLI chemotherapy	4.5×10^8	CR in 63 %	31 % at 2 years
	[33]	171	124 patients received pre-DLI chemotherapy	2.8×10^8	CR in 35 %	20 % at 3 year

(continued)

Table 1 (continued)

Disease	Ref.	Patients	Other treatments	DLI dose (per kg)	Responses	Survival
ALL	[28]	22	17 patients received chemotherapy	$2.9\ (0.3–11)\times 10^8$	CR in 9 patients after chemotherapy, 0 % CR after DLI alone	12 % AT 1 year
	[27]	15	Four patients received chemotherapy	$1–8.2\times 10^8$	CR in 18.2 %	18 % at 1.5 years
	[29]	7	Three patients in CR prior to DLI	0.9×10^8	Two out of four not in CR after chemotherapy went into CR	25 % at 3 years
	[34]	44	28 patients received chemotherapy	$0.01–8.8\times 10^8$	5 of 16 patients not receiving chemotherapy had CR	12.5 % at 2 years for patients receiving DLI
	[31]	23	None	2.1×10^8	CR in 25 %	5 % at 2 years
	[35]	10	All patients received chemotherapy	$2.9–7\times 10^8$	CR in 70 %	
Lymphoma	[36]	22/26 relapses	16/26 relapses treated with chemotherapy	$0.01–1.0\times 10^8$	CR in 77 %	Not provided
	[37]	14	Eight patients received chemotherapy	$0.01–1\times 10^8$	CR in 57 %	35 % at 2 years
	[38]	17	Seven patients received chemotherapy	$0.01–1\times 10^8$	CR in 76 %	88 %
Multiple myeloma	[27]	5	None	$1–8.2\times 10^8$	CR in 50 %	40 % at 2 years
	[39]	13	None	$0.01–3.3\times 10^8$	CR in 31 %	5–38 months
	[40]	27	13 patients received chemotherapy	$0.01–5\times 10^8$	CR in 30 %	40 % in 5 year
	[41]	25	Four patients received chemotherapy	$0.02–5.55\times 10^8$	CR in 28 %	48 % at 1 year
	[42]	63	None	$0.01–3\times 10^8$	Response seen in 38 %	50 % at 2 years

2.1.3 Other Hematologic Malignancies Including ALL, NHL, Myeloma, CLL, and HD

Pre-B cell or B cell ALL appear to respond even less favorably to DLI than myeloid leukemias perhaps due to the rapid proliferation of the leukemia cells and a variety of immunologic escape mechanisms. Multiple studies showed very low response rates to DLI for relapses after either related or unrelated SCT even when chemotherapy was given before DLI with survival rates of less than 20 % at 1–2 years of follow-up [27–29, 31, 34, 35].

For lymphoma patients with relapsed or progressive disease after allogeneic SCT, DLI with or without chemotherapy resulted in about 50–70 % response rates, but published studies are limited to relatively small number of patients [36–38]. Serial PET scanning after allogeneic SCT may allow more selective and earlier application of DLI leading to higher response rates [36]. In aggregate, these studies indicate that indolent lymphomas may have a better response rate to DLI compared to aggressive lymphomas. Myeloma also appears to be amenable to DLI with response rates of about 30–50 % and survival rates of about 40 % at 1–5 years of follow-up [27, 39–42]. For patients who achieve a PR after DLI, the median progression-free survival (PFS) is only about 7 months while for patients who achieve a CR, the median PFS is about 2 years [42]. Even so, the majority of patients who receive DLI for post-allogeneic SCT relapses of myeloma eventually develop disease progression.

There is very limited published experience for the use of DLI in patients with relapsed chronic lymphocytic leukemia (CLL) or Hodgkin's disease (HD). In one study of DLI for residual or relapsed lymphoid neoplasms after allogeneic SCT, three of four patients with CLL had a CR after DLI [44]. A CLL patient was among the 8 patients (of 18) who achieved a CR after receiving anti-CD3/anti-CD28 costimulated or "activated" DLI for relapsed disease, and this patient remained in CR for 53+ months [45]. Also, new and expanded $CD8^+$ T-cell clonotypes were demonstrated in serial peripheral blood samples taken from a CLL patient who received DLI for recurrent CLL, and the emergence of these clonotypes coincided with disease remission [46]. An anecdotal experience may also be illustrative: A patient with fludarabine-resistant CLL relapsed with nodal disease about 1 year after an unrelated allogeneic SCT. After no response to treatment with rituximab and lenalidomide, the patient received 1×10^7 $CD3^+$ T-cells/kg body weight from the unrelated donor and had a partial response. About 4 months later, a second DLI of 2.8×10^7 $CD3^+$ T-cells/kg body weight was administered. About 1 month later, acute skin GVHD developed which required a course of glucocorticoids. Coincident with the clinical GVHD, the lymphadenopathy regressed and a complete response ensued which has been sustained for more than 2 years. Despite limited data, it is fair to conclude that DLI has the potential to reinduce long-lasting clinical remissions for select patients with recurrent CLL after allogeneic stem transplantation. Data regarding the efficacy of DLI for recurrent HD after allogeneic SCT is also very limited. One study reported on 9 HD patients who received a median of

7.75×10^7 $CD3^+$ T-cells (range 0.5–28.5) [47]. The response rate was 44 % (4/9) with a median duration of 7 months (range 4–9). Three of the four responders developed GVHD and also received pre-DLI chemotherapy. The role of DLI for relapsed HD remains unclear.

2.1.4 Preemptive DLI in Hematologic Disease

The role of preemptive DLI in patients with hematologic malignancies was explored in a prospective study of 82 patients with a variety of hematological malignancies (AML, ALL, CML, and MDS) who were considered to be at high risk of relapse after partially T-depleted allogeneic SCT [48]. DLI was given prophylactically to 31 patients at a median of 22 weeks after transplantation. The first six patients received 0.7×10^8 CD3+ cells/kg body weight with five patients developing acute GVHD. The next 25 patients received a dose of 0.1×10^8 CD3+ cells/kg with eight patients developing acute GVHD and three patients developing limited chronic GVHD. The projected 3-year probability of disease-free survival was 77 % for the 35 patients who were eligible for DLI and 45 % for the 47 patients in the comparison group who were considered to be at high risk for relapse but did not receive DLI due to previous grade 2 or higher acute GVHD and/or chronic GVHD ($P = 0.024$). The relapse rate at 36 months after transplantation was 18 % in the patients who were eligible for treatment with DLI and 44 % in the comparison group ($P = 0.026$). Thus preemptive, low-dose DLI may be a worthy option for patients who are considered to be at high risk for relapse.

2.2 Strategies for Optimizing Clinical Benefit of DLI and Minimizing the Risks

2.2.1 Dosing of DLI

It has been postulated that a dose level or a range of T-cells exists which can induce disease remission without triggering GVHD. This dosing window is likely influenced by the type of hematological malignancy (indolent vs. aggressive) as well as the donor source (matched sibling vs. unrelated donor). One study included 22 patients with relapsed CML after SCT who were treated with escalating doses of DLI ranging from 1×10^5 to 5×10^8 $CD3^+$ T-cells/kg (8 dose levels) at a median of every 6 weeks (4–33 weeks) between infusions [49]. Remissions were seen at T-cell doses at or above 1×10^7 $CD3^+$ T-cells/kg. Nineteen of the 22 patients achieved disease remission (most became PCR negative) with 8 patients receiving just 1 dose of 1×10^7 CD3/kg, and only 1 patient out of these 8 developed chronic GVHD. However, 8 out of the 11 patients who responded and received a T-cell dose of $\geq 5 \times 10^7$/kg developed GVHD. Neither GVL nor GVHD effects were evident at T-cell doses

below 1×10^7 $CD3^+$ T-cells/kg. This study demonstrated that the incidence of GVHD correlated to the T-cell dose and for CML, the graft-vs.-leukemia effect can be partially separated from clinically significant GVHD. A large multicenter retrospective study evaluated three dosing regimens of less than 0.2, 0.2–2, and greater than 2×10^8 mononuclear cells/kg in 298 patients with CML and found no difference in response rates at these dose levels, but the incidence of GVHD was lower in patients who received the lower initial dose [50]. Another non-randomized study examined the effect of a bulk dose DLI regimen (BDR) vs. an escalating dose DLI regimen (EDR) in 48 patients with cytogenetic or hematologic relapse of CML after SCT. Twenty-eight patients received the BDR at a median of 1×10^8 cells/kg, whereas 20 received the EDR using a median total cell dose of 1.9×10^8 cells/kg starting at 1×10^7 cells/kg for HLA-related donors and 1×10^6 cells/kg for HLA-unrelated donors. The median interval between the sequential DLIs was 20 weeks. There was no statistical difference in the response rates of the two cohorts (67 % in the BDR and 91 % in the EDR); however, grade II–IV GVHD was seen in 45 % of the BDR compared to 10 % in the EDR. This study implies that a low dose of DLI followed by graduated dose escalation may be the preferred strategy for patients with CML and possibly other hematological malignancies if tumor growth kinetics allow.

2.2.2 Combination of DLI with Chemotherapy or Other Antineoplastic Agents

In addition to lowering the disease burden that must be targeted by the T-cells, pre-DLI cytoreductive chemotherapy may also deplete residual host T-cells and help create "immunological space" for the donor cells to expand. This is potentially a more effective approach for patients who are relatively resistant to DLI alone such as those with acute leukemia or advanced CML. In a prospective trial of 65 myeloid leukemia patients with hematologic relapse after HLA-matched BMT 65 patients were prospectively treated with cytarabine at a dose of 100 mg/m^2/day for 7 days and daunorubicin at 30 mg/m^2/day for 3 days followed by G-CSF-primed DLI at 10–14 days after the initiation of chemotherapy [51]. A complete response was seen in 27 patients albeit with treatment-associated mortality of 23 %. The overall survival was 19 % at 2 years. This study did not appear to show any increased incidence of GVHD with cytoreductive chemotherapy and DLI. In contrast, a small study in which 15 patients with relapsed non-CML malignancies who received cyclophosphamide at a dose of 50 mg/kg on day −6 and fludarabine 25 mg/m^2 for 5 consecutive days from −6 to −2 followed by DLI (1×10^8/kg) 48 h after the last dose of fludarabine were compared to 63 control patients who received DLI without chemotherapy suggested that cytoreductive therapy might contribute to worsening of GVHD [52]. All the patients who received chemotherapy developed lymphodepletion to promote donor lymphocyte expansion and a more effective graft-vs.-tumor effect but also developed significant acute GVHD. Mortality in the DLI-only group was due to either persistent disease or disease recurrence with only 5 % of deaths

due to GVHD. On the other hand, 5 of 11 deaths (45 %) in the chemotherapy + DLI group were attributed to GVHD, leading to premature termination of the study.

Another phase I–II study investigated the effect of low-dose thalidomide (100 mg/day) followed by DLI in 18 myeloma patients after allogeneic SCT with progressive or residual disease and previous failure of DLI alone. Complete remission was seen in 22 % of the patients with an overall response rate of 67 %. Two patients developed grade I acute GVHD of the skin, and two patients had chronic GVHD. This study indicated that low-dose thalidomide and DLI may have a clinically significant synergistic effect with a low incidence of GVHD [53].

2.2.3 DLI with CD8 Depletion

Earlier studies suggested that cytotoxic $CD8^+$ T-cells are the principal effectors of GVHD, therefore leading to studies of $CD8^+$-depleted stem cell grafts and eventually $CD8^+$-depleted DLI for disease relapse after SCT. A notable study included 40 patients with relapsed hematologic malignancies after SCT, who were treated with $CD8^+$-depleted DLI at 0.3, 1.0, and 1.5×10^8 $CD4^+$ cells/kg dose levels [54]. Fifteen of 19 patients (79 %) with early-phase relapsed CML responded to treatment, whereas 5 of 6 patients (83 %) with relapsed multiple myeloma and 1 patient with myelodysplasia also developed a response. Complete cytogenetic remission was seen in 87 % of CML patients, and a complete molecular response was seen in 78 % at 1 year after receiving DLI. Two CML patients who did not show a response at dose level 1 later achieved complete cytogenetic remission after a second infusion of CD8-depleted cells at dose level 2. All the patients who developed GVHD demonstrated tumor regression, but 48 % of patients who responded to treatment never developed GVHD. Acute GVHD was evident in 24 % of the patients, while chronic GVHD was seen in 16 %, with only one death due to either GVHD or infection. Also noted in this study was a delay in time to development of GVHD and disease response (median of 11 weeks) when compared to conventional DLI. Due to the relatively low risk of toxicity associated with the infusion of defined number of CD4(+) donor cells, further studies may be warranted to prevent relapse after allogeneic BMT in the setting of persistent minimal residual disease.

Another small randomized trial involving the administration of conventional DLI versus CD8+-depleted cells was conducted in patients with disease remission in an effort to prevent relapse [55]. Acute GVHD developed in six of the nine patients (67 %) undergoing conventional DLI as opposed to no cases of acute GVHD among nine patients receiving CD8-depleted DLI. In the CD8-depleted cohort, there were no toxic deaths and only one relapse. T-cell recovery patterns evaluated by T-cell receptor spectratyping were similar in both groups. This study showed that CD8-depleted DLI led to immune-mediated tumor responses without significant GVHD. Although CD8 depletion appears to reliably reduce GVHD, whether CD8+-depleted DLI will ultimately prove equally effective as a means of inducing GVL is not yet known.

2.2.4 DLI Using Lymphocytes Engineered to Express "Suicide Genes"

Investigators have sought to genetically engineer donor lymphocytes to express thymidine kinase (TK) "suicide" genes which can mediate lymphocyte inactivation upon exposure to ganciclovir. The thymidine kinase encoded by the herpes simplex virus type 1 phosphorylates ganciclovir to an active metabolite which inhibits DNA synthesis and causes cell death. Incorporation of the HSV TK gene into T-cells can lead to the killing of actively dividing cells particularly when these cells are mediating serious GHVD. In one study, 23 patients received TK gene-transduced donor T-cells for relapse of malignancy after SCT and 11/17 evaluable patients had significant clinical benefit including 6 complete responders [56]. Seven patients received ganciclovir which eliminated the TK+cells and appeared to selectively treat the GVHD.

3 Of Mice, Men, and Melanoma: Lessons from Mouse and Human Models of Autologous Immunotherapy

Autologous immunotherapy of cancer can be categorized into three major strategies: (1) general immune cell activation (e.g., IL-2 administration) based on the notion that tumor-directed T-cells exist in the patient but in an inactive state which can be overcome through pharmacologic manipulation; (2) active immunization of the patient with tumor-associated antigen vaccines designed to specifically elicit T-cell and or B-cell responses against the tumor; and (3) adoptive T-cell therapy (ACT) in which autologous T-cells are first removed from the tumor-bearing patient, then otherwise activated, expanded and/or genetically modified to enhance functionality, and then transferred back to the patient to attack the remaining cancer cells. As "stand-alone" therapies, the first two strategies have thus far yielded limited clinical benefits with an objective response rate of 3.3 % among more than 1,300 patients who received a variety of cancer vaccines both at the NIH Surgery Branch and in the published literature [57, 58]. In contrast, ACT has been shown to induce regression of cancer in 50–70 % of patients with advanced and refractory malignancy [59, 60] and offers the potential for sustained responses and application to a wide variety of human cancers.

Much of the early work and success in the field of autologous T-cell immunotherapy were focused on patients with advanced melanoma and EBV-driven tumors including lymphoma and nasopharyngeal carcinoma. Several important principles which would likely apply to the treatment of hematological malignancies with cellular immunotherapy have emerged from this body of work and are summarized below.

3.1 The Importance of Lymphodepletion Before Adoptive Transfers

In order to kill tumor cells in the patient, T-cells must (1) be present in sufficient number, (2) possess adequate affinity for the tumor antigen target, (3) traffic to the tumor bed, and (4) exert a cytotoxic effect on the cancer cells. In addition to the depletion of immune cells which usually accompanies repeated courses of chemoradiotherapy for cancer, a major impediment to effective cellular immunotherapy of cancer is the profound suppression of antitumor reactivity that occurs when T-cells encounter the tumor microenvironment. Indeed, a transgenic murine model in which >95 % of the CD8 cells were specific for a melanoma target antigen (gp100) failed to suppress growth of gp100+ melanoma tumors [61]. Early efforts to isolate, expand, and reinfuse tumor-infiltrating lymphocytes (TILs) to treat metastatic melanoma used either no preparative regimen or low-dose cyclophosphamide (25 mg/kg) yielding objective responses in about 30 % of patients, most of which were short-lived [62, 63]. Based on animal models that suggested that the results of ACT might be improved following more effective lymphodepletion, a series of consecutive trials were conducted that utilized increasingly intensive chemoradiotherapy. Using higher dose cyclophosphamide plus fludarabine (FluCy), FluCy plus low-dose (2 Gy) total body irradiation (TBI), and FluCy plus high-dose TBI (12 Gy), the rate of objective clinical responses after adoptive transfer of about 10^{10}–10^{11} tumor-reactive cultured TILs increased progressively to 49, 52, and 72 %, respectively, by RECIST criteria [64]. Furthermore, responses occurred in a variety of tissues and organs, and the majority of complete responses were durable. The mechanisms whereby intensive lymphodepletion leads to improved survival and clinical impact of adoptively transferred T-cells include (1) liberation of γ_c cytokines including IL-7, IL-15, and IL-21 from "sinks" associated with T/NK cell populations, (2) depletion of CD4+CD25+ regulatory T-cells (Tregs), and (3) enhanced tumor antigen presentation through tumor cell apoptosis and antigen-presenting cell (APC) activation [65, 66]. Whether lymphodepletion and the so-called homeostatic expansion should be routinely incorporated to augment adoptive T-cell transfer strategies requires additional study.

3.2 The Importance of Memory for Optimal ACT

While CD8+ cytotoxic T-cells appear to be the principal actors in the response to ACT, CD4+ T-cells likely provide critical help for CD8+ cells through elaboration of growth factors such as interleukin-2 (IL-2) and IL-21 and expression of CD40-ligand [67–71]. In a cellular vaccine model, CD4+ T-cells also played a broader role in orchestrating an effective antitumor response through recruitment of eosinophils and macrophages. Indeed anecdotally at least one patient with metastatic melanoma achieved a long-term complete remission after infusion of autologous CD4+ T-cell

clones that recognized the cancer–testis antigen (CTAg) NY-ESO-1 [72]. The major subsets from which CD8+ T-cells for ACT can be drawn include naïve T-cells (T_N) and memory T-cells (T_M) which can be separated into central memory (T_{CM}) and effector memory (T_{EM}) populations that exhibit distinctive phenotypes, homing properties, and function [73]. CD8+ T_{CM} cells express CD62L and CCR7 which cause homing to lymph nodes, and they activate and expand rapidly upon secondary exposure to cognate antigen. CD8+ T_{EM} cells are negative for CD62L, circulate to infected or inflamed tissues, and more rapidly exert effector functions upon antigen reexposure. Both types of CD8+ T-cells can generate potent effector T-cells (T_E) which kill tumor targets through lytic mechanisms that involve granzyme and perforin release. While highly cytolytic effector cells may exert more potent antitumor activity, memory CD8+ T-cells appear to be the preferred choice for ACT due to higher proliferation potential and survival in vivo [74]. Furthermore, in a primate model, adoptive transfer of effector CD8+ T-cells derived only from CD8+ T_{CM} persisted for a long term, reestablished a memory pool, and responded to rechallenge with a viral (CMV) antigen [75]. However, naïve CD8+ T (T_N)-cells possess characteristics such as higher CD27 expression and longer telomeres that may make them more suitable for ACT when using genetically modified T-cells which have been engineered to recognize and react to tumor targets [76]. The optimal T-cell subpopulations for adoptive transfer have not yet been definitively characterized, and protocols for in vitro expansion and differentiation have not been optimized for clinical use. Improved understanding of T-cell maturation and memory should help further improve ACT protocols.

It should also be noted that while most of the clinical experience of ACT for melanoma has involved TILs, antigen-specific CD8+ T-cell clones derived from the peripheral blood have also yielded durable objective clinical responses [77]. The ability to use tumor antigen-specific peripheral blood lymphocytes for ACT may expand the clinical reach of this form of immunotherapy to the significant proportion of patients whose tumors do not yield adequate TILs for culture and cloning. Recent studies using short-term cultures of enriched but unscreened (for tumor reactivity) CD8+ TILs may also simplify and accelerate the procedure for preparing TILs for successful ACT without sacrificing the high rate of objective responses observed in melanoma patients (50–60 %) [78, 79].

3.3 ACT Can Mediate Regression of Large Tumors: Strategies for Augmenting Responses

An important but perhaps unexpected lesson from studies in melanoma is that ACT can induce regression of very large tumor masses that are well vascularized and metastatic to multiple organs including the lung, liver, adrenal glands, muscle lymph nodes, and skin [64, 80]. Indeed, analysis of large ACT experiences has revealed little or no correlation between tumor bulk and clinical response [81]. Anecdotally, our group has also observed dramatic—albeit transient—regression of advanced,

refractory myeloma with nearly 100 % replacement of marrow cellularity by malignant plasma cells and plasmablasts in a patient who received about 5×10^{10} ex vivo costimulated autologous T-cells (unpublished observations). Serial marrow examinations over a period of about 5 weeks showed a progressive decline in marrow plasmacytosis from 100 to 15 % accompanied by a progressive increase in marrow-infiltrating CD8+ T-cells from <5 % to more than 70 %. Taken together, these observations appear to challenge the prevalent notion that cancer immunotherapy is primarily effective for patients with minimal residual disease or only applicable to the adjuvant setting. Factors that correlate to better clinical responses after ACT include long-term persistence of the transferred cells, longer telomere length, and re-expression of CD27 [80]. CD27 expression is a molecular feature which is associated with increased proliferation, IL-2 production, and more resistance to apoptosis of CD8+ T-cells in HIV-infected patients [82]. In a murine model of ACT for large tumors, higher T-cell dose, a T_{CM} phenotype, and post-transfer administration of IL-2, IL-7, IL-15, or IL-21 also predicted better tumor responses [83]. In another murine model, administration of antiangiogenic agents such as vascular endothelial growth factor (VEGF) antibody or VEGFR2 (VEGF-receptor) antibodies increased responses to ACT due in part to increased access of the transferred T-cells to the tumor bed [84].

3.4 ACT Can Be Used to Treat Viral Infections in Immunocompromised Hosts (e.g., EBV) and EBV-Driven Neoplasms

Another important application of adoptive T-cell transfer is in the treatment or the prevention of viral infections which arise as a result of loss of immune surveillance in patients who become severely immunocompromised in the course of intensive chemotherapy and/or allogeneic stem cell transplantation. For example, infusions of EBV-specific cytotoxic T lymphocytes (CTLs) generated through gene transfer led to durable (18+months) immunity against viral challenges [85]. CMV-specific T-cells which were generated by repetitive ex vivo stimulation with CMV antigen led to clearance of CMV viremia in 5/7 evaluable patients who had not responded to antiviral chemotherapy [86]. Newer culture techniques have extended this form of therapy to post-transplant adenoviral infections as well [87]. Given that EBV can cause life-threatening lymphoproliferative disorders after allogeneic stem cell transplantation including up to 25 % of pediatric recipients of T-cell-depleted unrelated or HLA-mismatched donor transplants, EBV-specific CTLs have also been tested in this setting. An early study of 39 patients who were at high risk for EBV-induced lymphoproliferative disorders received 2–4 infusions of polyclonal donor-derived T-cells that were selected and cultured for anti-EBV activity [88]. Six patients with high levels of EBV-DNA had 2–4 log reductions in viral DNA, and none developed lymphoma while two patients who did not receive EBV CTLs and

subsequently developed lymphoma exhibited complete responses after T-cell therapy. EBV-specific CTLs were successfully derived from 11 of 15 patients with relapsed EBV + Hodgkin disease and generated temporary clinical responses in 2 of 3 treated patients [89]. Immunoassays from this early study indicated that LMP2 was a frequent target of these CTLs and could elicit homing to tumors. Using gene-marked CTLs raised against EBV-transformed autologous lymphoblastoid cell lines as APCs and a novel strategy for accelerated expansion, 14 patients with relapsed HD were treated with ACT leading to complete responses in five patients, two of whom had measurable disease prior to cell transfer and remained in remission for >9 months and >27 months [90]. Five additional patients exhibited stable disease, and studies of the gene-marked cells clearly showed trafficking of the CTLs to tumor sites. The frequency of LMP2-directed CTLs could be increased about 100-fold by using LMP2 gene-modified APCs as stimulator cells, and these LMP2-specific and expanded CTLs were used to treat 16 patients with EBV + HD or NHL [91]. Nine of ten patients who were treated while in remission remained free of disease, while five of six patients with active disease just prior to ACT had an objective tumor response by RECIST criteria, four of which were complete and sustained for >9 months. One notable patient with marrow involvement with chemotherapy-resistant HD remained in remission for >34 months. ACT with donor-derived viral antigen-specific CTLs has also been used in the allogeneic transplant setting to treat reactivations of EBV, CMV, or adenovirus as well as EBV-driven lymphoproliferative disorders in 153 recipients while incurring acute GVHD in 6.5 % of patients, all of whom had earlier episodes [92]. Notably, there were no differences in the frequency of GVHD between patients who received CTLs from HLA-matched vs. HLA-mismatched donors. At least one patient with protracted and drug-resistant CMV encephalitis had viral suppression and clinical improvement after receiving graduated doses of unmanipulated donor lymphocytes while developing only grade II skin GVHD after the fifth infusion which was steroid responsive [93].

3.5 Safety and Tolerance of T-Cell Infusions

Adverse events early after infusions of autologous T-cells for ACT are generally mild and infrequent. The Baylor group recently conducted a review of 381 T-cell products given to 180 patients who were enrolled in 18 clinical trials over a 10-year period [94]. These patients received ex vivo-expanded T-cells that were selected and cultured for tumor or viral antigen specificity and/or were gene-modified. No grade 3–4 infusion reactions were identified during 24 h of observation after infusion. About 12.5 % of patients had grade 1–2 reactions within 24 h of infusion, including nausea/vomiting, hypotension, pain, dyspnea or hypoxia, fever, and chills. It should be noted that the cell doses in these studies were generally low (from 10^4/kg body weight up to $2 \times 10^8/m^2$). Early and later adverse effects of activated and expanded autologous T-cell transfers appear to be more frequent and more clinically significant in patients who receive higher T-cell doses, undergo more intensive

lymphodepletion (e.g., high-dose chemotherapy for autologous stem cell transplants), and/or receive T-cell products which are genetically modified to introduce new target specificities and functional properties. For example, a patient with bulky CLL died from multiorgan failure after receiving gene-modified T-cells engineered to express a chimeric antigen receptor (CAR) which recognized CD19, a common normal B-cell and B-cell lymphoma antigen [95]. A second patient with colon cancer metastatic to the lungs developed fulminant respiratory failure within 15 min of receiving T-cells which had been genetically modified to express a CAR that recognized ERBB2—the tumor-associated antigen which is targeted by the widely used monoclonal antibody Trastuzumab (Herceptin®) and died 5 days later [96]. The intracellular portion of this CAR contained signaling domains derived from CD28, CD3ζ, and 4-1BB which likely provided a strong activation and proliferation signal after antigen encounter. Additional toxicities associated with ACT in hematological neoplasms are discussed in later sections. Caution and vigilant clinical monitoring are clearly warranted for any T-cell products or T-cell stimulants that are being newly tested in humans. Even preclinical models including nonhuman primates failed to predict the nearly fatal widespread T-cell activation and cytokine storm that occurred after giving a superagonistic anti-CD28 monoclonal antibody (TGN1412) to normal human volunteers [97]. Historically, ACT has been avoided in patients with known brain metastases due to safety concerns and uncertainty about whether tumor-directed T-cells could successfully cross the blood–brain barrier. However, a recent analysis of 264 patients with metastatic melanoma who received ACT at the NCI Surgery Branch retrospectively identified 26 patients who had both untreated brain metastases and extracranial disease prior to ACT [98]. Seven of seventeen patients (41 %) who received TILs had a complete response in the brain accompanied by partial extracranial responses in six, while two of nine patients (22 %) who received gene-modified T-cells had a complete response, one of whom also had a partial extracranial response. One patient developed a subarachnoid hemorrhage in a brain tumor while thrombocytopenic but was successfully treated by resection. These data suggest that brain metastases are not beyond the reach of ACT and should not necessarily be a basis for routine exclusion from treatment. A recent trial involving transfer of CAR-modified T-cells into pediatric patients with relapsed childhood ALL shows that modified cells may cross the blood–brain barrier and further raises the possibility of ACT efficacy against CNS disease as well as the potential for CNS toxicity.

3.6 ACT with Gene-Modified T-Cells Is Effective and Potentially Widely Applicable

Despite the great promise of ACT and its demonstrated ability to induce regression of tumors in patients with advanced melanoma, there are at least two important limitations of this approach: (1) patients must have relatively large tumors from which TILs can be isolated and expanded; this procedure occurs successfully in about

50 % of eligible patients, and (2) in many other forms of cancer, tumor-reactive T-cells are much more difficult to identify, isolate, and expand. Work by Eshhar and others has shown that T-cells can be genetically engineered to express novel antigen recognition receptors composed of the variable binding domains of an immunoglobulin molecule fused to the constant, signaling domains of the T-cell receptor (TCR) [99]. These "chimeric TCR"- or "CAR"-expressing T-cells then become functionally redirected to the specific antigen which is recognized by the immunoglobulin portion of the molecule and can proliferate and mediate non-MHC-restricted cytotoxicity against cells expressing the antigenic target. An alternative approach is to isolate and clone native TCRs or generate "affinity-enhanced" TCRs for a specific tumor antigen epitope and then genetically modify T-cells to express these native or affinity-enhanced TCRs in order to redirect them to tumor cells that are known to express the tumor antigen target. This latter approach will be limited to patients who carry the HLA antigens (usually A-0201, A01, or other relatively common class I antigens) which are recognized by the TCRs. Advances in vector technology, specifically the advent of lentiviral vectors which can efficiently target both dividing and nondividing lymphocytes, have facilitated the recent clinical testing and development of these technologies.

Using a retroviral vector which was optimized to express the alpha and beta chains of an anti-MART-1 TCR, HLA-A0201$^+$ patients with refractory, metastatic melanoma received ACT with gene-modified autologous T-cells [100]. Among 15 patients who received short-term cultured cells (6–9 days of ex vivo stimulation with anti-CD3 antibody), all showed strong persistence of gene-modified cells with engraftment levels above 10 % of peripheral blood lymphocytes for 2 months or more after infusion. Two patients who had rapid progression of disease prior to ACT had partial responses by RECIST criteria which were sustained at 21 and 20 months of follow-up. Both of these patients had high levels of gene-marked cells at 1 year post treatment as well as evidence of proliferation in the peripheral blood. An emerging principle from both tumor and viral immunology is that higher avidity interactions between T-cells and target antigens may lead to more effective immune responses [101, 102]. In an effort to increase the affinity of native tumor antigen-specific TCRs for their target antigens, one or two amino acid substitutions have been introduced into the complementarity determining regions (CDRs) of TCRs for MART-1 (amino acids 27–35) and the CTAg NY-ESO-1 (amino acids 157–165), leading to enhanced TCR function without apparently sacrificing binding specificity [103]. A clinical trial of ACT using gene-modified autologous T-cells which were engineered to express an affinity-enhanced TCR for the NY-ESO-1 CTAg enrolled 17 patients with metastatic synovial cell sarcoma and melanoma [104]. Objective clinical responses by RECIST criteria were observed in 4/6 sarcoma patients and 5/11 melanoma patients, including 2 complete responses in the latter group which persisted for more than 1 year. Based on murine models, a safety concern that has been raised about the use of TCR gene-modified T-cells is the occurrence of serious autoimmune complications which may arise from the generation of new (self-directed) TCR specificities that result from mixed pairing of exogenous (transferred) and endogenous TCR chains [105]. However, no cases of GVHD nor

autoimmune pathology have been observed in more than 100 patients who received gene-modified T-cells that were engineered to express a variety of tumor antigen TCRs derived from both human and mouse origin [106]. This disparity again highlights the potential limitations of using animal models to predict the toxicities (and efficacy) of immunotherapeutic interventions in humans.

4 Clinical Studies of ACT for Hematologic Malignancies

4.1 Background and General Principles

Extensive rationale has led to the ongoing testing of ACT in the setting of hematologic malignancies. Immune cell depletion after chemotherapy, especially high-dose chemotherapy or radiotherapy, can be prolonged and leads to an increased risk for infections [12–14]. In addition, higher lymphocyte levels may be associated with lower rates of relapse and higher rates of survival after allogeneic or autologous stem cell transplantation for hematologic malignancies [107, 108]. Indeed one study of AML patients who received allogeneic transplants showed a 3-year likelihood of relapse of 16 % if the absolute lymphocyte count (ALC) was >200 cells/μl at day +29 vs. a relapse rate of 42 % for patients who exhibited an ALC of ≤ 200 cells/μl [107]. In a second study of allogeneic bone marrow transplantation for a variety of hematologic malignancies, the overall survival was 79 % at 1 year for patients who had an ALC at day 17 of ≥ 500/μl vs. 19 % for patients with an ALC <500 cells/μl ($p=0.002$) [108]. Porrata and colleagues examined 230 autograft recipients with myeloma or NHL and showed that day-15 ALC correlated to overall survival [15]. For 126 myeloma patients, an ALC ≥ 500 on day 15 was associated with median overall (OS) and progression-free survivals of 33 and 16 months, respectively, while an ALC <500 was associated with an OS of 12 months ($P<0.0001$) and a PFS of 8 months ($P<0.0003$) [15]. Among 104 NHL patients the median OS and PFS durations were also significantly longer for patients with an ALC of 500 cells/μl vs. patients with an ALC <500: For OS, not reached vs. 6 months, $P<0.0001$; for PFS, not reached vs. 4 months, $P<0.0001$. Additionally in this study, multivariate analysis revealed that the day-15 ALC level was an independent predictor of OS and PFS.

ACT may result in more predictable and robust patterns of immune cell recovery. However, initial or induction chemotherapy for hematological malignancies often results in profound immune cell depletion which may impair the ability to collect sufficient number of lymphocytes for transfer. Early studies explored whether ex vivo stimulation and expansion of patient-derived lymphocytes followed by adoptive transfer might influence in vivo immune recovery. An early phase I study of CD4+-enriched peripheral blood mononuclear cells (PBMCs) which were expanded ex vivo using anti-CD3 antibody for 4 days and then transferred into 31 patients who then received IL-2 for 7 days demonstrated a statistically significant increase in

CD4+ T-cells, CD4+ subsets, and CD4+/CD8+ ratio [109]. This study included 4 lymphoma patients and 14 melanoma patients, some with subcutaneous tumors to which 111-indium-labeled CD4+ T-cells showed trafficking. Another phase I trial included eight patients with various solid tumors who received multiple infusions of T-cells which were costimulated using anti-CD3/anti-CD28-coated bead cells in the presence of IL-2 [110]. PBMCs from recipients of the bead-costimulated T-cells showed enhanced production of interferon-γ and GM-CSF indicating possible functionality. However tumor responses in both of these studies were infrequent and partial perhaps due to inadequate lymphodepletion prior to T-cell transfers resulting in persistence of T regulatory cells and myeloid suppressor populations. In addition, T-cell responses against specific target antigens could not be evaluated.

In order to be useful in the treatment of hematological malignancies, ACT must likely enhance both T-cell numbers and function. Recent developments in the technology of ex vivo T-cell expansion have allowed about 100-fold expansion of lymphocytes obtained by leukapheresis, enabling even heavily pretreated patients to receive this form of therapy. While isolation and repetitive stimulation of tumor antigen-specific T-cells from peripheral blood or tumor samples may increase the likelihood of tumor recognition and targeting, the procedure is costly, labor intensive, and not infrequently unsuccessful. A second approach based on polyclonal stimulation of T-cells with immunomagnetic beads to which anti-CD3 and anti-CD28 monoclonal antibodies have been conjugated has consistently yielded high number of functional T-cells in support of numerous clinical trials. Key properties of this system are (1) the absence of "feeder" cell layers which facilitates conformity with FDA requirements, (2) ease of clinical scale-up to rapidly produce large number of mature T-cells, and (3) induction of telomerase to minimize the risk of replicative senescence [111, 112]. The rationale for this "polyclonal" approach is predicated in part on the notion that patient immune systems may already be "primed" to their tumors and that augmentation of this endogenous immune response will be clinically beneficial. Evidence for tumor priming seems to be particularly compelling in the area of hematological malignancies [23–25].

4.2 ACT Using Polyclonal T-Cell Populations

Early clinical applications of anti-CD3/anti-CD28-costimulated autologous T-cells to the treatment of hematological malignancies involved adoptive transfers after high-dose chemotherapy and autologous stem cell transplantation for patients with relapsed or refractory non-Hodgkin's B-cell lymphoma (NHL) and patients with CML who lacked a suitable donor for allogeneic transplantation. Sixteen patients with relapsed or refractory NHL received 2.5, 5.0, or 10×10^9 costimulated T-cells on day +14 after high-dose BCNU/cytarabine/etoposide/cyclophosphamide (BEAC) and CD34-selected autologous stem cell transplantation [113]. Five patients exhibited a delayed lymphocytosis between days 30 and 120 post transplant, and the procedure partially improved T-cell function as measured by IFN-γ production after

PMA or ionomycin stimulation. Four patients with chronic-phase CML participated in a small pilot study of anti-CD3/anti-CD28-costimulated T-cell transfers following autologous stem cell transplantation [114]. Three of the four patients are long-term survivors including one patient who remains in a complete molecular remission 13 years following autotransplantation, without having received any tyrosine kinase inhibitor therapy for her CML.

To test the feasibility of combining adoptive T-cell transfers with active immunizations and whether such a combined approach could induce vaccine-specific T-cell responses, a randomized trial was conducted in the setting of autologous stem cell transplantation for 54 patients with relapsed or refractory myeloma [115]. The selected vaccine was a heptavalent pneumococcal conjugate vaccine (®Prevnar, PCV) composed of saccharide antigens for the seven most common pneumococcal subtypes linked to a protein carrier (CRM-197) that is derived from diphtheria toxin. The choice of this vaccine allowed both antibody and T-cell responses to be evaluated, while the randomized design allowed different schedules of vaccination (pre- and post-transplant vs. post-transplant only) and T-cell infusion (day +12 vs. day +100 post transplant) to be compared. The optimal schedule (group 1) which yielded the most robust and only sustained antibody responses to the pneumococcal saccharide antigens and the most robust and sustained T-cell responses to the carrier protein was the following: pre-transplant immunization about 10 days before steady-state T-cell collection → early infusion of vaccine-primed and anti-CD3/CD28 antibody-costimulated autologous T-cells at day +12 post transplant → post-transplant booster immunizations at days 30 and 90 post transplantation. The T-cell/vaccine schedules for the other treatment groups including patients who did not receive costimulated T-cells until day +100 (groups 2 and 4) or received no priming (pre-transplant) vaccination (group 3) yielded significantly lower levels of vaccine-directed immune responses which were short-lived. Figure 1 illustrates the flow of this combined vaccine and T-cell strategy. The patients who were randomly assigned to receive up to 1×10^{10} costimulated T-cells at day +12 post transplant had significantly higher CD4 and CD8 T-cell counts at day +42 post transplant than the patients who received T-cells at day +100. In addition, only the patients who were randomized to receive pre- and post-transplant PCV immunizations along with the "early" day +12 infusion of costimulated T-cells generated and maintained protective levels of pneumococcal specific antibodies along with vaccine (CRM-197)-specific CD4+ T-cell responses as early as day +42 post transplant. This randomized pilot study provided convincing evidence that the severe quantitative and qualitative immune deficiencies which prevail after high-dose chemotherapy could be substantially rectified leading to clinically relevant immune function. A similar combination strategy using pre- and post-transplant immunizations using an influenza vaccine plus vaccine-primed and costimulated T-cells also proved effective for generating protective levels of anti-influenza antibodies early after autologous stem cell transplantation for myeloma [116].

To test whether this combination strategy of pre- and post-transplant immunizations plus early transfer of vaccine-primed and ex vivo-costimulated autologous T-cells could induce early immune responses to a cancer-related antigen, a

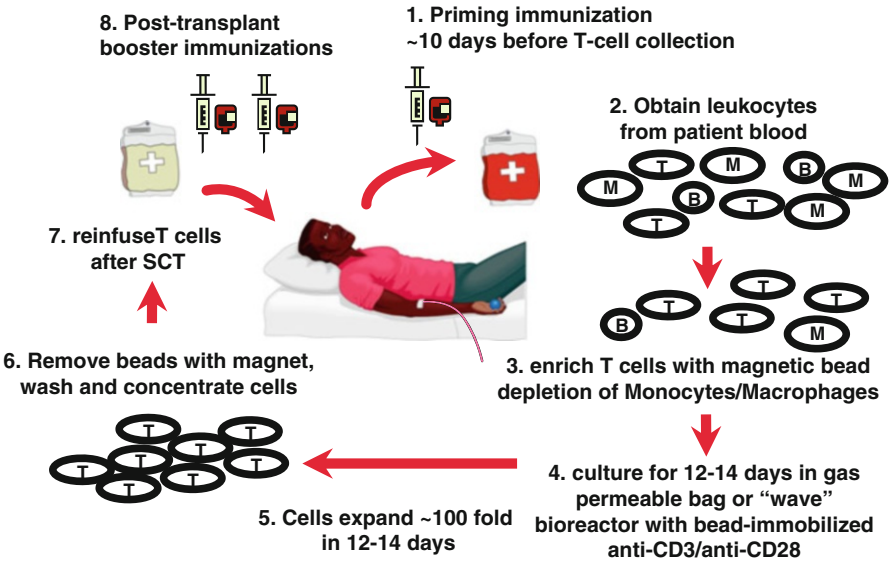

Fig. 1 Combination T-cell and vaccine immunotherapy for hematologic malignancies. Patients with hematologic malignancies who are candidates for autologous stem cell transplantation (ASCT) receive priming tumor and/or microbial antigen immunizations about 10 days before steady-state mononuclear cell collection from the peripheral blood (*1*,*2*). The mononuclear cells are enriched in T-cells by depletion of monocytes and macrophages which can inhibit ex vivo T-cell expansion (*3*). The T-cells are cultured for 12–14 days in gas-permeable bags or in a "wave" bioreactor system with anti-CD3/anti-CD28 monoclonal antibodies conjugated to immunomagnetic beads +/− low-dose IL-2 (*4*). The cells expand about 100-fold after which the magnetic beads are removed, the cells are concentrated, and then prepared for reinfusion after meeting release criteria for sterility and viability (*5*,*6*). Around day +2 after high-dose chemotherapy and autologous stem cell transplantation, patients receive the vaccine-primed and costimulated T-cell product. The product can be shipped fresh and infused on the same day or it can be viably frozen, shipped, and thawed/infused at a later time (*7*). Patients receive two or more post-transplant booster immunizations using the same tumor and/or microbial antigen vaccine that was administered earlier (*8*)

follow-up phase II two-arm trial was conducted using a tumor antigen vaccine composed of peptides derived from the human telomerase reverse transcriptase (hTERT) and the antiapoptotic protein survivin, two potential "universal" tumor antigens [117]. A total of 54 patients with myeloma were enrolled in this phase I/II study including 28 patients who were HLA-A2 positive and therefore eligible to receive the HLA-A2-restricted hTERT/survivin multipeptide tumor antigen vaccine. In an effort to further improve functional immune recovery this new study contained a variety of modifications: First, as a result of technical improvements in the T-cell expansion procedures, patients received up to 5×10^{10} costimulated T-cells which was ~5-fold higher than in the previous study. Second, costimulated T-cells were adoptively transferred on day +2 post transplant rather than day +12 to take better advantage of the stimulatory cytokine milieu induced by severe lymphopenia. Third, the multipeptide vaccine was emulsified in the adjuvant Montanide ISA 51

and coinjected with GM-CSF. Fourth, patients received a total of four vaccinations including one prior to T-cell collection and three vaccinations post transplant at days +14, +42, and +90. In this trial, 36 % of the A2-positive patients exhibited positive immune responses to the hTERT/survivin tumor antigen vaccine as assayed by tetramer analysis. Interestingly, the event-free survival for the group of A2-positive patients who received the tumor antigen vaccine was inferior to that observed in the A2-negative group although this difference appeared to be primarily due to a higher frequency of post-transplant maintenance therapy using thalidomide in the A2-negative (no vaccine) arm. This study also demonstrated that adoptive T-cell transfers resulted in significantly lower levels of regulatory T-cells (Tregs) and significantly higher Teffector/Treg ratios when compared to autograft recipients who did not receive T-cell transfers. Increased Teff/Treg ratios are associated with enhanced tumor necrosis in clinical trials involving immune modulation [118]. Non-myeloma polyclonal immunoglobulins appeared to recover more quickly and robustly in patients who received post-transplant T-cell transfers. Day +2 transfers of up to 5×10^{10} costimulated T-cells led to dramatically higher median CD4 and CD8 counts of about 1,500 cells/μl and nearly 3,000 cells/μl, respectively, at day +14 post transplant. Notably, ~16 % of patients also developed clinically significant autologous GVHD involving the gut and skin which required treatment with systemic glucocorticoids resulting in rapid and complete responses of the GVHD [119]. The patient who had the most severe case of autologous GVHD (grade II skin and grade III gut) remained in complete remission (CR) at 4 years post transplant despite enrolling in the study with advanced and treatment-refractory disease.

Strategies for increasing the frequency and potential clinical impact of post-transplant immune responses to a tumor antigen vaccine may include the use of more effective vaccine adjuvants to enhance priming and boosting of the T-cell responses as well as the incorporation of immunostimulatory drugs (e.g., lenalidomide, anti-CTLA4 antibodies, anti-PD1 antibodies). Along these lines, a recent study was conducted which included 27 patients who were autografted for myeloma. Using a similar pre- and post-transplant immunization scheme plus day +2 infusion of vaccine-primed and ex vivo-costimulated autologous T-cells, this study examined whether the addition of a toll-like receptor-3 (TLR-3) agonist called Poly-ICLC (®Hiltonol) to the vaccine formulation (in addition to GM-CSF and Montanide) would help elicit more robust immune responses [120]. The cancer antigen vaccine employed in this study was a multipeptide vaccine based on the CTAg called MAGE-A3. The vaccine (Orphan Drug Designation GL-0817) is composed of two HLA-A2-restricted class I epitopes and one relatively HLA-unrestricted class II epitope. Early clinical response rates have been encouraging, and importantly, 71 % of patients have exhibited functional vaccine-specific T-cell responses by IFN-γ production on CD4+ T-cells, CD8+ T-cells, or both. In this study, low-dose lenalidomide (10 mg per day) starting at day +100 post transplant was used as a maintenance drug and also as an immunomodulator based on extensive literature suggesting that it has immunostimulatory properties [121–123]. A recent randomized study also demonstrated that lenalidomide enhanced both B- and T-cell immune responses to the 7-valent pneumococcal conjugate vaccine (®Prevnar) in patients with

myeloma and appeared to increase myeloma-specific INF-γ-producing T-cells while decreasing Th-17 cells [124].

Potential drawbacks to using tumor antigen vaccines in order to generate tumor specificity are that the success of this approach depends on the existence of naturally occurring tumor-specific T-cell populations that are present in low frequency and even if expanded the T-cell receptors on these tumor antigen-specific T-cells are likely to exhibit low binding affinity as a result of normal T-cell ontogeny. Furthermore, the surface expression level of many tumor antigen epitopes is thought to be extremely low. In particular, the widely studied HLA-A2-restricted epitope NY-ESO$_{157–165}$ (SLLMWITQC), which is naturally expressed on primary myeloma cells, is estimated to have an expression density of only ~10–50 copies per cell, which is too low to activate conventional cytotoxic lymphocytes [125]. Some investigators have attempted to get around this problem by isolating and activating marrow-infiltrating lymphocytes ("MILs") from patients with myeloma which are akin to "TILs" in that these lymphocyte populations may be self-selected for enhanced tumor antigen specificity and affinity, although tolerized to the myeloma tumor by the immunologically suppressive microenvironment. Recent literature also suggests that the bone marrow is a specific homing site for effector memory T-cells, CD8+ memory cells being the preferred cell type for adoptive immunotherapy as discussed earlier [126]. Indeed, when T-cells were isolated from the marrow of myeloma patients and costimulated with anti-CD3/anti-CD28 to reverse tolerized function, these cells showed significantly higher myeloma-directed cytotoxicity as compared to activated peripheral blood lymphocytes taken from the same patients and also appeared to target clonogenic precursors [24]. A randomized clinical trial of activated MILs alone or in combination with an allogeneic GM-CSF-based myeloma cellular vaccine in the setting of autologous stem cell transplantation for myeloma is in progress.

4.3 Clinical Trials Using Gene-Modified Autologous T-Cells

As described earlier, another strategy to address the challenge of relying on tumor antigen vaccines and activation strategies to enhance endogenous cellular immune responses which are typically low in frequency and antigen affinity is to redirect T-cells to known tumor antigen targets through gene modification. The two major approaches that have been utilized for patients with hematological malignancies is to engineer T-cells to express affinity-enhanced TCRs or CARs, the latter of which are composed of binding domains from the variable regions of antibodies fused to the constant, signaling domains of the TCR (Fig. 2). In one ongoing study based on the first approach, patients receive gene-modified autologous T-cells at day +2 after autologous stem cell transplantation for myeloma [127]. Eligibility for the study requires that patients be HLA-0201 positive and that their myeloma cells express NY-ESO-1 or LAGE-1 by PCR. The T-cells were transduced with a lentiviral vector which encodes an affinity-enhanced TCR for the HLA-A201-restricted epitope

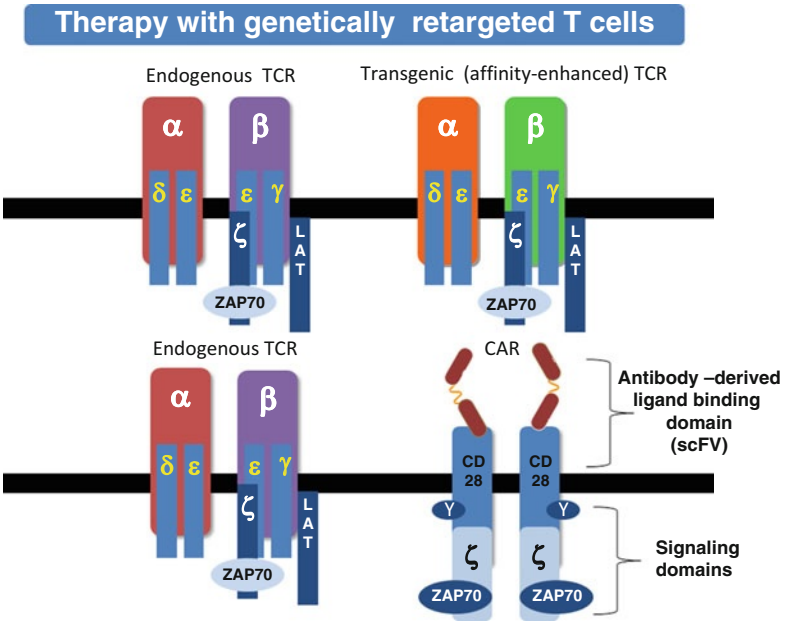

Fig. 2 Therapy with genetically retargeted T-cells. The *top panel* shows a genetically modified T-cell engineered to express an affinity-enhanced T-cell receptor (TCR). This transgenic TCR is coexpressed with the endogenous TCR. The *bottom panel* shows a genetically modified T-cell engineered to express a chimeric antigen receptor (CAR) along with the endogenous TCR. The CAR consists of a ligand or tumor antigen-binding domain derived from the variable regions of the heavy and light chains of an antibody molecule fused to signaling domains that may be derived from the CD3 ζ chain, CD28, 4-1BB, or a combination thereof. A simplified representation of the TCR complex is shown with the α and β subunits, components of CD3 (δ,ε,γ), and downstream signaling effectors (ZAP70 and the transmembrane adapter protein linker for the activation of T-cell—LAT)

$NY\text{-}ESO_{157–165}$ (SLLMWITQC) which is also shared by the LAGE-1 CTAg and then activated and expanded using anti-CD3/anti-CD28 immunomagnetic beads. To date, 16 patients have received the gene-modified T-cells, and 13 have reached the day 100 restaging timepoint. Infusions of the gene-modified T-cells have been well tolerated, and ten patients (77 %) have achieved a very good partial response (VGPR) or better, while 11/16 patients continue to show evidence of response with no myeloma progression. Complete and durable clinical responses have also been observed in patients with advanced, refractory, and extramedullary disease [127]. Importantly, the gene-modified T-cells persist for as long as 1 year post infusion and demonstrate marrow trafficking and antigen-specific targeting as NY-ESO-1/Lage-1 expression is extremely low or undetectable in patients with blood and/or marrow persistence of gene-modified T-cells [128].

In contrast to the approach of using affinity-enhanced TCRs, gene modification of T-cells using CARs offers the possibility of redirecting T-cells toward specific tumor antigens without major histocompatibility antigen (MHA) restriction.

Based on groundbreaking work by Eshhar, June, and others, clinical trials of CARs for hematological malignancies are in progress at multiple academic medical centers [99, 129]. The most advanced clinic trials have focused on CD19 which is restricted in its expression to normal and malignant B-cells. Major impediments to the clinical development of CAR technology have been the limited in vivo persistence and expansion of CAR-modified T-cells [130]. Preclinical work has established that addition of the CD137 (4-1BB) cytoplasmic signaling domain to the CD3-ζ chain results in significantly higher persistence, proliferation, and antitumor activity compared to CARs that carry the CD3-ζ chain alone [131]. Translational application of this work led to a pilot clinical trial using autologous T-cells genetically engineered to express an anti-CD19 CAR (CART-19 cells) for patients with relapsed, refractory CLL [132, 133]. Among the first three patients treated on this trial, two achieved a durable complete response and one had a durable partial response. The engineered T-cells expanded more than 1,000-fold in vivo, homed to the bone marrow, killed CD19-expressing target cells, and persisted for at least 6 months. In addition, while the CART19 CD8+ T-cells exhibited an effector memory phenotype ($CCR7^-$, $CD27^-$, $CD28^-$) during and soon after the tumor killing phase, by 6 months post infusion a portion of the CART19 $CD8^+$ T-cells showed a central memory phenotype with coexpression of CCR7 and increased levels of CD27 and CD28. One of the three patients who was described in greater detail had bulky adenopathy and extensive marrow involvement with CLL that carried a 17p deletion with loss of the TP53 locus, a cytogenetic feature which confers a very poor prognosis and is associated with resistance to chemotherapy. This patient received 3×10^8 T-cells over 3 days in escalating doses, of which 5 % were transduced for a total of 1.42×10^7 CART-19+ T-cells. At day 22 post infusion the patient developed dramatic clinical and laboratory signs of tumor lysis syndrome including transient kidney injury requiring hospitalization. This clinical syndrome coincided with peak (3-log) expansion of the CART-19+ T-cells at which time the CART19 cells comprised more than 20 % of the circulating lymphocytes. A complete regression of pathologic lymphadenopathy and marrow and blood involvement ensued which is now reported to be ongoing for 2 years.

This clinical trial experience was recently expanded to include ten patients including nine adults with refractory CLL (3/9 with P53 deletions) and one 7-year-old child with ALL in refractory relapse [134, 135]. All of the CLL patients received lymphodepleting chemotherapy prior to T-cell transfer while the ALL patients did not. The median T-cell dose was 7.5×10^8 (1.7–50) including 1.45×10^8 $CART19^+$ cells (0.14–5.9). With a median follow-up of nearly 6 months, four of nine evaluable patients had a CR (none of whom has relapsed) including three CLL patients and one ALL patient, while two CLL patients had partial responses lasting 3 and 5 months and three patients did not respond. In the four CR patients, the CART19+ cells expanded an average of 27-fold [21–40] in the blood with the peak expansion occurring between days 10 and 31 post infusion. An important and somewhat unexpected finding was that the CART19 cells trafficked to the cerebrospinal fluid in the child with ALL presumably due to the presence of unrecognized CNS involvement with leukemia. Of note, all responding patients developed a “cytokine release

syndrome" (CRS) characterized by high fevers and grade III/IV hypotension and hypoxia [136]. The child with ALL exhibited the most severe degree of CRS which culminated in grade IV hypotension and respiratory failure necessitating mechanical ventilatory and pressor support. After glucocorticoid administration led to no improvement, cytokine analysis revealed that IFN-γ, IL-6, IL-2, and TNFα levels were 6,040, 988, 163, and 17 times higher than baseline measured levels. The TNF and IL-6 receptor antagonists etanercept and tocilizumab were given to the patient followed by rapid and complete clinical improvement. Additional laboratory and clinical findings include dramatic elevations of the ferritin levels (44,000–605,000), hepatosplenomegaly unrelated to primary disease, and a moderate degree of disseminated intravascular coagulation (DIC). This constellation of findings suggested that the CRS syndrome had features of macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH). The 7-year-old ALL patient subsequently entered a complete blood/marrow and CNS remission which is ongoing at 8 months post treatment. This syndrome was subsequently recognized in three CLL patients and treated successfully with the IL-6 receptor antibody tocilizumab alone. Studies to define the optimal time to block the CRS so as not to interfere with the antitumor cellular immune response are under way. A long-term but expected consequence of successful treatment with the CART-19 cells is profound B-cell depletion and hypogammaglobulinemia.

Other groups have reported successful treatment of progressive CD19+ B-cell malignancies including follicular lymphoma using CD19-CAR T-cells in which the signaling domain was derived from CD3-ζ only [137, 138]. Using this construct six of eight patients obtained remissions and four had major elevations of inflammatory cytokines including IFNγ and TNF most likely derived from the gene-modified T-cells. Treatment-related toxicities correlated with the levels of these inflammatory cytokines.

5 Summary

Cellular immunotherapy is the latest to join the three principal systemic therapeutic modalities for hematologic malignancies of chemotherapy, targeted therapy, and antibody therapy. However, the potent cytotoxic potential of T-cells combined with their remarkable capacities for proliferation, trafficking, and sustainability ensures that their role in the treatment of advanced and aggressive blood cancers will likely expand. Although cellular immunotherapy has long been part of the curative mechanism of allogeneic stem cell transplantation, this form of T-cell therapy has been difficult to modulate and separate from serious complications such as GVHD and is limited to a minority of patients in need. The advent of effective and reliable expansion technologies for autologous T-cells and the ability to "redirect" these cells to specific tumor antigen targets through potent vaccine formulations and genetic engineering offer a highly effective and potentially safer approach for a wider spectrum of patients. Future work will likely follow these directions: (1) identification

of additional tumor antigens to serve as targets of new high-affinity TCRs or CARs; (2) application of immunomodulatory pharmacologic agents (e.g., IL-15, IL-7, anti-CTLA4, anti-PD1 antibodies, lenalidomide) to further enhance and sustain T-cell growth and function in vivo; and (3) refinement of strategies to ameliorate some of the toxicities associated with activated T-cell therapy including CAR-modified T-cells.

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