Leukemia (2015) **29,** 1–10

www.nature.com/leu

REVIEW

Cell-based strategies to manage leukemia relapse: efficacy and feasibility of immunotherapy approaches

A Rambaldi[1], E Biagi[2], C Bonini[3], A Biondi[2] and M Introna[1]

When treatment fails, the clinical outcome of acute leukemia patients is usually very poor, particularly when failure occurs after transplantation. A second allogeneic stem cell transplant could be envisaged as an effective and feasible salvage option in younger patients having a late relapse and an available donor. Unmanipulated or minimally manipulated donor T cells may also be effective in a minority of patients but the main limit remains the induction of severe graft-versus-host disease. This clinical complication has brought about a huge research effort that led to the development of leukemia-specific T-cell therapy aiming at the direct recognition of leukemia-specific rather than minor histocompatibility antigens. Despite a great scientific interest, the clinical feasibility of such an approach has proven to be quite problematic. To overcome this limitation, more research has moved toward the choice of targeting commonly expressed hematopoietic specific antigens by the genetic modification of unselected T cells. The best example of this is represented by the anti-CD19 chimeric antigen receptor (CD19.CAR) T cells. As a possible alternative to the genetic manipulation of unselected T cells, specific T-cell subpopulations with *in vivo* favorable homing and long-term survival properties have been genetically modified by CAR molecules. Finally, the use of naturally cytotoxic effector cells such as natural killer and cytokine-induced killer cells has been proposed in several clinical trials. The clinical development of these latter cells could also be further expanded by additional genetic modifications using the CAR technology.

Leukemia (2015) **29,** 1–10; doi:10.1038/leu.2014.189

INTRODUCTION

When treatment fails and disease relapses after allogeneic hematopoietic stem cell transplantation (HSCT), the prognosis is almost invariably very poor. Relapse remains the leading causes of death following HSCT[1] and strategies for a more successful treatment of this condition are urgently needed. Retreatment of the underlying disease by conventional chemotherapy or radiotherapy could be planned as a palliative treatment option or alternatively as a bridge to a second transplant or more innovative cellular therapy approaches that are all designed to maximize the immune response against the tumor. Donor lymphocyte infusion (DLI) remains the most commonly used approach, but in the past years several attempts have been made aiming at maximizing the allorecognition of the neoplastic clone while minimizing the severe risk of inducing graft-versus-host disease (GvHD).[2] The new cellular therapies with leukemia-specific T cells or with natural cytotoxic immune effector cells may offer new exciting treatment tools but their efficacy can still be limited and their preparation may not be feasible in most clinical centers. Even more exciting are the prospects opened up by the possibility of genetically manipulating T cells with leukemia-reactive chimeric antigen receptors (CARs). Although this increases enormously the antileukemic potential of T-cell therapy, on the other hand, these new technologies may pose new challenging problems in terms of feasibility and sustainability. This review describes the results obtained with all these procedures.

A SECOND ALLOGENEIC HSCT

A second allogeneic HSCT may represent an effective treatment option, but in the clinical practice it may be offered only to a minority of patients, whose disease recurs after the first transplant. In general, most patients experiencing an early relapse after transplant (within 6 months) should not be offered this treatment option, because they may not tolerate the severe toxicity of a second conditioning regimen.[3] For patients with a later relapse (>12 months), a second transplant may be considered but the nonrelapse mortality may exceed 30%, depending on the type of disease, previous therapies and age. In most cases, only a minority of fit younger patients can receive a second SCT for acute leukemia and myelodysplastic syndrome, and overall the clinical outcome remains disappointing. Considering the above stated limitation, it is possible that a second myeloablative conditioning regimen may overcome the pharmacological resistance of the leukemic cells expanding after a first transplantation. In patients younger than 20 years who relapsed more than 6 months after transplantation, a 5-year survival rate of 51% has been reported as compared with 3% observed in older patients or those who relapsed within 6 months after transplantation.[4] The clinical outcome of a second allogeneic transplantation from the original donor after a reduced-intensity conditioning was recently reported on a consecutive cohort of 104 patients. Of these, 67% achieved complete remission (CR) and the median overall survival (OS) was 11.6 months. The OS of patients achieving CR/CRi (CR with incomplete hematologic recovery) after the first

[1]Hematology and Bone Marrow Transplant Unit and Center of Cell Therapy 'G. Lanzani', Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; [2]Department of Pediatrics, M Tettamanti Research Center, Laboratory of Cell therapy 'S. Verri' University of Milano-Bicocca, San Gerardo Hospital, Monza, Italy and [3]Experimental Hematology Unit, San Raffaele Scientific Institute, Milano, Italy. Correspondence: Professor A Rambaldi, Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII Bergamo, Piazza OMS 1, Bergamo 24127, Italy.
E-mail: arambaldi@hpg23.it
Received 19 March 2014; revised 15 May 2014; accepted 27 May 2014; accepted article preview online 12 June 2014; advance online publication, 8 July 2014

reduced-intensity conditioning/HSCT was 18.8 months, as compared with 3.9 months for those who did not ($P<0.01$). By multivariate analysis a significant positive impact was associated with remission duration after initial HSCT ($P=0.026$) and the presence of acute GvHD after reduced-intensity conditioning/HSCT.[5] The role of donor change for a second allogeneic transplantation was studied in another recent retrospective registry study for relapsed acute leukemia after related or unrelated first HSCT. Independently from donor, 74% of patients achieved CR after the second HSCT, but half of these patients relapsed again. Overall survival at 2 years was 25% (39% after related; 19% after unrelated second transplant) but long-term survivors were observed even after two unrelated transplants. Multivariate analysis confirmed that remission duration after the first transplant and stage at second transplant are the key prognostic factors for OS from the second transplant. After both related and unrelated HSCT1, selecting a new donor for second transplant did not result in a relevant improvement in OS compared with HSCT2 from the original donor; however, donor change was not detrimental either.[6]

UNMANIPULATED DLI

After the first report showing ability of DLI to provide hematological remission in chronic myelogenous leukemia (CML) patients,[7] the use of DLI has also become common for other diseases, but the results are usually modest[8–19] and the exact definition of the optimal dose and timing are still open to question. In a recent retrospective study on 225 patients, multivariate analysis has shown that initial DLI dose of $>10\times10^7$ $CD3^+$/kg is associated with an increased risk of GvHD. Moreover, an initial DLI $CD3^+$ cell dose of 10×10^7 or higher did not decrease the risk of relapse and did not improve OS. Overall, these results support the use of $<10\times10^7CD3^+$ cells/kg as the initial cell dose for treatment of persistent or recurrent hematological malignancies after HSCT.[20,21] Giving another example, intense study has focused on the role that cyclophosphamide- and fludarabine-mediated lymph depletion may play in combination with DLI.[22,23] In contrast, DLI has proven to be of very limited efficacy in acute leukemia, with $<25\%$ survival after 2 years, whereas slightly more encouraging results have been reported for lymphoma and myeloma, but patient numbers are still too small and data would need confirmation in larger studies.[24]

MINIMALLY MANIPULATED DLI

Several groups have developed the idea that CD8-depleted DLI may still retain graft-versus-leukemia (GVL) while showing a much reduced GvHD activity.[25–27] Cases of complete molecular remissions have indeed been observed in CML, accompanied by the appearance of a high frequency of circulating host-reactive cytolytic $CD8^+$ T cells secreting interferon-γ.[28] Very interestingly, in one well-studied CML case, it was possible to document that the CD4 DLI had induced both CD8 and specific antibodies against CML66 endogenous HLA-B4403-restricted peptide. The rise in antibody titers and circulating $CD8^+$ cells coincided with disease remission. Indeed donor-derived CML66-reactive T cells were detected at low levels *in vivo* in the bone marrow before the administration of DLI, thus suggesting that $CD4^+$ DLI may result in rapid expansion of pre-existing marrow-resident leukemia-specific donor $CD8^+$ T cells, followed by a cascade of antigen-specific immune responses.[28,29] In a very recent extension of this hypothesis, it has been suggested that $CD4^+$ DLI may reverse the 'exhaustion' phenotype that characterizes the bone marrow-infiltrating $CD8^+$ T cells.[30] In an attempt to increase the GVL efficacy of DLI, 'immunosuppressive' $CD4/CD25/Foxp3^+$ T regulatory cells have also been specifically depleted in 17 adult patients who relapsed after allo-HSCT, being previously treated by at least one conventional DLI and had failed to respond. Two patients developed GvHD after the first infusion and experienced long-term remission of their malignancy. The remaining 15 patients did not respond to the first T regulatory cell-depleted DLI and received a second infusion. Four patients developed GvHD and two of them experienced long-lasting CRs. All in all, 5 patients were alive in CR, suggesting that T regulatory cell-depleted DLI can show effective GVL activity.[31]

Very recently, a large trial reported data on the prophylactic use of extensively *ex vivo* modified DLI to modulate the Th1/Th2 balance *in vivo* post transplant. Dedicated donor apheresis have undergone CD4-positive selection and subsequent anti-CD3 and anti-CD28 stimulation in the presence of interleukin-4 (IL-4) and rapamycin for 12 days. At the end, these cells (called T-Rapa) were administered at 2.5×10^7/kg on day 14 after allogeneic matched sibling donor HSCT receiving a low-intensity conditioning regimen. No major toxicity was reported following infusion but acute GvHD was observed in 10% of patients and late acute GvHD in additional 14/37 patients. Moreover, 17/37 evaluable patients had classical chronic GvHD and only 16/40 patients did not develop any form of GvHD. Interestingly, 37 of patients with a high-risk disease remained in sustained CR.[32]

LEUKEMIA-SPECIFIC T-CELL LINES

The seminal paper by Falkenburg *et al.*[33] raised the most exciting expectations by demonstrating that leukemia-reactive cytotoxic T lymphocytes lines (CTL), generated from human leukocyte antigen (HLA)-identical donors and administered at a total dose of 3.2×10^9 T lymphocytes in a single patient, were indeed able to completely eradicate *in vivo* an accelerated phase of CML. Many years later, we have learned that donor T cells recognize minor histocompatibility antigens that mediate either GVL or GvHD. These antigens have been cloned and characterized and the immune response against them can now be precisely monitored *in vivo*.[34] From these results, the idea was raised of generating *in vitro* minor histocompatibility antigen-specific T cells to be adoptively transferred to patients relapsing after transplantation. The results of the phase I study was nonetheless disappointing, as CTL lines could be generated only in 16 out of 27 donor recipient pairs, but only 11 patients were treated with GMP (good manufacturing practice)-compliant cells. Eight patients received from 1 to 7 CTL administrations, observing two CRs (one obtained after a combined administration of CTL and DLI), 2 patients had temporarily stable disease and in 4 patients no response was observed. Thus, the strategy remains logistically complex, time consuming and feasible only in a minority of cases.[35] With the aim of simplifying the methodology, the same group reported very recently the *in vitro* generation of clinical grade HA-1-specific T-cell lines from HA-1-negative donors. This strategy was applied successfully to 3 patients (out of 9 eligible), in a time frame of 4 to 5 weeks in order to obtain clinically relevant numbers of T cells, the predominant phenotype of T cells being that of CD8 effector memory subset. Even if no toxicity was reported, no clear clinical response occurred in the three treated patients.[36] In a very recent publication, the generation of a cell product containing HA-1-T-cell receptor-transduced virus-specific T cells using retroviral vectors has been described as a rapid procedure. This pure antigen-specific product may be safely administered early after allo-SCT, and exert GVL without GvHD activity.[37]

A rather similar approach has been also followed by stimulating recipient post-transplantation peripheral blood mononuclear cells 3 times with irradiated recipient pre-transplantation peripheral blood mononuclear cells, followed by subsequent restimulation with irradiated recipient-derived Epstein–Barr virus (EBV)-transformed lymphoblastic cell lines (EBV-LCL). The total duration of *in vitro* culture was from 10 to 13 weeks. The protocol was to

administer a series of 3 infusions at an escalating target dose over 11 days (from $3.3 \times 10^7/m^2$ to $3.3 \times 10^9/m^2$ and to higher doses for the patient not developing GvHD). Cells were prepared from 25 patients but only 7 received the cell therapy. A total of 46 infusions were administered and the highest dose administered to each patient ranged from 2.2×10^9 to 6.6×10^9 cells (the target maximum cell dose was achieved in only 3 out of 7 patients). Three patients developed severe grade 3 or 4 pulmonary toxicity, interpreted as secondary to T-cell therapy and correlated with the unexpected lung expression of minor antigens. GvHD requiring treatment occurred in three patients. Adoptively transferred CTLs were detected in the blood and the peak was observed within 3 to 5 days. Of the 7 patients, 5 achieved complete morphologic remissions. Of these, 3 achieved remission after infusion of T cells alone in the absence of additional cytoreductive therapy. PCR analysis was the direct demonstration that transferred T cells migrated to the bone marrow, even if all 5 of these patients subsequently relapsed (range, 4–43 months) and died.[38]

Passive transfer of T lymphocytes has been tested in a phase I study also by *in vitro* expansion of tumor-infiltrating T lymphocytes using anti-CD3/anti-CD28-coated magnetic beads in 8 patients with B-cell malignancy progressing after allo-HSCT. A median of 2×10^7 T cells/kg were infused. The expanded tumor-derived donor lymphocytes were well tolerated without any GvHD. Five patients were available for response and four of the five maintained stable disease. Two transiently positron emission tomography responses (both HL) and two mixed responses were observed in these refractory patients.[39]

PR1, WT1 and BCR/ABL were also used as stimulating peptides to generate antigen-specific CTLs by loading monocyte-derived dendritic cells for more than 1 month. Fourteen CML patients received allogeneic purified $CD34^+$ stem cells, followed by infusions of CTLs. A median dose of 1.8×10^6, 1.7×10^6 and 2.6×10^6 $CD8^+$ CTL/kg were administered on days 28, 56 and 112 after transplant, respectively. The infusion was associated with a prompt increase in the percentage of donor $CD4^+$ and $CD8^+$ T cells in the patient's peripheral blood. At the time of the third infusion, complete T-cell chimerism could be documented in all patients. In most patients, CTLs could be monitored *in vivo* by specific tetramer or pen tamer staining. Four out of five patients who received cytotoxic CTLs and in whom circulating CTLs could be monitored *in vivo* have remained BCR/ABL negative between 31 and 55 months after transplantation and none had clinical signs of GvHD. Overall, 13 patients were alive and 7 in molecular remission with a median follow-up of 45 months.[40]

LEUKEMIA-REDIRECTED GENETICALLY MODIFIED T CELLS

The innovative CAR strategy stands out because of the impressive results achieved in recent clinical trials performed using autologous[41–47] or donor-derived, allogeneic T lymphocytes.[48,49] CARs are artificial T-cell receptors composed by an extracellular antigen-binding domain derived from the fusion of the variable regions of the heavy and light chains of immunoglobulins, and an intracellular T cell-activating domain, usually the CD3ζ chain of the T-cell receptor complex.[50] Thus, CAR-redirected T cells are able to specifically recognize and kill tumor cells, as they combine the antigen-binding properties typical of an antibody molecule with a T cell-triggering domain. CARs being antibody-derived molecules have the main advantage of recognizing antigens in a non-HLA-restricted manner, bypassing the HLA downregulation as a tumor escape mechanism and the usual limitation of T cells for specific haplotypes. Moreover, a broader range of antigens can be targeted using CARs, such as carbohydrates and glycolipids. So, ideally, it is possible to design specific CARs for the targeting of any suitable molecule expressed on the surface of tumor cells, but conferring better biodistribution, immunological memory and persistence over time. The latter aspect can be improved to face a potential hostile tumor milieu by designing second- and third-generation CARs that carry one or even more additional co-stimulatory molecules. However, after initial clinical data using effector populations modified to express CAR molecules,[51] lack of persistence[52] and severe adverse events such as on-target cytokine storm[53,54] emerged as critical points for this advanced therapy. Consistent with such a combined success and emergence of harmful toxicity is the case of a 10-year-old girl with pre-B acute lymphoblastic leukemia (ALL) who had had a second relapse after umbilical cord transplant. This patient was treated by the administration of genetically modified autologous $CD3^+$ T cells/kg (for a total dose of 1.4×10^6 CTL019 cells per kg in a single dose) with specificity for the B-cell antigen CD19 coupled to CD137, a costimulatory receptor in the T cells (4-1BB) and CD3ζ (a signal transduction component of the T-cell antigen receptor signaling domains).[46] Approximately 1 month after infusion, morphologic remission of leukemia, a level of minimal residual disease $<0.01\%$ was achieved. She had grade 3 febrile neutropenia and encephalopathy and grade 4 elevated aspartate transaminase and alanine transaminase for a maximal duration of 6 days related to a cytokine release syndrome that evolved into the macrophage-activation syndrome. The fraction of CTL019 T cells in circulation progressively increased to 34% of T cells and the absolute lymphocyte counts increased substantially, expanding to levels that were more than 1000 times as high as the original engraftment levels. CTL019 cells were also observed in the cerebrospinal fluid and were persistent for 6 months. $CD19^+$ cells in bone marrow and blood were eliminated within 1 month after infusion. Unfortunately, 2 months after infusion, she had a clinical relapse that was apparent in the peripheral blood with leukemic cells that were CD19 negative, consistent with a potent antileukemic selective pressure of the chimeric antigen receptor T cells. The malignant clone was revealed by PCR as early as 23 days after infusion.[46] In another experience, 8 patients relapsing after allogeneic transplantation were treated with donor CD19.CAR-VSTs (viral specific T cells) 3 months to 13 years after HSCT. No infusion-related toxicities were observed and genetically modified T cells persisted for a median of 8 weeks in blood and up to 9 weeks at disease sites. Objective antitumor activity was evident in two of six patients with relapsed disease during the period of CD19.CAR-VST persistence, whereas two patients who received cells while in remission remain disease free.[48] All in all, T cells redirected by CD19-specific CAR have been reported to control the disease and induce remission in patients affected by relapsed, refractory otherwise incurable hematologic malignancies, and numerous trials are currently ongoing at different academic institutions[55–57] (Tables 1 and 2). Moreover, beside CD19, a large series of antigens targeted by CARs are now available, including the ganglioside GD2,[58] L1 CAM,[59] CD30,[60] the light chain of immunoglobulins,[61] HER2,[62] and CD44v6.[63]

CENTRAL MEMORY AND STEM MEMORY GENETICALLY MODIFIED T CELLS

Recently, early differentiated T cells redirected to CD44v6, an antigen expressed by acute myeloid leukemia (AML) and multiple myeloma, proved superior to effector cells, expressing the same CAR, in mediating antitumor activity *in vivo*.[63] It is thus envisaged that the choice of T-cell subsets and specific manipulation protocols for their genetic manipulation will play an important role for the complete exploitation of adoptive T-cell therapy. The ability of genetically modified T cells to expand and persist long-term after infusion appears a critical factor for clinical outcome,[64] and some evidence points to the differentiation state of the T lymphocytes infused as a major determinant of their *in vivo* persistence.[43,65,66] T cells can be classified in several distinct stages: mature T cells exit the thymus as naive cells (T_N), and, upon antigen encounter, expand and undergo a differentiation

Table 1. Ongoing clinical trials using autologous CAR T cells for hematologic malignancies, as of May 2014

Disease	*Target antigen (CAR signaling domain)*	*Patient age*	*Vector*	*Sponsor*	*Clinical Trial.gov ID*
ALL, NHL, CLL	CD19 (4-1BB–CD3ζ)	1–21 Years	Lentivirus	University of Pennsylvania	NCT01626495
		⩾18 Years			NCT01029366
		⩾18 Years			NCT01747486
		⩾18 Years			NCT02030847
ALL, NHL, CLL	CD19 (CD28-CD3ζ)	⩾18 Years	Retrovirus	Memorial Sloan Kettering cancer center	NCT01044069
		⩾18 Years			NCT01840566
		⩾18 Years			NCT01416974
		⩽ 26 Years			NCT01860937
	CD19 (CD28-CD3ζ & 4-1BB-CD3ζ)	⩾18 Years	Retrovirus/ lentivirus		NCT00466531
ALL, NHL, CLL	CD19 (CD28-CD3ζ & CD28-CD137-CD3ζ)	⩽ 75 Years	Retrovirus	Baylor College of Medicine	NCT01853631
	CD19 (CD28-CD3ζ)	Pediatric and adult			NCT00586391
NHL, HL	CD30 (CD28-CD3ζ)	Pediatric and adult			NCT01316146
		Pediatric and adult			NCT01192464[a]
NHL, MM, CLL	Ig κ light chain (CD28-CD3ζ)	⩾18 Years			NCT00881920
NHL, ALL	CD19 (CD28–CD3ζ–4-1BB)	⩾18 Years	Retrovirus	Uppsala University	NCT02132624
CLL, NHL	CD19 (CD3ζ)	18–80 Years	Transposon	MD Anderson Cancer Center	NCT01653717
		1–65 Years			NCT00968760
ALL, NHL, CLL	CD19 (CD3ζ)	1–30 Years	Retrovirus	National Cancer Institute	NCT01593696
	CD19 (CD28-CD3ζ)	18–68 Years			NCT00924326
NHL[b]	CD19/EGFRt (CD28-CD3ζ)	⩾18 Years	Lentivirus	City of Hope	NCT01815749
		⩾18 Years			NCT02051257
ALL	CD19/EGFRt (CD28-CD3ζ)	1–26 Years	Lentivirus	Seattle Children's Hospital	NCT01683279
	CD19/EGFRt (4-1BB–CD3ζ)	1–26 Years			NCT02028455
CLL, NHL, ALL	CD19 (CD3ζ)	> 18 Years	Lentivirus	Fred Hutchinson Cancer Research Center	NCT01865617
MF, CTCL	CD30	18–70 Years	Retrovirus	University of Cologne	NCT01645293
ALL, CLL, NHL	CD19 (CD137-CD3ζ and CD3ζ)	5–90 Years	Retrovirus	Chinese PLA General Hospital	NCT01864889
AML	CD33 (CD137-CD3ζ and CD3ζ)	5–90 Years			NCT01864902
MM	CD138 (CD137-CD3ζ and CD3ζ)	18–80 Years			NCT01886976
ALL, NHL	CD20 (4-1BB–CD3ζ)	18–90 Years			NCT01735604
MCL	CD19 (CD137-CD3ζ and CD3ζ)	50–80 Years			NCT02081937
AML, MDS, MM	Lewis-Y (Anti–Lewis-Y-CD28-CD3ζ)	⩾18 Years	Retrovirus	Peter MacCullum Cancer Center	NCT01716364

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; HL, Hodgkin's lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MF, mycosis fungoides; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma. [a]Autologous Epstein–Barr virus (EBV)-specific cytotoxic T lymphocytes (CTLs). [b]Central memory-enriched $CD8^+$ T cells.

Table 2. Ongoing clinical trials using allogeneic CAR T cells for hematologic malignancies, as of May 2014

Disease	*Target antigen (CAR signaling domain)*	*Patient age*	*Vector*	*Sponsor*	*Clinical Trial.gov ID*
ALL	CD19 (4-1BB–CD3ζ)	⩾18 Years	Lentivirus	University of Pennsylvania	NCT01551043
ALL[a]	CD19 (CD3ζ)	⩽19 Years	Retrovirus	Memorial Sloan Kettering cancer center	NCT01430390
ALL, CLL, NHL[b]	CD19 (CD3ζ)	Pediatric and adult	Retrovirus	Baylor College of Medicine	NCT00840853
ALL, NHL[c]	CD19 (CD3ζ)	1–75 Years	Transposon	MD Anderson Cancer Center	NCT01362452
ALL, NHL		1–65 Years			NCT01497184
NHL, CLL	CD19 (CD3ζ)	18–75 Years	Retrovirus	National Cancer Institute	NCT01087294
ALL, DLBCL, MCL, NHL, CLL[d]	CD19 (CD3ζ)	18–75 Years	Lentivirus	Fred Hutchinson Cancer Research Center	NCT01475058
ALL[e]	CD19 (CD3ζ)	⩽18 Years	Retrovirus	University College, London	NCT01195480
ALL, CLL, NHL	CD19 (CD137-CD3ζ and CD3ζ)	5–90 Years	Retrovirus	Chinese PLA General Hospital	NCT01864889
AML	CD33 (CD137-CD3ζ and CD3ζ)				NCT01864902

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin's lymphoma. [a]Epstein–Barr virus (EBV)-specific donor-derived cytotoxic T lymphocytes (CTLs). [b]Trivirus-specific donor-derived CTLs (against cytomegalovirus (CMV), EBV and adenovirus). [c]Donor-derived cord blood T cells. [d]Donor-derived CMV- or EBV-specific $CD62L^+$ T_{CM}. [e]EBV-specific CTLs.

program, resulting in the generation of effectors able to eliminate the pathogen and memory cells able to persist long term and to patrol the organism for antigen reappearance. Human memory T cells include distinct cellular subsets: CCR7^{pos} (and CD62L^{pos}) central memory T cells (T_{CM}), with low effector functions but high expansion potential and endowed with the ability to home to secondary lymphoid organs, and CCR7^{neg} (and CD62L^{neg}) effector memory T cells (T_{EM}), capable of immediate effector capacities, but prone to die.[67] Recently, a novel population of memory T lymphocytes named memory stem T cells (T_{SCM}), was described in mice[68] and humans.[69] T_{SCM} lymphocytes express CD45RA, CCR7, CD62L and interleukin-7 receptor-α, all molecules also expressed by T_N cells, from which T_{SCM} can be distinguished by the expression of CD95, CD45R0, IL-2Rβ and CXCR3. T_{SCM} lymphocytes display high survival and expansion capacities and the analysis of T-cell subset expression profiles place T_{SCM} on the top of the hierarchical T-cell memory differentiation tree.[69,70]

As long-term persistence of adoptively transferred T cells appears tightly linked to clinical responses, it might be assumed that manipulation protocols favoring the generation of long-living T_{SCM} and T_{CM} could be associated with higher clinical response rates than those observed with T_{EM} and T_E. The first clinical trials of adoptive T-cell therapy used manipulation conditions that favor the differentiation of T cells in effectors. However, follow-up of patients showed that clinical responses are associated with persistence of early differentiated adoptively transferred lymphocytes, expressing CD27 and CD28 and endowed with long telomeres.[65] These clinical observations were corroborated by studies in murine models and non-human primates, clearly indicating that T_{CM} cells are superior to T_{EM} *in vivo* in eradicating tumors.[71,72] A major advance in the field of immune gene therapy was the development of GMP-grade antibodies to CD3 and CD28 conjugated to paramagnetic beads pioneered by June and co-workers.[73] The simultaneous delivery of signal one (anti-CD3) and signal two (anti-CD28) promoted the activation of all T-cell subsets, including T_N cells, and their robust proliferation, while avoiding terminal differentiation.[74,75] A combination of standard DLI and escalating doses of activated DLI was administered to 18 patients who relapsed after transplant. Eight patients achieved CR, including six patients with acute leukemia. However, seven patients developed acute GvHD (five grade I–II and two grade III) and four developed chronic GvHD, indicating that activation might increase the GVT activity of donor lymphocytes, but is still limited by the risk of GvHD.[76] In the attempt to separate beneficial GVL from detrimental GvHD, T cells have been genetically modified to express a suicide gene, enabling their selective elimination if GvHD occurs. More than 120 patients have been treated to date with donor lymphocytes expressing the HSV-TK cells in several phase I–II clinical trials.[70] Suicide gene therapy proved to be safe and effective in controlling GvHD and the approach is currently tested in a phase III clinical trial. To overcome the immunogenicity of the TK viral protein, a chimeric gene incorporating the death domain of inducible caspase-9 has been recently developed. In this case, the administration of a chemical dimerizer permits killing of genetically modified cells. In a phase I/II clinical trial, *ex vivo* allogeneic-depleted inducible caspase-9-transduced donor lymphocytes were infused after haploidentical HSCT and the suicide machinery was effective in controlling grade I–II acute GvHD.[77]

The first suicide gene therapy manipulation protocols favored complete T-cell differentiation, and the majority of infused cells displayed an effector/effector memory phenotype. Although immunological monitoring of treated patients demonstrated persistence of TK cells, for up to 14 years after infusion (G Oliveira and C Bonini, unpublished observation), cells remained detectable at very low frequencies, suggesting that manipulation protocols enabling the infusion of less differentiated T cells might be beneficial. In the attempt to increase the fitness of adoptive T-cell therapy with genetically modified T cells, we activated purified human naive T cells with anti-CD3 and anti-CD28 antibody-conjugated beads, and transduced them in the presence of low-dose IL-7 and IL-15. With this protocol, a large number of genetically modified T_{SCM} cells can be generated.[70]

NK CELLS AND CIK CELLS

NK cells can show alloreactivity against neoplastic cells (but not normal tissues) that manifest themselves when KIR molecules on the surface of donor's natural killer (NK) cells are not engaged by certain HLA class I molecules on the surface of hosts' cells. In particular, KIR2DL1 are inhibited by HLA-C group 2 alleles characterized by a lys80 residue, KIR2DL2/DL3 by HLA-C group 1 alleles characterized by an asn80 residue and KIR3DL1 by the HLA-Bw4 group.[78] Many clinical studies have suggested some correlation between this NK allorecognition and the outcome of allogeneic transplants, particularly in the case of the haploidentical setting.[79] Following pilot experiences,[80,81] the safety, feasibility and engraftment of haploidentical NK cell infusions after an immunosuppressive regimen has been tested in 10 children with AML who had completed chemotherapy and were in first CR. After an *in vivo* lymph depletion with cyclophosphamide and fludarabine (KIR-HLA), mismatched NK cells were infused (median, 29×10^6/kg NK cells) followed by six doses of IL-2 (1×10^6 U/m^2). All patients had transient engraftment for a median of 10 days and a significant expansion of KIR-mismatched NK cells. Nonhematologic toxicity was limited, with no GvHD with a median follow-up of 964 days. All patients remained in remission, and the 2-year event-free survival was 100%.[82] One clinical attempt to induce a remission in a young man affected by AML who relapsed after a second haploidentical transplant was recently reported. He lacked the KIR3DL1 ligand Bw4 and the KIR2DL1 ligands belonging to the HLA-C2 family. Therefore, he was treated by a single infusion of donor NK cells at the dose of 3×10^7 /kg and IL-2. The NK composition of the donor population was by 14% KIR2DL1, 65% KIR2DL2/3 and 0% KIR3DL1. There was a progressive expansion of the infused NK cells starting on day 4 and peaking on day 21 (by at least fourfold) for the NK alloreactive population, that is, the KIR2DL1$^+$, KIR2DL2/DL3$^-$ subset. Analysis of chimerism showed the donor type at 61% before NK DLI and peaking at 99.4% at day $+73$ after the infusion. A medullary evaluation on day 34 appeared to show a complete response, but the patient subsequently relapsed and died. This report, in spite of clinical failure, shows that the administration of well-selected alloreactive purified NK cells could be considered, even if the efficacy may depend on the extent of the tumor burden.[83] In a phase II study including 16 patients with high-risk leukemia or multiple relapsed tumors, highly purified NK cells have been administered at days $+3$, $+40$ and $+100$ after haploidentical T cell-depleted SCT. Median doses were 1.2×10^7/kg containing 0.003×10^7 T cells/kg. Unfortunately, four patients developed acute GvHD $>$grade II, and in 3 out of 4 it was fatal. In ordinal regression, grade of acute GvHD was associated with cumulative dose of infused T cells but not with cumulative dose of infused NK cells. In spite of pre-emptive treatment, three patients experienced graft failure that was fatal in all cases. Furthermore, seven patients relapsed, of which six died. The presence or absence of a KIR ligand mismatch between donor and patient had no discernible effect on the risk of graft failure or relapse. Survival probabilities for the entire cohort were 44% and 25% at 1 and 2 years, respectively.[84] The possibility of using haploidentical NK cells has also been tested in a nontransplant setting, as pioneered by Miller *et al.*,[85] and more recently by Curti *et al*.[86] In this last experience, 13 elderly patients with AML received purified haplo-NK after immunosuppressive chemotherapy. Best responses were observed in patients with

either molecular relapse or morphological CR, underlying the possibility of implementing this strategy only in conditions of minimal residual disease.

Cytokine-induced killer (CIK) cells have also been considered for passive transfer in relapsed leukemia patients. CIK cells are T effector memory CD8 T lymphocytes that have acquired NK-like cytotoxicity in culture. CIK cell production can take place under strict GMP adherence in a 21-day expansion protocol making use of only three clinical grade reagents (anti-CD3 OKT3 antibody, interferon-γ and IL-2). Because of the very strong non- HLA-restricted NK-like cytotoxicity of CIK cells and, more importantly, on the basis of the preclinical observations that CIK almost completely lack GvHD activity, we have suggested that donor-derived CIK cells could be administered to lymphoma/leukemia patients who relapse after allo-HSCT. In our first study, 11 patients with AML ($n=4$), Hodgkin's disease ($n=3$), chronic myelomonocytic leukemia ($n=1$), pre-B ALL ($n=1$) and myelodysplastic syndrome ($n=2$), all of whom had relapsed after sibling ($n=6$) or matched unrelated donor ($n=5$) transplantations, entered the protocol. The median number of CIK infusions was 2 and the median number of total CIK cells was 12.4×10^6/kg. Infusions were well tolerated and no acute or late infusion-related reactions were recorded. Acute GvHD of grade I and II was observed in 4 patients 30 days after the last CIK infusion and these progressed into extensive chronic GvHD in two cases. Disease progression and death occurred in six patients. One patient had stable disease, one had an hematological improvement and three achieved CR.[87] Similarly, 18 patients with hematological malignancies received allogeneic CIK cells, following relapsed after allogeneic HSCT (with matched sibling in all cases). CIK were given at escalating doses of 1×10^7/kg, ($n=4$), 5×10^7/kg ($n=6$) and 1×10^8 /kg ($n=8$). Acute GvHD grade I–II was seen in two patients and one patient had limited chronic GvHD. After a median follow-up of 20 months (range 1–69), the median OS time was 28 months and the median event-free survival was 4 months. All deaths were because of leukemia relapse.[88] In a more recent experience, 24 patients with hematological malignancies who relapsed after allogeneic HSCT (15 from sibling and 9 from unrelated donors) were enrolled to receive allogeneic CIK cells. Only 20 patients had a donor available and 16 were actually infused with no response observed in 6 of them. Five additional patients fell into the 'unable to assess' group because of the concomitant use of other agents that could have induced a response. Finally, for five patients there was evidence to suggest antitumor activity of CIK cells (these included two ALL, two Hodgkin's disease and one AML patient). Interestingly, two of the responders had a response sustained for more than 2 years. Acute GvHD occurred in three patients and was in all cases easily treatable.[89] Another very provocative report has been published recently, even if on only two patients, suggesting the possibility of treating multiple myeloma patients with family haploidentical CIK cells, with no report of toxicity, except for low-grade fever.[90] This result, if confirmed, would open the way to wider possible use of CIK cells from a normal donor. Finally, our group published results on five patients with aggressive acute leukemia who had relapsed after cord blood transplantation and were treated with HLA-matched cord blood-derived CIK cells.[91] These CIK cells were obtained by *ex vivo* cell expansion using as starting material the washouts of the bags containing the cord blood unit, obtained at the end of the infusion, as reported by our group previously.[92] Using this protocol, we did not observe any acute or delayed adverse event and observed one partial remission in one patient, concomitant with the development of acute grade III GvHD.[91] On the basis of the results of our phase I study, at the end of 2009, we started a new phase II study (Table 3). Our study is an open-labeled, multicenter, exploratory phase IIA protocol to evaluate the safety (dose-finding) and efficacy of a sequential administration of DLI followed by *in vitro* expanded CIK cells to patients with hematologic malignancies relapsing after related or unrelated allogeneic HSCT. Two infusions of unmanipulated DLI (1×10^6/kg) are given with a minimum interval of 3 weeks. Three infusions of donor CIK cells were administered according to a dose-escalating program, starting 3 weeks after the second DLI. CIK administrations were separated by 3-week intervals. Maximal tolerated dose (in our protocol defined as grade IV a GvHD toxicity) was not reached even at the highest dosage (5×10^6/kg, 5×10^6/kg and 10×10^6/kg). As a consequence, this same dose was administered to additional 22 patients to evaluate the efficacy of the treatment. At the moment of writing this publication, we are analyzing the data, even if no significant acute toxicity has been reported and no severe (grade III–IV) GvHD observed (M Introna *et al.*, manuscript in preparation). Many clinical studies are currently conducted with CIK cells in hematological neoplastic conditions, as reported on the clinicalTrials.gov site (Table 3).

GENE MANIPULATION OF CIK CELLS AS A NOVEL PLATFORM FOR AML RELAPSE TREATMENT AFTER HSCT

The use of modified effector T-cell populations to improve the specificity and efficiency of the GVL effect *in vivo* has recently emerged as a critical and promising approach in the context of relapse after HSCT.[93,94] The success of CAR strategies in ALL prompted the development of molecules specific for AML targeting. In this context, preclinical studies were carried out investigating the specific targeting of the CD33 and CD123 AML antigens exploiting CAR-redirected CIK cells. Concerning the targeting of the CD33 antigen, a potent killing activity against AML was brought about by anti-CD33.CAR + CIK cells. However, an impairment in the normal hematopoietic reconstitution was observed because of the high expression level of CD33 on this cellular compartment.[95] In a quest for a more selective target antigen, studies in literature draw attention to the CD123 antigen. Indeed, CD123 is overexpressed by AML cells and at the same time less expressed by hematopoietic stem and progenitor cells.[96,97] Moreover, CD123 resulted in being a promising target in terms of trying to also hit leukemia stem cells (AML-LSCs), the main population responsible for chemoresistance and relapse.[98] The anti-CD123.CAR showed, *in vitro*, to enhance the antileukemia CIK functions, better sparing normal hematopoietic stem and progenitor cells compared with the anti-CD33.CAR. Furthermore, a limited killing of normal $CD123^+$ monocytes and CD123 low-expressing endothelial cells was observed.[99] Notably, the functional activity of anti-CD123.CAR on AML cells has also been corroborated by other research groups.[100] The *in vivo* functional activity of CIK cells redirected with third-generation anti-CD123.CAR was investigated in immunodeficient NOD/SCID/IL2r-γnull (NSG) mice xenotransplanted with primary AML blasts. The potent antitumor activity of these cells was confirmed in this setting. Upon secondary transplantation, AML cells were still sensitive to the anti-$CD123.CAR^+$ CIK treatment, indicating that no resistance mechanisms had occurred. Moreover, this treatment was again associated with a significant low toxicity against normal hematopoietic stem and progenitor cells observing a good level of engraftment of cord blood-derived $CD34^+$ cells upon transplantation in NSG mice.[101] Thus, in an attempt to design a clinical-grade stimulation protocol for producing CIK cells manipulated with third-generation anti-CD123.CAR, our group explored the use of the latest-generation Sleeping Beauty (SB) transposon-mediated gene transfer. Transposons are mobile genetic elements that can be inserted within the genome of an organism without the need of sequence homology. In particular, SB has been extensively reviewed as a good delivery system also in the context of CAR-mediated targeting of leukemia, as it has the major advantage of reducing time and costs of production, immunogenicity and propensity for gene toxicity.[102] Indeed, the design of the first in-human trial infusing CD19-specific T cells, modified using SB transposons system, was reported by

Table 3. Clinical studies applying CIK cells for hematologic malignancies

Disease	*Therapeutic approach*	*Patient age*	*Study status*	*Sponsor*	*Phase/ ClinicalTrial.govID*
MM, hematologic malignancy	CIK cells as posttransplant immunotherapy following allo-HSCT	18–75 Years	Ongoing, not recruiting participants	Stanford University	1/NCT00185757/
AML, MDS, high grade	CIK cells: as adjuvant therapy in minimal residual disease state after autologous PBSCT; as an adoptive immunotherapy in untreated disease state when conventional therapy with curative intent is not applicable	12– 75 Years	Completed	Singapore General Hospital	1/2/NCT00394381
Leukemia, MM	Autologous, *ex vivo* expanded CIK cells to reduce the relapse rate in autologous HSCT patients with high-risk hematologic malignancies	18–75 Years	Completed	Stanford University	1/2/NCT00477035
AML, ALL, CML, NHL, HL, MDS, MM	CIK cells as immunotherapy for relapse after allogeneic marrow transplant	12–60 Years	Currently recruiting participants	Singapore General Hospital	1/2/NCT00460694
CML	Autologous *ex vivo* expanded CIK cells as immunotherapy for CML patients on standard drug therapy	12–80 Years	Completed	Singapore General Hospital	2/NCT00815321
Hematologic malignancies	Unmanipulated DLIs and CIK cells for the treatment of molecular, cytogenetic or hematologic relapse after HSCT	18–65 Years	Currently recruiting participants	A.O. Ospedale Papa Giovanni XXIII	2/NCT01186809
Refractory anemia, myeloid leukemia, BM transplant failure, MDS, myelo-proliferative disorders, neural tube defects	Post-transplant infusion of allogeneic CIK cells as consolidative therapy after non-myeloablative allogeneic transplantation	$\geqslant$50 Years	Currently recruiting participants	Stanford University	2/NCT01392989
Solid tumors, B cell lymphoma	Decitabine alone and/or in combination with chemotherapy and/or CIK cell transfusion in relapsed or refractory patients	18–85 Years	Currently recruiting participants	Chinese PLA General Hospital	1/2/NCT01799083
Lymphomas	CD20 antibody usage followed by CIK transfusion in refractory and/or chemoresistant patients	18–90 Years	Not yet open for participant recruitment	Chinese PLA General Hospital	NCT01828008
Acute leukemia	CIK cells combined with genetically modified dendritic cells for patients relapsing after allo-HSCT	8–61 Years	Currently recruiting participants	Affiliated Hospital to Academy of Military Medical Sciences	1/2/NCT01956630

Abbreviations: ALL, acute lymphoblastic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; BM, bone marrow; CIK, cytokine-induced killer; CML, chronic myeloid leukemia; DLI, donor lymphocyte infusion; HL, Hodgkin's lymphoma; MDS, myelodysplastic syndrome; MF, mycosis fungoides; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; PBSCT, peripheral blood stem cell transplantation.

Kebriaei *et al.*[103] Modified CIK cells displayed stable expression of CD123-CAR, and exerted efficient lysis of leukemia blasts, cytokine secretion and proliferation. The use of SB coupled to electroporation minimally affected the phenotype of CIK cells in our hands and, most importantly, an optimized protocol of CIK cell differentiation was effective in inducing T-cell expansion with high and persistent surface expression of anti-CD123.CAR (Figure 1). However, recent findings by using fetal liver $CD34^+$ cells as source for *in vivo* modeling showed that CD123 CAR transduced into T cells eradicated human normal hematopoiesis in newborn NSG mice.[104]

CONCLUSIONS

The clinical outcome of patients relapsing after allogeneic transplantation is very poor. The choice of a second allogeneic transplant either from the same or a different donor can be advisable for patients relapsing at least 6 months later after the first transplant. The probability of dying because of a further relapse or transplant-related toxicity is so high that different cellular therapy approaches should always be taken into account. The use of unmanipulated or minimally manipulated DLI can be efficacious and should always be considered, particularly for the treatment of minimal residual disease. Besides the limited efficacy, the major drawback is still represented by the possibility of inducing a severe and often lethal GvHD. Over the past years, innovative approaches have been proposed and each of these offers a variety of benefits and limitations. The infusion of allogeneic MHC nonrestricted natural cytotoxic effector cells like $CD3^-$ NK cells or $CD3^+$ CIK cells can be safe, partially effective and relatively easy. The major limitation remains the lack of activity on resistant tumor cells (particularly ALL) and the need of repeated infusions to guarantee a reasonable prolonged *in vivo* activity. This limitation is particularly evident in the case of NK cells that can survive *in vivo* only for a limited number of days. The use of genetically modified T cells redirected to a common lineage-specific antigen like CD19 for lymphoid malignancies opens up new opportunities for the deepest molecular eradication of these diseases and potentially their definitive cure. However, several problems are still to be addressed before this approach can be considered feasible in most clinical centers. First, the highly selective pressure of these antigen-specific redirected cells may rapidly drive the emergence of antigen-negative leukemic cells that will rapidly expand into a complete resistant clone. This

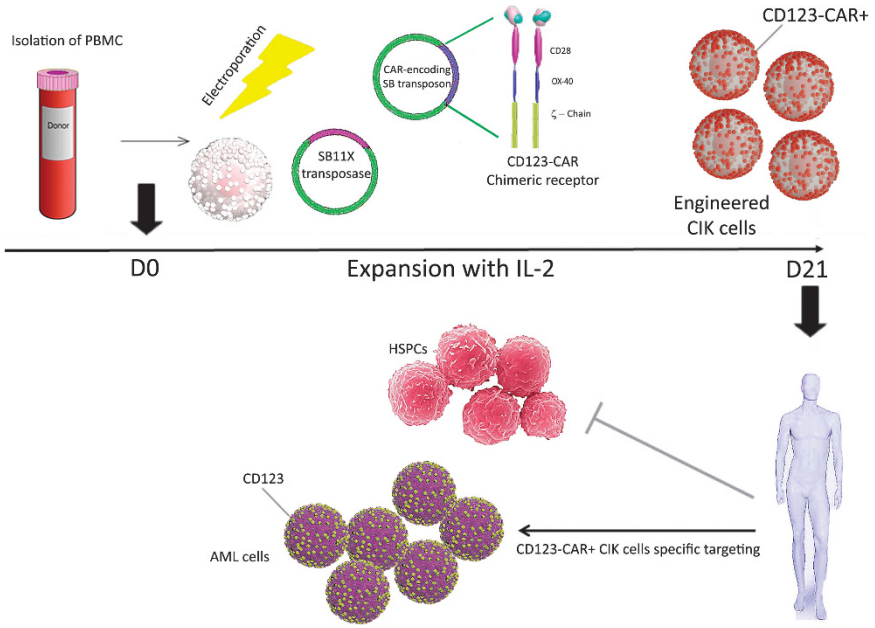

Figure 1. Design of CIK cell differentiation and modification protocol to induce the ectopic expression of anti-CD123 CAR by SB system for the clinical application. Peripheral blood mononuclear cells (PBMCs) from healthy donors are isolated and modified at day 0 by electroporation with the SB system composed of the SB11X transposase-encoding plasmid and the anti-CD123 CAR-encoding SB transposon. The SB11X transposase catalyzes the integration reaction of the third-generation (CD28-OX40-ζ chain) anti-CD123 CAR into the genome of the electroporated cells. After nucleofection, the cells are cultured for 21 days in the presence of IL-2, following the CIK cell differentiation protocol and becoming at the end CIK cells engineered to stably express anti-CD123 CAR. As a future clinical application, anti-CD123 CAR$^+$ CIK cells will be injected into the patients, where they will specifically target CD123$^+$ AML cells while sparing the hematopoietic stem cell compartment.

unfortunate event has been already described in several patients, and suggests the need for a wider antigen quest, possibly focused on molecules involved in ontogenesis and cancer progression. Second, although the antileukemic activity of CD19 CAR T cells is dramatically impressive for the treatment of lymphoid malignancies, no convincing clinical data are available for the treatment of myeloid malignancies, and the best antigen to be targeted has still to be established and tested. Moreover, the safety of these cells has not been yet convincingly proven. The cytokine storm induced by the potent antileukemic activity played by these genetically modified T cells can be difficult to manage. Finally, the laboratory requirements and the safety controls required to use retroviral vectors can hardly be met in the context of academic facilities. Until automation is introduced, the preparation of these cells will be only feasible in highly specialized laboratory facilities, most likely under the control of pharmaceutical companies. It is not difficult to imagine that the economic costs of these laboratory procedures would be extremely high, presenting questions of affordability for a significant number of patients. The development of innovative business models, the introduction of automation in the manufacturing process and the implementation of technologies enabling the reduction of time of manipulation and number of cells to be infused will be required in order to fully exploit this promising therapeutic approach.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported in part by grants from Associazione Italiana per la Ricerca contro il Cancro (AIRC) 'Special Program Molecular Clinical Oncology 5x1000' (Innate Immunity in Cancer to AR and AB, ref code 9962) and AIRC-IG8666 to AB and Associazione Italiana Lotta alla Leucemia (AIL), Sezione 'Paolo Belli', Bergamo, Italy.

REFERENCES

1 Savani BN, Mielke S, Reddy N, Goodman S, Jagasia M, Rezvani K. Management of relapse after allo-SCT for AML and the role of second transplantation. *Bone Marrow Transplant* 2009; **44**: 769–777.
2 van den Brink MR, Porter DL, Giralt S, Lu SX, Jenq RR, Hanash A *et al.* Relapse after allogeneic hematopoietic cell therapy. *Biol Blood Marrow Transplant* 2010; **16**: S138–S145.
3 Barrett AJ, Battiwalla M. Relapse after allogeneic stem cell transplantation. *Expert Rev Hematol* 2010; **3**: 429–441.
4 Eapen M, Giralt SA, Horowitz MM, Klein JP, Wagner JE, Zhang M-J *et al.* Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. *Bone Marrow Transplant* 2004; **34**: 721–727.
5 Leung AY, Tse E, Hwang YY, Chan TS, Gill H, Chim CS *et al.* Primary treatment of leukemia relapses after allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning second transplantation from the original donor. *Am J Hematol* 2013; **88**: 485–491.
6 Christopeit M, Kuss O, Finke J, Bacher U, Beelen DW, Bornhauser M *et al.* Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation from related and unrelated donors: the role of donor change. *J Clin Oncol* 2013; **31**: 3259–3271.
7 Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G *et al.* Donor leukocyte transfusion for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; **76**: 2462–2465.
8 Collins Jr RH, Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogenic bone marrow transplantation. *J Clin Oncol* 1997; **15**: 433–444.
9 Levine JE, Braun T, Penza SL, Beatty P, Cornetta K, Martino R *et al.* Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. *J Clin Oncol* 2002; **20**: 405–412.
10 Bhatia V, Porter DL. Novel approaches to allogeneic stem cell therapy. *Expert Opin Biol Ther* 2001; **1**: 3–15.
11 Porter DL, Luger SM, Duffy KM, Stadtmauer EA, Laport G, Schuster SJ *et al.* Allogeneic cell therapy for patients who relapse after autologous stem cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 230–238.
12 Salama M, Nevill T, Marcellus D, Parker P, Johnson M, Kirk A *et al.* Donor leukocyte infusions for multiple myeloma. *Bone Marrow Transplant* 2000; **26**: 1179–1184.

13 Collins Jr RH, Goldstein S, Giralt S, Levine J, Porter D, Drobyski W *et al.* Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant* 2000; **26**: 511–516.
14 Porter DL, Collins Jr RH, Hardy C, Kernan NA, Drobyski WR, Giralt S *et al.* Treatment of relapsed leukemia after unrelated donor marrow transplantation with unrelated donor leukocyte infusions. *Blood* 2000; **95**: 1214–1221.
15 Porter DL, Connors JM, Van Deerlin VMD, Duffy KM, McGarigle C, Saidman SL *et al.* Graft versus tumor induction with donor leukocyte infusions as primary therapy for patients with malignancies. *J Clin Oncol* 1999; **17**: 1234–1243.
16 Porter DL, Collins Jr RH, Shpilberg O, Drobyski WR, Connors JM, Sproles A *et al.* Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol Blood Marrow Transplant* 1999; **5**: 253–261.
17 Porter DL, Antin JH. The graft-versus-leukemia effects of allogeneic cell therapy. *Annu Rev Med* 1999; **50**: 369–386.
18 Klyuchnikov E, Holler E, Bornhauser M, Kobbe G, Nagler A, Shimoni A *et al.* Donor lymphocyte infusions and second transplantation as salvage treatment for relapsed myelofibrosis after reduced-intensity allografting. *Br J Haematol* 2012; **159**: 172–181.
19 Schmid C, Labopin M, Nagler A, Bornhauser M, Finke J, Fassas A *et al.* Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. *J Clin Oncol* 2007; **25**: 4938–4945.
20 Bar M, Sandmaier BM, Inamoto Y, Bruno B, Hari P, Chauncey T *et al.* Donor lymphocyte infusion for relapsed hematological malignancies after allogeneic hematopoietic cell transplantation: prognostic relevance of the initial CD3+ T cell dose. *Biol Blood Marrow Transplant* 2013; **19**: 949–957.
21 Bishop MR. Donor lymphocyte infusion: beauty is in the eye of the beholder. *Biol Blood Marrow Transplant* 2013; **19**: 849–850.
22 Miller JS, Weisdorf DJ, Burns LJ, Slungaard A, Wagner JE, Verneris MR *et al.* Lymphodepletion followed by donor lymphocyte infusion (DLI) causes significantly more acute graft-versus-host disease than DLI alone. *Blood* 2007; **110**: 2761–2763.
23 Guillaume T, Gaugler B, Chevallier P, Delaunay J, Ayari S, Clavert A *et al.* Escalated lymphodepletion followed by donor lymphocyte infusion can induce a graft-versus-host response without overwhelming toxicity. *Bone Marrow Transplant* 2012; **47**: 1112–1117.
24 Deol A, Lum LG. Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. *Cancer Treat Rev* 2010; **36**: 528–538.
25 Alyea EP, Soiffer RJ, Canning C, Neuberg D, Schlossman R, Pickett C *et al.* Toxicity and efficacy of defined doses of CD4(+) donor lymphocytes for treatment of relapse after allogeneic bone marrow transplant. *Blood* 1998; **91**: 3671–3680.
26 Giralt S, Hester J, Huh Y, Hirsch-Ginsberg C, Rondón G, Seong D *et al.* CD8-depleted donor lymphocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogenic bone marrow transplantation. *Blood* 1995; **86**: 4337–4343.
27 Alyea EP, Canning C, Neuberg D, Daley H, Houde H, Giralt S *et al.* CD8+ cell depletion of donor lymphocyte infusions using cd8 monoclonal antibody-coated high-density microparticles (CD8-HDM) after allogeneic hematopoietic stem cell transplantation: a pilot study. *Bone Marrow Transplant* 2004; **34**: 123–128.
28 Zorn E, Wang KS, Hochberg EP, Canning C, Alyea EP, Soiffer RJ *et al.* Infusion of CD4+ donor lymphocytes induces the expansion of CD8+ donor T cells with cytolytic activity directed against recipient hematopoietic cells. *Clin Cancer Res* 2002; **8**: 2052–2060.
29 Zhang W, Choi J, Zeng W, Rogers SA, Alyea EP, Rheinwald JG *et al.* Graft-versus-leukemia antigen CML66 elicits coordinated B-cell and T-cell immunity after donor lymphocyte infusion. *Clin Cancer Res* 2010; **16**: 2729–2739.
30 Bachireddy P, Hainz U, Rooney M, Pozdnyakova O, Aldridge J, Zhang W *et al.* Reversal of in situ T-cell exhaustion during effective human antileukemia responses to donor lymphocyte infusion. *Blood* 2014; **123**: 1412–1421.
31 Maury S, Lemoine FM, Hicheri Y, Rosenzwajg M, Badoual C, Cherai M *et al.* CD4+CD25+ regulatory T cell depletion improves the graft-versus-tumor effect of donor lymphocytes after allogeneic hematopoietic stem cell transplantation. *Sci Transl Med* 2010; **2**: 41ra52.
32 Fowler DH, Mossoba ME, Steinberg SM, Halverson DC, Stroncek D, Khuu HM *et al.* Phase 2 clinical trial of rapamycin-resistant donor CD4+ Th2/Th1 (T-Rapa) cells after low-intensity allogeneic hematopoietic cell transplantation. *Blood* 2013; **121**: 2864–2874.
33 Falkenburg JH, Wafelman AR, Joosten P, Smit WM, van Bergen CA, Bongaerts R *et al.* Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. *Blood* 1999; **94**: 1201–1208.
34 Kloosterboer FM, van Luxemburg-Heijs SA, van Soest RA, van Egmond HM, Barbui AM, Strijbosch MP *et al.* Minor histocompatibility antigen-specific T cells with multiple distinct specificities can be isolated by direct cloning of IFN-gamma-secreting T cells from patients with relapsed leukemia responding to donor lymphocyte infusion. *Leukemia* 2005; **19**: 83–90.
35 Marijt E, Wafelman A, van der Hoorn M, van Bergen C, Bongaerts R, van Luxemburg-Heijs S *et al.* Phase I/II feasibility study evaluating the generation of leukemia-reactive cytotoxic T lymphocyte lines for treatment of patients with relapsed leukemia after allogeneic stem cell transplantation. *Haematologica* 2007; **92**: 72–80.
36 Meij P, Jedema I, van der Hoorn MA, Bongaerts R, Cox L, Wafelman AR *et al.* Generation and administration of HA-1-specific T-cell lines for the treatment of patients with relapsed leukemia after allogeneic stem cell transplantation: a pilot study. *Haematologica* 2012; **97**: 1205–1208.
37 van Loenen MM, de Boer R, van Liempt E, Meij P, Jedema I, Falkenburg JHF *et al.* A Good Manufacturing Practice procedure to engineer donor virus-specific T cells into potent anti-leukemic effector cells. *Haematologica* 2014; **99**: 759–768.
38 Warren EH, Fujii N, Akatsuka Y, Chaney CN, Mito JK, Loeb KR *et al.* Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. *Blood* 2010; **115**: 3869–3878.
39 Hardy NM, Fellowes V, Rose JJ, Odom J, Pittaluga S, Steinberg SM *et al.* Costimulated tumor-infiltrating lymphocytes are a feasible and safe alternative donor cell therapy for relapse after allogeneic stem cell transplantation. *Blood* 2012; **119**: 2956–2959.
40 Bornhauser M, Thiede C, Platzbecker U, Kiani A, Oelschlaegel U, Babatz J *et al.* Prophylactic transfer of BCR-ABL-, PR1-, and WT1-reactive donor T cells after T cell-depleted allogeneic hematopoietic cell transplantation in patients with chronic myeloid leukemia. *Blood* 2011; **117**: 7174–7184.
41 Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA *et al.* Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* 2010; **116**: 4099–4102.
42 Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A *et al.* T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011; **3**: 95ra73.
43 Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; **365**: 725–733.
44 Till BG, Jensen MC, Wang J, Qian X, Gopal AK, Maloney DG *et al.* CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood* 2012; **119**: 3940–3950.
45 Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I *et al.* B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 2012; **119**: 2709–2720.
46 Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR *et al.* Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368**: 1509–1518.
47 Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG *et al.* CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* 2013; **5**: 177ra138.
48 Cruz CR, Micklethwaite KP, Savoldo B, Ramos CA, Lam S, Ku S *et al.* Infusion of donor-derived CD19-redirected virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study. *Blood* 2013; **122**: 2965–2973.
49 Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG *et al.* Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* 2013; **122**: 4129–4139.
50 Gross G, Gorochov G, Waks T, Eshhar Z. Generation of effector T cells expressing chimeric T cell receptor with antibody type-specificity. *Transplant Proc* 1989; **21**: 127–130.
51 Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA *et al.* A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006; **12**: 6106–6115.
52 Melenhorst JJ, Levine BL. Innovation and opportunity for chimeric antigen receptor targeted T cells. *Cytotherapy* 2013; **15**: 1046–1053.
53 Heslop HE. Safer CARS. *Mol Ther* 2010; **18**: 661–662.
54 Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol Ther* 2010; **18**: 666–668.
55 Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. *Nat Rev Clin Oncol* 2013; **10**: 267–276.
56 Fry TJ, Mackall CL. T-cell adoptive immunotherapy for acute lymphoblastic leukemia. *Hemaology Am Soc Hematol Educ Program* 2013; **2013**: 348–353.
57 Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood* 2014; **123**: 2625–2635.

58 Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD *et al.* Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* 2011; **118**: 6050–6056.
59 Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J *et al.* Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* 2007; **15**: 825–833.
60 Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE *et al.* Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. *Blood* 2007; **110**: 2620–2630.
61 Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C *et al.* T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. *Blood* 2006; **108**: 3890–3897.
62 Ahmed N, Ratnayake M, Savoldo B, Perlaky L, Dotti G, Wels WS *et al.* Regression of experimental medulloblastoma following transfer of HER2-specific T cells. *Cancer Res* 2007; **67**: 5957–5964.
63 Casucci M, Nicolis di Robilant B, Falcone L, Camisa B, Norelli M, Genovese P *et al.* CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. *Blood* 2013; **122**: 3461–3472.
64 Robbins PF, Dudley ME, Wunderlich J, El-Gamil M, Li YF, Zhou J *et al.* Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. *J Immunol* 2004; **173**: 7125–7130.
65 Huang J, Khong HT, Dudley ME, El-Gamil M, Li YF, Rosenberg SA *et al.* Survival, persistence, and progressive differentiation of adoptively transferred tumor-reactive T cells associated with tumor regression. *J Immunother* 2005; **28**: 258–267.
66 Kalos M. Biomarkers in T cell therapy clinical trials. *J Transl Med* 2011; **9**: 138.
67 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999; **401**: 708–712.
68 Zhang Y, Joe G, Hexner E, Zhu J, Emerson SG. Host-reactive CD8 + memory stem cells in graft-versus-host disease. *Nat Med* 2005; **11**: 1299–1305.
69 Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF *et al.* A human memory T cell subset with stem cell-like properties. *Nat Med* 2011; **17**: 1290–1297.
70 Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E *et al.* IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. *Blood* 2013; **121**: 573–584.
71 Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z *et al.* Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8 + T cells. *J Clin Invest* 2005; **115**: 1616–1626.
72 Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8 + T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest* 2008; **118**: 294–305.
73 Levine BL, Bernstein WB, Connors M, Craighead N, Lindsten T, Thompson CB *et al.* Effects of CD28 costimulation on long-term proliferation of CD4 + T cells in the absence of exogenous feeder cells. *J Immunol* 1997; **159**: 5921–5930.
74 Bondanza A, Valtolina V, Magnani Z, Ponzoni M, Fleischhauer K, Bonyhadi M *et al.* Suicide gene therapy of graft-versus-host disease induced by central memory human T lymphocytes. *Blood* 2006; **107**: 1828–1836.
75 Kaneko S, Mastaglio S, Bondanza A, Ponzoni M, Sanvito F, Aldrighetti L *et al.* IL-7 and IL-15 allow the generation of suicide gene-modified alloreactive self-renewing central memory human T lymphocytes. *Blood* 2009; **113**: 1006–1015.
76 Porter DL, Levine BL, Bunin N, Stadtmauer EA, Luger SM, Goldstein S *et al.* A phase 1 trial of donor lymphocyte infusions expanded and activated ex vivo via CD3/CD28 costimulation. *Blood* 2006; **107**: 1325–1331.
77 Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C *et al.* Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011; **365**: 1673–1683.
78 Moretta L, Moretta A. Killer immunoglobulin-like receptors. *Curr Opin Immunol* 2004; **16**: 626–633.
79 Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K *et al.* Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 1999; **94**: 333–339.
80 Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kuhne T *et al.* Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. *Leukemia* 2004; **18**: 1835–1838.
81 Koehl U, Sorensen J, Esser R, Zimmermann S, Gruttner HP, Tonn T *et al.* IL-2 activated NK cell immunotherapy of three children after haploidentical stem cell transplantation. *Blood Cells Mol Dis* 2004; **33**: 261–266.
82 Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T *et al.* NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *J Clin Oncol* 2010; **28**: 955–959.
83 Nguyen S, Beziat V, Norol F, Uzunov M, Trebeden-Negre H, Azar N *et al.* Infusion of allogeneic natural killer cells in a patient with acute myeloid leukemia in relapse after haploidentical hematopoietic stem cell transplantation. *Transfusion* 2011; **51**: 1769–1778.
84 Stern M, Passweg JR, Meyer-Monard S, Esser R, Tonn T, Soerensen J *et al.* Pre-emptive immunotherapy with purified natural killer cells after haploidentical SCT: a prospective phase II study in two centers. *Bone Marrow Transplant* 2012; **48**: 433–438.
85 Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK *et al.* Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; **105**: 3051–3057.
86 Curti A, Ruggeri L, D'Addio A, Bontadini A, Dan E, Motta MR *et al.* Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. *Blood* 2011; **118**: 3273–3279.
87 Introna M, Borleri G, Conti E, Franceschetti M, Barbui AM, Broady R *et al.* Repeated infusions of donor-derived cytokine-induced killer cells in patients relapsing after allogeneic stem cell transplantation: a phase I study. *Haematologica* 2007; **92**: 952–959.
88 Laport GG, Sheehan K, Baker J, Armstrong R, Wong RM, Lowsky R *et al.* Adoptive immunotherapy with cytokine-induced killer cells for patients with relapsed hematologic malignancies after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011; **17**: 1679–1687.
89 Linn YC, Niam M, Chu S, Choong A, Yong HX, Heng KK *et al.* The anti-tumour activity of allogeneic cytokine-induced killer cells in patients who relapse after allogeneic transplant for haematological malignancies. *Bone Marrow Transplant* 2012; **47**: 957–966.
90 Zhou X, Zhu J, Sun H, Shao L, Xu M, Guo H. Family haploidentical donor-derived cytokine-induced killer cell biotherapy combined with bortezomib in two patients with relapsed multiple myeloma in a non-allogeneic transplant setting. *Leuk Lymphoma* 2013; **54**: 209–211.
91 Introna M, Pievani A, Borleri G, Capelli C, Algarotti A, Mico C *et al.* Feasibility and safety of adoptive immunotherapy with CIK cells after cord blood transplantation. *Biol Blood Marrow Transplant* 2010; **16**: 1603–1607.
92 Introna M, Franceschetti M, Ciocca A, Borleri G, Conti E, Golay J *et al.* Rapid and massive expansion of cord blood-derived cytokine-induced killer cells: an innovative proposal for the treatment of leukemia relapse after cord blood transplantation. *Bone Marrow Transplant* 2006; **38**: 621–627.
93 Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. *Nat Rev Cancer* 2013; **13**: 525–541.
94 Fischbach MA, Bluestone JA, Lim WA. Cell-based therapeutics: the next pillar of medicine. *Sci Transl Med* 2013; **5**: 179ps177.
95 Marin V, Pizzitola I, Agostoni V, Attianese GM, Finney H, Lawson A *et al.* Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: improvement of their immune activity by expression of CD33-specific chimeric receptors. *Haematologica* 2010; **95**: 2144–2152.
96 Terakura S, Yamamoto TN, Gardner RA, Turtle CJ, Jensen MC, Riddell SR. Generation of CD19-chimeric antigen receptor modified CD8 + T cells derived from virus-specific central memory T cells. *Blood* 2012; **119**: 72–82.
97 Jin L, Lee EM, Ramshaw HS, Busfield SJ, Peoppl AG, Wilkinson L *et al.* Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. *Cell Stem Cell* 2009; **5**: 31–42.
98 Snauwaert S, Vandekerckhove B, Kerre T. Can immunotherapy specifically target acute myeloid leukemic stem cells? *Oncoimmunology* 2013; **2**: e22943.
99 Tettamanti S, Marin V, Pizzitola I, Magnani CF, Giordano Attianese GM, Cribioli E *et al.* Targeting of acute myeloid leukaemia by cytokine-induced killer cells redirected with a novel CD123-specific chimeric antigen receptor. *Br J Haematol* 2013; **161**: 389–401.
100 Mardiros A, Dos Santos C, McDonald T, Brown CE, Wang X, Budde LE *et al.* T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. *Blood* 2013; **122**: 3138–3148.
101 Pizzitola I, Anjos-Afonso F, Rouault-Pierre K, Lassailly F, Tettamanti S, Spinelli O *et al.* Chimeric antigen receptors against CD33/CD123 antigens efficiently target primary acute myeloid leukemia cells *in vivo*. *Leukemia* 2014; e-pub ahead of print 7 February 2014; doi:10.1038/leu.2014.62.
102 Hackett PB, Largaespada DA, Cooper LJ. A transposon and transposase system for human application. *Mol Ther* 2010; **18**: 674–683.
103 Kebriaei P, Huls H, Jena B, Munsell M, Jackson R, Lee DA *et al.* Infusing CD19-directed T cells to augment disease control in patients undergoing autologous hematopoietic stem-cell transplantation for advanced B-lymphoid malignancies. *Hum Gene Ther* 2012; **23**: 444–450.
104 Gill S, Tasian SK, Ruella M, Shestova O, Li Y, Porter DL *et al.* Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. *Blood* 2014; **123**: 2343–2354.