

Review Article

A long road of T-cells to cure cancer: from adoptive immunotherapy with unspecific cellular products to donor lymphocyte infusions and transfer of engineered tumor-specific T-cells

Alexandros Spyridonidis, Maria Liga

Hematology Division, BMT Unit, University Hospital of Patras, 26500 Rio, Greece

Received March 25, 2012; accepted May 3, 2012; Epub May 18, 2012; Published June 15, 2012

Abstract: The notion that immunocompetent cells, contained within adult bone marrow or peripheral blood, are capable of mediating an antitumor effect was first validated experimentally in 1957. T-cell immunotherapy for malignant disease is now routinely used in the context of allogeneic bone marrow transplantation. After 50 years of investigations into the use of T-cells for cancer therapy, adoptive cellular immunotherapy for cancer has progressed from the delivery of unspecific cellular products to the transfer of engineered tumor-specific T-cells. Adoptive cellular immunotherapy for cancer has now reached a stage of increasing feasibility and efficacy.

Keywords: Immunocompetent cells, antitumor effect, T-cell immunotherapy, allogeneic bone marrow transplantation, tumor-specific T-cells

History of adoptive immunotherapy of malignancy

The idea that immunocompetent cells are capable of mediating an antitumor effect was first validated experimentally in 1957 by Barnes and Loutit [1] who showed that leukemic animals that were lethally irradiated and reconstituted with allogeneic bone marrow had a lower tumor burden following transplantation than animals that were reconstituted with syngeneic marrow. In 1973, Bortin and colleagues [2, 3] attempted to quantify the immunologic antitumor effect, which they called "Graft versus Leukemia, GvL-effect," of donor lymphocytes. These observations in animal models led Mathé and colleagues [4] to speculate that leukocyte transfusions could mediate antitumor effects in cancer-bearing recipients. To test this hypothesis, pooled white cell products were transfused into non-transplanted patients with end-stage acute leukemia, which resulted in responses [5]. Meanwhile, a series of clinical observations provided evidence for GvL activity in humans. These included a higher relapse rate in recipi-

ents of syngeneic compared with allogeneic transplants [6], a reduction in relapse rates in patients with graft-vs-host disease (GvHD) compared with those free of GvHD [7], induction of remission in patients after withdrawal of immunosuppression [8], and higher relapse rates in recipients receiving T-cell-depleted grafts compared with unmanipulated grafts [9].

The first patient to receive donor lymphocyte infusions (DLI) for a hematologic malignancy in relapse after Bone Marrow Transplantation (BMT) was a boy with acute lymphocytic leukemia (ALL) that was resistant to chemotherapy and cytokines [10]. He ultimately obtained a sustained complete remission of his disease by receiving multiple transfusions of lymphocytes from his sister, the original bone marrow donor. Then, Kolb and colleagues [11] reported on 3 patients with relapsed chronic myeloid leukemia (CML) who failed to respond to treatment with interferon-*alpha* but who obtained complete remissions with the combination of IFN-*alpha* plus DLI. Thus, the era of adoptive immunotherapy to treat post-transplant relapse of hema-

tologic malignancies was born.

Pathobiology of graft-versus-leukemia effect

Several pathways by which the GvL effect may eliminate tumor cells have been suggested [12]. Extensive analysis in both human and animal studies has shown that GvL activity is mediated primarily by T-cells and NK cells, although other cells can also contribute through direct or indirect mechanisms. Cytotoxic T-cells execute their function by direct killing of leukemia cells via the perforin/granzyme system or by triggering the target cell's own apoptotic pathways via cytokines, such as tumor necrosis factor-alpha and IFN-gamma. In some cases, donor T-cells can recognize a tumor-specific antigen (eg, Bcr/Abl in CML) or a minor histocompatibility antigen, such as a polymorphic antigen with restricted expression on hematopoietic cells (eg, HA-1/2); however, the major contributors to the GvL activity of donor T-cells are likely alloreactive T-cells recognizing alloantigens on tumor cells and normal tissue cells in the recipient. Clinical studies support this notion and have demonstrated an inverse correlation between GvHD (especially chronic GvHD) and the risk of post-transplantation relapse. However, DLI-induced leukemia responses can occur in the absence of GvHD and it is still difficult to separate GvL from GvH mechanistically.

Efficacy of donor lymphocyte infusions

The best responses to DLI occur in patients with CML. There are important issues that we have learned from the DLI treatment strategy in relapsed CML. First, the most powerful predictor of the response to DLI is disease stage. Thus, DLI induces cytogenetic complete remissions in 81% of patients whose relapse was detectable only by cytogenetic analysis, but in only 33% of patients whose disease had transformed to either accelerated phase or blast crisis [13, 14]. Second, the T-cell dose, expressed as the number of CD3+ cells per kilogram of recipient body weight, seems to influence response rates, at least in relapsed CML. In two elegant studies Mackinnon and colleagues [13, 14] found that after HLA-identical sibling BMT for CML a total CD3 dose $>10^7$ cells/kg given in an escalation manner (and not as a single bulk dose regimen) provide the best response rates while GvHD rates remain low. Third, GvL after DLI in CML is prolonged. The time to complete cytogenetic

response and molecular response following the initial infusion of DLI is 8-16 weeks and >6 months, respectively. Patients with other malignancies respond less frequently to DLI. Response rates of 25-50% have been reported in relatively indolent diseases like multiple myeloma, chronic lymphocytic leukemia and other low grade lymphomas [15]. In acute myeloid (AML) leukemia, remissions have been documented even less frequently (about 21%) [16]. Remissions after DLI are generally of short duration, and long-term survival is $<20\%$ [17].

In two large retrospective series of DLI published in the late 1990s, response rates in AML were only 15-29% [18, 19]. DLI from unrelated donors maybe associated with a higher response rate than that seen from related donors, with one series reporting 42% of patients achieving a complete response after unrelated DLI [20]. When DLI has been used for recurrent or persistent AML or MDS after non-meloablative transplantation (Seattle protocol), the response rates have been low and the 1-year survival rate was only 11% for AML and 8% for MDS [21]. The most recent comprehensive assessment of DLI in AML was conducted by the European Group for Blood and Marrow Transplantation and published in 2007 [22]. In this retrospective analysis, estimated survival at 2 years was superior in the 171 patients receiving DLI compared with the 228 patients who did not receive DLI (21% vs 9%), despite the fact that a smaller number of patients went on to second allogeneic haematopoietic stem cell transplantation in the DLI group compared with the non-DLI group (13 vs 33). After adjustment for all the clinical variables in the two groups, DLI administration ($P=0.04$) appeared to be associated with improved outcome, together with younger patient age ($P=0.008$) and relapse occurring more than 5 months following transplantation ($P<0.0001$). Among AML patients who received DLI, marrow blast count at relapse, female gender, favourable cytogenetics and remission at the time of DLI were predictive of survival in multivariable analysis. For patients who received DLI in remission and had favourable cytogenetics, 2-year overall survival was estimated at 56%. In contrast, patients who received DLI during active disease had an OS of 9% to 20% (overall 15%), depending on other risk factors.

Reasons for reduced DLI efficacy in acute

myeloid leukemia

Limited efficacy of DLI for relapsed AML may be probably related to the rapid leukemia cell growth which may outpace the cytotoxicity of donor leukocytes. After donor cell infusion, the T-cells must recognize antigen, become activated, proliferate to a critical number of effectors and target the malignant cells before cell killing can occur. That interval may be too long and patients with relapsed acute leukemia may progress rapidly and die before a GvL effect is evident. Alternatively, lack of response to DLI in AML may be due to intrinsic differences in susceptibility of different tumor types to adoptive immunotherapy (lack of expression of recognizable tumor-specific antigens, lack of costimulatory molecules on malignant cells, inhibition of T cell activation or function or weak tumor cell killing). Other immune escape mechanisms of leukemic cells from GvL include defects in presentation of antigenic peptides, presence of areas of immunological sanctuary in the recipient (e.g. a significant fraction of patients relapsing after an initial response to DLI have done so in extramedullary sites) and downregulation of HLA molecules. In a very elegant paper published in *New England Journal of Medicine*, Vago et al. [23] reported that after haploidentical transplantation, leukemic cells can escape from the donor's antileukemic T-cells through the loss of the mismatched HLA haplotype.

DLI's are not always the same

Most commonly, the DLI product is obtained by leukapheresis of unstimulated peripheral blood, containing approximately 2×10^8 mononuclear cells, of which about 50% are CD3+ cells, but also includes B-cells, dendritic cells and others. The composition of DLI is altered when G-CSF is given to the donors prior to apheresis or DLI are isolated from the G-CSF primed stem cell graft. G-CSF priming in normal donors increases the yield of CD3+ cells by almost threefold and changes the cytokine profile resulting in Th2-type differentiation, increase of Tregs and expansion of immature antigen presenting cells (APCs). Whether G-CSF mobilized DLI will affect GVHD or GvL remains to be determined.

Regarding dosing of DLI, no obvious dose-response relationship has been identified for diseases other than CML [18-20]. Similarly, there appears to be no relationship between DLI dose and GVHD. Although many investigators

use a lower dose for unrelated DLI than for matched sibling DLI, limited retrospective data have identified neither a higher risk of GVHD for unrelated DLI nor a relationship between unrelated DLI dose and either response or toxicity.

Next generation DLI's

Several unique methods explore the dual goals of improving the efficacy of DLI without increasing GvHD/Transplant Related Mortality (TRM). Small numbers of patients have been treated with each of these novel strategies and no solid conclusions can be made. These methods, however, appear promising and deserve further exploration. These include: (1) Ex vivo activation and expansion of donor T-cells through costimulation (activated DLI) [24]. (2) Generation and infusion of minor histocompatibility antigen-specific T-cells [25]. (3) Infusion of selected T-cell subsets (CD8+ cell depletion or CD4+ cell selection) [26]. (4) Promote the in vivo clonal expansion of leukemia-reactive T-cells (e.g. via IL-2 administration) [27]. (5) Enhancement of AML antigen presentation to T-cells (e.g. via administration of GM-CSF) [28]. (6) Inactivation of alloreactive T-cells (that is, through transduction of suicide genes into donor T-cells, photochemical inactivation, chemotherapy inactivation, irradiation) [29, 30]. (7) Infusion of regulatory cells as an attempt to minimize post DLI-GvHD. (8) Generation and infusion of Th2-type T-cells. (9) Lymphodepletion followed by DLI (eg promote in vivo expansion of the infused lymphocytes by providing lymphoid space and eliminating suppressive regulatory T-cells) [31]. (10) Epigenetic modulation with low-dose 5-azacytidine followed by DLI (e.g. enhance immune effects through upregulation of HLA or cancer testis antigens on leukemic blasts) [32, 33]. (11) Biological markers predicting the safety and efficacy of DLI. (e.g. CTLA-4 Single Nucleotide Polymorphism may be used as a surrogate marker for DLI outcome after allogeneic hematopoietic cell transplantation for acute leukemia) [34]. (12) Since immunotherapy seems to be more effective in stages of impending, rather than overt relapse, perhaps the most appropriate platform for DLIs is in a prophylactic setting [35-37].

Adoptive immunotherapy using cancer specific T-cells

In order to make T-cell products more specific and potent, researchers sought to generate

Adoptive cellular immunotherapy for cancer

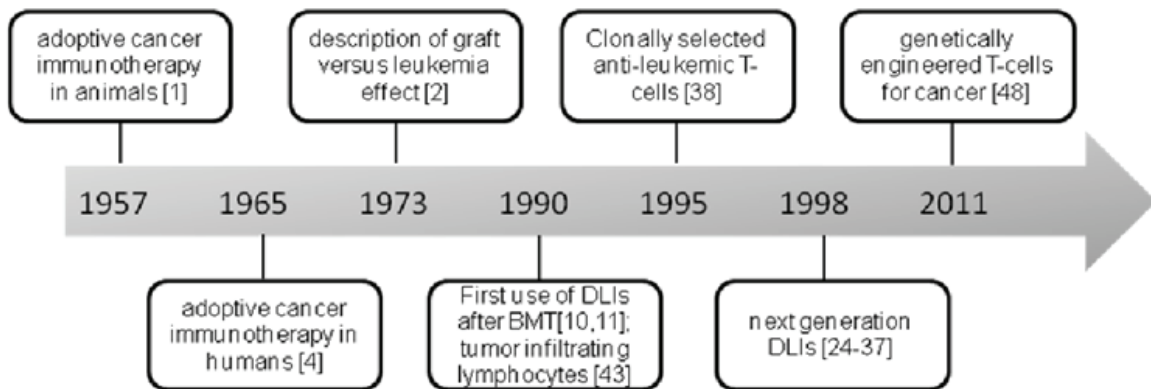


Figure 1. The long road of T-cells to cure cancer

leukemia specific T-cells by in vitro stimulation of donor cells with patients' leukemia. Falkenburg and colleagues [38] were the first to show proof of principle that clonally selected antileukemic T-cells could induce long remissions in patients with CML relapsing after bone marrow transplantation. Accumulating clinical data provide evidence in support of the effectiveness of adoptive T-cell therapy to control cancer [39-42]. The group of Rosenberg pioneered the use of tumor infiltrating lymphocytes (TIL) to patients with melanoma [43] and Heslop et al. used virus-specific T-cells from peripheral blood to control and eliminate post-transplant EBV-specific lymphoproliferative disease [44]. These early clinical trials have helped to understand the hallmarks of T-cell therapies and provided critical insights to the manufacturing of a therapeutically robust product, such as selection of specific tumor antigens (e.g. PR1, WT1) [45] and generation of T-cells with high avidity for specific tumor antigens and with specific phenotypes (e.g. both CD4 and CD8 T-cells, central memory subsets [46]). Next generation antigen-specific T-cells which show resistance to TGF-beta [47] or T-cells that were introduced to express a tumor-specific T-cell receptor (TCR) [42] or a chimeric antigen receptor (CAR, composed of a tumor targeting antibody fused to an activation molecule) [48] have been shown to overcome tolerance and are tested in trials presently under way at a number of academic institutions.

Conclusion

Although many hurdles remain unresolved, adoptive cellular immunotherapy for cancer has

now reached a stage of increasing feasibility and efficacy [49]. Development of facilities and experienced staffs for Good Manufacturing Practice (GMP) production of cellular immunotherapeutic products is currently extremely expensive. As T-cell manufacturing processes become more robust, closed and automated [50], it is anticipated that smaller facilities will be able to consistently manufacture a GMP cell product, leading to the wider dissemination of T-cell based therapies to patients (**Figure 1**).

Acknowledgments

This work has been supported by the Research Committee of Patras University within the framework of K. Karatheodoris scholarships (to AS). The authors declare no conflict of interest.

Address correspondence to: Dr. Spyridonidis A, Associate Professor, Head of the BMT Unit, Hematology Division, University Hospital of Patras, 26500 Rio, Patras, Greece E-mail: spyridonidis@upatras.gr

References

- [1] Barnes DW and Loutit JF. Treatment of murine leukaemia with x-rays and homologous bone marrow. II. *Br J Haematol* 1957; 3: 241-252.
- [2] Bortin MM, Rimm AA and Saltzstein EC. Graft versus leukemia: quantification of adoptive immunotherapy in murine leukemia. *Science* 1973; 179: 811-813.
- [3] Bortin MM, Truitt RL, Rimm AA and Bach FH. Graft-versus-leukaemia reactivity induced by alloimmunisation without augmentation of graft-versus-host reactivity. *Nature* 1979; 281: 490-491.
- [4] Mathe G, Amiel JL, Schwarzenberg L, Cattani A and Schneider M. Adoptive immunotherapy of

Adoptive cellular immunotherapy for cancer

- acute leukemia: experimental and clinical results. *Cancer Res* 1965; 25: 1525-1531.
- [5] Schwarzenberg L, Mathe G, Schneider M, Amiel JL, Cattani A and Schlumberger JR. Attempted adoptive immunotherapy of acute leukaemia by leucocyte transfusions. *Lancet* 1966; 2: 365-368.
- [6] Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, Rimm AA, Ringden O, Rozman C, Speck B, Truitt RL, Zwaan FE, Bortin MM. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; 75: 555-562.
- [7] Weiden PL, Sullivan KM, Flournoy N, Storb R and Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* 1981; 304: 1529-1533.
- [8] Collins RH Jr, Rogers ZR, Bennett M, Kumar V, Nikein A and Fay JW. Hematologic relapse of chronic myelogenous leukemia following allogeneic bone marrow transplantation: apparent graft-versus-leukemia effect following abrupt discontinuation of immunosuppression. *Bone Marrow Transplant* 1992; 10: 391-395.
- [9] Goldman JM, Gale RP, Horowitz MM, Biggs JC, Champlin RE, Gluckman E, Hoffmann RG, Jacobsen SJ, Marmont AM, McGlave PB, Messner HA, Rimm AA, Rozman C, Speck B, Tura S, Weiner RS, Bortin MM. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann Intern Med* 1988; 108: 806-814.
- [10] Slavin S, Naparstek E, Nagler A, Ackerstein A, Kapelushnik J and Or R. Allogeneic cell therapy for relapsed leukemia after bone marrow transplantation with donor peripheral blood lymphocytes. *Exp Hematol* 1995; 23: 1553-1562.
- [11] Kolb HJ, Mittermuller J, Clemm C, Holler E, Lederose G, Brehm G, Heim M and Wilmanns W. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; 76: 2462-2465.
- [12] van den Brink MR and Burakoff SJ. Cytolytic pathways in haematopoietic stem-cell transplantation. *Nat Rev Immunol* 2002; 2: 273-281.
- [13] Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, Castro-Malaspina H, Childs BH, Gillio AP, Kernan NA, Small TN, Young JW, O'reilly RJ. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* 1995; 86: 1261-1268.
- [14] Dazzi F, Szydlo RM, Craddock C, Cross NC, Kaeda J, Chase A, Olavarria E, van Rhee F, Kanfer E, Apperley JF and Goldman JM. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood* 2000; 95: 67-71.
- [15] Zeiser R, Bertz H, Spyridonidis A, Houet L and Finke J. Donor lymphocyte infusions for multiple myeloma: clinical results and novel perspectives. *Bone Marrow Transplant* 2004; 34: 923-928.
- [16] Loren AW and Porter DL. Donor leukocyte infusions for the treatment of relapsed acute leukemia after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2008; 41: 483-493.
- [17] Porter DL, Collins RH Jr, Shpilberg O, Drobyski WR, Connors JM, Sproles A and Antin JH. Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol Blood Marrow Transplant* 1999; 5: 253-261.
- [18] Collins RH Jr, Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R, Goodman SA, Wolff SN, Hu W, Verfaillie C, List A, Dalton W, Ognoskie N, Chetrit A, Antin JH and Nemunaitis J. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 1997; 15: 433-444.
- [19] Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, Ljungman P, Ferrant A, Verdonck L, Niederwieser D, van Rhee F, Mittermuller J, de Witte T, Holler E and Ansari H. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 1995; 86: 2041-2050.
- [20] Porter DL, Collins RH Jr, Hardy C, Kernan NA, Drobyski WR, Giralt S, Flowers ME, Casper J, Leahey A, Parker P, Mick R, Bate-Boyle B, King R and Antin JH. Treatment of relapsed leukemia after unrelated donor marrow transplantation with unrelated donor leukocyte infusions. *Blood* 2000; 95: 1214-1221.
- [21] Bethge WA, Hegenbart U, Stuart MJ, Storer BE, Maris MB, Flowers ME, Maloney DG, Chauncey T, Bruno B, Agura E, Forman SJ, Blume KG, Niederwieser D, Storb R and Sandmaier BM. Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood* 2004; 103: 790-795.
- [22] Schmid C, Labopin M, Nagler A, Bornhauser M, Finke J, Fassas A, Volin L, Gurman G, Maertens J, Bordignon P, Holler E, Ehninger G, Polge E, Gorin NC, Kolb HJ and Rocha V. 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.
- [23] Vago L, Perna SK, Zanussi M, Mazzi B, Bar-

- lassina C, Stanghellini MT, Perrelli NF, Cosentino C, Torri F, Angius A, Forno B, Casucci M, Bernardi M, Peccatori J, Corti C, Bondanza A, Ferrari M, Rossini S, Roncarolo MG, Bordignon C, Bonini C, Ciceri F and Fleischhauer K. Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med* 2009; 361: 478-488.
- [24] Porter DL, Levine BL, Bunin N, Stadtmauer EA, Luger SM, Goldstein S, Loren A, Phillips J, Nasta S, Perl A, Schuster S, Tsai D, Sohal A, Veloso E, Emerson S and June CH. A phase 1 trial of donor lymphocyte infusions expanded and activated ex vivo via CD3/CD28 costimulation. *Blood* 2006; 107: 1325-1331.
- [25] Marijt WA, Heemskerck MH, Kloosterboer FM, Goulmy E, Kester MG, van der Hoorn MA, van Luxemburg-Heys SA, Hoogeboom M, Mutis T, Drijfhout JW, van Rood JJ, Willemze R and Falkenburg JH. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci USA* 2003; 100: 2742-2747.
- [26] Alyea EP, Soiffer RJ, Canning C, Neuberg D, Schlossman R, Pickett C, Collins H, Wang Y, Anderson KC and Ritz J. Toxicity and efficacy of defined doses of CD4(+) donor lymphocytes for treatment of relapse after allogeneic bone marrow transplant. *Blood* 1998; 91: 3671-3680.
- [27] Nadal E, Fowler A, Kanfer E, Apperley J, Goldman J and Dazzi F. Adjuvant interleukin-2 therapy for patients refractory to donor lymphocyte infusions. *Exp Hematol* 2004; 32: 218-223.
- [28] Schmid C, Schleuning M, Aschan J, Ringden O, Hahn J, Holler E, Hegenbart U, Niederwieser D, Dugas M, Ledderose G and Kolb HJ. Low-dose ARAC, donor cells, and GM-CSF for treatment of recurrent acute myeloid leukemia after allogeneic stem cell transplantation. *Leukemia* 2004; 18: 1430-1433.
- [29] Ciceri F, Bonini C, Marktel S, Zappone E, Servida P, Bernardi M, Pescarollo A, Bondanza A, Peccatori J, Rossini S, Magnani Z, Salomoni M, Benati C, Ponzoni M, Callegaro L, Corradini P, Bregni M, Traversari C and Bordignon C. Antitumor effects of HSV-TK-engineered donor lymphocytes after allogeneic stem-cell transplantation. *Blood* 2007; 109: 4698-4707.
- [30] Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, Liu H, Cruz CR, Savoldo B, Gee AP, Schindler J, Krance RA, Heslop HE, Spencer DM, Rooney CM and Brenner MK. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011; 365: 1673-1683.
- [31] Miller JS, Weisdorf DJ, Burns LJ, Slungaard A, Wagner JE, Verneris MR, Cooley S, Wangen R, Fautsch SK, Nicklow R, Defor T and Blazar BR. Lymphodepletion followed by donor lymphocyte infusion (DLI) causes significantly more acute graft-versus-host disease than DLI alone. *Blood* 2007; 110: 2761-2763.
- [32] Lubbert M, Bertz H, Wasch R, Marks R, Ruter B, Claus R and Finke J. Efficacy of a 3-day, low-dose treatment with 5-azacytidine followed by donor lymphocyte infusions in older patients with acute myeloid leukemia or chronic myelomonocytic leukemia relapsed after allografting. *Bone Marrow Transplant* 2010; 45: 627-632.
- [33] Craddock C. Epigenetic manipulation of the immune response: a novel treatment strategy in hematologic malignancies. *Cytotherapy* 2011; 13: 516-517.
- [34] Metaxas Y, Bertz H, Spyridonidis A, Spyropoulou-Vlachou M, Porzelius C and Finke J. CT60 single-nucleotide polymorphism as a surrogate marker for donor lymphocyte infusion outcome after allogeneic cell transplantation for acute leukemia. *Bone Marrow Transplant* 2012; 47: 411-415.
- [35] Spyridonidis A, Liga M, Triantafyllou E, Themeli M, Marangos M, Karakantza M and Zoumbos N. Pharmacokinetics and clinical activity of very low-dose alemtuzumab in transplantation for acute leukemia. *Bone Marrow Transplant* 2011; 46: 1363-1368.
- [36] Schmid C, Schleuning M, Ledderose G, Tischer J and Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. *J Clin Oncol* 2005; 23: 5675-5687.
- [37] Rettinger E, Willasch AM, Kreyenberg H, Borkhardt A, Holter W, Kremens B, Strahm B, Woessmann W, Mauz-Koerholz C, Gruhn B, Burdach S, Albert MH, Schlegel PG, Klingebiel T and Bader P. Preemptive immunotherapy in childhood acute myeloid leukemia for patients showing evidence of mixed chimerism after allogeneic stem cell transplantation. *Blood* 2011; 118: 5681-5688.
- [38] Faber LM, van der Hoeven J, Goulmy E, Hooftman-den Otter AL, van Luxemburg-Heijs SA, Willemze R and Falkenburg JH. Recognition of clonogenic leukemic cells, remission bone marrow and HLA-identical donor bone marrow by CD8+ or CD4+ minor histocompatibility antigen-specific cytotoxic T lymphocytes. *J Clin Invest* 1995; 96: 877-883.
- [39] Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, Robbins PF, Huang J, Citrin DE, Leitman SF, Wunderlich J, Restifo NP, Thomasian A, Downey SG, Smith FO, Klapper J, Morton K, Laurencot C, White DE and Rosenberg SA. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol* 2008; 26: 5233-5239.
- [40] Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, Huls MH, Liu E, Gee AP, Mei Z, Yvon E, Weiss HL, Liu H, Rooney CM, Heslop HE

- and Brenner MK. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008; 14: 1264-1270.
- [41] Bollard CM, Gottschalk S, Leen AM, Weiss H, Straathof KC, Carrum G, Khalil M, Wu MF, Huls MH, Chang CC, Gresik MV, Gee AP, Brenner MK, Rooney CM and Heslop HE. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. *Blood* 2007; 110: 2838-2845.
- [42] Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA and Rosenberg SA. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 2006; 314: 126-129.
- [43] Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, Merino MJ, Culver K, Miller AD, Blaese RM, Anderson WF. Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* 1990; 323: 570-578.
- [44] Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, Bollard CM, Liu H, Wu MF, Rochester RJ, Amrolia PJ, Hurwitz JL, Brenner MK and Rooney CM. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* 2010; 115: 925-935.
- [45] Greiner J, Schmitt M, Li L, Giannopoulos K, Bosch K, Schmitt A, Dohner K, Schlenk RF, Pollack JR, Dohner H and Bullinger L. Expression of tumor-associated antigens in acute myeloid leukemia: Implications for specific immunotherapeutic approaches. *Blood* 2006; 108: 4109-4117.
- [46] Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C and 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.
- [47] Bollard CM, Rossig C, Calonge MJ, Huls MH, Wagner HJ, Massague J, Brenner MK, Heslop HE and Rooney CM. Adapting a transforming growth factor beta-related tumor protection strategy to enhance antitumor immunity. *Blood* 2002; 99: 3179-3187.
- [48] Porter DL, Levine BL, Kalos M, Bagg A and June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; 365: 725-733.
- [49] Fox BA, Schendel DJ, Butterfield LH, Aamdal S, Allison JP, Ascierto PA, Atkins MB, Bartunkova J, Bergmann L, Berinstein N, Bonorino CC, Borden E, Bramson JL, Britten CM, Cao X, Carson WE, Chang AE, Characiejus D, Choudhury AR, Coukos G, de Grujil T, Dillman RO, Dolstra H, Dranoff G, Durrant LG, Finke JH, Galon J, Gollob JA, Gouttefangeas C, Grizzi F, Guida M, Hakansson L, Hege K, Herberman RB, Hodi FS, Hoos A, Huber C, Hwu P, Imai K, Jaffee EM, Janetzki S, June CH, Kalinski P, Kaufman HL, Kawakami K, Kawakami Y, Keilholtz U, Khleif SN, Kiessling R, Kotlan B, Kroemer G, Lapointe R, Levitsky HI, Lotze MT, Maccalli C, Maio M, Marschner JP, Mastrangelo MJ, Masucci G, Melero I, Nelief C, Murphy WJ, Nelson B, Nicolini A, Nishimura MI, Odunsi K, Ohashi PS, O'Donnell-Tormey J, Old LJ, Ottensmeier C, Papamichail M, Parmiani G, Pawelec G, Proietti E, Qin S, Rees R, Ribas A, Ridolfi R, Ritter G, Rivoltini L, Romero PJ, Salem ML, Scheper RJ, Seliger B, Sharma P, Shiku H, Singh-Jasuja H, Song W, Straten PT, Tahara H, Tian Z, van Der Burg SH, von Hoegen P, Wang E, Welters MJ, Winter H, Withington T, Wolchok JD, Xiao W, Zitvogel L, Zwierzina H, Marincola FM, Gajewski TF, Wigginton JM and Disis ML. Defining the Critical Hurdles in Cancer Immunotherapy. *J Transl Med* 2011; 9: 214.
- [50] Vera JF, Brenner LJ, Gerdemann U, Ngo MC, Sili U, Liu H, Wilson J, Dotti G, Heslop HE, Leen AM and Rooney CM. Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). *J Immunother* 2010; 33: 305-315.