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Review

Cellular Immunotherapy: Using Alloreactivity to Induce Anti-Leukemic Responses without Prolonged Persistence of Donor Cells

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Abstract: A goal of cancer immunologists is to harness cellular immune responses to achieve anti-cancer responses. One of the strongest activating stimuli for the immune system is the encounter with cells expressing allogeneic HLA molecules. While alloreactive responses can negatively impact of the outcome of hematopoietic stem cell transplant because of graft-versus-host disease (GVHD), these same responses can have anti-leukemic effects. Donor lymphocyte infusions have been used in an attempt to harness alloreactive responses to achieve anti-leukemic responses. Because this protocol is usually carried out in the absence of recipient anti-donor responses, this protocol often induces GVHD as well as anti-leukemic responses. A recent study indicated the infusion of large number of haploidentical donor cells (1–2 × 10^8 $CD3^+$ cells/kg) into patients with refractory hematological malignancies (100 cGy total body irradiation) resulted in 14 (7 major) responses/26 patients. A rapidly developing cytokine storm was observed, while no persisting donor cells could be detected at two weeks after infusion eliminating the possibility of GVHD. Characterization of the effector mechanisms responsible for the anti-leukemic responses in this protocol, should guide new approaches for achieving enhanced anti-leukemic responses using this protocol.

Keywords: alloreactivity; haploidentical; cellular immunotherapy; donor cell infusion

1. Introduction

Alloreactivity is a property of the immune system in which a large fraction of T cells selected to recognize foreign peptides presented by self major histocompatibility complex (MHC) molecules are also able to bind to allogeneic MHC molecules presenting endogenous peptides, often in a tissue dependent fashion [1]. Traditionally, alloreactivity as a therapeutic modality has centered on allogeneic stem cell transplantation (allo-SCT) in which a donor's immune system is supplanted into a patient with a hematological malignancy. These donor immune cells recognize residual cancer cells as foreign and consequentially target the malignancy. In the allo-SCT setting, conditioning of the recipient with high doses of chemotherapy and/or radiotherapy prior to transplant significantly impairs the responsiveness of the recipient immune system. The lack of recipient immunity allows the donor alloreactive T cells present in the inoculum to initially engraft and later generate anti-recipient responses. These responses cause the graft-versus-leukemia (GVL) effect but also frequently result in graft-versus-host disease (GVHD). GVHD, with its attack on the skin, liver and gut of the recipient, can be severe enough to cause death [2]. While GVHD can be deleterious, an early retrospective study demonstrated that the alloreactive responses inducing GVHD also mediated anti-leukemic responses, preventing leukemic relapse and indicating that it was difficult to separate these two responses [3].

These findings have led investigators to an ongoing quest to generate the GVL responses without the GVHD responses. Usually this has focused on harnessing donor anti-recipient immune responses to achieve the anti-leukemic responses typically via donor-lymphocyte-infusions (DLI). DLI involves the infusion of mature donor lymphocytes into transplant recipients after donor cell tolerance establishment by the recipient following hematopoietic stem cell transplantation [4]. In this setting, little or no recipient anti-donor responses would be expected. DLI was shown to have a significant anti-leukemic response. However GVHD still occurred following donor lymphocyte infusion (DLI). Retrospective analysis of $CD3^+$ cell dose given with DLI following allogeneic hematopoietic cell transplantation found that $CD3^+$ cell doses $\geq 10 \times 10^7$ $CD3^+$ cells/kg was associated with 55% incidence of GVHD, >1 to $<10 \times 10^7$ $CD3^+$ cells/kg with 45% GVHD and $\leq 1 \times 10^7$ $CD3^+$ cells/kg with 21% GVHD. Despite the high incidence of GVHD the use of $CD3^+$ cell doses of $>10 \times 10^7$ did not result in decreased risk of relapse [5].

These approaches have focused on harnessing donor anti-recipient responses to achieve anti-leukemic responses. However in several settings it was demonstrated that anti-leukemic responses could be achieved without engraftment of the donor cells, a situation which should eliminate the possibility of GVHD developing [6] and supporting the concept that (at least prolonged) donor anti-recipient responses are not necessarily required to achieve anti-leukemic responses. In a murine mixed chimera model, it was demonstrated that recipient anti-donor responses were able to induce responses to tumor cells derived from the recipient strain [7–11]. Recipient IFN-γ as well as invariant NKT cells were required for responses in this model. A limitation of this system is that the immune manipulations to the recipient mice occurred prior to the actual injection of tumor cells. Therefore this

protocol avoids factoring in the impact that the growing tumor could have on host immune responses in the tumor bearing individuals.

2. Cellular Therapy; Clinical Experiences

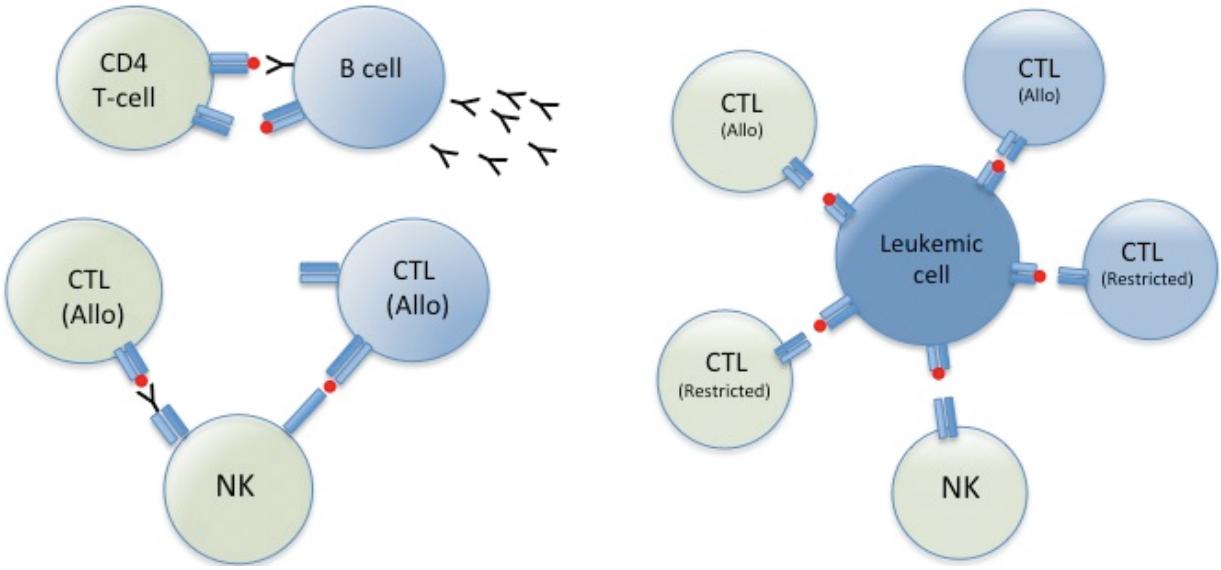
Anti-leukemic responses without donor cell persistence have been observed in clinical trials conducted by our group and others. White blood cells collected from G-CSF mobilized haploidentical donors were infused in high numbers (1–2 × 10^8 $CD3^+$ cells/kg) into refractory cancer patients who had received 100 cGy of total body irradiation prior to the infusion [12]. A cytokine storm with high levels (>1,000 pg/mL) of IL-6, MCP-1, MIP-1β and lower levels (10–100 pg/mL) of IL-5, IL-7, IL-8, IL-10, IL-13, and IFN-γ was rapidly generated (median time of onset of 14 hours) in these recipients. The cytokine storm caused high fever in all patients and skin rash, diarrhea, liver dysfunction, effusions, respiratory distress and edema in subsets of patients. The symptoms of the cytokine storm were allowed to persist for 2 days, if possible, and then remitted rapidly by administration of methylprednisolone. While no responses were seen in patients with solid tumors, 14 (7 major)/26 patients with refractory hematological malignancies showed responses. Patients with AML, acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) were all included in the study. Amongst the 7 major responders, three had AML and attained a complete response (CR) while four had NHL with 2 attaining a CR and an additional 2 with partial response (PR). In AML patients, 7 of 12 patients demonstrated a transient response with a resolution of peripheral blasts and/or >50% reduction in bone marrow blasts two weeks post cellular infusion. These responses occurred despite absence of donor chimerism 2 weeks after infusion in patients. Two patients (out of 41 total as patients with solid tumors were also included) demonstrated donor cell engraftment and developed GVHD or GVHD like symptoms [12]. Neither of these patients showed a clinical response to therapy. This was a much lower frequency of GVHD than was observed when similar numbers of donor lymphocytes were given in a DLI protocol [5]. Presumably this was because the limited amount of conditioning of the recipient allowed the development of recipient anti-donor responses capable of eliminating the donor cells in almost all of the patients.

A similar approach was described in a study by Guo and colleagues, who randomized acute myeloid leukemia (AML) patients 60 years of age and older into groups that received chemotherapy alone or chemotherapy along with haploidentical G-CSF mobilized peripheral blood stem cells. In the chemotherapy alone group, the complete response rate was 43% while the chemotherapy plus peripheral blood stem cells resulted in 80% complete response rate [13]. The two year progression-free survival was 10% for the chemotherapy alone and 39% for the chemotherapy plus peripheral blood stem cells. In a subsequent study by Guo *et al.*, patients with cytogenetic low and intermediate risk AML who attained complete remission during induction were infused HLA mismatched donor G-CSF mobilized peripheral blood stem cells on three separate occasions following consolidation with cytarabine [14]. The 6-year leukemia free survival was 84% in the low risk group while the intermediate risk group had a 6 year leukemic free survival rate of 59%. These results compare favorably to the overall survival of 55% and 24% in similar risk groups [15]. In each of these studies no patients developed GVHD.

3. Cellular Therapy; Mechanism of Action

The possible role of various effector cell populations was not evaluated in our initial study [12]. Patients whose immune system had been significantly diminished by conditioning prior to a hematopoietic stem cell transplant and then developed tolerance to the donor cells would not be expected to generate immune responses when mature donor lymphocytes were infused. In contrast, the patients in our study had only received 100 cGy total body irradiation. Thus it would be expected that recipient leukocytes could still respond effectively in this setting potentially resulting in multiple donor and recipient subpopulations being activated and acting as effectors in this protocol (Figure 1). Competing donor anti-recipient and recipient anti-donor responses could also result in additional layers of immune regulation in the cellular immunotherapy protocol that would not be seen in protocols using donor lymphoid infusion in patients receiving a hematopoietic stem cell transplant.

Figure 1. (**A**) Donor/Recipient Alloreactive Responses. Possible alloreactive responses of donor cells (green) and recipient cells (blue) include induction of recipient alloantibody production, donor NK cell mediated elimination of antibody coated donor cells and activated recipient cells and activation of donor and recipient alloreactive CTL as a result of recognition of allogeneic APC. (**B**) Anti-Leukemic Responses. Possible anti-leukemic responses include donor alloreactive or restricted CTL, alloreactive NK cells or cross-reactive alloreactive recipient CTL or restricted recipient CTL.



(**A**) (**B**)

A series of murine studies examined the effector cells responsible for the elimination of a large number (one spleen equivalent) of allogeneic splenocytes when injected intravenously into naïve recipients [16,17]. Allogeneic splenocytes were eliminated by day 3 in these studies. It was found that recipient $CD8^+$ cells were responsible for elimination of the allogeneic donor cells. In the absence of recipient $CD8^+$ cells, anti-donor alloantibodies were responsible for elimination of the allogeneic donor cells. Further studies found that the donor $CD4^+$ cells were important for triggering the recipient $CD8^+$ cells by acting as antigen presenting cells. Thus it would be predicted that recipient alloreactive $CD8^+$

cells would play an important role in the elimination of the donor cells in cellular immunotherapy and thus could contribute to anti-leukemic responses.

The activation of the alloreactive recipient T lymphocytes could also mediate anti-leukemic responses using two possible TCR mediated mechanisms. The cross reactivity of TCR has been demonstrated by increased alloreactivity by memory anti-viral T cells and increased anti-viral responses when T cells were stimulated with allogeneic cells [18,19]. In a similar fashion, the TCR recognizing the allogeneic MHC molecules could cross-react with the MHC molecules expressed on the leukemic cells presenting peptides derived from the leukemic cells. Alternatively it has been observed that alloreactive T cells contain a disproportionate amount of T cells expressing dual TCR [20,21]. These TCRs have the same β chain but two different α chains. In this scenario, one TCR could serve as the TCR subject to selection permitting the other TCR to bind to unselected antigens. Thus one of the TCRs of dual TCR expressing T cells could react with allogeneic MHC molecules and the second TCR could bind to the leukemic cells allowing the alloreactive cell to bind to the leukemic cell.

Elevated levels of a number of cytokines/chemokines have been found in the plasma as a result of the cytokine storm following infusion of haploidentical cells [12]. These cytokines could facilitate previously generated anti-leukemic memory T cells to be activated, possibly by overcoming inhibitory barriers, and to regain their ability to lyse leukemic cells. The presence of these cytokines could also nonspecifically induce the expression of cytolytic effector molecules such as granzymes and perforin in both $CD8^+$ and $CD4^+$ cells. These activated cytolytic lymphocytes could also mediate lysis using non-TCR mediated recognition of leukemic cells using receptors such as NKG2D and LFA-1 to bind to the leukemic cells [22–25].

Another effector population that could be contributing to anti-leukemic response is alloreactive NK cells. Alloreactive NK cells are a subset of haploidentical or allogeneic donor NK cells which lack expression of inhibitory KIR molecules specific for the HLA molecules expressed by the recipient, thereby allowing these donor NK cells to recognize and lyse these recipient cells including leukemic cells without receiving inhibitory signals [26]. Based on this functional activity, infusion of alloreactive NK cells or expanded NK cells has been proposed as a treatment for cancer. Selecting for the appropriate KIR expressed by the donor NK cells could potentially improve the effectiveness of this therapy [27,28]. While the use of NK cells may contribute to anti-leukemia responses, NK cells have also been shown to be able to lyse activated T cells especially $CD4^+$ cells, thereby downregulating immune responses [28–31]. Donor alloreactive NK cells could lyse donor alloreactive T cells as well as activated recipient T cells [32]. Thus the NK cells present in the donor cell infusion for cellular immunotherapy could be detrimental because their anti-T cell responses could eliminate activated recipient T cells that were potentially mediating anti-leukemic responses. Characterization of the anti-leukemic effectors in cellular immunotherapy would provide greater insight as to whether it would be more advantageous to deplete NK cells from the donor cells to be infused or to expand and enhance NK cells to achieve anti-leukemia activity as a separate infusion.

Because donor and recipient share one HLA haplotype in a haploidentical setting, it is possible that restricted T cells of donor origin could be activated. A recent study by Cobbald, *et al.* showed that HLA B7 molecules expressed on AML cells contained a greatly increased number of phosphopeptides that for the most part were unique to AML [33] These authors went on to show that normal donors expressing HLA B7 had memory T cells able to respond to HLA B7 presenting these leukemic specific

phosphopeptides thereby inducing cells able to lyse leukemic cells. These effector cells were not present in the leukemic patient, suggesting that they had been eliminated or inhibited. Thus $CD3^+$ cells obtained from a normal donor would be expected to contain memory T cells able to respond to the phosphopeptides presented by APC and the leukemic cells. Thus exposure to activated donor T cells for even a limited time following infusion could potentially generate anti-leukemic responses.

The encounter with allogeneic lymphoid cells can also result in an allogeneic effect. Usually donor $CD4^+$ cells are able to directly activate alloreactive recipient B and $CD8^+$ cells because allorecognition brings the allogeneic donor $CD4^+$ cells into close proximity with these cells [11,34]. The result is rapid activation of both donor and recipient cells resulting in expression of costimulatory ligands and production of cytokines, which facilitates the rapid production of alloantibodies by recipient B cells and activation of the recipient $CD8^+$ cells. The alloantibodies binding to the donor cells could trigger antibody dependent cellular cytotoxicity (ADCC) and contribute to the elimination of the donor cells. The activation of the recipient $CD8^+$ cells could trigger anti-leukemic responses.

Leukemic cells often use a variety of mechanisms to deflect or inhibit the immune responses. Leukemic cells have been shown to be capable of expressing or secreting inhibitory factors/ligands. Often T cells in leukemic patients express increased expression of the inhibitory receptors PD-1, CTLA-4, LAG-3, and Tim3 [35]. Likewise, leukemic cells often express the ligands for the inhibitory receptors. For example, the encounter with activated T cells has been shown to induce the increased expression of the PD-L1 molecule on leukemic cells [36]. The activation of T cells by cellular immunotherapy could result in the upregulation of the inhibitory receptor, PD-1(CD279), on T cells as well as upregulation of PD-L1(CD274) on the leukemic cells. Thus activation of T cells in these patients could subsequently result in inhibition of T cell responses instead of increased T cell responses. In the patients in which there is upregulation of both PD-1 and PD-L1, anti-PD-1 could be administered to the patients to block inhibitory signals induced by this interaction, thereby enhancing anti-leukemic responses. The ability to identify the leukemic cells that will upregulate PD-L1 in responses to activated T cells prior to the infusion of donor cells would facilitate identification of which patients would benefit from use of anti-PD-1 administered at the same time as the infusion of donor cells.

Leukemic cells have also been shown to secrete inhibitory factors such indoleamine-2,3-deoxygenase (IDO) which depletes tryptophan from the milieu thereby inhibiting T cell responses [37,38]. It has also been observed that leukemic cells can express increased levels of inhibitors of granzyme B (SPI6 in mice and PI-9 in humans) inhibiting the ability of cytolytic cells to lyse the leukemic cells [39]. Leukemic cells have also been found to downregulate expression of MHC antigens, inducing increased presence of inhibitory cells such as T regulatory cells and myeloid suppressor cells and even causing depletion of leukemia reactive T cells [40–46].

The results of the initial trial carried out in refractory cancer patients indicated that only a fraction of the patients with refractory hematological malignancies responded to cellular immunotherapy and those that did respond, responded with varying degrees. Is it possible to enhance both the effectiveness and the number of patients that respond? A number of approaches could potentially be taken to improve the responses. Infusion of chimeric antigen receptor modified (CAR) T cells has also been found to induce a cytokine storm and response was associated with the presence of the cytokine storm [47,48]. In this study administration of anti-IL-6 was found to break the fever effectively. Thus

the use of anti-IL-6 in cellular immunotherapy protocol may allow for fever cessation without the broadly suppressive effect of corticosteroids. In addition there are a number of immunomodulatory antibodies in varying phases of clinical development. They include antibodies that block the function of inhibitory receptors such as CD279 (PD-1) and CTLA-4 (CD152), namely the commercially available nivolumab and ipilumumab, respectively [35,49]. Alternatively, agonistic antibodies to CD137 and CD134 trigger increased responses by the activated T cells and NK cells expressing these activating receptors [50–52]. The use of these antibodies at appropriate time-points could potentially give a boost to the anti-leukemic responses.

Another possible approach for improved anti-leukemic responses is to enhance the generation of $CD4^+$ cytolytic effector cells. Because $CD4^+$ CTL are directed toward peptides presented by MHC class II antigens (DR, DP, DQ) they could be especially effective toward MHC class II expressing leukemias. $CD4^+$ cells as cytolytic effector cells targeted toward HLA-DP antigens have been shown to generate a strong anti-myeloid leukemia response [53,54]. Because of a recombinational hotspot between DR, DQ and DP, it was observed that unrelated donors and recipients to be frequently mismatched at DP even though they shared HLA A, B, C, DR and DQ alleles. Usually only a limited number of $CD4^+$ cells exhibiting cytolytic effector function are detected in immune responses. When $CD4^+$ cells are differentiating in the thymus, they upregulate expression of a transcription factor called ThPOK. When present, this transcription factor inhibits expression of the cytolytic effector molecules preventing the $CD4^+$ cells from expressing the cytolytic effector pathway and directing these cells down non-cytolytic pathways [55,56]. Antigen stimulated $CD4^+$ lymphocytes downregulate the expression of thPOK permitting the development of $CD4^+$ CTL [57]. In a mouse model it was demonstrated that $CD4^+$ CTL development was dependent on STAT2-dependent type I interferon signaling and IL-2 driven upregulation of T-bet and Blimp-1 [58]. A better understanding of how to regulate expression of these transcription factors could lead to the development of approaches that increase the levels of $CD4^+$ CTL.

4. Conclusions

To extend the findings of the previous trial [12], the cellular immunotherapy trial has been reopened. Initially refractory leukemic patients will be infused with $1–2 \times 10^8$ $CD3^+$ cells/kg obtained by pheresis from a haploidentical donor. The cellular immunotherapy protocol has been modified by collecting the white blood cells from the donor without prior G-CSF treatment and without the recipient receiving 100 cGy of total body irradiation prior to the infusion [59]. In addition to examining the clinical responses, laboratory studies geared toward identifying the effector cells responsible for the anti-leukemic responses will be initiated. These studies include obtaining blood samples from the recipient at sequential timepoints after infusion. After removing an aliquot of the blood for obtaining DNA, the remaining blood will be spun to obtain plasma. Then PBMNC will be isolated using Ficoll-Hypaque discontinuous centrifugation. Both plasma and PBMNC will be frozen for future analysis. The PBMNC obtained from these samples will be used for phenotypic characterization of donor and recipient cells to measure survival of donor leukocytes, expression of cytolytic effector molecules, inhibitory and activating receptors, and phenotypic changes in the leukemic cells. The plasma obtained from these samples will be used to measure the levels of a variety

of cytokines and chemokines as well as levels of cytolytic effector molecules. The DNA will be used to perform additional typing for HLA DP and KIR loci. The results obtained to date have indicated that elimination of haploidentical donor cells prevents GVHD development while inducing anti-leukemic responses. The results of these studies will help define the anti-leukemic effector mechanisms. As the results of these studies become available, modifications will be made to the protocol to potentially improve anti-leukemic responses. These protocol changes will have to be tested to make sure that they do not interfere with the ability of recipient immune cells to eliminate the donor cells while still stimulating the anti-leukemic responses. This approach is unique in that permits the development of anti-leukemic responses without the development of GVHD.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. D'Orsogna, L.J.; Nguyen, T.H.; Claas, F.H.; Witt, C.; Mifsud, N.A. Endogenous-peptide-dependent alloreactivity: New scientific insights and clinical implications. *Tissue Antigens* **2013**, *81*, 399–407.
2. Blazar, B.R.; Murphy, W.J.; Abedi, M. Advances in graft-versus-host disease biology and therapy. *Nat. Rev. Immunol.* **2012**, *12*, 443–458.
3. Weiden, P.L.; Flournoy, N.; Thomas, E.D.; Prentice, R.; Fefer, A.; Buckner, C.D.; Storb, R. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N. Engl. J. Med.* **1979**, *300*, 1068–1073.
4. Kolb, H.J. Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood* **2008**, *112*, 4371–4383.
5. Bar, M.; Sandmaier, B.M.; Inamoto, Y.; Bruno, B.; Hari, P.; Chauncey, T.; Martin, P.J.; Storb, R.; Maloney, D.G.; Storer, B.; *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.
6. Dey, B.R.; McAfee, S.; Colby, C.; Cieply, K.; Caron, M.; Saidman, S.; Preffer, F.; Shaffer, J.; Tarbell, N.; Sackstein, R.; *et al*. Anti-tumour response despite loss of donor chimaerism in patients treated with non-myeloablative conditioning and allogeneic stem cell transplantation. *Br. J. Haematol.* **2005**, *128*, 351–359.
7. Rubio, M.T.; Kim, Y.M.; Sachs, T.; Mapara, M.; Zhao, G.; Sykes, M. Antitumor effect of donor marrow graft rejection induced by recipient leukocyte infusion in mixed chimeras prepared with nonmyeloablative conditioning: Critical role for recipient-derived IFN-gamma. *Blood* **2003**, *102*, 2300–2307.
8. Rubio, M.T.; Saito, T.I.; Kattleman, K.; Zhao, G.; Buchli, J.; Sykes, M. Mechanisms of the antitumor responses and host-versus-graft reactions induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: A critical role for recipient CD4+ T cells and recipient leukocyte infusion-derived IFN-gamma-producing CD8+ T cells. *J. Immunol.* **2005**, *175*, 665–676.

9. Saito, T.I.; Li, H.W.; Sykes, M. Invariant NKT cells are required for antitumor responses induced by host-versus-graft responses. *J. Immunol.* **2010**, *185*, 2099–2105.
10. Saito, T.I.; Rubio, M.T.; Sykes, M. Clinical relevance of recipient leukocyte infusion as antitumor therapy following nonmyeloablative allogeneic hematopoietic cell transplantation. *Exp. Hematol.* **2006**, *34*, 1271–1277.
11. Symons, H.J.; Levy, M.Y.; Wang, J.; Zhou, X.; Zhou, G.; Cohen, S.E.; Luznik, L.; Levitsky, H.I.; Fuchs, E.J. The allogeneic effect revisited: Exogenous help for endogenous, tumor-specific T cells. *Biol. Blood Marrow Transplant.* **2008**, *14*, 499–509.
12. Colvin, G.A.; Berz, D.; Ramanathan, M.; Winer, E.S.; Fast, L.; Elfenbein, G.J.; Quesenberry, P.J. Nonengraftment haploidentical cellular immunotherapy for refractory malignancies: Tumor responses without chimerism. *Biol. Blood Marrow Transplant.* **2009**, *15*, 421–431.
13. Guo, M.; Hu, K.X.; Yu, C.L.; Sun, Q.Y.; Qiao, J.H.; Wang, D.H.; Liu, G.X.; Sun, W.J.; Wei, L.; Sun, X.D.; *et al*. Infusion of HLA-mismatched peripheral blood stem cells improves the outcome of chemotherapy for acute myeloid leukemia in elderly patients. *Blood* **2011**, *117*, 936–941.
14. Guo, M.; Hu, K.X.; Liu, G.X.; Yu, C.L.; Qiao, J.H.; Sun, Q.Y.; Qiao, J.X.; Dong, Z.; Sun, W.J.; Sun, X.D.; *et al*. HLA-mismatched stem-cell microtransplantation as postremission therapy for acute myeloid leukemia: Long-term follow-up. *J. Clin. Oncol.* **2012**, *30*, 4084–4090.
15. Byrd, J.C.; Mrozek, K.; Dodge, R.K.; Carroll, A.J.; Edwards, C.G.; Arthur, D.C.; Pettenati, M.J.; Patil, S.R.; Rao, K.W.; Watson, M.S.; *et al.* Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: Results from Cancer and Leukemia Group B (CALGB 8461). *Blood* **2002**, *100*, 4325–4336.
16. Fast, L.D. Recipient CD8+ cells are responsible for the rapid elimination of allogeneic donor lymphoid cells. *J. Immunol.* **1996**, *157*, 4805–4810.
17. Fast, L.D. Recipient elimination of allogeneic lymphoid cells: Donor CD4(+) cells are effective alloantigen-presenting cells. *Blood* **2000**, *96*, 1144–1149.
18. D'Orsogna, L.J.; van den Heuvel, H.; van der Meer-Prins, E.M.; Roelen, D.L.; Doxiadis, II; Claas, F.H. Stimulation of human EBV- and CMV-specific cytolytic effector function using allogeneic HLA molecules. *J. Immunol.* **2012**, *189*, 4825–4831.
19. Brehm, M.A.; Daniels, K.A.; Priyadharshini, B.; Thornley, T.B.; Greiner, D.L.; Rossini, A.A.; Welsh, R.M. Allografts stimulate cross-reactive virus-specific memory CD8 T cells with private specificity. *Am. J. Transplant.* **2010**, *10*, 1738–1748.
20. Morris, G.P.; Allen, P.M. Cutting edge: Highly alloreactive dual TCR T cells play a dominant role in graft-versus-host disease. *J. Immunol.* **2009**, *182*, 6639–6643.
21. Morris, G.P.; Uy, G.L.; Donermeyer, D.; Dipersio, J.F.; Allen, P.M. Dual receptor T cells mediate pathologic alloreactivity in patients with acute graft-versus-host disease. *Sci. Transl. Med.* **2013**, *5*, 188ra174.
22. Lask, A.; Goichberg, P.; Cohen, A.; Goren-Arbel, R.; Milstein, O.; Aviner, S.; Feine, I.; Ophir, E.; Reich-Zeliger, S.; Hagin, D.; *et al.* TCR-independent killing of B cell malignancies by anti-third-party CTLs: The critical role of MHC-CD8 engagement. *J. Immunol.* **2011**, *187*, 2006–2014.

23. Lask, A.; Ophir, E.; Or-Geva, N.; Cohen-Fredarow, A.; Afik, R.; Eidelstein, Y.; Reich-Zeliger, S.; Nathansohn, B.; Edinger, M.; Negrin, R.S.; *et al*. A new approach for eradication of residual lymphoma cells by host nonreactive anti-third-party central memory CD8 T cells. *Blood* **2013**, *121*, 3033–3040.
24. Tietze, J.K.; Wilkins, D.E.; Sckisel, G.D.; Bouchlaka, M.N.; Alderson, K.L.; Weiss, J.M.; Ames, E.; Bruhn, K.W.; Craft, N.; Wiltrout, R.H.; *et al*. Delineation of antigen-specific and antigen-nonspecific CD8(+) memory T-cell responses after cytokine-based cancer immunotherapy. *Blood* **2012**, *119*, 3073–3083.
25. Franciszkiewicz, K.; Le Floc'h, A.; Boutet, M.; Vergnon, I.; Schmitt, A.; Mami-Chouaib, F. CD103 or LFA-1 engagement at the immune synapse between cytotoxic T cells and tumor cells promotes maturation and regulates T-cell effector functions. *Cancer Res.* **2013**, *73*, 617–628.
26. Ruggeri, L.; Capanni, M.; Urbani, E.; Perruccio, K.; Shlomchik, W.D.; Tosti, A.; Posati, S.; Rogaia, D.; Frassoni, F.; Aversa, F.; *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* **2002**, *295*, 2097–2100.
27. Cooley, S.; Weisdorf, D.J.; Guethlein, L.A.; Klein, J.P.; Wang, T.; Le, C.T.; Marsh, S.G.; Geraghty, D.; Spellman, S.; Haagenson, M.D.; *et al.* Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood* **2010**, *116*, 2411–2419.
28. Sivori, S.; Carlomagno, S.; Falco, M.; Romeo, E.; Moretta, L.; Moretta, A. Natural killer cells expressing the KIR2DS1-activating receptor efficiently kill T-cell blasts and dendritic cells: Implications in haploidentical HSCT. *Blood* **2011**, *117*, 4284–4292.
29. Waggoner, S.N.; Cornberg, M.; Selin, L.K.; Welsh, R.M. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* **2012**, *481*, 394–398.
30. Cook, K.D.; Whitmire, J.K. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. *J. Immunol.* **2013**, *190*, 641–649.
31. Rabinovich, B.A.; Li, J.; Shannon, J.; Hurren, R.; Chalupny, J.; Cosman, D.; Miller, R.G. Activated, but not resting, T cells can be recognized and killed by syngeneic NK cells. *J. Immunol.* **2003**, *170*, 3572–3576.
32. Olson, J.A.; Leveson-Gower, D.B.; Gill, S.; Baker, J.; Beilhack, A.; Negrin, R.S. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. *Blood* **2010**, *115*, 4293–4301.
33. Cobbold, M.; De La Pena, H.; Norris, A.; Polefrone, J.M.; Qian, J.; English, A.M.; Cummings, K.L.; Penny, S.; Turner, J.E.; Cottine, J.; *et al.* MHC class I-associated phosphopeptides are the targets of memory-like immunity in leukemia. *Sci. Transl. Med.* **2013**, *5*, 203ra125.
34. Katz, D.H.; Davie, J.M.; Paul, W.E.; Benacerraf, B. Carrier function in anti-hapten antibody responses. IV. Experimental conditions for the induction of hapten-specific tolerance or for the stimulation of anti-hapten anamnestic responses by "nonimmunogenic" hapten-polypeptide conjugates. *J. Exp. Med.* **1971**, *134*, 201–223.
35. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **2012**, *12*, 252–264.

36. Dolen, Y.; Esendagli, G. Myeloid leukemia cells with a B7-2(+) subpopulation provoke Th-cell responses and become immuno-suppressive through the modulation of B7 ligands. *Eur. J. Immunol.* **2013**, *43*, 747–757.
37. Corm, S.; Berthon, C.; Imbenotte, M.; Biggio, V.; Lhermitte, M.; Dupont, C.; Briche, I.; Quesnel, B. Indoleamine 2,3-dioxygenase activity of acute myeloid leukemia cells can be measured from patients' sera by HPLC and is inducible by IFN-gamma. *Leuk. Res.* **2009**, *33*, 490–494.
38. Curti, A.; Aluigi, M.; Pandolfi, S.; Ferri, E.; Isidori, A.; Salvestrini, V.; Durelli, I.; Horenstein, A.L.; Fiore, F.; Massaia, M.; *et al*. Acute myeloid leukemia cells constitutively express the immunoregulatory enzyme indoleamine 2,3-dioxygenase. *Leukemia* **2007**, *21*, 353–355.
39. Fritsch, K.; Finke, J.; Grullich, C. Suppression of granzyme B activity and caspase-3 activation in leukaemia cells constitutively expressing the protease inhibitor 9. *Ann. Hematol.* **2013**, *92*, 1603–1609.
40. Zhang, L.; Chen, X.; Liu, X.; Kline, D.E.; Teague, R.M.; Gajewski, T.F.; Kline, J. CD40 ligation reverses T cell tolerance in acute myeloid leukemia. *J. Clin. Invest.* **2013**, *123*, 1999–2010.
41. Matsushita, H.; Vesely, M.D.; Koboldt, D.C.; Rickert, C.G.; Uppaluri, R.; Magrini, V.J.; Arthur, C.D.; White, J.M.; Chen, Y.S.; Shea, L.K.; *et al*. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature* **2012**, *482*, 400–404.
42. Vago, L.; Perna, S.K.; Zanussi, M.; Mazzi, B.; Barlassina, C.; Stanghellini, M.T.; Perrelli, N.F.; Cosentino, C.; Torri, F.; Angius, A.; *et al.* Loss of mismatched HLA in leukemia after stem-cell transplantation. *N. Engl. J. Med.* **2009**, *361*, 478–488.
43. Almand, B.; Clark, J.I.; Nikitina, E.; van Beynen, J.; English, N.R.; Knight, S.C.; Carbone, D.P.; Gabrilovich, D.I. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J. Immunol.* **2001**, *166*, 678–689.
44. Shenghui, Z.; Yixiang, H.; Jianbo, W.; Kang, Y.; Laixi, B.; Yan, Z.; Xi, X. Elevated frequencies of CD4(+) CD25(+) CD127lo regulatory T cells is associated to poor prognosis in patients with acute myeloid leukemia. *Int. J. Cancer.* **2011**, *129*, 1373–1381.
45. Szczepanski, M.J.; Szajnik, M.; Czystowska, M.; Mandapathil, M.; Strauss, L.; Welsh, A.; Foon, K.A.; Whiteside, T.L.; Boyiadzis, M. Increased frequency and suppression by regulatory T cells in patients with acute myelogenous leukemia. *Clin. Cancer Res.* **2009**, *15*, 3325–3332.
46. Wang, X.; Zheng, J.; Liu, J.; Yao, J.; He, Y.; Li, X.; Yu, J.; Yang, J.; Liu, Z.; Huang, S. Increased population of CD4(+)CD25(high), regulatory T cells with their higher apoptotic and proliferating status in peripheral blood of acute myeloid leukemia patients. *Eur. J. Haematol.* **2005**, *75*, 468–476.
47. Grupp, S.A.; Kalos, M.; Barrett, D.; Aplenc, R.; Porter, D.L.; Rheingold, S.R.; Teachey, D.T.; Chew, A.; Hauck, B.; Wright, J.F.; *et al.* Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* **2013**, *368*, 1509–1518.
48. Teachey, D.T.; Rheingold, S.R.; Maude, S.L.; Zugmaier, G.; Barrett, D.M.; Seif, A.E.; Nichols, K.E.; Suppa, E.K.; Kalos, M.; Berg, R.A.; *et al.* Cytokine release syndrome after blinatumomab treatment related to abnormal macrophage activation and ameliorated with cytokine-directed therapy. *Blood* **2013**, *121*, 5154–5157.
49. Sliwkowski, M.X.; Mellman, I. Antibody therapeutics in cancer. *Science* **2013**, *341*, 1192–1198.

50. Houot, R.; Kohrt, H.; Levy, R. Boosting antibody-dependant cellular cytotoxicity against tumor cells with a CD137 stimulatory antibody. *Oncoimmunology* **2012**, *1*, 957–958.
51. Kohrt, H.E.; Houot, R.; Weiskopf, K.; Goldstein, M.J.; Scheeren, F.; Czerwinski, D.; Colevas, A.D.; Weng, W.K.; Clarke, M.F.; Carlson, R.W.; *et al.* Stimulation of natural killer cells with a CD137-specific antibody enhances trastuzumab efficacy in xenotransplant models of breast cancer. *J. Clin. Invest.* **2012**, *122*, 1066–1075.
52. Marabelle, A.; Kohrt, H.; Sagiv-Barfi, I.; Ajami, B.; Axtell, R.C.; Zhou, G.; Rajapaksa, R.; Green, M.R.; Torchia, J.; Brody, J.; *et al*. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. *J. Clin. Invest.* **2013**, *123*, 2447–2463.
53. Rutten, C.E.; van Luxemburg-Heijs, S.A.; Halkes, C.J.; van Bergen, C.A.; Marijt, E.W.; Oudshoorn, M.; Griffioen, M.; Falkenburg, J.H. Patient HLA-DP-specific CD4+ T cells from HLA-DPB1-mismatched donor lymphocyte infusion can induce graft-versus-leukemia reactivity in the presence or absence of graft-versus-host disease. *Biol. Blood Marrow Transplant.* **2013**, *19*, 40–48.
54. Stevanovic, S.; van Bergen, C.A.; van Luxemburg-Heijs, S.A.; van der Zouwen, B.; Jordanova, E.S.; Kruisselbrink, A.B.; van de Meent, M.; Harskamp, J.C.; Claas, F.H.; Marijt, E.W.; *et al.* HLA class II upregulation during viral infection leads to HLA-DP-directed graft-versus-host disease after CD4+ donor lymphocyte infusion. *Blood* **2013**, *122*, 1963–1973.
55. Jones-Mason, M.E.; Zhao, X.; Kappes, D.; Lasorella, A.; Iavarone, A.; Zhuang, Y. E protein transcription factors are required for the development of CD4(+) lineage T cells. *Immunity* **2012**, *36*, 348–361.
56. Rui, J.; Liu, H.; Zhu, X.; Cui, Y.; Liu, X. Epigenetic silencing of CD8 genes by ThPOK-mediated deacetylation during CD4 T cell differentiation. *J. Immunol.* **2012**, *189*, 1380–1390.
57. Mucida, D.; Husain, M.M.; Muroi, S.; van Wijk, F.; Shinnakasu, R.; Naoe, Y.; Reis, B.S.; Huang, Y.; Lambolez, F.; Docherty, M.; *et al*. Transcriptional reprogramming of mature CD4(+) helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. *Nat. Immunol.* **2013**, *14*, 281–289.
58. Hua, L.; Yao, S.; Pham, D.; Jiang, L.; Wright, J.; Sawant, D.; Dent, A.L.; Braciale, T.J.; Kaplan, M.H.; Sun, J. Cytokine-Dependent Induction of CD4+ T cells with Cytotoxic Potential during Influenza Virus Infection. *J. Virol.* **2013**, *87*, 11884–11893.
59. Reagan, J.L.; Fast, L.D.; Safran, H.; Nevola, M.; Winer, E.S.; Castillo, J.J.; Butera, J.N.; Quesenberry, M.I.; Young, C.T.; Quesenberry, P.J. Cellular immunotherapy for refractory hematological malignancies. *J. Transl. Med.* **2013**, *11*, 150.