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Natural killer cell immunotherapy for cancer: a new hope

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Abstract

Recently there has been a substantial gain in our understanding of the role NK-cells play in mediating innate host immune responses. Although NK cells have long been known to mediate antigen independent tumor cytotoxicity, the therapeutic potential of NK cell-based immunotherapy has yet to be realized. Manipulating the balance between inhibitory and activating NK receptor signals, sensitization of tumor target cells to NK cell-mediated apoptosis, and recent discoveries in NK-cell receptor biology have fueled translational research that has led to clinical trials investigating a number of novel methods to potentiate NK cytotoxicity against human malignancies.

Keywords

NK cells; immunotherapy; innate immunity; allogeneic transplant; killer IgG like receptors (KIR); renal cell carcinoma

Introduction

The failure of conventional chemotherapy to improve survival in a large percentage of patients with advanced solid tumors has prompted immune-based cancer therapies. Optimal T-cell based immunotherapy for cancer requires targeting an antigen (Ag) that is both highly expressed and restricted to the tumor. Unfortunately, at present, most tumor Ag do not meet these criteria [1,2]. Recently, research has focused on the potential of natural killer (NK) cells to treat cancer. The ability of NK cells to kill tumor cells without the need to recognize a tumor-specific Ag provides advantages over T cells and makes them appealing as potential effectors for immunotherapy.

Functionally, NK cells do not rearrange genes encoding for specific Ag receptors; rather, their recognition of targets is regulated through a balance of activating and inhibitory signals [3]. In addition, NK cells have the ability to kill target cells directly, as well as mediate antibody (Ab)-dependent cellular cytotoxicity (ADCC) via the membrane receptor Fc γ RIII (CD16), which binds to the Fc portion of IgG Ab [4]. NK cells can also directly induce tumor apoptosis via the perforin granzyme pathway, or through death receptor ligands, such

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as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), or Fas ligand expressed on their cell surfaces, which directly trigger tumor death via their respective receptors (Figure 1)[3].

The mechanism by which an NK cell recognizes a target cell, with subsequent activation or inhibition of killing, is complex [1]. Under physiologic circumstances, NK cell recognition of target cells is predominantly mediated by paired inhibitory and activating signals through NK receptors (NKR), as well as various adhesion and costimulatory molecules [5,6]. The best characterized activating and inhibitory receptors belong to the killer cell immunoglobulin (Ig)-like receptor (KIR) superfamily, which primarily recognizes human leukocyte Ag (HLA)-A, B, and C, and the C-type lectin CD94/NKG2 heterodimers, which are ligated by HLA-E (Figure 2) [6,7].

Even in the presence of an activating ligand, inhibitory ligands can initiate overriding signals that culminate in a net suppression of NK cell function [3]. The inactivation of NK cells by self-HLA molecules is a potential mechanism by which malignant cells evade host NK cell-mediated immunity [3]. This may limit the ability of both endogenous and adoptively infused autologous NK cells to induce anti-tumor effects against tumors [8].

Inhibitory receptors and the ‘missing self’ hypothesis

In the ‘missing self’ hypothesis, recognition of self-major histocompatibility complex (MHC) class I molecules is defined as a dominant mechanism by which NK cells are impaired from killing host cell tumor cells. The absence of MHC class I molecules on some tumor cells (missing self) triggers their elimination by cytotoxic NK cells. KIR with two Ig domains (KIR2D) identify HLA-C allotypes. KIR2DL2 recognizes an epitope shared by group 1 HLA-C allotypes, identified according to amino acid residues present at positions 77 and 80 in the alpha-1 helix of the HLA-C molecule. Group 1 HLA-C alleles each have a Ser77 and Asn80. KIR2DL1 recognizes an epitope shared by the reciprocal group 2 HLA-C allotypes, which have Asn77 and Lys80 in the alpha-1 helix. KIR3DL1, with three Ig domains, recognizes an epitope shared by HLA-Bw4 alleles. Finally, KIR3DL2, a homodimer of molecules with three Ig domains, recognizes HLA-A3 and -A11 (Figure 2).

Expression of KIR genes varies considerably among individuals and among individual NK cells. Inhibitory receptors are germline-encoded by a set of polymorphic genes that segregate independently from MHC genes. The method by which NK cells develop tolerance to self is complex. It is believed that, during development, each NK cell precursor makes a random choice of which KIR genes it will express, with NK cells expressing a self-MHC class I-specific inhibitory receptor becoming ‘licensed’ for functional competence, whereas those lacking such a receptor remaining functionally inert [9]. Consequently, NK cells from any given individual will be alloreactive toward cells from others that lack their KIR ligands and, conversely, will be tolerant of cells from another individual who has the same or additional KIR ligands. In support of this concept, allogeneic NK cells mismatched for KIR ligands (i.e. NK cells possessing KIR in which the MHC ligands are absent on the tumor) are more cytotoxic *in vitro* against leukemia cells [10] and to solid tumor cells, such

as renal cell carcinoma (RCC) and melanoma, compared with KIR ligand-matched autologous and/or allogeneic counterparts [11].

KIR incompatibility and allogeneic HCT

In vivo evidence of NK cell-mediated anti-leukemia effects

The most compelling evidence in humans to support the activity of NK cells against cancer comes in patients with acute myeloid leukemia (AML) undergoing mismatched allogeneic hematopoietic stem cell transplantation. Ruggeri *et al.* investigated the impact of NK cell alloreactivity in 60 patients undergoing an HLA haplotype-mismatched hematopoietic stem cell transplant, focusing on the role of the three major NK KIR families with specificity for HLA-C group 1, HLA-C group 2 and HLA-Bw4 alleles. The cytolytic function of donor NK cell clones, obtained from recipients after transplantation, against recipient lymphocytes and allogeneic leukemic cells was evaluated *in vitro*. KIR epitope-mismatched NK cell clones were more cytotoxic against recipient target cells compared with the KIR epitope-matched NK cell clones. Lysis followed the rules of NK cell alloreactivity, being blocked only by the MHC class I KIR ligand, which was missing in the recipient. These *in vitro* data correlated with the clinical finding that transplants from KIR epitope-incompatible donors in recipients with AML led to an improvement in engraftment, a reduction in graft-versus-host disease (GvHD) and a significant decrease in relapse.

Updated data by this same group [12,13] on 112 haplo-identical T-cell depleted transplants for high-risk AML showed a highly statistically significant event-free and overall survival advantage for patients receiving haplo-identical transplants where NK alloreactivity existed in the graft-versus-leukemia (GvL) direction ($n = 34$; 60% event-free survival at 5 years) compared with transplants without alloreactivity ($n = 58$; 5% event-free survival at 5 years; $P < 0.0005$). Remarkably, graft rejection and GvHD were not observed in any recipients receiving NK alloreactive transplants [12,13]. The near-complete absence of morbidity and mortality from GvHD was probably multi-factorial and explained by (1) the low burden of donor T cells infused with the graft, (2) the absence or low-level expression of activating NK receptor ligands (e.g. MICA/B and ULBP) on the cell surface of normal host tissues and (3) donor haplo-identical NK cells killing recipient dendritic cells (DC), thus preventing the donor T-cell priming to host alloantigens required to induce GvHD, as shown by experimental data [14].

These seminal findings show that, in the setting of an HLA-mismatched hematopoietic cell transplant, NK cells can serve as both GvL effectors and facilitators of engraftment independent of T-cell mediated graft-versus-host (GvH) reactions [10]. Furthermore, these observations provide the first evidence that alloreactive donor NK cells can favorably alter the outcome of HCT.

Experimental evidence that alloreactive NK cells prevent GvHD and mediate GvT effects

Based on the above clinical observations, investigators [15] used an MHC-mismatched murine model to confirm the hypothesis that allogeneic transplants utilizing KIR-incompatible donors result in the *in vivo* expansion of alloreactive KIR-incompatible donor-

derived NK cells, which kill host T cells that mediate graft rejection and host Ag-presenting cells (APC) necessary to prime alloreactive T cells, thus reducing GvHD. While most human KIR are specific for classic MHC class I molecules, rodents and other species have evolved convergent receptors of the C-type lectin superfamily, termed Ly49. Subgroups of MHC-specific Ly49 receptors are expressed on murine NK cells, regulating their function analogous to KIR on human NK cells. In experimental murine models of MHC-mismatched transplantation, the infusion of alloreactive NK cells lacking Ly49 inhibitory receptors specific for recipient MHC molecules did not cause GvHD, even when infused in large numbers into lethally irradiated recipients. Furthermore, in non-lethally irradiated recipients, alloreactive NK cells (but not non-alloreactive NK cells) reduced recipient T-cell and granulocyte counts in the marrow and spleen to levels observed after lethal irradiation, establishing that alloreactive NK cells can induce lymphohematopoietic ablation without causing GvHD. Alloreactive NK cells also accelerated the loss of host APC in the bone marrow, spleen and gut compared with mice conditioned with total body irradiation (TBI) or TBI plus non-alloreactive NK cells. Taken together, these data indicate that alloreactive NK cells prevent GvHD by elimination of recipient APC.

Data showing reduced AML relapse in humans undergoing HLA-mismatched transplantation from KIR-incompatible donors suggests alloreactive NK cells are also capable of mediating GvL effects. In light of these observations, human alloreactive NK clones were infused into human AML-engrafted non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. If left untreated, or given non-alloreactive human NK clones, mice died significantly earlier compared with cohorts receiving alloreactive NK cells [14]. These clinical observations in humans, together with correlative mouse models, have established that alloreactive NK cell populations arising from transplanted hematopoietic stem cells from HLA-mismatched donors induce potent GvL effects while preventing rejection and GvHD.

Animal models have also shown that adoptively infused alloreactive NK cells can reduce GvHD and mediate graftversus-solid tumor effects in the setting of MHC-matched T-cell replete hematopoietic stem cell transplants. In one model, adoptively infused Ly49 ligand-mismatched NK cells in tumor-bearing animals were shown to reduce significantly the incidence of skin GvHD compared with allogeneic HCT recipients receiving Ly49 ligand-matched NK cells or no NK cells (Figure 3). Ly49 ligand-mismatched NK cells not only decreased GvHD but also mediated anti-tumor effects that significantly prolonged survival in BALB/c recipients transplanted with established pulmonary metastasis from RENCA tumors [16,17].

Additional transplant clinical studies

Subsequent to the seminal observations by Ruggeri *et al.*, there have been reports both confirming [16,17] and contradicting [18,19] a beneficial effect of alloreactive NK cells on transplant outcome in humans. One study [16] of 130 patients with hematologic malignancies undergoing an unmanipulated HSCT from unrelated donors reported improved transplant outcomes in the setting of KIR incompatibility; patients were divided into those with and without KIR ligand incompatibility with respect to their donors. At 4.5 years,

patients with KIR ligand incompatibility compared with those without KIR ligand incompatibility had a higher probability of overall survival (87% versus 48%; $P=0.006$) and disease-free survival (87% versus 39%; $P=0.0007$). Transplant-related mortality for the two groups equaled 6% and 40% ($P=0.01$), respectively. Relapse rates for patients receiving transplants from a donor with or without KIR ligand incompatibility were 6% and 21%, respectively ($P=0.07$) [16].

These findings, showing a beneficial effect of NK cell alloreactivity in mismatched transplants, led investigators to evaluate whether HLA-mismatched transplants with KIR incompatibility would improve transplant outcome over fully HLA-matched unrelated transplants. Two studies unfortunately did not substantiate this hypothesis: Davies *et al.* [20] reported, in a single institution analysis, that KIR mismatching was associated with a worse outcome in recipients of T-cell replete URD transplants mismatched for at least one class I HLA locus. Similarly, a CIBMTR analysis of outcomes after 1571 unrelated donor transplantations for myeloid malignancies reported treatment-related mortality (TRM), treatment failure and overall mortality were higher in recipients receiving an allograft mismatched at an HLA-A, -B, or -C locus compared with fully HLA-matched URD, with KIR incompatibility in mismatched transplant recipients having no impact on outcome. Furthermore, leukemia recurrence was similar between HLA-matched and -mismatched transplant recipients and was not impacted by KIR incompatibility [19]. Finally, one haplo-identical transplant study that used less T-cell depletion than Ruggeri *et al.* reported higher acute GvHD and inferior survival in KIR-mismatched patients [21]. These discrepant clinical observations highlight the complexity of KIR interactions and further raise the possibility that the beneficial effects of KIR incompatibility mediated by NK cells could potentially be offset by alloreactive T cells or other immune effectors. T cells in stem cell grafts have been shown to affect expression of KIR on NK cells, and T-regulatory T cells have been shown to suppress NK cell cytotoxicity [22,23]. Taken altogether, these observations suggest that the benefit of KIR-incompatible NK cell populations may be most evident in T-cell lymphopenic hosts and/or in recipients of T-cell depleted transplants.

The genes encoding KIR and HLA molecules map to different chromosomes. HLA and KIR genotyping has shown that, even in the setting of an HLA-matched allogeneic HCT, a significant percentage of patients will lack an HLA ligand for one or more inhibitory KIR that are genotypically present in the donor. This observation led to a retrospective analysis exploring whether GvHD and disease relapse might be reduced in HLA-matched transplant recipients who lack a KIR ligand for one or more donor KIR. Lack of HLA ligand for donor-inhibitory KIR (missing KIR ligand) had no effect on disease-free survival, overall survival or relapse in patients receiving transplants for CML and ALL. In contrast, patients with AML and MDS lacking an HLA-C or -B ligand for donor-inhibitory KIR had a significantly lower relapse rate and improved disease-free and overall survival, with patients lacking two HLA ligands for donor-inhibitory KIR having the best disease-free survival and overall survival [24]. These data indicate that the lack of HLA-C or -B ligands for donor-inhibitory KIR can contribute to improved outcomes for patients with AML and MDS. Further, although not shown directly, they raise the possibility that, even in the setting of a fully HLA-matched allogeneic transplant, KIR-incompatible NK cell populations from the donor might mediate beneficial anti-leukemic effects.

NK cell-based tumor immunotherapy outside the context of an allogeneic transplant

NK cell-based therapy outside the context of an allogeneic HCT is being explored and may open new avenues for the treatment of AML relapse and other malignant diseases. Miller *et al.* treated 43 patients bearing either advanced solid malignancies or poor prognosis AML with immunosuppressive conditioning followed by HLA haplo-identical NK cell infusions and interleukin (IL)-2. Remarkably, five out of 19 AML patients achieved complete remission, including three of four AML patients who received NK cell infusions from donors with a KIR ligand mismatch in the GvH direction.

Hurdles to overcome

Until recently, the inability to expand large numbers of NK cells *in vitro* has precluded investigators from pursuing phase I trials evaluating an NK cell dose–response relationship. Cytokines, including IL-15 and IL-2, alone or combined with other growth factors, typically result in minimal NK cell expansion *in vitro*. However, the addition of feeder cell populations, such as Epstein–Barr virus-transformed lymphoblastoid cells (EBV-LCL), or gene-modified K562 cells expressing NK-stimulatory molecules, such as 4-1BB ligand and IL-15, can dramatically enhance NK cell expansions *in vitro* and have recently been used for the large-scale expansion of clinical-grade NK cells [25–27].

Using irradiated EBV-LCL and IL-2, we have developed a method for expanding large numbers of clinical-grade NK cells *in vitro* in Baxter Lifecell bAg under good manufacturing practice (GMP) conditions. A pure population of predominantly CD56⁺/CD16⁺/CD3[−] NK cells expanded an average of 490-fold over 21 days. Importantly, these NK cell populations were highly activated and had higher TRAIL, FasL and NKG2D expression and significantly higher TRAIL and perforin granzyme-mediated cytotoxicity against tumors compared with resting NK cells [28]. This *ex vivo* NK cell expansion technique is currently being utilized in a clinical trial evaluating the anti-tumor activity of adoptively infused NK cells in combination with bortezomib [29].

Methods to enhance autologous NK cell anti-tumor immunity

Overcoming KIR-mediated inactivation of NK cells

The adoptive infusion of allogeneic KIR-incompatible NK cells has recently been explored as a method to offset inhibitory KIR-mediated suppression of NK cell function. However, when MHC-mismatched allogeneic NK cells are adoptively infused in patients with cancer, intense host immunosuppression must first be given before any measurable NK cell engraftment can be achieved [30]. Therefore, outside the setting of an allogeneic transplant, KIR ligand-mismatched NK cells might be of limited therapeutic use because differences in MHC molecules would eventually lead to their rejection by a patient's immune system [31]. Investigators have explored alternative methods to potentiate autologous NK cell tumor cytotoxicity, including the use of KIR Ab to disrupt the function of specific inhibitory KIR. The blocking of NK cell MHC class I-specific inhibitory receptors increases NK cell

effector function against tumor cells *in vivo* in mice, and this approach is currently being tested in a phase I clinical trial in humans with AML [32].

Enhancing tumor susceptibility to NK cell killing

An alternative approach to offset KIR ligand inhibition that augments NK cell tumor killing would be to render tumor cells more susceptible to NK cell tumor attack. NK cells lyse tumor targets indirectly through cytokines or directly through perforin/granzyme, or through molecules such as Fas ligand and TRAIL, which directly trigger death receptor pathways, inducing tumor apoptosis [33-35]. Death receptors, including Fas, TNFR1, TRAIL-R1/DR4, TRAIL-R2/DR5, DR3 and DR6, share a conserved death domain that is triggered by adaptor molecules that activate executioner caspases and initiate apoptosis. Recently, several agents that influence the surface expression of these death receptors, including the proteasome inhibitor bortezomib (PS-341; Velcade) and the histone deacetylase inhibitor depsipeptide (FK228), have been described [36]. We and others have found that pretreatment of malignant cells *in vitro* with depsipeptide or bortezomib enhances NK-mediated tumor cytotoxicity mediated by TRAIL by up-regulating tumor surface expression of the TRAIL death receptor DR5 and as a consequence of drug-induced augmentation in tumor caspase 8 activity (Figure 4). More recently, we have also found that bortezomib enhances tumor susceptibility to NK cell killing via perforin/granzyme. NK cells expanded from patients with metastatic RCC were significantly more cytotoxic against the patient's own tumor cells pre-treated with either bortezomib or depsipeptide compared with untreated tumors. Most tumors sensitized to NK cells had high MHC class I surface expression that was not affected by bortezomib treatment, suggesting bortezomib could offset KIR ligand-mediated inhibition of autologous NK cells. Importantly, these *in vitro* observations could be reproduced *in vivo* in tumor-bearing mice, where we found treatment with bortezomib followed by syngeneic NK cell infusions delayed tumor progression and prolonged survival compared with controls receiving NK cells or bortezomib alone [37]. This anti-tumor effect was further potentiated by eradicating regulatory T cells (Treg) prior to adoptive NK cell infusions. Importantly, IL-2-activated and/or EBV-LCL-expanded NK cells substantially upregulated surface expression of TRAIL, which further augmented bortezomib-induced sensitization to TRAIL. These findings suggest that drug-induced sensitization to TRAIL and perforin granzyme could be used as a novel strategy to potentiate anti-cancer effects of both allogeneic and autologous adoptively infused NK cells in patients with cancer. Based on these findings, a clinical trial evaluating the anti-tumor activity of *ex vivo*-expanded adoptively infused NK cells in combination with bortezomib and Treg depletion in patients with cancer is currently being pursued at the National Heart Lung and Blood Institute (NHLBI, National Institutes of Health, Bethesda, MD, USA).

Directing chimeric Ag receptor-transduced NK cells against tumor-specific Ag

The development of an efficient method to modify NK cells genetically could be useful to introduce transgenes with long-term expression into human NK cells. A chimeric Ag receptor consisting of a CD20-specific scFv Ab fragment has been transduced into the human NK-92 cell line. While the *in vitro* activity of the retargeted NK-92 cells against CD20-negative targets remained unchanged, these gene-modified NK cells displayed markedly enhanced cytotoxicity against CD20⁺ target cells compared with the parental

NK-92 human cell line [38]. Imai *et al.* [25] have also shown that the cytotoxicity of *ex vivo*-expanded NK cells against CD19-expressing malignant B cells could be dramatically enhanced by NK cell transduction with chimeric receptors directed against CD19, a molecule widely expressed by malignant B cells.

Enhancing NK cell activation via activating receptors

NK cells do not always kill MHC class I-deficient cells. Therefore, under certain circumstances, the presence of MHC class I is neither necessary nor sufficient to protect cells from NK cell lysis. These findings imply the existence of activating receptors on NK cells whose engagement by tumor cell ligands can trigger NK killing. The best characterized NK cell-activating receptor in the context of human cancer is NKG2D. NKG2D is constitutively expressed by all NK cells. Its surface expression requires association with an adaptor protein, DAP10. DAP10 is phosphorylated upon NKG2D ligand engagement, resulting in recruitment and activation of phosphatidylinositol-3 kinase and perforin-dependent cytotoxicity. NKG2D recognizes at least six ligands on the surface of human cells, each with MHC class I homology: three transmembrane proteins, MICA, MICB and ULBP4, and three glycoposphatidylinositol-anchored proteins, ULBP1–3. Enhancing the NKG2D receptor–ligand system would be an attractive target for enhancing the therapeutic activity of NK cells against cancer. Although the best regimen for optimizing NKG2D-dependent killing has not yet been determined *in vivo*, encouraging studies have provided a number of candidate approaches. Histone deacetylase inhibitors (HDACi), including sodium valproate and depsipeptide, have been reported to induce NKG2D ligand up-regulation on tumors, sensitizing them to NKG2D-dependent NK cell cytotoxicity *in vitro* [39,40]. Whether these agents would potentiate the anti-tumor effects of innate or adoptively infused NK cells *in vivo* remains under investigation.

Enhancing NK cell-mediated ADCC

Human NK cells can also lyse Ab-coated target cells through the process of ADCC. A large proportion of human NK cells express CD16, the low-affinity Fcγ receptor IIIa (FCGR3A), which binds to the constant (Fc) region of Ig. Thus CD16 enables NK cells to recognize Ab-coated tumor cells, resulting in NK cell degranulation and perforin-dependent killing [41]. ADCC is proposed as a mechanism that contributes at least in part to the efficacy of tumor-directed Ab therapies, such as rituximab (a-CD20) and trastuzumab (a-Her2/Neu) [42]. Improving ADCC responses is desirable because it is thought to be an important anti-tumor mechanism for some Ab-based therapies. Binyamin *et al.* [43] showed enhancement of ADCC responses by blocking NK cell inhibitory receptors; in a cell line model of lymphoma therapy, the combination of rituximab with an Ab (DX9) that blocks inhibitory self-recognition or anti-KIR3DL1 yielded increased NK cell-mediated target cell lysis.

Lenalidomide, an immunomodulatory agent, has been shown to enhance T and NK cell activation markers in patients with advanced cancers [44]. In an *in vivo* SCID mouse lymphoma model, NK cell expansion during treatment with lenalidomide has been associated with increased cytotoxic effects of rituximab [45]. In other studies, lenalidomide has been shown to enhance the cytotoxicity of an anti-CD40 monoclonal Ab (SGN-40) in CD40-expressing human multiple myeloma cell lines [46], as well the ADCC of rituximab-

treated NHL cell lines and primary B-CLL cells *in vitro*. Based on these observations, innovative protocols that incorporate the use of agents designed to augment NK cell ADCC *in vivo* in recipients of tumor-targeted monoclonal Ab therapy will probably be tested in humans with cancer.

Conclusions

Based on other immunotherapeutic approaches for cancer, it is reasonable to assume that NK cell-based strategies would have their greatest benefit when used in the setting of minimal residual disease or as an adjunct to other nonimmune-based therapies. Retrospective data showing a role for donor NK cells mediating anti-leukemic effects following allogeneic SCT could lead to transplant regimens that specifically select donors based on KIR incompatibility and/or the presence of favorable donoractivating NK receptor allotypes. Infusion of activated NK cells in cancer patients treated with KIR-specific Ab the disrupt inhibitory KIR function and/or bispecific Ab targeting NCR, NKG2D and /or CD16 might also be of therapeutic value. Pre-clinical tumor models have shown that combination therapies using Ab targeting tumor Ag that bind with high affinity to FcγRIII may significantly potentiate NK cell tumor killing via ADCC. Yet despite these advances in our knowledge of methods to potentiate NK cell anti-tumor function, it remains to be determined whether adoptively infused *ex vivo*-expanded NK cells are capable of homing to tumors. Strategies aimed at altering NK cell trafficking to tumors or secondary lymphoid organs may be necessary before the full therapeutic potential of NK cell-based immunotherapy for cancer can be realized.

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Figure 1.

Figure 2.

Figure 3. GvHD incidence and overall survival in RENCA tumor-bearing BALB/c mice after allogeneic hematopoietic cell transplantation from MHC-matched B10.d2 donors given a single infusion of either ligand-matched (non-alloreactive; Ly49G2⁺) or ligand-mismatched (alloreactive; Ly49C⁺) Ly49. Modified from Lundqvist et al. [31].

Figure 4.

Cell-surface phenotype and cytotoxicity after treatment with depsipeptide or bortezomib. Human renal cell carcinoma cells were treated with depsipeptide or bortezomib and analyzed for cell surface expression of HLA-ABC, MIC-A/B, DR4 (TRAIL-R1), Fas and DR5 (TRAIL-R2) by flow cytometry. Black lines, isotype control; blue lines, untreated cells; green lines, bortezomib-treated cells; red lines, depsipeptide-treated cells. NK cells were tested against untreated, bortezomib-treated or depsipeptide-treated renal cell carcinoma cells, respectively.