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Improving natural killer cell cancer immunotherapy

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Abstract

Purpose of review—Natural killer (NK) cells are innate lymphoid cells specialized to eliminate malignant cells via direct cytotoxicity and immunoregulatory cytokine production. As such, NK cells are ideal as cellular therapy for cancer patients, and a number of studies have provided proof-of-principle that adoptively transferred NK cells can induce remissions in patients with leukemia. A clear understanding of the mechanisms underlying NK cell anti-tumor responses, including target cell recognition, activation status, and negative regulatory signals will improve NK cellular therapy for cancer patients.

Recent findings—Clinical studies have demonstrated the safety and preliminary efficacy of NK cell adoptive transfer, especially in hematologic malignancies. A variety of NK cell sources, isolation techniques, activation approaches, and ex vivo expansion strategies are under investigation. New approaches have been developed and are being tested to optimize NK cell therapy, including ways to better target NK cells to malignant cells, increase their functional competence, facilitate expansion in patients, and limit inhibitory signals or cells.

Summary—NK cells represent a promising cellular immunotherapy for the treatment of cancer. In addition to adoptive cellular therapy, adjunct treatments that optimize NK cell targeting and function will enhance their potency and broaden their potential use to many cancer types.

Keywords

Natural Killer Cells; Immunotherapy; Cancer

Introduction

For over a century it has been understood that the immune system is involved in controlling tumor growth [1]. Cancer immunotherapy strategies seek to harness the immune system's implicit ability to recognize and eliminate malignant cells, mediated by T cells, natural killer (NK) cells, NK-T cells, B cells, dendritic cells, and macrophages [2]. As the original immune-based cellular therapy, allogeneic hematopoietic cell transplantation (HCT) has provided long-term, disease-free survival to patients with hematologic cancers [3]. This

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Conflicts of interest

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procedure suppresses a patient's immune system to allow engraftment of the allogeneic donor's immune system, which in turn eliminates "foreign" cancer cells, commonly referred to as a graft versus leukemia effect (GVL). The drawback of this treatment approach is the recognition of normal patient cells as "foreign," thereby causing graft versus host disease (GVHD) – a major life-threatening complication. Thus, one rational approach to improve allogeneic HCT is to isolate specific anti-tumor immune cells, primarily T and NK cells, and utilize them for cell-based therapy. Indeed, the success of chimeric antigen receptor (CAR) modified T cells for the treatment of B cell malignancies has demonstrated the promise of this reductionist cellular immunotherapy approach. Similarly, NK cells have been isolated from allogeneic donors, and utilized to induce remissions, primarily in patients with acute myeloid leukemia (AML).

NK cells are innate lymphoid cells that circulate through most tissues, and are specialized to eliminate virus-infected and malignantly transformed target cells [4,5]. NK cells contribute to cancer immunoediting [6], and are frequently deficient or dysfunctional in cancer patients [7–9], suggesting that NK cells represent a significant immunoevasion requirement for cancer genesis and progression. Further, a large epidemiologic study demonstrated that low NK cell function predicted for an increased risk of developing cancer [10]. In the setting of allogeneic HCT, HLA-haploidentical NK cells can recognize AML blasts, which predicts for improved outcomes in high risk AML [11–14]. Adoptive NK cell therapy studies utilizing the HLA-disparity between the donor NK cells and patient AML to target NK cells to blasts show promise [15,16]. Further, immunogenetic studies of killer-cell immunoglobulin-like receptors (KIR) in patients who have undergone HCT have correlated certain KIR haplotypes or activating receptor expression with disease relapse [17–19]. A number of parameters are currently being investigated to improve NK cell adoptive immunotherapy, including the donor cell source, the use of large-scale ex vivo expansion, use of off-the-shelf cell lines, and NK cell differentiation from progenitors [20–23]. Moreover, strategies are now being tested to optimize NK cell responses, including targeting NK cells more effectively to the tumor, enhancing NK cell anti-tumor functional status, and removing inhibitory signals or cells [23] . The focus of this review is to summarize recent advancements in the adoptive NK cellular therapy of cancer, and highlight promising NK cell immunotherapy combination strategies.

What is an NK Cell?

NK cells are innate lymphoid cells that can recognize and eliminate malignant cells. NK cell functions that are responsible for tumor surveillance and clearance include cytokine/chemokine secretion and cytotoxicity [4,5,24]. These cytokines (e.g. IFN-γ and TNF) and chemokines (e.g., MIP-1α) are important for shaping the immune response to the tumors and for recruiting additional effector cells to the site of malignancy [4]. NK cells also release perforin and granzymes into specialized cytotoxic synapses to induce target cell death. Following activation, NK cells may also express the death receptor ligands TRAIL and FasL, which bind to their receptors on target cells to trigger apoptosis. Human NK cells are phenotypically identified as $CD56^+$ cells lacking T (CD3, TCR) and B (CD19) cell lineage markers, constitute approximately 10% of human blood lymphocytes, and consist of two

developmentally-related but functionally-distinct subsets of human NK cells, $CD56^{dim}$ and $CD56^{bright}$ NK cells [25].

In order to maintain proper tolerance to healthy tissues and effectively eliminate diseased cells, NK cells utilize germ-line encoded activating and inhibitory receptors [26]. During development, NK cells must express at least one inhibitory receptor specific for self MHC class I (MHC-I) to attain functional competence (e.g. licensing) [27]. In a mature, licensed NK cell, the balance of signals received through these receptors determines the fate of the engaged cell, where more activating receptor signaling results in target killing and cytokine production. NK cells recognize diseased cells that have lost inhibitory receptor ligand expression ("missing self") and upregulated activating receptor ligands ("abnormal or induced self"). KIR and C-type lectin inhibitory receptors (CD94/NKG2A) recognize MHC-I and MHC-I like molecules that are expressed on most normal healthy tissues, and provide the negative signals that prevent NK cell autoimmunity. Activating receptors expressed by NK cells, such as NKG2D, NKp46, and natural cytotoxicity receptors, recognize ligands that are upregulated on stressed or malignant cells [26]. NK cell receptors are variably and stochastically expressed on individual NK cells, resulting in thousands of NK cell specificities [28]. NK cell responses are not static; activated NK cells upregulate both inhibitory molecules (e.g., LAG-3, TIM-3, PD-1) and co-activating receptors (e.g., CD137). While induced inhibitory receptors are important in resolving a normal immune response and protecting healthy tissues, they also represent a means by which malignant cells evade NK cell responses [29–33]. Although immune checkpoints are well-defined in T cell anti-tumor responses, relevant activation-induced NK cell inhibitory checkpoints remain under investigation. In addition, recent studies revealed that NK cells can exhibit memory of prior activation [34] and NK cell memory is an area of active investigation [35]. Thus, NK cells are specialized effectors poised to respond to malignant cells, but in many diseases require modulation of trigger/recognition, functional capacity, or negative regulators for an optimal response.

Adoptive NK cell Immunotherapy

Since donor NK cell alloreactivity against leukemia correlated with improved clinical outcomes in AML patients with HLA-haploidentical HCT [11–14], most studies of NK cell adoptive immunotherapy have been performed in this disease. The recognition of an AML blast was linked to functionally-competent NK cells triggered via missing-self or induced/abnormal-self signaling [17–19]. In the first clinical trial investigating NK cell adoptive transfer outside of HCT, Miller et. al. enriched NK cells by depleting T cells (~40% NK cells in the final product), activated them overnight with IL-2, and administered them to lymphodepleted (fludarabine/cyclophosphamide) AML patients [15]. Key parameters of this and other reported studies of NK cellular therapy are summarized in Table 1. Overall, 5 of 19 (26%) of AML patients achieved a complete remission (CR) on this trial. Curti et. al., administered purified CD56+CD3− NK cells from HLA-haploidentical donors selected for a KIR-KIR ligand mismatch using a similar treatment protocol. Of the 5 patients with active AML, 1 patient (20%) obtained a CR [14]. More recently, Bachanova et. al. reported a series of patients treated with modifications of the Miller et al. platform, and demonstrated that the provision of IL-2-diptheriatoxin to deplete regulatory T cells (Tegs) enhanced NK cell

expansion and increased the frequency of CR (50%). General conclusions from these studies included 1) lymphodepletion is important for donor NK cell expansion and resulted in increased host IL-15 production, 2) in vivo expansion of donor NK cells correlated with AML CR frequencies, 3) allogeneic NK cells did not cause GVHD, 4) patient Tregs limit NK cell expansion and anti-leukemia activity in vivo. Multiple groups are examining alternative sources of NK cells, such as ex vivo expansion, differentiation from progenitors, and even immortalized NK cell lines [21,36–39]. While these studies provide proof-of-principle that allogeneic NK cell adoptive transfer may induce remissions in leukemia patients, it is clear that new, complementary approaches are needed to achieve lasting responses in patients (Table 2).

There remain a large number of open questions in the field, including the optimal setting in which to administer adoptive NK cell therapy. Although NK cell adoptive immunotherapy has the benefit of no GVHD, the “window of opportunity” for NK cells to clear leukemia is a few weeks, since host T cells eliminate allogeneic donor NK cells as they recover from Flu/Cy. In contrast, adding NK cell infusions to HCT procedures may allow for persistence in the host, enhance GVL and reduce GVHD, but does not address the key adverse events associated with the HCT per se. Thus, the most recent research efforts have investigated multiple approaches to enhance NK cell anti-tumor responses, which may have implications in NK cell adoptive immunotherapy in either setting, and may also be used to stimulate endogenous patient NK cell responses. The remainder of the review focuses on progress in the three major strategies to enhance NK cell-mediated tumor clearance.

Providing a trigger: enhancing NK cell tumor recognition

While initial studies utilized KIR ligand mismatch to facilitate donor NK cell recognition of recipient leukemias, advances in our understanding of NK cell triggering via CD16 by monoclonal antibody therapies has led to the development of novel single-chain variable fragments (scFv) fusion proteins designed to enhance targeting the NK cell to the tumor. Bispecific and trispecific killer cell engagers (BiKE and TriKE) that cross-link CD16 expressed on NK cells and 1-2 tumor antigens on target cells have been reported in pre-clinical studies [50]. The CD16xCD33 BiKE triggered NK cells via CD16 to kill and produce cytokines in response to CD33-expressing cell lines and primary leukemia samples in vitro [50]. Gleason et al. have recently tested the effectiveness of the CD16xCD33 BiKE to target NK cells to myelodysplastic syndrome (MDS) patient samples ($CD33^+$ MDS targets) [40]. Moreover, it was demonstrated that the CD16xCD33 BiKE triggers patient NK cells to lyse $CD33^+$ MDS blasts and immunosuppressive $CD33^+$ myeloid derived suppressor cells ($CD33^+$ MDSCs) in intact patient samples. Thus, CD33-scFv BiKEs could be utilized to limit endogenous myeloid regulators of NK cells, thereby enhancing allogeneic, and potentially autologous, NK cell responses. Rothe et al. reported a bispecific NK cell-specific targeting agent, AFM13, in a phase 1 study for treating rel/ref Hodgkin lymphoma [41,51]. AFM13 is a tetravalent chimeric antibody construct (TandAb) that contains two binding domains for CD16A, the NK cell specific isoform of the CD16 receptor, and CD30, which is expressed on hematologic malignancies, including Hodgkin lymphoma. In this study, patients were treated with escalating doses of AFM13 (0.01 to 7.0 mg/kg, three patients per dose) administered once a week for 4 weeks. AFM13 was well tolerated, and 3 of 26

patients experienced a partial response. Furthermore, the authors demonstrated enhanced activation marker expression on NK cells after AFM13 infusion providing a proof-of-principle that NK cells can be targeted and enhanced by AFM13 in vivo. By specifically using the CD16A scFv the large sink of $CD16^+$ non-NK cells is eliminated (e.g. $CD16B^+$ neutrophils), potentially improving the potency of this class of agents. Finally, a number of clinical monoclonal antibodies partially rely on ADCC mediated tumor clearance, including trastuzumab, rituximab, and cetuximab [52–55]. Efforts are being made to optimize monoclonal antibodies to enhance ADCC and antibody-dependent cytokine release (ADCR) by NK cells [42,56]. Because these responses are dependent on IgG Fc interaction with Fc receptors, de Romeuf et al. generated a chimeric monoclonal antibody which promoted optimal FcγRIIIA (CD16A) binding and signaling [56]. In two separate studies, Le Garff-Tavernier et. al., compared ADCC of CLL or lymphoplasmacytic lymphoma opsonized with the Fc-optimized anti-CD20 monoclonal antibody, ublituximab, or with the first-generation anti-CD20 antibody, rituximab [43,57]. In both studies, NK cell engagement with the ublituximab resulted in increased degranulation and target killing compared to rituximab [43,57]. All of these agents rely on CD16-triggered NK cell functions, however, a number of additional activating receptors (e.g. NKG2D) expressed by NK cells could be targeted in a similar fashion, thereby producing potent activating signals to enhance NK cell effector functions. Apart from these antibody-type targeting agents, there is interest in using CAR-modified NK cells for enhancing tumor specificity [44,45,58,59]. Haploidentical NK cells could be enriched from donor PBMCs, transduced to express tumor-specific CAR with NK cell activating receptor signaling domains (i.e., DAP10 and DAP 12) [45]. Because aplasia of normal hematopoietic stem cells and progenitors induced by long-lasting CAR T cells is currently one of the major hurdles in successfully using this technology for treating cancers like AML [60,61], the shorter persistence of allogeneic CAR NK cells may provide a viable alternative to CAR T cells.

Press the gas pedal: function-enabling NK cells

NK cell functional capacity can be enhanced in a variety of ways to improve NK cell mediated tumor clearance. Constitutive and induced expression of cytokine receptors by NK cells provides opportunities to use cytokines for immunomodulation [62]. Classically, IL-2 has been used to activate and support NK cells in vivo for patients receiving NK cell adoptive therapy. One major drawback is the exquisite sensitivity of Tregs to IL-2 via their high affinity IL-2Rαβγ, which may expand and limit NK cell responses [62]. IL-15 is the critical cytokine for NK cell homeostasis and function, and has been a long-standing attractive alternative to IL-2 to augment NK cell and CD8 T cell number and function [48,63,64]. Recently, the results of a first-in-human phase 1 clinical trial of rhIL-15 administered to advanced cancer patients demonstrated safety with clear NK and T cell immunomodulation [65]. This study provides the first evidence that IL-15-based agents are feasible in the clinic with favorable adverse event profiles at biologically active doses. Alternative forms of IL-15 based therapy have also been developed and are now in clinical trials. ALT-803 is an IL-15 super agonist mutein complexed with a fusion of IL-15Rα sushi domains to an IgG1 Fc domain, resulting in increased stability and in vivo half-life of this protein complex [66]. ALT-803 is currently being evaluated in a number of clinical trials in

cancer patients, and more recently in combination with therapeutic mAbs. IL-12, IL-18, and IL-21 are also being explored as NK cell activating cytokine adjuvants [62]. Beyond the scope of this review, several groups are utilizing cytokines and artificial stimulator cells to expand and activate NK cells prior to adoptive transfer (Table 3) [62,67].

Recent advances in NK cell biology have identified that NK cells exhibit a memory of prior activation [34], which includes enhanced responses to leukemia. For example, brief IL-12, IL-15, and IL-18 combined pre-activation of human NK cells resulted in differentiation of memory-like NK cells [68,69], with enhanced IFN-γ secretion and cytotoxicity upon restimulation with cytokines or tumor targets [35,68]. This approach is now being tested in a first-in-human study of cytokine induced memory-like NK cells in patients with relapsed or refractory AML (NCT01898793). Phase 1 testing of haploidentical NK cells primed ex vivo with CTV-1 lysate has been completed, and preliminary reports show that this approach is safe (NCT01520558) [70]. Lenalidomide is an immunomodulatory agent that has been demonstrated to enhance ADCC by NK cells [46,71]. Multiple trials are utilizing lenalidomide in combination with anti-tumor monoclonal antibodies, in order to enhance NK cell mediated ADCC and to improve clinical outcomes for patients (Table 2). In addition to targeting NK cells to tumors, monoclonal antibodies can also signal through costimulatory receptors to improve NK cell function. Using anti-41BB agonistic antibodies is one such strategy which is currently being tested in the clinic (Table 2) [72].

Take your foot off the brake: blocking NK cell inhibition

Similar to all other immune cells, NK cells have endogenous checks on their activity. To fully optimize NK cell anti-tumor responses in vivo, approaches are required to limit NK cell suppression, either from inhibitory molecules expressed by the NK cell (i.e. KIR) [73], or by suppressor cell types (i.e. regulatory T cells, myeloid derived suppressor cells) [74,75] (Figure 1). As previously mentioned, Tregs represent one obstacle to proper NK cell expansion after adoptive therapy plus IL-2 [47]. In order to limit Tregs during IL-2 administration, Bachanova et al. utilized an IL-2 diphtheria toxin fusion protein (IL2DT) to selectively deplete recipient Tregs [16]. In addition to extrinsic NK cell inhibition by regulatory cells, inhibitory receptors expressed by NK cells can also suppress their activities, such as the constitutively expressed KIR or NKG2A, as well as the induced co-inhibitory receptors (i.e. TIM-3, PD-1) [26,76]. IPH2101 is a mAb blocking common KIRs that bind to HLA-C alleles with the aim of disrupting the inhibitory HLA-KIR signal and enhancing NK cell function [77,78,49]. Initial clinical studies using IPH2101 did not demonstrate any major responses while using this as a single agent [77,78]. In a recent study, IPH2101 combined safely with lenalidomide in the absence of steroids with objective responses observed in patients with rel/ref multiple myeloma [49], providing support for the concept that combined immunotherapy strategies are required to unleash the most potent NK cell response.

Conclusions

The great promise of NK cell anti-tumor immune effects continues to expand as basic aspects of their biology are unraveled and translated to the clinic. Adoptively transferred NK

cells mediate anti-leukemia responses and can result in remissions, but many open questions remain regarding optimal purification, ex vivo manipulations, and in vivo support tactics. New immunotherapy approaches that combine allogeneic NK cell therapy with strategies to 1) improve targeting/triggering, 2) augment anti-tumor responses, and 3) limit inhibition will be the key to enhance the efficacy of NK cell adoptive therapy to a wider variety of malignancies.

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Key Points

- NK cell adoptive immunotherapy may induce complete remissions in patients with acute myeloid leukemia
- NK cell recognition and targeting may be enhanced using therapeutic mAbs, bi- and tri-specific agents, and chimeric antigen receptors
- NK cells are primed for anti-tumor responses by cytokines, activating receptor mAbs, and immunomodulatory agents
- Checkpoints on NK cell anti-tumor responses include inhibitory receptor signals and suppressive cells that may be targeted to enhance NK cell therapy

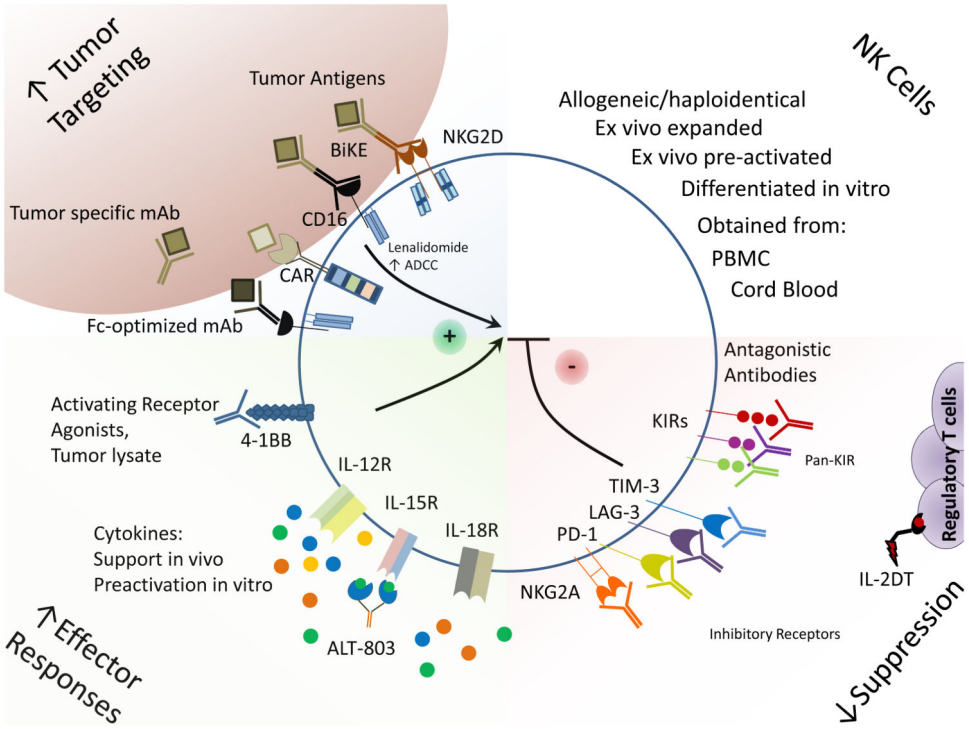

Figure 1. Combinatorial strategies to improve NK cell adoptive immunotherapy

NK cell adoptive therapy can be improved by combination approaches aimed at enhancing NK cell tumor specificity, enhancing NK cell effector capacity, and by reducing NK cell inhibition. Tumor-specificity can be increased by utilizing bispecific killer engagers (BiKE) or ADCC-optimized, tumor-specific monoclonal antibodies. NK cell effector capacity can be improved by in vitro preactivation with cytokines as well as in vivo with cytokine support, either with recombinant cytokines or with cytokine receptor super-agonists (e.g. ALT-803). Furthermore, activating receptor agonists can also be employed in vivo to improve NK cell activation and effector responses. Antibody checkpoint blockade therapy specific for inhibitory receptors expressed by NK cells will limit cell intrinsic immunosuppression. Finally, efforts to deplete regulatory T cells during NK cell adoptive therapy, e.g., using IL-2 diphtheria toxin fusion protein (IL2DT), are promising and currently under investigation.

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Table 1

Summary of recent NK cell adoptive therapy clinical trials. Flu, intravenous fludarabine; Cy,intravenous cyclophosphamide; IL2DT, IL-2-diphtheria fusion protein; A, Adult; P, Pediatric; CD3−, CD3 depletion; CD56+, CD56, positive selection; CD19−, CD19 depletion; NR, not reported. CR rates include those patients with active disease at the time of therapy.

Study	Conditioning	Post-Infusion Therapy IL-2 ($\times 10^6$ IU/m^2)	KIR-KIR ligand Mismatch	Purification	NK Dose ($\times 10^6$/kg)	Donor Chimerism	Donor NK cell number (per μl)	Peak Expansion (Days)	CR	GVHD	Reference
Rubnitz et al., 2010	Flu-Cy	1.0	Yes	CD3-CD56+	29	7 % (NK)	5.8	14	NA	No	[13]
Curti et al., 2011	Flu-Cy	1.0	Yes	CD3-CD56+	2.5	NR	NR	7-10	1/5	No	[14]
Miller et al., 2005	Flu-Cy	1.75-10	No	CD3-	9	25 % (PBMC)	NR	14	5/19	No	[15]
Bachanova et al., 2014	Flu-Cy	9.0						7			[16]
Cohort 1			17%	CD3-	9.6	NR	NR		5/19	No	
Cohort 2			50%	CD3-CD56+	3.4	NR	NR			No	
Cohort 3	+IL2DT		56%	CD3-CD19−	26	49 % (PBMC)	190		8/15	No	

Table 2

Recently published studies aimed at enhancing NK cell tumor responses by increasing NK cell activity, increasing NK cell specificity, and/or decreasing NK cell suppression.

Preclinical studies					
Disease	**Cell Product/ Agent**	**Description**	**Outcomes**	**NK Cell Strategy**	**Reference**
Hematologic malignancy	NK cells	Preactivation of cord blood NK cells by cytokines	Cord blood NK cells pre-activated with cytokine combinations (IL-15 + IL-2 or IL-18) are more active than those pre-activated with IL-2 alone	↑Activity Preclinical	[36]
MDS	Bispecific Killer Engager (BiKE)	CD16xCD33 BiKE to target NK cells to CD33+ MDS	CD16xCD33 BiKE increases activity of intact MDS patient NK cells, ex vivo	↑Specificity ↓ Suppression Preclinical	[40]
Hodgkin Lymphoma	AFM13 Bispecific Ab construct (TandAb)	CD30/CD16A TandAb to recruit NK cells to lyse CD30+ tumors	Tetravalent bispecific CD30/CD16A is optimized for NK cell ADCC and engagement the TandAb increased NK cell cytotoxicity in a CD30/CD16A dependent manner	↑Specificity Preclinical	[41]
CLL & WM Lymphoma	Ublituximab Fc-Optimized mAb	ADCC optimized α-CD20 compared to standard α-CD20	ADCC optimized anti CD20 antibody Ublituximab enhanced degranulation and cytotoxicity of NK cells in response to opsonized targets compared to rituximab treated controls	↑Specificity Preclinical	[42, 43]
Prostate Cancer	DAP12-based CAR NK cells	YTS NK Cell line was transduced to express an α-PSCA-DAP12 CAR	DAP12 CAR-YTS NK cells were more cytotoxic than CD3ζ CAR YTS NK cells. Primary NK cells engineered with the DAP12-CAR recognized and killed PSCA+ tumor cells in vitro	↑Specificity Preclinical	[44]
Multiple Myeloma	CD3/CD28 CAR NK Cells	NK cell lines engineered to express CS1-specific CAR	Two NK cell lines, NK-92 and NKL, killed tumor targets in a CS1 dependent manner	↑Specificity Preclinical	[45]
Carcinoma	Anti-41BB, cetuximab	α-41BB enhances NK cell function against cetuximab (α-EGFR) coated tumor targets	Cetuximab triggers ADCC by NK cells which is enhanced by ligation of the 41BB agonistic mAb	↑Activity (α-41 BB) ↑Specificity (cetuximab) Preclinical	[46]
Melanoma	α-TIM-3 mAb	Hyporesponsive NK cells from patients treated with α-TIM-3 mAb	TIM-3 blockade restores anti-tumor responses of melanoma patient-derived NK cells, in vitro	↓ Suppression Preclinical	[47]
Clinical studies					
Disease	**Cell Product/ Agent**	**Description**	**Outcomes**	**NK Cell Strategy**	**Reference**
AML	IL-2 diphtheria toxin fusion, NK cells, IL-2	IL-2DPT pretreatment + IL-2 preactivated haplo-identical NK cells	IL-2DP effectively depleted Tregs and improved engraftment of IL-2 preactivated, haploidentical NK cells in AML patients	↑Activity ↓ Suppression Clinical	[16]
Melanoma Renal Cell	Recombinant human IL-15	Determine the safety of intravenous bolus IL-15	IL-15 can be administered safely, however alternative strategies will likely increase efficacy and reduce	↑Activity Clinical	[48]

Preclinical studies					
Disease	**Cell Product/ Agent**	**Description**	**Outcomes**	**NK Cell Strategy**	**Reference**
cancer		administration	toxicity		
Rel/Ref Multiple Myeloma	Anti-KIR antibody IPH2101 and Lenalidomide	α-KIR mAb plus lenalidomide to treat patients	IPH2101 plus lenalidomide is safe, tolerable and evidence suggests may lengthen progression free survival.	↑Activity ↓ Suppression Clinical	[49]

Table 3

Ongoing clinical trials utilizing NK cells and NK cell functional modulation to treat cancer.

Clinical Diagnosis	ClinicalTrials.gov Identifier	Trial Phase	Cell Product / Agent	Institute(s) Involved
AML and MDS	NCT01898793	Phase I	Cytokine Induced Memory Like NK Cells	Washington University School of Medicine
AML	NCT01370213	Phase II	IL-2 activated NK cells prior to αβ-depleted haploidentical hematopoietic cell transplant	University of Minnesota, Washington University School of Medicine, Emory University, and Ohio State University
AML and MDS	NCT01385423	Phase I	NK cells followed by rh-IL15	University of Minnesota
Pediatric patients with leukemia or solid tumors	NCT01287104	Phase I	Ex vivo expanded NK cells early after hematopoietic cell transplant	National Cancer Institutes (NCI)
Leukemia	NCT01823198	Phase I/II	NK cells with HCT for high risk Myeloid malignancy	MD Anderson Cancer Center
Hematologic malignancies	NCT00789776	Phase I/II	NK cell infusion after hematopoietic cell transplant	Fred Hutchinson Cancer Center, Children's Hospital of Milwaukee and Medical College of Wisconsin
Leukemia	NCT01619761 NCT02280525	Phase I	Expanded cord blood NK cells	MD Anderson
Hematologic malignancies	NCT01853358	Phase I	IL-2 activated NK cells	Institut Paoli-Calmettes, France
Hematologic malignancies	NCT01904136	Phase I/II	IL-2 activated NK cells with hematopoietic cell transplant	MD Anderson Cancer Center
AML	NCT01787474	Phase I	IL-21 expanded NK cells	MD Anderson Cancer Center
Multiple Myeloma	NCT01040026	Phase I/II	Ex vivo expanded allogeneic NK cells with autologous hematopoietic cell transplant	University Hospital, Basel, Switzerland
Lymphoma	NCT01956695	Phase I	Efficacy of Lenalidomide with rituximab in rel/ref primary CNS lymphoma	Institute Curie, France
Leukemia	NCT01807611	Phase II	KIR mismatched haploidentical NK cell transplant	St. Jude Children's Research Hospital
AML	NCT00900809	Phase I	NK cell line (NK-92, Neukoplast)	UPMC Cancer Center
Lymphoma	NCT01729104	Phase I/II	Carfilzomib plus lenalidomide and rituximab in the treatment of rel/ref MCL	MD Anderson Cancer Center
ALL	NCT02185781	Phase I	Expanded autologous NK cells	Policlinico Umberto I di Roma

Clinical Diagnosis	ClinicalTrials.gov Identifier	Trial Phase	Cell Product / Agent	Institute(s) Involved
Leukemia	NCT02420938	Phase II	Urelumab with rituximab for rel/ref or high-risk CLL	MD Anderson Cancer Center
AML	NCT01520558	Phase I/II	CNDO-109-AANK for AML in First Complete Remission	Moffitt Cancer Center, University of Minnesota, Washington University School of Medicine, Medical University of South Carolina
Hematologic malignancies	NCT01885897	Phase I/II	Safety and efficacy of ALT-803 for relapse of malignancy after Allogeneic SCT	University of Minnesota
iNHL	NCT02384954	Phase I/II	Safety and efficacy of ALT-803 for rel/ref iNHL plus rituximab	Washington University School of Medicine
Multiple Myeloma	NCT02099539	Phase I/II	Safety and efficacy of ALT-803 for rel/ref multiple myeloma	University of Minnesota, Washington University School of Medicine, Roswell Park Cancer Center, Thomas Jefferson University
Breast and Gastric Cancer	NCT02030561	Phase II	NK cells + Trastuzumab	National University Hospital, Singapore