HHS Public Access

Author manuscript

Curr Opin Immunol. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Curr Opin Immunol. 2018 April ; 51: 146–153. doi:10.1016/j.coi.2018.03.013.

Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering

May Daher[1] and **Katayoun Rezvani**[1]

[1]Department of Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, University of Texas, Houston, Texas

Abstract

Recent advances in the field of cellular therapy have focused on autologous T cells engineered to express a chimeric antigen receptor (CAR) against tumor antigens. Remarkable responses have been observed in patients receiving autologous CD19- redirected T cells for the treatment B-lymphoid malignancies. However, the generation of autologous products for each patient is logistically challenging and expensive. Extensive research efforts are ongoing to generate an off-the-shelf cellular product for the treatment of cancer patients. Natural killer cells (NK) are attractive contenders since they have potent anti-tumor activity, and their safety in the allogeneic setting obviates the need for an autologous source. In this review, we discuss advantages and limitations of NK cellular therapy, and novel genetic engineering strategies that may be applied to overcome some of the limitations. Next-generation engineered NK cells are showing great promise in the preclinical setting and it is likely that in the next few years CAR- engineered NK cells will be incorporated into the current armamentarium of cell-based cancer therapeutics.

Keywords

Natural killer cells; cellular therapy; genetic engineering; cancer immunotherapy

Introduction

In the past decade, the field of cellular therapy has emerged as a powerful treatment modality for advanced cancers refractory to conventional therapy. Genetic engineering of immune cells has evolved from a promising concept to a practical solution for the treatment of a number of previously refractory types of cancer. Indeed, in the past few months, the Food and Drug Administration (FDA) approved the first gene-modified chimeric antigen receptor (CAR) T-cell therapy (tisagenlecleucel) for relapsed B-cell acute lymphoblastic leukemia (ALL) in children and young adults [1]. This was quickly followed by the approval of the second gene-modified CAR T-cell therapy (axicabtagene ciloleucel) for patients with

Corresponding author: Katayoun Rezvani, MD PhD, Stem Cell Transplantation & Cellular Therapies, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd. Unit 0423; KRezvani@mdanderson.org, Fax: 713-745-2517.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

certain types of relapsed large B-cell non Hodgkin lymphoma (NHL) [2,3]. These exciting new therapies mark a paradigm shift in the treatment of certain hematologic malignancies and are expected to cause a ripple effect in the field of cellular therapy and gene editing to target other cancers.

Despite its success, CAR modified autologous T-cell therapy has some undeniable limitations [4]. From a clinical standpoint, some of the patients are heavily pretreated and therefore lymphopenic, which makes it difficult to collect sufficient numbers of autologous lymphocytes to generate a clinically relevant dose of CAR-T cells for therapy. Furthermore, despite the high response rates, a significant number of patients still experience relapse as the cancer cells develop mechanisms to escape recognition by CAR-T cells [5]. From a logistic standpoint, the generation of autologous CAR-T cells is cumbersome and therefore restrictive for large-scale clinical application. The process is also lengthy and time consuming and therefore not applicable for patients with rapidly progressing malignancies. An off-the-shelf, ready to use, allogeneic source of CAR-T cells is therefore attractive, however allogeneic T cells are notorious for causing graft- versus-host (GVHD), even after HLA matching [6].

Natural killer (NK) cells are very effective at mediating cytotoxicity against tumor cells and unlike T-cells, kill their targets in a non-antigen specific manner and without the need for prior sensitization [7]. Allogeneic NK cells lack the potential to cause GVHD [8,9], and could be made available as an off-the-shelf allogeneic product for immediate clinical use. Therefore, genetic engineering of NK cells by introducing a CAR to redirect their specificity is an active field of investigation. Notably, engineered CAR-NK cells retain their diverse arrays of activating and inhibitory receptors [10], which in principle should make relapse due to downregulation of the CAR target antigen less likely than it is with CAR-T cells [11]. Therefore, the inherent qualities of NK cells make them attractive candidates for genetic engineering for the therapy of cancer.

In this review, we detail recent advances in the field of NK cell engineering for cancer immunotherapy and discuss advantages and limitations of these strategies.

NK Cell Biology and Role in Cancer Immune Surveillance

As their name indicates, NK cells are a subset of effector lymphocytes involved in innate “natural” immunity and being potent “killers”, they represent the first line of defense against pathogens and malignant cells [12]. They are characterized by CD56 and CD16 expression and lack of T cell receptor (TCR) and CD3 expression on their surface. NK cells can be further subdivided into two distinct subsets depending on their level of CD56 expression. The most common subtype present in the peripheral blood is $CD16^{+}CD56^{dim}$ and represents the more mature and highly cytotoxic phenotype; the second subtype is $CD16^{-}CD56^{bright}$, which characterizes a less mature immunoregulatory population mainly found in lymphoid tissues [13]. Unlike T cells, NK cells do not need prior antigen sensitization to kill their target cells. NK cells can mediate cytotoxicity through a number of mechanisms, the most important of which are degranulation and antibody dependent cellular cytotoxicity (ADCC), mediated by CD16 binding to the Fc portion of IgG1 opsonized on the surface of target cells

[14]. Unlike other immune cells, which require time to acquire cytolytic activity, NK cells are “ready to kill”. In fact, when NK cells form an immunologic synapse with their target cells, they release preformed cytolytic granules containing perforin and granzyme, leading to target cell lysis. In addition, engaged NK cells release molecules of the tumor necrosis factor (TNF) family, which induce death ligands (such as FAS ligand and TRAIL) on their surface. In turn, these ligands bind to death receptors on target cells, initiating an enzymatic cascade through caspases leading to apoptosis [14,15]. Activated NK cells also produce interferon (IFN)-γ, which activates dendritic cells and macrophages and has pleiotropic effects on the adaptive immune response [15]. In fact, the activity of NK cells is intricately complex and depends on the delicate integration of signals from multiple activating and inhibitory receptors, cytokines and chemokines [16].

NK cells can distinguish between normal and tumor cells through several mechanisms. To prevent the killing of healthy cells, NK cells primarily use inhibitory receptors, such as killer cell immunoglobulin-like receptors (KIRs) and CD94-NKG2A, that bind to major histocompatibility complex (MHC) class I molecules which are constitutively present on normal cells. Malignant cells can be recognized and killed by NK cells as they often downregulate or lose the expression of MHC-class I molecules [17] and/or by activating signals provided by multiple activating receptors on the surface of NK cells, which recognize stress ligands on the tumor cells. The vast array of cytokines and chemokines in the tumor microenvironment also play a role in the final disposition of NK cells to kill the transformed cells [18].

In the past few decades, numerous studies have implicated an important role for NK cells in tumor surveillance. In fact, quantitative and qualitative deficiencies of NK cells have been shown to contribute to cancer risk [19–21]. In addition, in experimental animal models, specific depletion of NK cells has been shown to cause more aggressive tumor progression and metastasis [22]. These studies point to a crucial role for NK cells in the immune surveillance of cancer.

Limitations of Adoptive NK cellular Therapy

Despite the lure of NK cells for adoptive therapy of cancer and their favorable safety profile, their efficacy in human trials has been modest at best. A number of factors limit the application of NK cell immunotherapy for the treatment of cancer. First, adoptively transferred NK cells have limited persistence in vivo, which while desirable from a safety standpoint, may hinder their efficacy [23]. Also, NK cell migration and their ability to penetrate tumor tissues have been reported to be inferior to that of T cells, which raises concerns regarding their usefulness in the setting of solid tumors [24]. Thus, strategies to increase infiltration of NK cells into tumors would be a plausible strategy to enhance antitumor efficacy. In addition, tumors develop mechanisms to evade NK cell surveillance such as upregulation of HLA molecules to disguise as normal cells, or downregulation of ligands for activating NK cell receptors [25]. The tumor microenvironment is also a major barrier to the effectiveness of NK cells. For instance, regulatory T cells (Treg cells) and myeloid-derived suppressor cells (MDSCs) frequently found at the tumor site can inhibit the function of NK cells [26]. Activated platelets in the malignant milieu have also been shown

to suppress NK cell cytotoxicity through a range of mechanisms [7]. Furthermore, the tumor microenvironment is rich in immunosuppressive cytokines and metabolites such as TGF-β, adenosine, prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase (IDO), which have been linked to NK cell dysfunction [7]. Finally, until relatively recently, the genetic manipulation of NK cells was considered to be challenging, yielding low efficiency and cell viability. However, recent optimizations in viral transduction, gene editing and electroporation technologies have renewed interest in strategies to enhance NK cell activity through genetic engineering [27]. These include approaches to make these cells persist longer, home to tumor sites, have enhanced cytotoxicity against tumors and be more adept at circumventing the immunosuppressive microenvironment (Figure 1).

Genetic modification of NK cells to enhance their function for cancer immunotherapy

Genetic modification to improve NK cell persistence

The in vivo persistence and proliferation of NK cells following adoptive transfer has been shown to correlate with clinical responses [28]. Several groups have attempted to enhance the in vivo persistence of NK cells by genetically reprogramming them to produce cytokines such as IL-2 or IL-15, which are critical for NK cell survival and development (Figure 1A). Retroviral transduction of NK cell lines or primary NK cells with *IL-2* or *IL-15* improved their in-vivo persistence and activity in tumor-bearing mice without the addition of exogenous cytokines [24]. Our group has shown that retroviral transduction of ex vivo expanded NK cells with a vector encoding a CAR against CD19 and the IL15 gene dramatically increased the in vivo persistence and anti-tumor activity of CAR-NK cells in a murine mouse model of lymphoma [10].

Genetic modification to improve NK cell homing and tumor penetration

Homing of NK cells to tumor sites is critical for their efficacy in cancer immunotherapy. NK cells that acquire expression of the chemokine receptor CCR7 via trogocytosis were reported to preferentially home to lymph nodes [29]. Another group showed that ex vivo expansion of NK cells results in increased expression of CXCR3 on their surface and improved migration and anti-tumor activity in a xenograft mouse model of CXCL10- transfected melanoma tumor [30]. Since then, several groups have explored genetic engineering of NK cells to improve their homing (Figure 1B). For instance, electroporation of NK cells with mRNA coding for the chemokine receptor CCR7 was shown to improve their migration toward the lymph node-associated chemokine CCL19 [31]. In another report, viral transduction of human primary NK cells to express CXCR2 improved their ability to migrate to renal cell carcinoma tumor sites [32]. Similarly, another group showed that engineering NK cells to express CXCR4 conferred specific chemotaxis to CXCL12/SDF-1α secreting glioblastoma cells and improved tumor regression and survival in a mouse model of glioblastoma [33].

Genetic modification to protect NK cells from the tumor microenvironment

One of the hallmarks of cancer is an aberrant chronic inflammatory state that is maintained by complex interactions between malignant cells, stromal cells and immune cells [34]. This

ineffective inflammatory milieu favors tumor evasion from host defenses, partly due to the release of immunosuppressive molecules by immunomodulatory cells such us Tregs, MDSCs, and type 2 macrophages (M2). TGF-β is a potent immunosuppressive cytokine that plays an important role in NK cell suppression within the malignant milieu. To overcome this well-described suppressive pathway, several groups have engineered NK cells with dominant negative TGF-β receptors to enhance the activity of adoptively transferred NK cells against multiple cancer types including glioblastoma, breast cancer and lung cancer [35–37]. Our group recently reported that that genetic disruption of TGF-β receptor 2 (TGF -βR2) by CRlSPR-CAS9 gene editing can render NK cells resistant to the suppressive effect of TGF-β and enhance their in vivo activity in a xenograft mouse model of acute myeloid leukemia [38]. Adenosine is another critical immunosuppressive metabolite in the tumor microenvironement and is generated from ATP by the ectonucleotidases CD39 and CD73 in response to hypoxia and extracellular stress [39]. Adenosine signals via the high affinity A2A adenosine receptor (A2AR) and hampers NK cell and T cell function [39]. NK cells deficient in A2AR displayed enhanced proliferation, maturation and better tumor control in murine models of melanoma, fibrosarcoma and breast adenocarcinoma [40,41].

Chronic inflammation and prolonged exposure to tumor antigens also directly contribute to dysfuntion of effector lymphocytes. Upregulation of checkpoint molecules such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) was first reported in exhausted T cells. These discoveries led to development of checkpoint inhibitors targeting CTLA-4 and the PD-1/PDL-1 axis that have revolutionized the treatment of certain cancers (reviewed in [42]). Checkpoint molecules have also been found to be expressed on NK cells in the setting of cancer. Several groups have demonstrated that PD1 mediates functional exhaustion of NK cells in certain cancers, and that blocking the PD-1/ PDL-1 axis can restore their function (reviewed in [43]). The expression of other checkpoint molecules such as CTLA-4, TIM- 3, LAG-3, TIGIT on NK cells in the setting of malignancy is less well explored and necessitates further elucidation.

In essence, the tumor microenvironment plays a critical role in immune escape from NK cell surveillance, and reprogramming NK cells to circumvent these immune evasion mechanisms is a promising strategy to improve the efficacy of adoptive NK cell therapy (Figure 1C).

Genetic modification to improve NK cell cytotoxicity

The panoply of activating and inhibitory receptors on NK cells and the myriad of mechanisms by which NK cells mediate cytotoxicity provide ample opportunities to engineer NK cells using approaches aimed at skewing the signaling balance towards activation. These strategies include blocking inhibitory receptors, potentiating activation receptors and ADCC, and redirecting the specificity of NK cells by introducing chimeric antigen receptors (Figure 1D).

Targeting inhibitory receptors—NKG2A-CD94 is an important inhibitory receptor universally expressed on NK cells. It binds HLA-E, a member of the nonpolymorphic MHC class Ib molecules upregulated on cells from many cancers, and inhibits NK cell function[44]. Knockdown of NKG2A in NK cells using shRNA or siRNA triggers a NK cell

"missing-self" response and leads to increased NK cell cytotoxicity against HLA-E expressing cancer cell lines in vitro and in vivo[45,46]. Monalizumab (previously IPH2201) is anti-NKG2A checkpoint inhibitor currently under clinical investigation in phase I/II clinic studies. NK cells also constitutively express inhibitory KIRs (iKIRs) that bind to HLA class I molecules and prevent NK cell activation towards healthy autologous cells. In the setting of haploidentical stem cell transplantation for hematologic malignancies, a KIR ligand-mismatched donor favors NK cell alloreactivity and is associated with improved relapse-free survival [47]. Thus, KIR blockade using an anti-KIR antibody (Lirilumab) is an attractive approach that has been shown to improve NK cell cytotoxicity in vitro and in preclinical models of myeloma and lymphoma [48,49]. This drug is currently being tested in twelve different clinical trials in combination with other biologics or immunotherapeutic agents for the treatment of hematologic malignancies and solid tumors (clinicaltrials.gov).

Enhancing the function of activating receptors and ADCC—NKG2D is an activating receptor that plays a critical role in NK cell anti-tumor response [16,50]. Retroviral transduction of NK cells with a chimeric receptor composed of NKG2D and the two signaling molecules DAP10 and CD3z enhanced NK cell function and cytotoxicity against multiple tumor cell lines and resulted in better tumor control in a mouse model of osteosarcoma [51]. Another innovative approach to enhance NK- mediated killing is to transduce NK cells with a chimeric receptor specific for TRAIL- receptor 1 to target TRAIL on target cells [52]. In addition, several groups are exploring ways to increase NK-mediated ADCC towards tumor cells by the concomitant use of monoclonal antibodies, bispecific killer cell engagers (BiKEs) or trispecific killer cell engagers (TriKEs) or by engineering NK cells to express a high affinity CD16 (Reviewed in [53,54]).

CAR-engineered NK cells

The majority of published data on adoptive NK cell therapy have explored the use of the NK cell line NK-92, a human NK cell line originally derived from a patient with NHL [55,56]. Promising pre-clinical data led to early-phase clinical trials of adoptive therapy using NK-92 cells to treat patients with advanced cancers [56,57]. Despite the safety profile of NK-92 cells, efficacy remains limited, even with multiple infusions. Therefore, several groups are investigating the use of CAR-engineering to redirect the specificity and enhance the antitumor activity of NK-92 cells. There are several potential advantages of using CAR engineered NK-92 cells over primary NK cells [58]. The NK-92 cell line is a well-characterized, uniform and reproducible source of NK cells. It offers an unlimited source of NK cells, which is attractive for cellular therapy. In addition, given the homogeneity of the product, transduction efficiency is more consistent in NK-92 cells compared to primary NK cells. In recent years, several groups have engineered NK-92 cells to express various CARs targeting both hematologic and solid malignancies, including CD19 and CD20 for B cell leukemia and lymphoma, CD38 and CS-1 for multiple myeloma, HER-2 for epithelial cancers, wild-type EGFR and mutant form EGFRvIII for brain metastasis and glioblastoma, ganglioside protein D2 (GD2) for neuroectodermal tumors, GPA7 for melanoma, and CD5 for T cell malignancies (Reviewed in [27,55]). Despite its attractive features, NK-92 cellular therapy has a number of important shortcomings. Most importantly, since this cell line is

derived from a patient with NHL, there is legitimate concern about its potential for tumorogenicity [59]. Therefore, for safety purposes, NK-92 cells are irradiated before infusion, which will significantly affect their in vivo proliferation, persistence and efficacy [55,59]. Finally, NK- 92 cells lack expression of several activating receptors such as NKp44 and NKp46, and do not express endogenous Fc receptors and thus, are not capable of mediating ADCC [27]. To counteract the latter drawback, an NK-92 cell derivative expressing the high affinity variant of FcgRIII has been developed [60,61].

Given the above-mentioned limitations of CAR engineered NK cell lines, several groups, including ours, have developed approaches to introduce CARs into primary NK cells to target B cell malignancies, breast and ovarian cancer, colon cancer, prostate cancer and sarcoma (Reviewed in [27]). Most of these published reports are utilizing primary NK cells derived from peripheral blood, cord blood or human pluripotent stem cells (HPSCs) [62].

Our group has developed a GMP compliant pipeline for the generation of CAR-NK cells from umbilical cord blood and is leading the first in-human clinical trial to test the safety and efficacy of off-the-shelf CB-NK cells engineered to express a CAR against CD19, to ectopically produce IL-15 to support NK cell proliferation and persistence in vivo, and to express a suicide gene, inducible caspase 9, to address any potential safety concerns (NCT03056339).

What does the future hold?

The ideal product for adoptive cellular therapy is one that is safe, off-the-shelf, universal and easy to generate in sufficient quantities for clinical use. Universal NK cells generated from cord blood, HPSCs or NK cell lines are currently being explored by a number of groups. It is likely that in the near future, we will witness an array of combinatorial gene manipulation strategies for therapeutic applications of NK cells, including CAR transduction and CRISPR/CAS9 or TALEN gene editing, to redirect their antigen specificity, improve their persistence and their trafficking, enhance their cytotoxicity while preserving their safety, and increase their resistance to the immunosuppressive TME. Moreover, notwithstanding the hurdles and uncertainties that these approaches will likely encounter as a result of the FDA’s regulatory path remaining undefined, their increasing adoption and efficacy may well provide a paradigm shift in the field of cancer immunotherapy.

Concluding remarks

The past several years has seen ‘breakthrough’ advances in the engineering of immune effector cells as therapy for cancer. CARs have been used extensively to redirect the specificity of T-cells against lymphoid malignancies with striking positive clinical results which led to the FDA approval of CD19-redirected CAR T cell therapy for relapsed B ALL in children and young adults [1] and for patients with certain types of relapsed NHL [2,3]. However, a number of obstacles still limit the widespread use of this promising new therapy in patients: (1) the generation of an autologous CAR T-cell product for each individual patient is expensive and logistically challenging; (2) allogeneic T-cells (even if HLA-matched) carry a significant risk of GVHD mediated through their native T-cell receptor; and

(3) the infused immune effector cells are susceptible to the inhibitory effects of checkpoint molecules and the TME. NK cells constitute a valuable, safe and versatile allogeneic product for immunotherapy. With the recent advances in the field of NK cell biology and genetic engineering, it is likely that NK cellular therapy will be incorporated into the current armamentarium of cell-based cancer therapeutics.

Acknowledgments

Funding

The authors' research efforts are funded in part by grants by from the NIH (R01 CA211044–01), the Cancer Prevention Research Institute of Texas (RSG-15–218- 01- LIB) and the American Cancer Society (ACS RSG-15–218-01-LIB) and the generous philanthropic support of the MD Anderson Cancer Center Moon Shots programs. The authors wish to apologize to colleagues whose work could not be adequately cited or discussed due to space constraints.

References

1. First-Ever CAR T-cell Therapy Approved in U.S. Cancer Discov 2017, 7:OF1.
2. FDA Approves Second CAR T-cell Therapy. Cancer Discov 2017.
3. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, et al.: Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med 2017.
4. Ruella M, Kenderian SS: Next-Generation Chimeric Antigen Receptor T-Cell Therapy: Going off the Shelf. BioDrugs 2017, 31:473–481. [PubMed: 29143249]
5•. Ruella M, Maus MV: Catch me if you can: Leukemia Escape after CD19-Directed T Cell Immunotherapies. Comput Struct Biotechnol J 2016, 14:357–362. [PubMed: 27761200] This paper reviews some of the mechanisms of immune escape after CAR- CD19 T cell therapy and the lessons that we can learn for the design of better CAR directed cellular therapies.
6. Yang Y, Jacoby E, Fry TJ: Challenges and opportunities of allogeneic donor-derived CAR T cells. Curr Opin Hematol 2015, 22:509–515. [PubMed: 26390167]
7. Morvan MG, Lanier LL: NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer 2016, 16:7–19. [PubMed: 26694935]
8. Locatelli F, Moretta F, Brescia L, Merli P: Natural killer cells in the treatment of high-risk acute leukaemia. Semin Immunol 2014, 26:173–179. [PubMed: 24613727]
9. Simonetta F, Alvarez M, Negrin RS: Natural Killer Cells in Graft-versus-Host- Disease after Allogeneic Hematopoietic Cell Transplantation. Front Immunol 2017, 8:465. [PubMed: 28487696]
10••. Liu E, Tong Y, Dotti G, Shaim H, Savoldo B, Mukherjee M, Orange J, Wan X, Lu X, Reynolds A, et al.: Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia 2017.This study highlights a very effective engineering strategy of umbilical cord blood NK cells using retroviral transduction of a vector containing IL-15 gene and CAR-CD19 to enhance NK cell in-vivo persistence and cytotoxicity in a lymphoma mouse model.
11. Ruella M, June CH: Chimeric Antigen Receptor T cells for B Cell Neoplasms: Choose the Right CAR for You. Curr Hematol Malig Rep 2016, 11:368–384. [PubMed: 27475429]
12. Campbell KS, Hasegawa J: Natural killer cell biology: an update and future directions. J Allergy Clin Immunol 2013, 132:536–544. [PubMed: 23906377]
13. Malmberg KJ, Carlsten M, Bjorklund A, Sohlberg E, Bryceson YT, Ljunggren HG: Natural killer cell-mediated immunosurveillance of human cancer. Semin Immunol 2017, 31:20–29. [PubMed: 28888619]
14. Guillerey C, Huntington ND, Smyth MJ: Targeting natural killer cells in cancer immunotherapy. Nat lmmunol 2016, 17:1025–1036.
15. Lanier LL: Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008,9:495–502. [PubMed: 18425106]

16. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S: Innate or adaptive immunity? The example of natural killer cells. Science 2011, 331:44–49. [PubMed: 21212348]

17. Wu J, Lanier LL: Natural killer cells and cancer. Adv Cancer Res 2003, 90:127–156. [PubMed: 14710949]

18. Moretta L, Bottino C, Pende D, Vitale M, Mingari MC, Moretta A: Human natural killer cells: Molecular mechanisms controlling NK cell activation and tumor cell lysis. Immunol Lett 2005, 100:7–13. [PubMed: 16109445]

19. Orange JS: Natural killer cell deficiency. J Allergy Clin Immunol 2013, 132:515–525; quiz 526. [PubMed: 23993353]

20. Wood SM, Ljunggren HG, Bryceson YT: Insights into NK cell biology from human genetics and disease associations. Cell Mol Life Sci 2011, 68:3479–3493. [PubMed: 21874350]

21•. Rouce RH, Shaim H, Sekine T, Weber G, Ballard B, Ku S, Barese C, Murali V, Wu MF, Liu H, et al.: The TGF-beta/SMAD pathway is an important mechanism for NK cell immune evasion in childhood B-acute lymphoblastic leukemia. Leukemia 2016, 30:800–811. [PubMed: 26621337] The authors of this study demonstrate the importance of the tumor microenvironment and particularly the TGF-beta canonical pathway in the immune escape of B-cell ALL from NK surveillance in pediatric patients, which supports the rationale of blocking the TGF-beta pathway as a genetic engineering strategy.

22. Gross E, Sunwoo JB, Bui JD: Cancer immunosurveillance and immunoediting by natural killer cells. Cancer J 2013, 19:483–489. [PubMed: 24270347]

23. Burga RA, Nguyen T, Zulovich J, Madonna S, Ylisastigui L, Fernandes R, Yvon E: Improving efficacy of cancer immunotherapy by genetic modification of natural killer cells. Cytotherapy 2016, 18:1410–1421. [PubMed: 27421740]

24. Carlsten M, Childs RW: Genetic Manipulation of NK Cells for Cancer Immunotherapy: Techniques and Clinical Implications. Front Immunol 2015, 6:266. [PubMed: 26113846]

25. Groth A, Kloss S, von Strandmann EP, Koehl U, Koch J: Mechanisms of tumor and viral immune escape from natural killer cell-mediated surveillance. J Innate Immun 2011, 3:344–354. [PubMed: 21576922]

26. Murray S, Lundqvist A: Targeting the tumor microenvironment to improve natural killer cell-based immunotherapies: On being in the right place at the right time, with resilience. Hum Vaccin Immunother 2016, 12:607–611. [PubMed: 26402368]

27. Rezvani K, Rouce R, Liu E, Shpall E: Engineering Natural Killer Cells for Cancer Immunotherapy. Mol Ther 2017, 25:1769–1781. [PubMed: 28668320]

28. Davis ZB, Felices M, Verneris MR, Miller JS: Natural Killer Cell Adoptive Transfer Therapy: Exploiting the First Line of Defense Against Cancer. Cancer J 2015, 21:486–491. [PubMed: 26588681]

29. Somanchi SS, Somanchi A, Cooper LJ, Lee DA: Engineering lymph node homing of ex vivo-expanded human natural killer cells via trogocytosis of the chemokine receptor CCR7. Blood 2012, 119:5164–5172. [PubMed: 22498742]

30. Wennerberg E, Kremer V, Childs R, Lundqvist A: CXCL10-induced migration of adoptively transferred human natural killer cells toward solid tumors causes regression of tumor growth in vivo. Cancer Immunol Immunother 2015, 64:225–235. [PubMed: 25344904]

31•. Carlsten M, Levy E, Karambelkar A, Li L, Reger R, Berg M, Peshwa MV, Childs RW: Efficient mRNA-Based Genetic Engineering of Human NK Cells with High-Affinity CD16 and CCR7 Augments Rituximab-Induced ADCC against Lymphoma and Targets NK Cell Migration toward the Lymph Node Associated Chemokine CCL19. Front Immunol 2016, 7:105. [PubMed: 27047492] This study employs two different strategies of genetic engineering of NK cells, one to improve ADCC by introducing a high affinity CD16, the other is to improve NK cell trafficking and homing to lymph nodes by introducing the chemokine receptor CCR7.

32. Kremer V, Ligtenberg MA, Zendehdel R, Seitz C, Duivenvoorden A, Wennerberg E, Colon E, Scherman-Plogell AH, Lundqvist A: Genetic engineering of human NK cells to express CXCR2 improves migration to renal cell carcinoma. J Immunother Cancer 2017, 5:73. [PubMed: 28923105]

33. Muller N, Michen S, Tietze S, Topfer K, Schulte A, Lamszus K, Schmitz M, Schackert G, Pastan I, Temme A: Engineering NK Cells Modified With an EGFRvIII-specific Chimeric Antigen Receptor to Overexpress CXCR4 Improves Immunotherapy of CXCL12/SDF-1alpha-secreting Glioblastoma. J Immunother 2015, 38:197–210. [PubMed: 25962108]
34. Vitale M, Cantoni C, Pietra G, Mingari MC, Moretta L: Effect of tumor cells and tumor microenvironment on NK-cell function. Eur J Immunol 2014, 44:1582–1592. [PubMed: 24777896]
35. Yang B, Liu H, Shi W, Wang Z, Sun S, Zhang G, Hu Y, Liu T, Jiao S: Blocking transforming growth factor-beta signaling pathway augments antitumor effect of adoptive NK-92 cell therapy. Int Immunopharmacol 2013, 17:198–204. [PubMed: 23806302]
36. Yvon ES, Burga R, Powell A, Cruz CR, Fernandes R, Barese C, Nguyen T, Abdel- Baki MS, Bollard CM: Cord blood natural killer cells expressing a dominant negative TGF-beta receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy 2017, 19:408–418. [PubMed: 28109751]
37. Zhao Y, Hu J, Li R, Song J, Kang Y, Liu S, Zhang D: Enhanced NK cell adoptive antitumor effects against breast cancer in vitro via blockade of the transforming growth factor-beta signaling pathway. Onco Targets Ther 2015, 8:1553–1559. [PubMed: 26124672]
38. Daher MBR, Shaim H, Gokdemir E, Uprety N, Kontoyiannis A, Mendt MC, Imahashi N, Kerbauy LN, Wei Inng Lim FL, Li L, et al.: The TGF-0/SMAD Signaling Pathway As a Mediator of NK Cell Dysfunction and Immune Evasion in Myelodysplastic Syndrome. In ASH annual meeting. Atlanta, GA: Blood; 2017:53 vol 130.
39. Hoskin DW, Mader JS, Furlong SJ, Conrad DM, Blay J: Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review). Int J Oncol 2008, 32:527–535. [PubMed: 18292929]
40. Young A, Ngiow SF, Gao Y, Patch AM, Barkauskas DS, Messaoudene M, Lin G, Coudert JD, Stannard KA, Zitvogel L, et al.: A2AR adenosine signaling suppresses natural killer cell maturation in the tumor microenvironment. Cancer Res 2017.
41. Mittal D, Young A, Stannard K, Yong M, Teng MW, Allard B, Stagg J, Smyth MJ: Antimetastatic effects of blocking PD-1 and the adenosine A2A receptor. Cancer Res 2014, 74:3652–3658. [PubMed: 24986517]
42•. Sharma P, Allison JP: Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell 2015, 161:205–214. [PubMed: 25860605] This is a comprehensive review of the importance of immune checkpoints in cancer immune evasion and the value of targeting them to potentially cure certain malignancies.
43. Beldi-Ferchiou A, Caillat-Zucman S: Control of NK Cell Activation by Immune Checkpoint Molecules. Int J Mol Sci 2017, 18.
44. Borrego F, Masilamani M, Kabat J, Sanni TB, Coligan JE: The cell biology of the human natural killer cell CD94/NKG2A inhibitory receptor. Mol Immunol 2005, 42:485–488. [PubMed: 15607803]
45. Figueiredo C, Seltsam A, Blasczyk R: Permanent silencing of NKG2A expression for cell-based therapeutics. J Mol Med (Berl) 2009, 87:199–210. [PubMed: 19002424]
46. Furutani ESS, Smith A, Berg M and Childs R: siRNA Inactivation of the Inhibitory Receptor NKG2A Augments the Anti-Tumor Effects of Adoptively Transferred NK Cells In Tumor-Bearing Hosts. In *ASH annual meeting*. Blood; 2010:1015 116.
47. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, 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. [PubMed: 11896281]
48••. Kohrt HE, Thielens A, Marabelle A, Sagiv-Barfi I, Sola C, Chanuc F, Fuseri N, Bonnafous C, Czerwinski D, Rajapaksa A, et al.: Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. Blood 2014, 123:678–686. [PubMed: 24326534] The authors show that blocking inhibitory KIRs is an effective strategy to enhance NK cell anti-tumor activity by skewing the balance of NK signaling towards activation.

49. Nijhof IS, Lammerts van Bueren JJ, van Kessel B, Andre P, Morel Y, Lokhorst HM, van de Donk NW, Parren PW, Mutis T: Daratumumab-mediated lysis of primary multiple myeloma cells is enhanced in combination with the human anti-KIR antibody IPH2102 and lenalidomide. Haematologica 2015, 100:263–268. [PubMed: 25510242]

50. Raulet DH: Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003, 3:781–790. [PubMed: 14523385]

51••. Chang YH, Connolly J, Shimasaki N, Mimura K, Kono K, Campana D: A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells. Cancer Res 2013, 73:1777–1786. [PubMed: 23302231] The authors of this study describe a novel strategy of engineering NK cells with a construct that co-expresses DAP10 with NKG2D/CD3z CAR and demonstrated enhanced cytotoxicity against multiple cancer cell lines.

52. Kobayashi E, Kishi H, Ozawa T, Hamana H, Nakagawa H, Jin A, Lin Z, Muraguchi A: A chimeric antigen receptor for TRAIL-receptor 1 induces apoptosis in various types of tumor cells. Biochem Biophys Res Commun 2014, 453:798–803. [PubMed: 25445592]

53. Ochoa MC, Minute L, Rodriguez I, Garasa S, Perez-Ruiz E, Inoges S, Melero I, Berraondo P: Antibody-dependent cell cytotoxicity: immunotherapy strategies enhancing effector NK cells. Immunol Cell Biol 2017, 95:347–355. [PubMed: 28138156]

54. Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM: NK Cell-Mediated Antibody- Dependent Cellular Cytotoxicity in Cancer Immunotherapy. Front Immunol 2015, 6:368. [PubMed: 26284063]

55. Suck G, Odendahl M, Nowakowska P, Seidl C, Wels WS, Klingemann HG, Tonn T: NK-92: an 'off-the-shelf therapeutic' for adoptive natural killer cell-based cancer immunotherapy. Cancer Immunol Immunother 2016, 65:485–492. [PubMed: 26559813]

56. Tonn T, Schwabe D, Klingemann HG, Becker S, Esser R, Koehl U, Suttorp M, Seifried E, Ottmann OG, Bug G: Treatment of patients with advanced cancer with the natural killer cell line NK-92. Cytotherapy 2013, 15:1563–1570. [PubMed: 24094496]

57. Arai S, Meagher R, Swearingen M, Myint H, Rich E, Martinson J, Klingemann H: Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial. Cytotherapy 2008, 10:625–632. [PubMed: 18836917]

58. Klingemann H, Boissel L, Toneguzzo F: Natural Killer Cells for Immunotherapy - Advantages of the NK-92 Cell Line over Blood NK Cells. Front Imunol 2016, 7:91.

59•. Zhang C, Oberoi P, Oelsner S, Waldmann A, Lindner A, Tonn T, Wels WS: Chimeric Antigen Receptor-Engineered NK-92 Cells: An Off-the-Shelf Cellular Therapeutic for Targeted Elimination of Cancer Cells and Induction of Protective Antitumor Immunity. Front Immunol 2017, 8:533. [PubMed: 28572802] This is an up-to-date review of the preclinical experience with CAR engineered NK-92 cell lines to target cancer.

60. Jochems C, Hodge JW, Fantini M, Fujii R, Morillon YM, 2nd, Greiner JW, Padget MR, Tritsch SR, Tsang KY, Campbell KS, et al.: An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele. Oncotarget 2016, 7:86359–86373. [PubMed: 27861156]

61. Jochems C, Hodge JW, Fantini M, Tsang KY, Vandeveer AJ, Gulley JL, Schlom J: ADCC employing an NK cell line (haNK) expressing the high affinity CD16 allele with avelumab, an anti-PD-L1 antibody. Int J Cancer 2017, 141:583–593. [PubMed: 28477372]

62. Lowe E, Truscott LC, De Oliveira SN: In Vitro Generation of Human NK Cells Expressing Chimeric Antigen Receptor Through Differentiation of Gene- Modified Hematopoietic Stem Cells. Methods Mol Biol 2016, 1441:241–251. [PubMed: 27177671]

Highlights

- NK cells play a critical role in cancer immune surveillance
- NK cellular therapy is safe but has some limitations
- Genetic engineering strategies are being employed to overcome these limitations

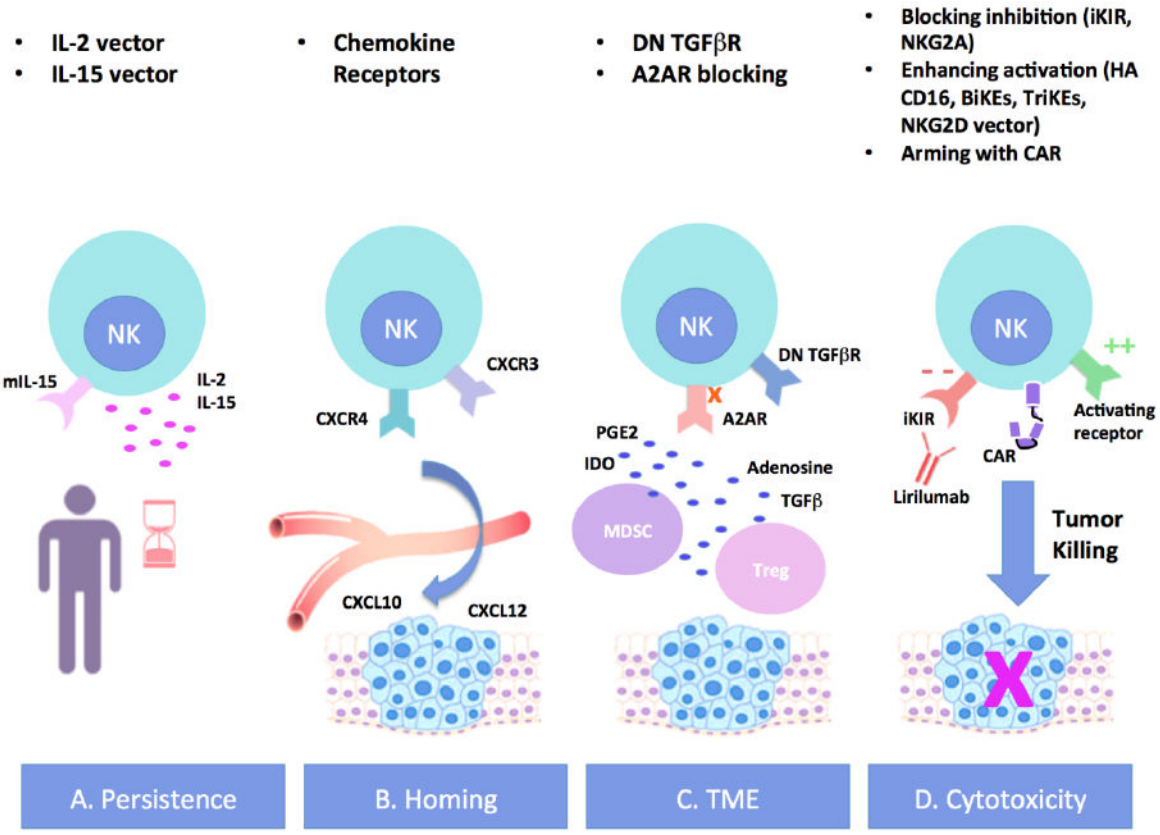

Figure 1. NK cell engineering strategies to enhance the effectiveness of NK cellular therapy.

To overcome some of the limitations of NK cellular therapy genetic engineering strategies are employed to improve (A) NK cell expansion and persistence, (B) NK cell trafficking and homing to tumor sites, (C) NK cell ability to circumvent the immunosuppressive tumor microenvironment, and (D) NK cell cytotoxicity against tumors. A bbreviations: m IL −15: membrane bound IL-15. DN TGFβR: Dom inant negative TGFβ recep tor. A2AR: A2A adenosine receptor. MDSC: myeloid derived su pp ressor cell. Treg: regu latory T cell. TME: Tumor microenvironment. iKIR: inhibitory killer imm unoglobulin recep tor. BiKE: biscpecific killer cell engager. TriKE: trispecific killer cell engager. CAR: chimeric antigen receptor.