# A Look under the Hood of the Engineered Human Natural Killer Cells

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**Abstract:** Notwithstanding multimodal approaches including chemotherapeutic agents and radiation have been used for decades as major strategies to successfully treat cancer patients; however, the emergence of drug or radiation resistance led to a significant incidence of tumor relapse and hence limits their effectiveness. Therefore, the need for novel and effective strategies which are clinically vital; not only for improved efficacy to eliminate resistant tumor cells but also to permit less-toxic doses and potentially overcome resistance, was and still a hopeful goal. Natural killer (NK) cells comprise 5–10% of peripheral blood lymphocytes (PBLs). Owing to the fact that NK cells have an importance role in anti-tumour immunity as demonstrated by several elegant studies, therefore, this NK-cell activity has been exploited as the basis of cancer immunotherapy strategies. Nevertheless, tumor cells can effectively escape NK cell-mediated apoptosis through a cocktail of different mechanisms. Thus, to enhance NK cell effector function against tumors, different approaches have been recently developed to achieve an *ex vivo* NK-cell enhancement. One adoptive transfer approach uses expanded allogeneic NK cells, which are MHC class I-resistant. A second approach uses stable allogeneic NK cell lines, which is more practical for large-scale production and safety. A third approach is the genetic modification of NK cells or NK cell lines to highly express cytokines, Fc receptors and/or chimeric tumor-antigen receptors. Therapeutic NK cells can be derived from different sources, including peripheral or cord blood cells, stem cells or induced pluripotent stem cells (iPSCs). Here, we summarize the recent developments in genetic engineering of NK-cell-based biopharmaceuticals, and covering the usefulness, effectiveness, and safety for their clinical applications.

**Keywords:** Natural killer cells, Engineered NKs, Adoptive transfer, Allogenic, Chimeric receptors.

## INTRODUCTION

The immune system is honoured century ago to have the capacity to fight against tumours [1]; however, a well recognized hallmark of cancer is that the artful manner the cancer cells use to evade destruction by immune cells [2]. Immunotherapy is a novel promising approach for the treatment of malignant tumours. Titanic efforts are made to improve the use of immunotherapy against malignancies. Currently, there is a plethora of immunotherapy approaches; however, a general approach involves stimulating patient immune cells *ex vivo* to enhance an anti-tumour response when administered back into the patient. Current strategies are striving to improve anti-tumour responses to a wide spectrum of malignant tumours, by enhancing tumour antigen presentation to naive or memory T cells and activating other effector cells, such as natural killer (NK) cells [3]. It should not be forgotten, however that the ultimate goal in this odyssey is to protect patients from future tumour relapses by inducing a long-term memory response, while delaying or inhibiting tumour growth [4]. Until now there are three main branches of immunotherapy; non-specific stimulation, active immunotherapy and adoptive transfer. Non-specific stimulation, such as interleukin-2 (IL-2) or interferon-α (IFN-α) treatment, aims to enhance the immune system against cancer (*e.g.*, melanoma and kidney cancer), however this treatment alone has shown to be ineffective [5]. Active immunotherapy provokes the host's immune system to generate a response against the cancer, with the use of vaccination. Low toxicity and the potential of a lasting effect (cellular memory) are the major pros of this treatment; however the effectiveness of vaccinations remains varied [6]. The last branch is adoptive, or cell transfer immunotherapy, which involves isolating immune cells that can fight against cancer cells. These immune effector cells are growing and modifying *ex vivo*, and then adoptively transferring into the patients [7]. So far, a wide spectrum of therapeutic agents have been investigated in the cancer immunotherapy field, including cytokines, monoclonal antibodies, adoptive cell transfers (T, NK and NKT) and Toll-like receptor (TLR) agonists [8-10]. Indeed, adoptive transfer of NK cells has held great promise for over three decades since the original observation that isolated NK cells could kill malignant cells [11], and hence its use in the clinical trials as a powerful immunotherapy for the treatment of malignant diseases is ongoing [12].

In this article, we will review the recent advances in genetic engineering of NK-cell-based biopharmaceuticals, and covering the usefulness, effectiveness, and safety for their clinical applications.

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The NK cells: natural fighters against cancer cellsNK cells were initially abandoned when they regarded as an "experimental artifact" in T cell cytotoxicity assays. Rolf Kiessling and Eva Klein in 1975 [11, 13], and Herberman and colleague [14, 15] were the first who discovered the NK cells in mice more than 35 years ago, and who also named them natural killer cells. The odyssey of identifying human NK cells starts with describing them as non-adherent, non-phagocytic, FcγR+, large granular lymphocytes (LGL) [16]. However, later it was realized that not only NK cells shared the LGL phenotype but also some NK cells displayed normal small lymphocyte morphology, depending on their activation status [17]. NKR-P1 [18] and NK1.1 [19] antigens made it possible to define murine NK cells roughly as $NK1.1^+$ $TCR^-$ $sIg^-CD16^+$. Today, human NK cells are defined as lymphocytes that are distinguished by $CD3^-CD56^+$. They comprise approximately 10-15% of all circulating lymphocytes and are also found in tissues, including the liver, and placenta. Resting NK cells that circulate in the blood are able to infiltrate into most tissues that contain pathogen-infected or malignant cells after their activation by cytokines [20].

Klas Kärre presented the first piece of the puzzle as the non-self hypothesis in 1981. He suggested that NK cells kill target cells lacking expression of self major histocompatability (MHC) class-I molecules although the mechanism was unclear at that time [21, 22]. This model was later confirmed by the discovery of inhibitory receptors on NK cells.

Natural killer cells express both inhibitory and activating receptors, the immunoglobulin super family (killer-cell immunoglobulin-like receptors (KIR) and natural cytotoxicity receptors (NCR)) and the C-type lectin superfamily [23]. The balance of signals that NK cells receive will determine whether or not NK cells turn activated [24]. NK cells become activated when ligands expressed on tumor cells engaged the activating receptor, while keeping the inhibitory receptor unoccupied [23]. The ample evidence from a mouse xenograft tumor model demonstrated that NK cells can effectively eradicate tumor cells through direct or indirect mechanisms [25-28]. Direct mechanisms include cytoplasmic granule release [29], death receptor-induced apoptosis [30], effector molecule production (*e.g.* IFN-γ) [31] or antibody dependent cellular cytotoxicity (ADCC) [27]. Indirect mechanisms of killing tumor cells include the crosstalk between the NK cells and dendritic cells (DC) to enhance the tumor antigen uptake and presentation [32-34], and hence facilitating the generation of antigen-specific cytotoxic T lymphocytes (CTL) responses. Furthermore, IFN-γ produced by activated NK cells can induce switching of $CD8^+$ T cells to CTLs, control differentiation of $CD4^+$ T cells toward a Th1 response and promote CTL differentiation [35, 36]. A second role of the cytokines produced by activated NK cells might also orchestrate antitumor antibody production by B cells [27].

## THE NK CELLS AS AN ANTICANCER BIOPHARMACEUTICAL

A biopharmaceutical is a pharmaceutical product that is biological in nature and manufactured using biotechnology [37]. Therefore, production of *ex vivo* NK cells and their use for the treatment of malignant diseases may be considered as an anticancer biopharmaceutical. Tumor cells use different pathways to escape from NK-cell recognition, such as silencing expression of adhesion molecules, ligands for activating receptors, up regulating MHC class I, soluble MIC, FasL, secreting immunosuppressive factors such as IL-10, TGF-β and resisting Fas- or perforin-mediated apoptosis [38, 39]. Decreased cytotoxicity, defective expression of activating receptors, over expression of inhibitory receptors, defective proliferation, and defective cytokine production are hallmarks of NK cells abnormalities in cancer patients [31]. Therefore, NK-cell based immunotherapeutic strategies (Figure **1**) have been proposed as an as an anticancer biopharmaceutical. A broad spectrum of various approaches has been applied from the use of monoclonal antibodies or recombinant cytokines to adoptive transfer of *ex vivo* activated or genetically modified donor NK cells [40].

## MODULATION OF ENDOGENOUS NK CELL ACTIVITY

Early studies have been clearly shown that NK cell activation with IL-2 can enhance the proliferation and cytotoxic activity against NK-resistant targets that [41-43]. Several elegant studies on animal models have supported further the efficiency of IL-2 treatment approach for cancer immunotherapy [44-53]. In the clinical setting, Rosenberg and colleagues [54, 55] have demonstrated the potent effect of IL-2 in the treatment of cancer patients by activating the cytotoxicity of the NK cells [56], and this effect is dependent on the dose and schedule of IL-2 administration [57]. IL-2 has been attempted for the treatment of various tumor types, and was shown significantly increase number of circulating NK cells

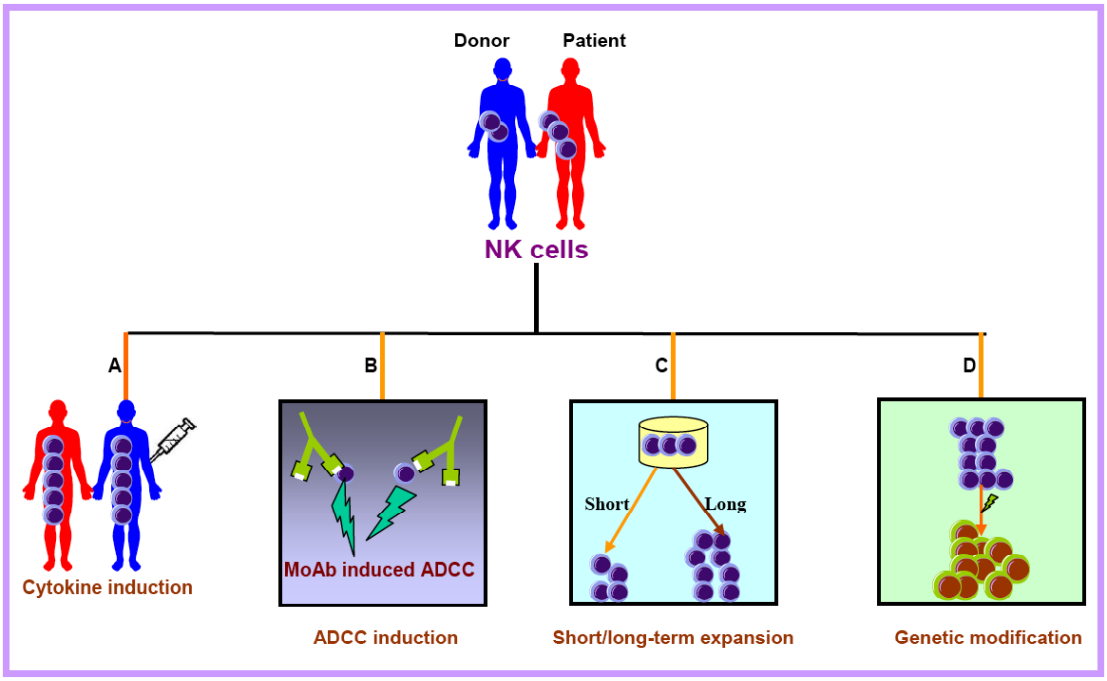


**Figure 1**: *In vivo* and *ex vivo* modulation of NK cell activity.
Autologous (patient) or donor NK cells can be transferred to fight against the tumor cells after an *in vivo* or *ex vivo* manipulations. An *in vivo* modification of natural killer (NK) cell activity can be achieved through (**A**) infusion of cytokines and or (**B**) tumor specific monoclonal antibodies (MoAbs) to enhance the Antibody-dependent cellular cytotoxicity (ADCC) responses. The adoptive transfer of the *ex vivo* modified autologous or allogenic NK cells after short or long-term (**C**) expansion is another approach to eradicate the cancer cells. Genetic modification of NK cells (**D**) is a recent promise option.

and their cytotoxicity against different types of NK resistant cancers [58-63]. In addition, using a coktail IL-2, IL-12, IL-15, IL-18, IL-21, and type I IFN have shown to stimulate NK cell cytotoxicity *in vitro* and show synergy when used in combination [64, 65]. Increase of the NK cell proliferation capacity, cytokine production, and up regulation of Kp44, perforin, granzymes, FasL expression are hallmarks of IL-2 therapy induced NK cell activity in cancer patients.85 Transferring of IL-2 activated NK cells has showed greater success than the adminstering of IL-2 systemically [66-68].

Overall, data from the reports demonstrated that notwithstanding IL-2 treatment is a promising approach, however is not the optimal strategy for treating cancer patients, and that combination therapy is needed. Furthermore, toxicity of IL-2 and induction of NK cell apoptosis by IL-2 are the dark face of the moon and can lead to a poor clinical outcome.

## ADOPTIVE TRANSFER OF NK CELLS

In the clinical setting, the number, purity, and activation status of NK cells to be used are vital factors to be considered. The selection of NK cell isolation method from peripheral blood (PB) is crucial for normal expression of cell surface markers, intracellular cytokines, perforin and granzyme B, and preserving their proliferative and cytotoxic capacity, and therefore their use for adoptive immunotherapy [69-75]. Given that NK cells present in PB only in low number, therefore obtaining a large number of NK cells to be used in the clinical studies is a difficult task. Thus, many reports showed successful *ex vivo* expansion of NK cells for adoptive immunotherapy applications, and some of these NK cell-based products have already been used in the clinic, which showed to exert specific *in vitro* cytotoxic activity against different human tumor cells [75-89]. These studies clearly showed that every NK cell expansion protocol and every different donor does not yield expanded NK cells with similar phenotype. Thus, factors such as distribution of KIR expressing populations and expression of activating and inhibitory receptors are vital and need to be checked as they have a significant effect on the their clinical applicability and efficiency.

## ADOPTIVE TRANSFER OF AUTOLOGOUS NK CELLS

Different animal studies revealed that adoptive transfer of NK cells proved to be efficient and successful [90]. Additionally, activated NK cells isolated from acute myeloid leukemia (AML) patients

demonstrated high cytotoxicity against autologous AML blasts *in vivo* in an NOD/SCID model [91]. Various clinical studies have investigated the impact of autologous NK cells infusion in different types of cancers on the clinical outcome. The clinical outcomes varied from no improvements to fully improved and complete remission (CR) of the patients [61, 77, 80, 86, 92-94].

## ADOPTIVE TRANSFER OF ALLOGENEIC NK CELLS

Efficient adoptive transfer of allogenic NK cells requires absence of one or more killer immunoglobulin like receptors (KIR) ligands in the recipient but present in the donor. NK cells that express inhibitory KIR for which there is no ligand on recipient cells would give the best chances for anti-tumor reactivity and hence for clinical responses [95-97]. The greatest attention of using the KIR-ligand mismatched in the setting of NK cell-based immunotherapy occurred after the distinguished retrospective analysis of haplotype mismatched hematopoietic stem cell transplants (HSCT) by Ruggeri *et al*. which revealed delayed relapse, better engraftment and protection from graft versus host disease (GvHD) in leukemia patients [96, 98]. Further studies have shown that NK cells from healthy donors and cancer patients have higher cytotoxic activity against various KIR-ligand mismatched tumor cell lines when compared to KIR-ligand matched targets [99]. Several groups performed clinical trials with infusion of allogeneic NK cells.[72, 97, 100-108] Of these studies, Shi *et al*. treated 10 multiple myeloma (MM) patients with haploidentical NK cells before autlogous stem cell transplantation (ASCT), and interestingly found that the allogeneic NK cells survived in the PB of the patient at about 7 days until eventually they were undetectable by day 14. This finding has been confirmed by Miller *et al* who observed an *in vivo* expansion of the allogenic adoptively transferred NK cells, and a complete remission rate of 50% was also reported. This may indicate the clinical benefit of the adoptive allogenic NK cell trasfer without long-term engraftment, although the cells were undetectable after 14 days. NK cell lines such as 92287 are another alternative in NK cell-based tumor immunotherapy. This cell line lacks KIR but expresses several activating receptors [109]. NK-92 cell line has been used in mouse studies [110, 111] and as direct infusions to patients [112, 113]. These experiments suggest that infusion of NK-92 may be safe and potentially beneficial.

## GENETIC MODIFICATION OF NK CELLS: A NEW NAVIGATION IN CANCER IMMUNOTHERAPY

### Gene Therapy

The delivery of the gentic material (DNA or RNA) into target cells for the purpose of of preventing or treating a disease. The first gene transfer into human cells was described in 1990; a four-year old patient with adenosine deaminase deficiency was the first who received gene therapy [114]. This trial opened the door widely for many other gene therapy clinical trials with their highs and lows. A distinguished study by Rosenberg *et al* in 1990 has motivated the interest in genetically modifying immune effector cells in order to use them in cancer immunotherapy. Rosenberg and colleagues have *ex vivo* introduced the foreign genes into the hematopoitic cells to be adoptively later transferred [115]. Initially, T cells were the only investigated effector cells in the cancer immunotherapy; however, more players including NK cells are now considered as new “weapons of mass destruction” and hence great efforts are made to genetically engineering them for the cancer immunotherapy.

### Gene Delivery Vectors

The delivery of the gene-of-interest (GOI) into the cell using viral vectors was launched in 1986 by Rogers and Pfuderer [116]. Since then, viruses have been widely used as gene delivery vehicles. Given that viruses vectors have the limitations of pathogenicity and immunogenicity; other researchers prefer to use non-viral delivery methods. Every method (viral versus non-viral) of delivering nucleic acids needs special considerations and has pros and cons [117, 118].

A defining feature of viral vectors vis-à-vis non-viral delivery of genes is that in general they are highly efficient. However, using viruses for gene delivery has its own cons. To overcome the virus pathogenicity and to ensure that the virus will not be replicating and spreading, all viral genes and sequences except those necessary for packaging of the viral genome are removed, and thus will leave a space for therapeutic genes to be inserted. Changing viral promoter elements and envelope proteins is highly recommended to enhance safety and ensure the tropism of the virus to the target cell type [119, 120]. The commonly used viral vectors include gamma retrovirus, lentivirus, adenovirus, or herpes vectors. Every vector has its own characteristics in terms of genome size, coating of the particle, infection mode,

persistence and immunogenicity. The high level of stable transgene expression and long term experience is the major advantages of the retroviral vectors. Contrary, a disadvantage of these vectors is that transduction can be performed only on efficiently dividing cells [121]. Lentiviral vectors are another type of gene delivery vectors which are capable of integrating also into non-dividing cells. The permanent gene expression and lower risk of damaging insertions are two features of the lentiviral vectors; however, an integration bias without oncogenic selection has recently been reported [122].

### Genetic Modification of NK Cells

Gene transfer into NK cells may pave the way for new opportunities for the immunotherapy cancer in both autologous and allogeneic settings. The introduction of chimeric antigen receptors (CAR) is very recently stepping on the scene as a novel means of genetically modifying NK cells to redirect them to attack the tumor targets, and investigations are ongoing to use them for clinical applications [123]. Furthermore, induction of NK cell proliferation or survival using cytokine gene therapy is an additional approach of genetic engineering of NK cells [124]. Stable transduction using retroviral [124-129] or lentiviral [130-136] vectors is preferred over the transient methods such as electroporation [123, 137, 138] or nucleofection [139] in terms of long-term effects. Liu *et al*. have demonstrated that transfection of the CD18 gene into the CD18-deficient NK cell line (YT-1) causes restoration of the cytotoxic capacity of the cell line against a B cell lymphoma line [140]. Another study showed that upon genetic modification of the NK cell lines with the IL-15 gene leads to increases in the proliferative rate and cytotoxic capacity [141, 142]. IL-12 is another cytokine gene therapy in which the gene was transferred into mouse NK cells which results in an increased in their survival capacity and *in vivo* anti-tumor activity [143]. Activation of T cells which increases the chance of GvHD [144] and stimulation of immunosuppressive T regulatory cells [145] are both unwelcomed side effects of the systemic IL-2 administration [146, 147]. Since IL-2 is a potent enhancer of the NK cells and to avoid the unwelcomed IL-2 side effects, IL-2 gene was transduced into the NK cell lines (*e.g.,* NK-92 )[124, 148] and an increased in the cytotoxic activity against tumor cell lines *in vitro* was observed. This manipulation of NK92 cells enabled them to secrete IL-2 independently on an exogenous feeding, and the cells showed an increased in the proliferation capacity and antitumor activity in mice models [124, 149]. However, the risk of activating other immune effector cells by secreted IL-2 from the transduced NK cells still remains, and therefore a study investigated an alternative approach for IL-2 delivery by keeping the effect only in NK cells in a controlled and localized manner [129]. This approach may be useful for the future engineering of the NK cells. Another approach to reprogram the NK cells for cancer immunotherapy is the CAR receptor expression on the NK cells, which enhances the NK cell activity through retargeting of the NK cells to tumor cells. Generally a single-chain variable fragment receptor specific for a certain tumor-associated antigen and is fused to the intracellular signalling moiety CD3ζ chain. This approach has been used by several groups and proved an effective tactic to increase the NK cell reactivity against different tumor antigens. Chimeric receptors against CEA [150], CD33 [151], and Her2/neu [128, 152, 153], have been successfully delivered to NK cell lines and were displayed to markedly increased NK cell cytotoxicity both *in vitro* and *in vivo*. To further extend the importance of these findings, Pegram *et al*. have reprogrammed the gene of primary mouse cells to express a chimeric receptor against Her2/neu and found that the adoptive transfer of these cells to mice bearing Her2+ tumors inhibits tumor progression *in vivo* [154]. Kruschinski *et al*. have also reprogrammed primary NK cells from human donors to express a chimeric receptor against Her2/neu and observed high cytotoxic activity against Her2+ cell lines both *in vitro* and in xenograft models with $RAG^{2-/-}$ mice [155]. In addition, Imai *et al*. have found that reprogramming of autologous NK cells to express a chimeric receptor against CD19; a molecule widely expressed by malignant B cells, results in efficient killing of autologous leukemic cells *in vitro* [156]. Transduction of NK-92-MI cells with a CAR (CD138); a highly expressed antigen on multiple myeloma (MM) cells, has been shown to significantly enhance the anti-MM efficacy of NK cells, and the first to be tested in the clinical trials [157]. Taken together, these data indicate a new avenue of the adoptive transfer of chimeric antigen-specific bearing NK cells, which proved at least *in vitro* to enhance the NK cytotoxic abilities, and thus might be a novel navigation in the field of cancer immunotherapy. However, limitations such as efficiency of gene delivery to NK cells and safety of the vectors are still challenging for their clinical applications.

## CONCLUSIONS

Notwithstanding that NK cell based therapies showed little clinical success, but it still holds great promise for the treatment of cancer patients. Better understanding of the NK cell biology and function will

open new vistas to reprogram the NK cells to enhance the NK cytotoxic functions, and thus might be a novel navigation in the field of cancer immunotherapy.

## REFERENCES


[1] Ehrlich P. Über den jetzigen stand der karzinomforschung. Ned Tijdschr Genees 1909; 5: 273-290.

[2] Floor SL, Dumont JE, Maenhaut C, Raspe E. Hallmarks of cancer: Of all cancer cells, all the time? Trends Mol Med 2012; 18: 509-515. http://dx.doi.org/10.1016/j.molmed.2012.06.005

[3] Klein O, Schmidt C, Knights A, Davis ID, Chen W, Cebon J. Melanoma vaccines: Developments over the past 10 years. Expert Rev Vaccines 2011; 10: 853-873. http://dx.doi.org/10.1586/erv.11.74

[4] Boudreau JE, Bonehill A, Thielemans K, Wan Y. Engineering dendritic cells to enhance cancer immunotherapy. Mol Ther 2011; 19: 841-853. http://dx.doi.org/10.1038/mt.2011.57

[5] Vivancos P, Granena A, Jr., Sarra J, Granena A. Treatment with interleukin-2 (il-2) and interferon (ifn(alpha 2b)) after autologous bone marrow or peripheral blood stem cell transplantation in onco-hematological malignancies with a high risk of relapse. Bone Marrow Transplant 1999; 23: 169-172. http://dx.doi.org/10.1038/sj.bmt.1701532

[6] Fillon M. Promising immunotherapy technique. J Natl Cancer Inst 2012; 104: 1528-1529. http://dx.doi.org/10.1093/jnci/djs449

[7] Meier T, Uhlik M, Chintharlapalli S, Dowless M, Van Horn R, Stewart J, et al. Tasisulam sodium, an antitumor agent that inhibits mitotic progression and induces vascular normalization. Mol Cancer Ther 2011; 10: 2168-2178. http://dx.doi.org/10.1158/1535-7163.MCT-11-0323

[8] Borghaei H, Smith MR, Campbell KS. Immunotherapy of cancer. Eur J Pharmacol 2009; 625: 41-54. http://dx.doi.org/10.1016/j.ejphar.2009.09.067

[9] Baxevanis CN, Perez SA, Papamichail M. Cancer immunotherapy. Crit Rev Clin Lab Sci 2009; 46: 167-189. http://dx.doi.org/10.1080/10408360902937809

[10] Zhao E, Xu H, Wang L, Kryczek I, Wu K, Hu Y, Wang G, Zou W. Bone marrow and the control of immunity. Cell Mol Immunol 2012; 9: 11-19. http://dx.doi.org/10.1038/cmi.2011.47

[11] Kiessling R, Klein E, Wigzell H. "Natural" Killer cells in the mouse. I. Cytotoxic cells with specificity for mouse moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975; 5: 112-117. http://dx.doi.org/10.1002/eji.1830050208

[12] Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, Jonson AL, Panoskaltsis-Mortari A, Curtsinger J, McKenna D, Dusenbery K, Bliss R, Downs LS, Miller JS. A phase ii study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy 2011; 13: 98-107. http://dx.doi.org/10.3109/14653249.2010.515582

[13] Kiessling R, Klein E, Pross H, Wigzell H: "Natural" Killer cells in the mouse. Ii. Cytotoxic cells with specificity for mouse moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 1975; 5: 117-121. http://dx.doi.org/10.1002/eji.1830050209

[14] Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. Ii. Characterization of effector cells. Int J Cancer 1975; 16: 230-239. http://dx.doi.org/10.1002/ijc.2910160205

[15] Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer 1975; 16: 216-229. http://dx.doi.org/10.1002/ijc.2910160204

[16] Trinchieri G: Biology of natural killer cells. Adv Immunol 1989; 47: 187-376. http://dx.doi.org/10.1016/S0065-2776(08)60664-1

[17] Dvorak AM, Galli SJ, Marcum JA, Nabel G, der Simonian H, Goldin J, Monahan RA, Pyne K, Cantor H, Rosenberg RD, Dvorak HF. Cloned mouse cells with natural killer function and cloned suppressor t cells express ultrastructural and biochemical features not shared by cloned inducer t cells. J Exp Med 1983; 157: 843-861. http://dx.doi.org/10.1084/jem.157.3.843

[18] Chambers WH, Vujanovic NL, DeLeo AB, Olszowy MW, Herberman RB, Hiserodt JC. Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine-activated killer cells. J Exp Med 1989; 169: 1373-1389. http://dx.doi.org/10.1084/jem.169.4.1373

[19] Ryan JC, Turck J, Niemi EC, Yokoyama WM, Seaman WE. Molecular cloning of the nk1.1 antigen, a member of the nkr-p1 family of natural killer cell activation molecules. J Immunol 1992; 149: 1631-1635.

[20] Glas R, Franksson L, Une C, Eloranta ML, Ohlen C, Orn A, Karre K. Recruitment and activation of natural killer (nk) cells in vivo determined by the target cell phenotype. An adaptive component of nk cell-mediated responses. J Exp Med 2000; 191: 129-138. http://dx.doi.org/10.1084/jem.191.1.129

[21] Karre K. Nk cells, mhc class i molecules and the missing self. Scand J Immunol 2002; 55: 221-228. http://dx.doi.org/10.1046/j.1365-3083.2002.01053.x

[22] Ljunggren HG, Karre K: In search of the 'missing self': Mhc molecules and nk cell recognition. Immunol Today 1990; 11: 237-244. http://dx.doi.org/10.1016/0167-5699(90)90097-S

[23] Murphy WJ, Parham P, Miller JS. Nk cells--from bench to clinic. Biol Blood Marrow Transplant 2012; 18: S2-7. http://dx.doi.org/10.1016/j.bbmt.2011.10.033

[24] Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of h-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature 1986; 319: 675-678. http://dx.doi.org/10.1038/319675a0

[25] Hayakawa Y, Smyth MJ: Innate immune recognition and suppression of tumors. Adv Cancer Res 2006; 95: 293-322. http://dx.doi.org/10.1016/S0065-230X(06)95008-8

[26] Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM: In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci U S A 2000; 97: 2731-2736. http://dx.doi.org/10.1073/pnas.050588297

[27] Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer 2002; 2: 850-861. http://dx.doi.org/10.1038/nrc928

[28] Wu J, Lanier LL: Natural killer cells and cancer. Adv Cancer Res 2003; 90: 127-156. http://dx.doi.org/10.1016/S0065-230X(03)90004-2

[29] Trapani JA, Davis J, Sutton VR, Smyth MJ. Proapoptotic functions of cytotoxic lymphocyte granule constituents in vitro and in vivo. Curr Opin Immunol 2000; 12: 323-329. http://dx.doi.org/10.1016/S0952-7915(00)00094-7

[30] Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ, Smyth MJ. Increased susceptibility to tumor initiation and metastasis in tnf-related apoptosis-inducing ligand-deficient mice. J Immunol 2002; 168: 1356-1361.

http://dx.doi.org/10.4049/jimmunol.168.3.1356

[31] Sutlu T, Alici E. Natural killer cell-based immunotherapy in cancer: Current insights and future prospects. J Intern Med 2009; 266: 154-181. http://dx.doi.org/10.1111/j.1365-2796.2009.02121.x

[32] Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S: Functions of natural killer cells. Nat Immunol 2008; 9: 503-510. http://dx.doi.org/10.1038/ni1582

[33] Bryceson YT, March ME, Ljunggren HG, Long EO. Activation, coactivation, and costimulation of resting human natural killer cells. Immunol Rev 2006; 214: 73-91. http://dx.doi.org/10.1111/j.1600-065X.2006.00457.x

[34] Bapsy PP, Sharan B, Kumar C, Das RP, Rangarajan B, Jain M, Suresh Attili VS, Subramanian S, Aggarwal S, Srivastava M, Vaid A. Open-label, multi-center, non-randomized, single-arm study to evaluate the safety and efficacy of dendritic cell immunotherapy in patients with refractory solid malignancies, on supportive care. Cytotherapy 2014; 16: 234-244. http://dx.doi.org/10.1016/j.jcyt.2013.11.013

[35] Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, Sallusto F. Induced recruitment of nk cells to lymph nodes provides ifn-gamma for t(h)1 priming. Nat Immunol 2004; 5: 1260-1265. http://dx.doi.org/10.1038/ni1138

[36] Mocikat R, Braumuller H, Gumy A, Egeter O, Ziegler H, Reusch U, Bubeck A, Louis J, Mailhammer R, Riethmuller G, Koszinowski U, Rocken M. Natural killer cells activated by mhc class i(low) targets prime dendritic cells to induce protective cd8 t cell responses. Immunity 2003; 19: 561-569. http://dx.doi.org/10.1016/S1074-7613(03)00264-4

[37] Rader RA. (re)defining biopharmaceutical. Nat Biotechnol 2008; 26: 743-751. http://dx.doi.org/10.1038/nbt0708-743

[38] Waldhauer I, Steinle A. Nk cells and cancer immunosurveillance. Oncogene 2008; 27: 5932-5943. http://dx.doi.org/10.1038/onc.2008.267

[39] Lion E, Willemen Y, Berneman ZN, Van Tendeloo VF, Smits EL. Natural killer cell immune escape in acute myeloid leukemia. Leukemia 2012; 26: 2019-2026. http://dx.doi.org/10.1038/leu.2012.87

[40] Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001; 2: 293-299. http://dx.doi.org/10.1038/86297

[41] Robinson BW, Morstyn G. Natural killer (nk)-resistant human lung cancer cells are lysed by recombinant interleukin-2-activated nk cells. Cell Immunol 1987; 106: 215-222. http://dx.doi.org/10.1016/0008-8749(87)90165-1

[42] Lotzova E, Savary CA, Herberman RB. Induction of nk cell activity against fresh human leukemia in culture with interleukin 2. J Immunol 1987; 138: 2718-2727.

[43] Lotzova E, Savary CA, Herberman RB: Inhibition of clonogenic growth of fresh leukemia cells by unstimulated and il-2 stimulated nk cells of normal donors. Leuk Res 1987; 11: 1059-1066. http://dx.doi.org/10.1016/0145-2126(87)90158-5

[44] Bubenik J, Perlmann P, Indrova M, Simova J, Jandlova T, Neuwirt J. Growth inhibition of an mc-induced mouse sarcoma by tcgf (il 2)-containing preparations. Preliminary report. Cancer Immunol Immunother 1983; 14: 205-206. http://dx.doi.org/10.1007/BF00205362

[45] Lafreniere R, Rosenberg SA. Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin 2. Cancer Res 1985; 45: 3735-3741.

[46] Rosenberg SA, Mule JJ, Spiess PJ, Reichert CM, Schwarz SL. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. J Exp Med 1985; 161: 1169-1188. http://dx.doi.org/10.1084/jem.161.5.1169

[47] Maekawa R, Matsumoto M, Kitagawa T, Harada M, Sato K. Effect of recombinant interleukin 2 (r-il2) on in vivo growth of murine myeloma x5563. Cancer Immunol Immunother 1986; 23: 25-30. http://dx.doi.org/10.1007/bf00205551

[48] Thompson JA, Peace DJ, Klarnet JP, Kern DE, Greenberg PD, Cheever MA. Eradication of disseminated murine leukemia by treatment with high-dose interleukin 2. J Immunol 1986; 137: 3675-3680.

[49] Vaage J. Local and systemic effects during interleukin-2 therapy of mouse mammary tumors. Cancer Res 1987; 47: 4296-4298.

[50] Rutten VP, Klein WR, De Jong WA, Misdorp W, Den Otter W, Steerenberg PA, De Jong WH, Ruitenberg EJ. Local interleukin-2 therapy in bovine ocular squamous cell carcinoma. A pilot study. Cancer Immunol Immunother 1989; 30: 165-169. http://dx.doi.org/10.1007/BF01669425

[51] Maas RA, Van Weering DH, Dullens HF, Den Otter W. Intratumoral low-dose interleukin-2 induces rejection of distant solid tumour. Cancer Immunol Immunother 1991; 33: 389-394. http://dx.doi.org/10.1007/BF01741599

[52] Den Otter W, Maas RA, Koten JW, Dullens HF, Bernsen M, Klein WR, Rutten VP, Steerenberg PA, Balemans L, Ruitenberg EJ, et al. Effective immunotherapy with local low doses of interleukin-2. In vivo 1991; 5: 561-565.

[53] Alici E, Konstantinidis KV, Sutlu T, Aints A, Gahrton G, Ljunggren HG, Dilber MS. Anti-myeloma activity of endogenous and adoptively transferred activated natural killer cells in experimental multiple myeloma model. Exp Hematol 2007; 35: 1839-1846. http://dx.doi.org/10.1016/j.exphem.2007.08.006

[54] Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 1987; 316: 889-897. http://dx.doi.org/10.1056/NEJM198704093161501

[55] Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 1985; 313: 1485-1492. http://dx.doi.org/10.1056/NEJM198512053132327

[56] Phillips JH, Gemlo BT, Myers WW, Rayner AA, Lanier LL. In vivo and in vitro activation of natural killer cells in advanced cancer patients undergoing combined recombinant interleukin-2 and lak cell therapy. J Clin Oncol 1987; 5: 1933-1941.

[57] Gratama JW, Bruin RJ, Lamers CH, Oosterom R, Braakman E, Stoter G, Bolhuis RL. Activation of the immune system of cancer patients by continuous i.V. Recombinant il-2 (ril-2) therapy is dependent on dose and schedule of ril-2. Clin Exp Immunol 1993; 92: 185-193. http://dx.doi.org/10.1111/j.1365-2249.1993.tb03378.x

[58] Margolin K. Cytokine therapy in cancer. Expert Opin Biol Ther 2008; 8: 1495-1505. http://dx.doi.org/10.1517/14712598.8.10.1495

[59] Belldegrun A, Tso CL, Kaboo R, Pang S, Pierce W, deKernion JB, Figlin R. Natural immune reactivity-associated therapeutic response in patients with metastatic renal cell carcinoma receiving tumor-infiltrating lymphocytes and interleukin-2-based therapy. J Immunother Emphasis Tumor Immunol 1996; 19: 149-161.

http://dx.doi.org/10.1097/00002371-199603000-00008

[60] Miller JS, Tessmer-Tuck J, Pierson BA, Weisdorf D, McGlave P, Blazar BR, Katsanis E, Verfaillie C, Lebkowski J, Radford J, Jr., Burns LJ: Low dose subcutaneous interleukin-2 after autologous transplantation generates sustained in vivo natural killer cell activity. Biol Blood Marrow Transplant 1997; 3: 34-44.

[61] Burns LJ, Weisdorf DJ, DeFor TE, Vesole DH, Repka TL, Blazar BR, Burger SR, Panoskaltsis-Mortari A, Keever-Taylor CA, Zhang MJ, Miller JS. Il-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: A phase i/ii trial. Bone Marrow Transplant 2003; 32: 177-186. http://dx.doi.org/10.1038/sj.bmt.1704086

[62] Kalwak K, Ussowicz M, Gorczynska E, Turkiewicz D, Toporski J, Dobaczewski G, Latos-Grazynska E, Ryczan R, Noworolska-Sauren D, Chybicka A. Immunologic effects of intermediate-dose il-2 i.V. After autologous hematopoietic cell transplantation in pediatric solid tumors. J Interferon Cytokine Res 2003; 23: 173-181. http://dx.doi.org/10.1089/107999003765027375

[63] Gottlieb DJ, Prentice HG, Mehta AB, Galazka AR, Heslop HE, Hoffbrand AV, Brenner MK. Malignant plasma cells are sensitive to lak cell lysis: Pre-clinical and clinical studies of interleukin 2 in the treatment of multiple myeloma. Br J Haematol 1990; 75: 499-505. http://dx.doi.org/10.1111/j.1365-2141.1990.tb07789.x

[64] Seidel MG, Freissmuth M, Pehamberger H, Micksche M. Stimulation of natural killer activity in peripheral blood lymphocytes of healthy donors and melanoma patients in vitro: Synergism between interleukin (il)-12 and il-15 or il-12 and il-2. Naunyn Schmiedebergs Arch Pharmacol 1998; 358: 382-389. http://dx.doi.org/10.1007/PL00005268

[65] DeBlaker-Hohe DF, Yamauchi A, Yu CR, Horvath-Arcidiacono JA, Bloom ET. Il-12 synergizes with il-2 to induce lymphokine-activated cytotoxicity and perforin and granzyme gene expression in fresh human nk cells. Cell Immunol 1995; 165: 33-43. http://dx.doi.org/10.1006/cimm.1995.1184

[66] Boiardi A, Silvani A, Ruffini PA, Rivoltini L, Parmiani G, Broggi G, Salmaggi A. Loco-regional immunotherapy with recombinant interleukin-2 and adherent lymphokine-activated killer cells (a-lak) in recurrent glioblastoma patients. Cancer Immunol Immunother 1994; 39: 193-197. http://dx.doi.org/10.1007/BF01533386

[67] Hayes RL, Koslow M, Hiesiger EM, Hymes KB, Hochster HS, Moore EJ, Pierz DM, Chen DK, Budzilovich GN, Ransohoff J. Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. Cancer 1995; 76: 840-852. http://dx.doi.org/10.1002/1097-0142(19950901)76:5<840::AID-CNCR2820760519>3.0.CO;2-R

[68] Keilholz U, Scheibenbogen C, Brado M, Georgi P, Maclachlan D, Brado B, Hunstein W. Regional adoptive immunotherapy with interleukin-2 and lymphokine-activated killer (lak) cells for liver metastases. Eur J Cancer 1994; 30A: 103-105. http://dx.doi.org/10.1016/S0959-8049(05)80028-0

[69] Smetak M, Kimmel B, Birkmann J, Schaefer-Eckart K, Einsele H, Wilhelm M, Kunzmann V. Clinical-scale single-step cd4(+) and cd8(+) cell depletion for donor innate lymphocyte infusion (dili). Bone Marrow Transplant 2008; 41: 643-650. http://dx.doi.org/10.1038/sj.bmt.1705942

[70] Leung W, Iyengar R, Leimig T, Holladay MS, Houston J, Handgretinger R. Phenotype and function of human natural killer cells purified by using a clinical-scale immunomagnetic method. Cancer Immunol Immunother 2005; 54: 389-394. http://dx.doi.org/10.1007/s00262-004-0609-6

[71] Iyengar R, Handgretinger R, Babarin-Dorner A, Leimig T, Otto M, Geiger TL, Holladay MS, Houston J, Leung W. Purification of human natural killer cells using a clinical-scale immunomagnetic method. Cytotherapy 2003; 5: 479-484. http://dx.doi.org/10.1080/14653240310003558

[72] Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kuhne T, Favre G, Gratwohl A. Purified donor nk-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia 2004; 18: 1835-1838. http://dx.doi.org/10.1038/sj.leu.2403524

[73] Lang P, Pfeiffer M, Handgretinger R, Schumm M, Demirdelen B, Stanojevic S, Klingebiel T, Kohl U, Kuci S, Niethammer D. Clinical scale isolation of t cell-depleted cd56+ donor lymphocytes in children. Bone Marrow Transplant 2002; 29: 497-502. http://dx.doi.org/10.1038/sj.bmt.1703406

[74] McKenna DH, Jr., Sumstad D, Bostrom N, Kadidlo DM, Fautsch S, McNearney S, Dewaard R, McGlave PB, Weisdorf DJ, Wagner JE, McCullough J, Miller JS. Good manufacturing practices production of natural killer cells for immunotherapy: A six-year single-institution experience. Transfusion 2007; 47: 520-528. http://dx.doi.org/10.1111/j.1537-2995.2006.01145.x

[75] Koehl U, Esser R, Zimmermann S, Tonn T, Kotchetkov R, Bartling T, Sorensen J, Gruttner HP, Bader P, Seifried E, Martin H, Lang P, Passweg JR, Klingebiel T, Schwabe D. Ex vivo expansion of highly purified nk cells for immunotherapy after haploidentical stem cell transplantation in children. Klin Padiatr 2005; 217: 345-350. http://dx.doi.org/10.1055/s-2005-872520

[76] Torelli GF, Guarini A, Palmieri G, Breccia M, Vitale A, Santoni A, Foa R: Expansion of cytotoxic effectors with lytic activity against autologous blasts from acute myeloid leukaemia patients in complete haematological remission. Br J Haematol 2002; 116: 299-307. http://dx.doi.org/10.1046/j.1365-2141.2002.03277.x

[77] Escudier B, Farace F, Angevin E, Charpentier F, Nitenberg G, Triebel F, Hercend T. Immunotherapy with interleukin-2 (il2) and lymphokine-activated natural killer cells: Improvement of clinical responses in metastatic renal cell carcinoma patients previously treated with il2. Eur J Cancer 1994; 30A: 1078-1083. http://dx.doi.org/10.1016/0959-8049(94)90460-X

[78] Hercend T, Farace F, Baume D, Charpentier F, Droz JP, Triebel F, Escudier B. Immunotherapy with lymphokine-activated natural killer cells and recombinant interleukin-2: A feasibility trial in metastatic renal cell carcinoma. J Biol Response Mod 1990; 9: 546-555.

[79] Miller JS, Klingsporn S, Lund J, Perry EH, Verfaillie C, McGlave P. Large scale ex vivo expansion and activation of human natural killer cells for autologous therapy. Bone Marrow Transplant 1994; 14: 555-562.

[80] Lister J, Rybka WB, Donnenberg AD, deMagalhaes-Silverman M, Pincus SM, Bloom EJ, Elder EM, Ball ED, Whiteside TL. Autologous peripheral blood stem cell transplantation and adoptive immunotherapy with activated natural killer cells in the immediate posttransplant period. Clin Cancer Res 1995; 1: 607-614.

[81] Pierson BA, Europa AF, Hu WS, Miller JS. Production of human natural killer cells for adoptive immunotherapy using a computer-controlled stirred-tank bioreactor. J Hematother 1996; 5: 475-483. http://dx.doi.org/10.1089/scd.1.1996.5.475

[82] Carlens S, Gilljam M, Chambers BJ, Aschan J, Guven H, Ljunggren HG, Christensson B, Dilber MS. A new method for in vitro expansion of cytotoxic human cd3-cd56+ natural killer cells. Hum Immunol 2001; 62: 1092-1098. http://dx.doi.org/10.1016/S0198-8859(01)00313-5

[83] Luhm J, Brand JM, Koritke P, Hoppner M, Kirchner H, Frohn

C. Large-scale generation of natural killer lymphocytes for clinical application. J Hematother Stem Cell Res 2002; 11: 651-657.
http://dx.doi.org/10.1089/15258160260194794

[84] Guven H, Gilljam M, Chambers BJ, Ljunggren HG, Christensson B, Kimby E, Dilber MS. Expansion of natural killer (nk) and natural killer-like t (nkt)-cell populations derived from patients with b-chronic lymphocytic leukemia (b-cll): A potential source for cellular immunotherapy. Leukemia 2003; 17: 1973-1980.
http://dx.doi.org/10.1038/sj.leu.2403083

[85] Klingemann HG, Martinson J. Ex vivo expansion of natural killer cells for clinical applications. Cytotherapy 2004; 6: 15-22.
http://dx.doi.org/10.1080/14653240310004548

[86] Ishikawa E, Tsuboi K, Saijo K, Harada H, Takano S, Nose T, Ohno T. Autologous natural killer cell therapy for human recurrent malignant glioma. Anticancer Res 2004; 24: 1861-1871.

[87] Berg M, Lundqvist A, McCoy P, Jr., Samsel L, Fan Y, Tawab A, Childs R. Clinical-grade ex vivo-expanded human natural killer cells up-regulate activating receptors and death receptor ligands and have enhanced cytolytic activity against tumor cells. Cytotherapy 2009; 11: 341-355.
http://dx.doi.org/10.1080/14653240902807034

[88] Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, Eldridge P, Leung WH, Campana D. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res 2009; 69: 4010-4017.
http://dx.doi.org/10.1158/0008-5472.CAN-08-3712

[89] Torelli GF, Guarini A, Maggio R, Alfieri C, Vitale A, Foa R. Expansion of natural killer cells with lytic activity against autologous blasts from adult and pediatric acute lymphoid leukemia patients in complete hematologic remission. Haematologica 2005; 90: 785-792.

[90] Basse PH, Whiteside TL, Herberman RB. Cancer immunotherapy with interleukin-2-activated natural killer cells. Mol Biotechnol 2002; 21: 161-170.
http://dx.doi.org/10.1385/MB:21:2:161

[91] Siegler U, Kalberer CP, Nowbakht P, Sendelov S, Meyer-Monard S, Wodnar-Filipowicz A. Activated natural killer cells from patients with acute myeloid leukemia are cytotoxic against autologous leukemic blasts in nod/scid mice. Leukemia 2005; 19: 2215-2222.
http://dx.doi.org/10.1038/sj.leu.2403985

[92] deMagalhaes-Silverman M, Donnenberg A, Lembersky B, Elder E, Lister J, Rybka W, Whiteside T, Ball E. Posttransplant adoptive immunotherapy with activated natural killer cells in patients with metastatic breast cancer. J Immunother 2000; 23: 154-160.
http://dx.doi.org/10.1097/00002371-200001000-00018

[93] Krause SW, Gastpar R, Andreesen R, Gross C, Ullrich H, Thonigs G, Pfister K, Multhoff G. Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: A clinical phase i trial. Clin Cancer Res 2004; 10: 3699-3707.
http://dx.doi.org/10.1158/1078-0432.CCR-03-0683

[94] Milani V, Stangl S, Issels R, Gehrmann M, Wagner B, Hube K, Mayr D, Hiddemann W, Molls M, Multhoff G. Anti-tumor activity of patient-derived nk cells after cell-based immunotherapy--a case report. J Transl Med 2009; 7: 50.
http://dx.doi.org/10.1186/1479-5876-7-50

[95] Ruggeri L, Mancusi A, Capanni M, Martelli MF, Velardi A. Exploitation of alloreactive nk cells in adoptive immunotherapy of cancer. Curr Opin Immunol 2005; 17: 211-217.
http://dx.doi.org/10.1016/j.coi.2005.01.007

[96] Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002; 295: 2097-2100.
http://dx.doi.org/10.1126/science.1068440

[97] Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, McKenna D, Le C, Defor TE, Burns LJ, Orchard PJ, Blazar BR, Wagner JE, Slungaard A, Weisdorf DJ, Okazaki IJ, McGlave PB: Successful adoptive transfer and in vivo expansion of human haploidentical nk cells in patients with cancer. Blood 2005; 105: 3051-3057.
http://dx.doi.org/10.1182/blood-2004-07-2974

[98] Ruggeri L, Mancusi A, Burchielli E, Aversa F, Martelli MF, Velardi A. Natural killer cell alloreactivity and haplo-identical hematopoietic transplantation. Cytotherapy 2006; 8: 554-558.
http://dx.doi.org/10.1080/14653240601078721

[99] Igarashi T, Wynberg J, Srinivasan R, Becknell B, McCoy JP, Jr., Takahashi Y, Suffredini DA, Linehan WM, Caligiuri MA, Childs RW. Enhanced cytotoxicity of allogeneic nk cells with killer immunoglobulin-like receptor ligand incompatibility against melanoma and renal cell carcinoma cells. Blood 2004; 104: 170-177.
http://dx.doi.org/10.1182/blood-2003-12-4438

[100] Koehl U, Sorensen J, Esser R, Zimmermann S, Gruttner HP, Tonn T, Seidl C, Seifried E, Klingebiel T, Schwabe D. Il-2 activated nk cell immunotherapy of three children after haploidentical stem cell transplantation. Blood Cells Mol Dis 2004; 33: 261-266.
http://dx.doi.org/10.1016/j.bcmd.2004.08.013

[101] Shi J, Tricot G, Szmania S, Rosen N, Garg TK, Malaviarachchi PA, Moreno A, Dupont B, Hsu KC, Baxter-Lowe LA, Cottler-Fox M, Shaughnessy JD, Jr., Barlogie B, van Rhee F. Infusion of haplo-identical killer immunoglobulin-like receptor ligand mismatched nk cells for relapsed myeloma in the setting of autologous stem cell transplantation. Br J Haematol 2008; 143: 641-653.
http://dx.doi.org/10.1111/j.1365-2141.2008.07340.x

[102] Barkholt L, Alici E, Conrad R, Sutlu T, Gilljam M, Stellan B, Christensson B, Guven H, Bjorkstrom NK, Soderdahl G, Cederlund K, Kimby E, Aschan J, Ringden O, Ljunggren HG, Dilber MS. Safety analysis of ex vivo-expanded nk and nk-like t cells administered to cancer patients: A phase i clinical study. Immunotherapy 2009; 1: 753-764.
http://dx.doi.org/10.2217/imt.09.47

[103] Huenecke S, Zimmermann SY, Kloess S, Esser R, Brinkmann A, Tramsen L, Koenig M, Erben S, Seidl C, Tonn T, Eggert A, Schramm A, Bader P, Klingebiel T, Lehrnbecher T, Passweg JR, Soerensen J, Schwabe D, Koehl U. Il-2-driven regulation of nk cell receptors with regard to the distribution of cd16+ and cd16- subpopulations and in vivo influence after haploidentical nk cell infusion. J Immunother 2010; 33: 200-210.
http://dx.doi.org/10.1097/CJI.0b013e3181bb46f7

[104] Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, Pui CH, Leung W. Nkaml: A pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. J Clin Oncol 2010; 28: 955-959.
http://dx.doi.org/10.1200/JCO.2009.24.4590

[105] Rizzieri DA, Storms R, Chen DF, Long G, Yang Y, Nikcevich DA, Gasparetto C, Horwitz M, Chute J, Sullivan K, Hennig T, Misra D, Apple C, Baker M, Morris A, Green PG, Hasselblad V, Chao NJ. Natural killer cell-enriched donor lymphocyte infusions from a 3-6/6 hla matched family member following nonmyeloablative allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2010; 16: 1107-1114.
http://dx.doi.org/10.1016/j.bbmt.2010.02.018

[106] Brehm C, Huenecke S, Quaiser A, Esser R, Bremm M, Kloess S, Soerensen J, Kreyenberg H, Seidl C, Becker PS, Muhl H, Klingebiel T, Bader P, Passweg JR, Schwabe D, Koehl U. Il-2 stimulated but not unstimulated nk cells induce selective disappearance of peripheral blood cells: Concomitant results to a phase i/ii study. PLoS One 2011; 6:

e27351.
http://dx.doi.org/10.1371/journal.pone.0027351

[107] Nguyen S, Beziat V, Norol F, Uzunov M, Trebeden-Negre H, Azar N, Boudifa A, Bories D, Debre P, Vernant JP, Vieillard V, Dhedin N. Infusion of allogeneic natural killer cells in a patient with acute myeloid leukemia in relapse after haploidentical hematopoietic stem cell transplantation. Transfusion 2011; 51: 1769-1778.
http://dx.doi.org/10.1111/j.1537-2995.2010.03058.x

[108] Curti A, Ruggeri L, D'Addio A, Bontadini A, Dan E, Motta MR, Trabanelli S, Giudice V, Urbani E, Martinelli G, Paolini S, Fruet F, Isidori A, Parisi S, Bandini G, Baccarani M, Velardi A, Lemoli RM. Successful transfer of alloreactive haploidentical kir ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. Blood 2011; 118: 3273-3279.
http://dx.doi.org/10.1182/blood-2011-01-329508

[109] Maki G, Klingemann HG, Martinson JA, Tam YK. Factors regulating the cytotoxic activity of the human natural killer cell line, nk-92. J Hematother Stem Cell Res 2001; 10: 369-383.
http://dx.doi.org/10.1089/152581601750288975

[110] Yan Y, Steinherz P, Klingemann HG, Dennig D, Childs BH, McGuirk J, O'Reilly RJ. Antileukemia activity of a natural killer cell line against human leukemias. Clin Cancer Res 1998; 4: 2859-2868.

[111] Tam YK, Miyagawa B, Ho VC, Klingemann HG. Immunotherapy of malignant melanoma in a scid mouse model using the highly cytotoxic natural killer cell line nk-92. J Hematother 1999; 8: 281-290.
http://dx.doi.org/10.1089/106161299320316

[112] Tonn T, Becker S, Esser R, Schwabe D, Seifried E. Cellular immunotherapy of malignancies using the clonal natural killer cell line nk-92. J Hematother Stem Cell Res 2001; 10: 535-544.
http://dx.doi.org/10.1089/152581601752509145

[113] 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.
http://dx.doi.org/10.1080/14653240802301872

[114] Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, Shearer G, Chang L, Chiang Y, Tolstoshev P, Greenblatt JJ, Rosenberg SA, Klein H, Berger M, Mullen CA, Ramsey WJ, Muul L, Morgan RA, Anderson WF. T lymphocyte-directed gene therapy for ada- scid: Initial trial results after 4 years. Science 1995; 270: 475-480.
http://dx.doi.org/10.1126/science.270.5235.475

[115] Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 1990; 323: 570-578.
http://dx.doi.org/10.1056/NEJM199008303230904

[116] Rogers S, Pfuderer P. Use of viruses as carriers of added genetic information. Nature 1968; 219: 749-751.
http://dx.doi.org/10.1038/219749a0

[117] Pathak A, Patnaik S, Gupta KC: Recent trends in non-viral vector-mediated gene delivery. Biotechnol J 2009; 4: 1559-1572.
http://dx.doi.org/10.1002/biot.200900161

[118] Li SD, Huang L. Gene therapy progress and prospects: Non-viral gene therapy by systemic delivery. Gene Ther 2006; 13: 1313-1319.
http://dx.doi.org/10.1038/sj.gt.3302838

[119] Trobridge G, Josephson N, Vassilopoulos G, Mac J, Russell DW. Improved foamy virus vectors with minimal viral sequences. Mol Ther 2002; 6: 321-328.
http://dx.doi.org/10.1006/mthe.2002.0672

[120] Heinkelein M, Dressler M, Jarmy G, Rammling M, Imrich H, Thurow J, Lindemann D, Rethwilm A. Improved primate foamy virus vectors and packaging constructs. J Virol 2002; 76: 3774-3783.
http://dx.doi.org/10.1128/JVI.76.8.3774-3783.2002

[121] Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, Vogel AN, Kalos M, Riley JL, Deeks SG, Mitsuyasu RT, Bernstein WB, Aronson NE, Levine BL, Bushman FD, June CH. Decade-long safety and function of retroviral-modified chimeric antigen receptor t cells. Sci Transl Med 2012; 4: 132ra153.
http://dx.doi.org/10.1126/scitranslmed.3003761

[122] Biffi A, Bartolomae CC, Cesana D, Cartier N, Aubourg P, Ranzani M, Cesani M, Benedicenti F, Plati T, Rubagotti E, Merella S, Capotondo A, Sgualdino J, Zanetti G, von Kalle C, Schmidt M, Naldini L, Montini E. Lentiviral vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection. Blood 2011; 117: 5332-5339.
http://dx.doi.org/10.1182/blood-2010-09-306761

[123] Grund EM, Muise-Helmericks RC. Cost efficient and effective gene transfer into the human natural killer cell line, nk92. J Immunol Methods 2005; 296: 31-36.
http://dx.doi.org/10.1016/j.jim.2004.10.008

[124] Nagashima S, Mailliard R, Kashii Y, Reichert TE, Herberman RB, Robbins P, Whiteside TL. Stable transduction of the interleukin-2 gene into human natural killer cell lines and their phenotypic and functional characterization in vitro and in vivo. Blood 1998; 91: 3850-3861.

[125] Becknell B, Trotta R, Yu J, Ding W, Mao HC, Hughes T, Marburger T, Caligiuri MA. Efficient infection of human natural killer cells with an ebv/retroviral hybrid vector. J Immunol Methods 2005; 296: 115-123.
http://dx.doi.org/10.1016/j.jim.2004.11.012

[126] Guven H, Konstantinidis KV, Alici E, Aints A, Abedi-Valugerdi M, Christensson B, Ljunggren HG, Dilber MS. Efficient gene transfer into primary human natural killer cells by retroviral transduction. Exp Hematol 2005; 33: 1320-1328.
http://dx.doi.org/10.1016/j.exphem.2005.07.006

[127] Alici E, Sutlu T, Sirac Dilber M. Retroviral gene transfer into primary human natural killer cells. Methods Mol Biol 2009; 506: 127-137.
http://dx.doi.org/10.1007/978-1-59745-409-4_10

[128] Uherek C, Tonn T, Uherek B, Becker S, Schnierle B, Klingemann HG, Wels W. Retargeting of natural killer-cell cytolytic activity to erbb2-expressing cancer cells results in efficient and selective tumor cell destruction. Blood 2002; 100: 1265-1273.

[129] Konstantinidis KV, Alici E, Aints A, Christensson B, Ljunggren HG, Dilber MS. Targeting il-2 to the endoplasmic reticulum confines autocrine growth stimulation to nk-92 cells. Exp Hematol 2005; 33: 159-164.
http://dx.doi.org/10.1016/j.exphem.2004.11.003

[130] Tran J, Kung SK. Lentiviral vectors mediate stable and efficient gene delivery into primary murine natural killer cells. Mol Ther 2007; 15: 1331-1339.
http://dx.doi.org/10.1038/sj.mt.6300184

[131] Baeriswyl V, Wodnar-Filipowicz A, Kalberer CP. The effect of silencing nkg2d through rna interference on receptor functions in interleukin-2-activated human natural killer cells. Haematologica 2006; 91: 1538-1541.

[132] Figueiredo C, Seltsam A, Blasczyk R. Permanent silencing of nkg2a expression for cell-based therapeutics. J Mol Med (Berl) 2009; 87: 199-210.
http://dx.doi.org/10.1007/s00109-008-0417-0

[133] Karimi M, Cao TM, Baker JA, Verneris MR, Soares L, Negrin RS. Silencing human nkg2d, dap10, and dap12 reduces cytotoxicity of activated cd8+ t cells and nk cells. J Immunol 2005; 175: 7819-7828.
http://dx.doi.org/10.4049/jimmunol.175.12.7819

[134] Kung SK. Introduction of shrnas into primary nk cells with lentivirus. Methods Mol Biol 2010; 612: 233-247. http://dx.doi.org/10.1007/978-1-60761-362-6_16

[135] Micucci F, Zingoni A, Piccoli M, Frati L, Santoni A, Galandrini R: High-efficient lentiviral vector-mediated gene transfer into primary human nk cells. Exp Hematol 2006; 34: 1344-1352. http://dx.doi.org/10.1016/j.exphem.2006.06.001

[136] Tran J, Mahmood S, Carlyle JR, Kung SK. Altering the specificity of nk: Target cell interactions by genetic manipulation of nk receptor expression on primary mouse nk cells. Vaccine 2010; 28: 3767-3772. http://dx.doi.org/10.1016/j.vaccine.2010.03.013

[137] Schoenberg K, Trompeter HI, Uhrberg M. Delivery of DNA into natural killer cells for immunotherapy. Methods Mol Biol 2008; 423: 165-172. http://dx.doi.org/10.1007/978-1-59745-194-9_11

[138] Boissel L, Betancur M, Wels WS, Tuncer H, Klingemann H. Transfection with mrna for cd19 specific chimeric antigen receptor restores nk cell mediated killing of cll cells. Leuk Res 2009; 33: 1255-1259. http://dx.doi.org/10.1016/j.leukres.2008.11.024

[139] Trompeter HI, Weinhold S, Thiel C, Wernet P, Uhrberg M. Rapid and highly efficient gene transfer into natural killer cells by nucleofection. J Immunol Methods 2003; 274: 245-256. http://dx.doi.org/10.1016/S0022-1759(02)00431-3

[140] Liu JH, Wei S, Blanchard DK, Djeu JY. Restoration of lytic function in a human natural killer cell line by gene transfection. Cell Immunol 1994; 156: 24-35. http://dx.doi.org/10.1006/cimm.1994.1150

[141] Zhang J, Sun R, Wei H, Tian Z. Characterization of interleukin-15 gene-modified human natural killer cells: Implications for adoptive cellular immunotherapy. Haematologica 2004; 89: 338-347.

[142] Jiang W, Zhang J, Tian Z. Functional characterization of interleukin-15 gene transduction into the human natural killer cell line nkl. Cytotherapy 2008; 10: 265-274. http://dx.doi.org/10.1080/14653240801965156

[143] Goding SR, Yang Q, Knudsen KB, Potter DM, Basse PH. Cytokine gene therapy using adenovirally transduced, tumor-seeking activated natural killer cells. Hum Gene Ther 2007; 18: 701-711. http://dx.doi.org/10.1089/hum.2007.052

[144] Roychowdhury S, Blaser BW, Freud AG, Katz K, Bhatt D, Ferketich AK, Bergdall V, Kusewitt D, Baiocchi RA, Caligiuri MA. Il-15 but not il-2 rapidly induces lethal xenogeneic graft-versus-host disease. Blood 2005; 106: 2433-2435. http://dx.doi.org/10.1182/blood-2005-04-1597

[145] Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on il-2. Nat Rev Immunol 2004; 4: 665-674. http://dx.doi.org/10.1038/nri1435

[146] Maas RA, Dullens HF, Den Otter W. Interleukin-2 in cancer treatment: Disappointing or (still) promising? A review. Cancer Immunol Immunother 1993; 36: 141-148. http://dx.doi.org/10.1007/BF01741084

[147] Ardizzoni A, Bonavia M, Viale M, Baldini E, Mereu C, Verna A, Ferrini S, Cinquegrana A, Molinari S, Mariani GL, et al. Biologic and clinical effects of continuous infusion interleukin-2 in patients with non-small cell lung cancer. Cancer 1994; 73: 1353-1360. http://dx.doi.org/10.1002/1097-0142(19940301)73:5<1353::AID-CNCR2820730508>3.0.CO;2-H

[148] Tam YK, Maki G, Miyagawa B, Hennemann B, Tonn T, Klingemann HG. Characterization of genetically altered, interleukin 2-independent natural killer cell lines suitable for adoptive cellular immunotherapy. Hum Gene Ther 1999; 10: 1359-1373. http://dx.doi.org/10.1089/10430349950018030

[149] Miller JS, Tessmer-Tuck J, Blake N, Lund J, Scott A, Blazar BR, Orchard PJ. Endogenous il-2 production by natural killer cells maintains cytotoxic and proliferative capacity following retroviral-mediated gene transfer. Exp Hematol 1997; 25: 1140-1148.

[150] Schirrmann T, Pecher G. Human natural killer cell line modified with a chimeric immunoglobulin t-cell receptor gene leads to tumor growth inhibition in vivo. Cancer Gene Ther 2002; 9: 390-398. http://dx.doi.org/10.1038/sj.cgt.7700453

[151] Schirrmann T, Pecher G. Specific targeting of cd33(+) leukemia cells by a natural killer cell line modified with a chimeric receptor. Leuk Res 2005; 29: 301-306. http://dx.doi.org/10.1016/j.leukres.2004.07.005

[152] Demirtzoglou FJ, Papadopoulos S, Zografos G. Cytolytic and cytotoxic activity of a human natural killer cell line genetically modified to specifically recognize her-2/neu overexpressing tumor cells. Immunopharmacol Immunotoxicol 2006; 28: 571-590. http://dx.doi.org/10.1080/08923970601066971

[153] Meier R, Piert M, Piontek G, Rudelius M, Oostendorp RA, Senekowitsch-Schmidtke R, Henning TD, Wels WS, Uherek C, Rummeny EJ, Daldrup-Link HE. Tracking of [18f]fdg-labeled natural killer cells to her2/neu-positive tumors. Nucl Med Biol 2008; 35: 579-588. http://dx.doi.org/10.1016/j.nucmedbio.2008.02.006

[154] Pegram HJ, Jackson JT, Smyth MJ, Kershaw MH, Darcy PK. Adoptive transfer of gene-modified primary nk cells can specifically inhibit tumor progression in vivo. J Immunol 2008; 181: 3449-3455. http://dx.doi.org/10.4049/jimmunol.181.5.3449

[155] Kruschinski A, Moosmann A, Poschke I, Norell H, Chmielewski M, Seliger B, Kiessling R, Blankenstein T, Abken H, Charo J. Engineering antigen-specific primary human nk cells against her-2 positive carcinomas. Proc Natl Acad Sci U S A 2008; 105: 17481-17486. http://dx.doi.org/10.1073/pnas.0804788105

[156] Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood 2005; 106: 376-383. http://dx.doi.org/10.1182/blood-2004-12-4797

[157] Jiang H, Zhang W, Shang P, Zhang H, Fu W, Ye F, Zeng T, Huang H, Zhang X, Sun W, Man-Yuen Sze D, Yi Q, Hou J. Transfection of chimeric anti-cd138 gene enhances natural killer cell activation and killing of multiple myeloma cells. Mol Oncol 2013; 8: 297-310. http://dx.doi.org/10.1016/j.molonc.2013.12.001

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