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Genetically modified NK cells with enhanced reactivity – potential in treating therapy-resistant cancers

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ABSTRACT

Treatment-resistant cancers are a major challenge in clinical oncology, which requires the development of innovative treatment strategies. NK cells are a unique population of lymphoid cells with an innate ability to identify and eliminate virus-infected cells and cancer cells. The aim of this paper is to discuss the natural anticancer properties of NK cells, as well as the methods of their acquisition and modification aimed at enhancing anticancer activity. In particular, the mechanisms of cytotoxicity and the ability of NK cells to modulate the anti-tumour immune response were demonstrated. Problems related to the use of unmodified NK cells in cancer therapy are discussed. Methods of obtaining and modifying the anticancer activity of NK cells, including genetic modifications and metabolic reprogramming with the use of cytokines, were presented. Techniques such as chimeric antigen receptor (CAR) engineering and receptor-based modifications are discussed in detail. The possibilities of enhancing antitumor reactivity and NK cell persistence through costimulatory signalling, checkpoint inhibition and the use of cytokines are discussed. Preclinical and clinical studies demonstrating the efficacy of genetically engineered natural killer cells against a variety of treatment-resistant cancers have been reviewed with promising results. The development of allogeneic ready-to-use natural killer cell products has the potential to provide patients with immediate treatment options, bypassing the limitations of autologous cell therapies. Challenges and issues related to genetically modified NK cell therapies are discussed. Manufacturing scalability, potential side effects, and long-term safety concerns are important factors to consider during clinical translation. Ongoing research and clinical trials are discussed, highlighting the need for further validation and optimization of these therapies.

INTRODUCTION

Natural Killer cells play a crucial role within the innate immune system, serving as a frontline defence against both viral infections and malignant cells. Their unique ability to detect and eliminate aberrant cells without prior sensitization makes them highly attractive candidates for immunotherapy. However, despite their innate cytotoxic capabilities, NK (Natural Killer) cells encounter significant hurdles when targeting and eradicating therapy-resistant cancers. In recent years, the field of genetic engineering has emerged as a promising avenue to enhance NK cells reactivity and empower them to overcome these formidable barriers presented by resistant malignancies.

The aim of this work is to explore the intricacies of NK cell biology, uncovering their functions and mechanisms of action, to provide a detailed analysis of the multifaceted strategies used by cancer cells to develop resistance to therapies, shedding light on the intricate mechanisms at play, as well as to discuss the natural anticancer properties of NK cells. Additionally, we will explore the exciting field of genetic modifications that can significantly improve NK cell function and effectiveness against treatment-resistant cancers. In an era characterized by innovative breakthroughs in genetic engineering that are changing the landscape of cancer immunotherapy, this review aims to provide a comprehensive overview of the current state of NK cell-based therapies and the promising avenues they open in the ongoing battle against therapy-resistant cancers. By leveraging the powerful possibilities of genetic modification, we have the potential to revolutionize this field and instil newfound hope in patients facing enormous therapeutic challenges.

The work is of a review nature. The basis for assessing the current state of knowledge was a systematic review of the literature based on the following databases: PubMed, Science Direct and Wiley Online Library and other sources and materials related to the topic of the work in a direct or indirect way. In order to isolate all publications related to the topic of the work, selected sources were selected based on the use of keywords and keywords such as: "NK cells cancer", "NK cells cancer immunotherapy", "NK cells cancer therapy", "CAR–NK cells cancer", "NK cells cancer treatment". Based on this methodology, experimental and clinical studies were identified, the results of which were published in the years 1990-2023, in order to review and synthesize conclusions.

TREATMENR-RESISTANT TUMORS

Cancer treatment resistance remains a significant challenge in the field of oncology. Although the early successes of chemotherapy were promising, the emergence of drug resistance in cancer cells quickly overshadowed these achievements, leading to the adoption of combination chemotherapy (Vasan, 2019).

This approach has proven effective for some types of cancer, such as breast cancer and testicular cancer (Bonadonna, 1976; Bosl, 1986). However, it should be noted that the effectiveness of chemotherapy is limited in almost 90% of cases due to the development of drug resistance in cancer cells. This not only weakens the effectiveness of treatment, but also increases the aggressiveness of the cancer and facilitates its metastasis to other parts of the body (Vasan, 2019; Emran, 2022). In response to these challenges, new therapeutic strategies have emerged in oncology targeting fundamental cellular characteristics associated with carcinogenesis. These strategies include targeted therapies, which encompass a range of approaches including the use of tyrosine kinase inhibitors, nuclear receptor antagonists, and agents specifically designed to target and disrupt molecular pathways that promote cancer growth. Importantly, targeted therapies have shown significant effectiveness in certain types of cancer (Vasan, 2019). Another breakthrough in cancer treatment is immunotherapy. Monoclonal antibodies, such as those directed against immune checkpoints such as CTLA-4 (cytotoxic T cell antigen 4) and PD-1/PD-L1 (programmed death receptor 1/programmed death ligand 1), have revolutionized cancer therapy by blocking negative regulators or checkpoints within the immune system (Leach, 1996; Iwai, 2002). In some cases, these immunotherapies have achieved remarkable anti-tumor responses, giving patients new hope (Vasan, 2019). Additionally, new therapies such as dendritic cell therapy, CAR-T cell therapy, CAR-NK therapy, and many others have begun to achieve early successes (Neelapu, 2017; Liau, 2016; Liu, 2020). Despite these significant advances, it must be acknowledged that resistance remains a persistent challenge in the field of cancer therapy. Scientists and clinicians continue to explore innovative approaches to overcoming mechanisms of resistance and increasing the effectiveness of therapies, aiming to improve outcomes for people fighting cancer.

LIMITATIONS OF CONVENTIONAL THERAPIES AND MECHANISMS OF RESISTANCE

MDR (Multidrug Resistance) in cancer cells is a multifaceted phenomenon driven by a constellation of complex factors, each of which contributes to the resistance of these cells to chemotherapy. One of the hallmark aspects of MDR is the increased efflux of therapeutic agents through ABC transporters (ATP-binding cassette transporters), for example P-gp (P-glycoprotein) and BCRP (breast cancer resistance protein). These integral proteins exert significant control over the distribution, absorption, and elimination of a variety of chemicals, thereby protecting cells from the cytotoxic consequences of elevated intracellular drug concentrations. However, their presence may also interfere with drug delivery, leading to decreased bioavailability and decreased intracellular drug concentration (Mesci, 2019). Moreover, genetic mutations, widely considered a key cause of chemotherapy treatment failure, play a key role in this complex landscape. Mutations in key genes such as MYC, RAS and TP53 significantly influence the development of drug resistance (Duesberg, 2000). Furthermore, changes in the regulatory networks governed by miRNAs (microRNAs) and lncRNAs (long non-coding RNAs) further highlight the genetic complexity of MDR (Chen, 2016; Chen, 2019). Moreover, during carcinogenesis, the epigenome undergoes a series of profound changes, including global loss of DNA methylation, local hypermethylation, and pervasive changes in histone modification patterns. These epigenetic changes likely contribute significantly to the emergence of multidrug resistance (Kanwal, 2012). The tumor microenvironment also plays a key role in inducing drug resistance. In this complex environment, the antitumor functions of the immune system are disrupted, drug absorption is impaired, and cancer cell proliferation is promoted. Conditions such as hypoxia and autophagy in the tumor microenvironment further enhance drug resistance, significantly reducing the effectiveness of therapeutic interventions against cancer cells (Liang, 1996; van Vuuren, 2019). Moreover, additional factors, including selective therapeutic pressure that can lead to increased genomic instability and changes in tumor growth kinetics, as well as physical barriers, further contribute to the complex phenomenon of drug resistance (Sharma, 2019). This complex interplay of diverse factors highlights the powerful and challenging nature of drug resistance in the context of cancer.

BIOLOGY AND FUNCTIONS OF NK CELLS

NK cells are a population of cells characterized by a larger size compared to T and B lymphocytes and the presence of unique cytoplasmic granules (Chu, 2022). They originate from CD34+ hematopoietic stem cells located in the bone marrow and are widely distributed in various tissues, including the bloodstream, liver, and spleen (Yu, 2013). NK cells are part of the innate immune system and play a key role in monitoring and eliminating virus-transformed or infected cells; their effect is independent of previous immunization (Kwaśnik, 2020). Unlike T cells, they lack the TCR (T-cell receptor) and the associated CD3 complex responsible for signal transduction. NK cells are identified by the presence of the CD56

surface antigen and the lack of CD3 expression. They also express receptors such as CD16 and CD57 (Dalle, 2005). NK cells can be divided into two main subpopulations based on CD56 surface expression, each with distinct functions. CD56+ CD16-, characterized by high levels of CD56 and lack of CD16 expression, produce primarily cytokines. Conversely, CD56+CD16+ cells express moderate CD56 but high CD16 expression, resulting in greater cytotoxicity. Approximately 90% of NK cells in peripheral blood belong to the CD56+ CD16+ group (Kwasnik, 2020; Yu, 2013). NK cells have two primary functions: cytotoxicity and immune regulation. In the absence of prior activation, NK cells can identify and eliminate abnormal cells by releasing perforin and granzymes (Vivier, 2008). Stimulation of NK cells by KARs (killer activating receptors) triggers the expression and release of death ligands such as TNFα (tumour necrosis factor alpha), TRAIL (TNF-related apoptosis-inducing ligand), and FasL (Fas ligand), initiating the apoptosis pathway (Martínez- Lostao, 2015). Additionally, through their CD16 receptor, they can recognize cells coated with antibodies, initiating antibody-dependent cellular cytotoxicity (ADCC) and cytokine production (Kwaśnik, 2016). In their role as regulatory cells, NK cells produce a variety of cytokines and chemokines, including IL-10 (interleukin 10), IFN-γ (interferon-gamma), CCL3 (chemokine (CC motif) ligand 3), CCL4 (chemokine (CC motif)) ligand 4), CCL5 (chemokine (CC motif) ligand 3) and lymphotactin (Vivier, 2008). One study suggests that human NK cells may have memory-like properties. In support of this idea, studies have shown that when NK cells are initially activated with IL-12 (interleukin 12), IL-15 (interleukin 15), and IL-18 (interleukin 18), followed by a rest period of 1–3 weeks, they can induce a strong response characterized by increased production of IFN-γ upon subsequent exposure to cytokines or leukemic cells (Romme, 2012). This dual role of NK cells as both cytotoxic effectors and immune regulators highlights their importance in the body's defence mechanisms against cancer and infection.

THE ROLE OF NK CELLS IN THE ANTI-CANCER IMMUNE RESPONSE – MODIFYING IMPACT OF THE TUMOUR MICROENVIRONMENT

Anticancer mechanisms mediated by NK cells can be divided into direct and indirect actions. Direct mechanisms include several strategies used by NK cells to directly target cancer cells. First, they induce apoptosis in malignant cells by releasing perforin and granzyme upon direct contact. This approach is mainly used by cells with the CD56+CD16+ phenotype, effectively eliminating target cells, including those with reduced MHC (Major Histocompatibility Complex) class I expression (Chu, 2022). Second, NK cells with a CD56+CD16- phenotype initiate apoptosis without direct contact by binding membrane TNF family molecules to tumour cell ligands (Chu, 2022; Myers, 2021). Additionally, NK cells facilitate antibody-dependent cellular cytotoxicity (ADCC) (Hatjiharissi, 2007). Furthermore, NK cells produce cytokines, especially IFN-γ, which inhibit tumour angiogenesis and activate a specific immune response (Chu, 2022; Smyth, 2007). Turning to indirect mechanisms, NK cells possess immunomodulatory abilities by influencing various immune cells, including macrophages, T cells, and B cells, resulting in the production of numerous cytokines, growth factors, and chemokines (Chu, 2022). Upon activation, NK cells release IFN-γ, promoting the differentiation of CD8+ T cells into cytotoxic T cells and CD4+ T cells into Th1 (T helper 1) cells. Additionally, NK cells play a role in eliminating tumour cells and delivering tumour antigens to dendritic cells, causing dendritic cell maturation and antigen presentation (Nguyen-Pham, 2012). In the tumour microenvironment (TME), several mechanisms contribute to immunosuppression by hindering NK cell activity.

IMMUNOSUPRESIVE CYTOKINES IN TUMOUR MICROENVIRONMENT

An example is TGFβ (Transforming Growth Factor-beta), a strong immunosuppressive cytokine in the TME. TGFB inhibits NK cell function through mechanisms such as phosphorylation of Smad2/3 (maternal against decapentaplegic homolog 2/maternal against decapentaplegic homolog 3), limiting IFN-γ secretion, and influencing NK cell chemokine receptors (Du, 2021; Yu, 2006). TGFβ, mainly from tumour and regulatory T cells, downregulates NKG2D expression, inhibits the mTOR (mammalian target of rapamycin) pathway, and induces FBP1 (fructose-1,6-bisphosphatase) expression, collectively reducing NK cell activity. Additionally, cytokines such as IL-6 (interleukin 6) and potentially IL-8 (interleukin 8) contribute to impaired NK cell function by activating the STAT3 (signal transducer and activator of transcription 3) pathway (Wu, 2019).

IMMUNOSUPRESIVE METABOLIC COMPONENTS IN TUMOUR MICROENVIRONMENT

In the metabolic realm, cancer cells compete with activated NK cells for vital resources like glucose and glutamine, which are crucial for ATP (Adenosine triphosphate) generation and their rapid growth. Additionally metabolic factors such as indoleamine 2,3-dioxygenase, adenosine, and PGE2 (Prostaglandin E2) exert suppressive influences over NK cell proliferation and functional capacities (Du, 2021).

ABNORMAL LIGAND EXPPRESSION OF CANCER CELLS BOOSTS IMMUNE ESCAPE

A complex interaction involves the shedding of NKG2D ligands by cancer cells, facilitated by the ADAMs (disintegrin and metalloproteinase) family. This shedding leads to the proteolytic cleavage of MICA (MHC class I polypeptide–related sequence A), resulting in a reduction of MICA surface density (Waldhauer, 2008). Furthermore, cancer cells release soluble ligands like MICA/B, which bind to NKG2D. This binding hinders the interaction between NK cells and their target cells, subsequently downregulating NKG2D expression on the surface of NK cells, facilitating immune evasion (Du, 2021).

DYSFUNCTIONAL KREG CELLS REDUCE EFFECTOR CELL CYTOTOXICITY

In solid tumours, CD56bright NKreg (Natural Killer cell regulator) cells reshape the immunosuppressive microenvironment. They do this through high CD94-NKG2A expression and low CD16 expression, reducing cytotoxicity. These NKreg cells also secrete immunomodulatory factors, including IL-10 (Interleukin 10) and TGFβ. Moreover, their NKG2D and NKp46 (Natural Killer cell p46) receptors inhibit T cells proliferation and function (Fu, 2014).

INTERFERENCE BY OTHER IMMUNE CELLS IN TUMOUR MICROENVIRONMENT

Various immunosuppressive immune cells in the TME hinder NK cell activity. These include Tregs (regulatory T cells), MDSCs (myeloid-derived suppressor cells), TAMs (tumour-associated macrophages), and CAFs (tumour-associated fibroblasts). They use a variety of tactics, such as secreting immunosuppressive substances such as TGFβ and metabolites, disrupting interactions between NK cells and cancer cells through competition or decoy mechanisms, and releasing vesicles containing IL-37 (interleukin 37), which modifies NK cell function. Additionally, circulating platelet-coated tumour cells release TGFβ or express inhibitory receptor ligands, impeding NK cell activation and disrupting activating ligand expression (Placke, 2012; Chu, 2021; Vitale, 2014). Together, these mechanisms illustrate the complex interactions of factors in the TME that collectively contribute to suppressing NK cell activity in the context of cancer.

MECHANISMS OF REGULATION OF NK CELLS ACTIVITY

NK cells feature a balance of inhibitory and stimulatory receptors on their surface, crucial for regulating their immune responses. KIRs (Killer cell immunoglobulin-like receptors) are among these receptors, recognizing specific MHC class I alleles. NK cells also express NCRs (Natural cytotoxicity receptors) on their surface, facilitating cytotoxic mechanisms against cells with reduced or absent MHC class I expression. CD94/NKG2 lectin-like receptors constitute another essential group of NK cell receptors. CD94/NKG2 receptors detect non-classical HLA-E (Human Leukocyte Antigen E) class I molecules commonly found on cancer cells (Kwasnik, 2020; Borrego, 1998).

We can divide receptors into inhibitory and activating ones:

- inhibitory receptors include KIRs and C-type lectin receptors such as CD94/NKG2A/B. These receptors play a crucial role in maintaining NK cell quiescence (Chu, 2022);
- conversely, activating receptors play a pivotal role in stimulating NK cell responses and promoting their cytotoxic activities. They complement the inhibitory signals to ensure a balanced response. Activating receptors comprise cytotoxicity receptors (NKp44, NKp46, NKp30), C-type lectin receptors (NKG2E/H, CD94/NKG2C, NKG2F, NKG2D), and select KIRs (KIR-3DS and KIR-2DS) (Chu, 2022).

NK cells perform a complex assessment of the combination of these stimulatory and inhibitory signals to discern their target cells effectively. The ultimate outcome of NK cell activation depends on the unique attributes of the target cells, allowing NK cells to selectively eliminate aberrant cells while sparing healthy ones.

MODIFIED NK CELLS IN THE TREATMENT OF TREATMENT-RESISTANT CANCERS

NK cells have potential for clinical use despite resistance from cancer cells. In recent years, research in the field of NK cell-based cancer immunotherapy has flourished. Recent developments primarily focus on various strategies, including cytokine supplementation, monoclonal antibodies, modifications of the intrinsic signalling pathway, adoptive transfer, and genetic engineering of NK cells. These innovative approaches have opened up new opportunities in the quest to harness the full potential of NK cells in the fight against treatment-resistant cancers (Hodgins, 2019; Daher, 2018; Cheng 2013).

SOURCES AND METHODS OF OBTAINING NK CELLS

The use of NK cells in cancer therapy poses significant challenges due to their impaired functionality in cancer patients and limitations associated with autologous production (Platonova, 2011). To address these issues, allogeneic NK cells are favoured and can be derived from a variety of sources, including peripheral blood mononuclear cells, umbilical cord blood, immortalized cell lines, hematopoietic stem, and progenitor cells (HSPCs), and induced pluripotent stem cells (iPSCs). Each source has distinct advantages and disadvantages, influencing their genetic and functional characteristics (Laskowski, 2022). Primary NK cells can come from peripheral blood (PB-NK cells) or umbilical cord blood (CB-NK cells). CB-NK cells are readily available in blood banks, while PB-NK cells require donor-specific collection via apheresis (Laskowski, 2022; Dolstra 2017; Sharipo, 2022). Both sources have demonstrated successful applications in CAR (chimeric antigen receptor) redirected therapies. The selection of an appropriate NK cell source can be tailored to the specific requirements of patient populations and various diseases (Laskowski 2022). It is worth noting that donor-to-donor variability and interactions between HLA-KIR genotypes can significantly influence NK cell profiles and consequently influence clinical outcomes. The use of donor-derived NK cells may provide benefits, particularly when administered despite HLA-KIR genotype differences to counteract immune evasion by tumour cells (Ciurea, 2022). Selection of the NK cell source and consideration of genetic factors are critical aspects in the design of effective NK cell-based anticancer therapies.

METHODS OF ENHANCING THE ANTICANCER ACTIVITY OF NK CELLS

GENETIC MODIFICATIONS

Genetic manipulation of NK cells is extremely promising in the field of cancer immunotherapy, especially in the case of cancers that are resistant to destruction by NK cells (Kwaśnik, 2020). NK cell functionality is closely related to cytokines such as IL-2 (interleukin 2) and IL-15 (interleukin 15). However, systemic administration of these cytokines may induce an undesirable expansion of regulatory T cells. To circumvent these side effects, an interesting approach was used: direct introduction of the IL-2 gene into NK cells using various methods, using retroviruses and cDNA molecules (Kwasnik, 2020; Maddineni, 2022). This innovative strategy has yielded impressive results, especially when using NK-92 cells. The NK-92 cell line is characterized by abundant activating receptors but lacks some inhibitory receptors. This unique profile of NK-92 cells makes them suitable for clinical translation as they can be easily cultured *in vitro* (Ishikawa, 2018). These genetically modified NK-92 cells exhibit exceptional cytotoxicity and, even more remarkably, they possess the ability to effectively attack cancer cells regardless of specific tumour antigens. This has been convincingly demonstrated both in controlled laboratory conditions (*in vitro*) and in living organisms (*in vivo*) (Jochems, 2016; Tonn, 2013). Nevertheless, a limitation arises due to the inability of NK-92 cells to mediate antibody-dependent cellular cytotoxicity (ADCC). To address this limitation, Jochems and colleagues genetically modified NK-92 cells to introduce the high-affinity molecule CD16, a key factor in ADCC that is not normally found in NK-92 cells. In laboratory experiments, haNK (NK cells expressing a high-affinity Fc receptor) demonstrated potent cytotoxicity against various types of cancer, including lung and breast cancer. Moreover, the haNK system was further adapted to express a PD-L1-targeting CAR (Programmed Death-Ligand 1) called t-haNK, which showed promising efficacy both *in vitro* and *in vivo*, effectively controlling PD-L1-dependent tumours in mouse models (Klingemann, 2016; Jochems, 2016).

However, the use of NK-92 cell lines has specific limitations. First of all, these cell lines have limited expansion potential *in vivo*, which limits their ability to generate strong and durable effector responses. This limited expansion capacity ultimately reduces the maximum efficacy achievable with NK-92 cell therapies (Kang, 2021). Similar strategies that were used through IL-2 gene insertion were also used for the IL-15 gene, leading to increased NK cell proliferation and increased cytotoxicity (Kwasnik, 2020).

Another method of modification focuses on increasing the ability of NK cells to detect cancer cells by creating CARs (chimeric antigen receptors). CAR-developed T-cell therapies are at the forefront of immuno-oncology applications. While CAR-T cells can indeed achieve long-lasting responses due to their prolonged persistence and potential to differentiate into memory T-cell subsets, CAR-NK cells have the added advantage of being able to eliminate tumour cells regardless of CAR functionality. Additionally, CAR-T cells may be associated with significant associated toxicities, including cytokine release syndrome and neurotoxicity. In contrast, CAR-NK cells typically exhibit less toxicity, mainly due to their limited lifespan in circulation (Xie, 2020; Neelapu, 2017). CAR-NK cells are carefully designed to express receptors capable of recognizing specific tumour-associated antigens, resulting in increased cytotoxicity (Kwasnik, 2020). The evolution of CAR technology has led to various generations of CARs, including first-generation CARs with a basic structure and a single signalling region (Jensen, 2010); a second-generation CAR with an additional co-stimulatory domain such as CD28 (Maher, 2002); Third-generation CARs containing multiple co-stimulatory domains (Carpenito, 2009); and fourth-generation CARs combining multiple co-stimulatory domains with cytokine signals (Chu, 2022). CARs have been meticulously designed to target a wide range of antigens such as Her2/neu (erbB-2 receptor tyrosine-protein kinase/neurogenic differentiation factor 1), CD33, CD20, CD19, DAP12 (DNAX activating protein 12), FLT3 (FMS-Like Tyrosine Kinase 3) and/or CD33 (Kruschinski, 2009; Schirrmann, 2005; Muller, 2008; Imai, 2005; Töpfer, 2015; Tang, 2018). Notably, NK cells have inherent specificity and transient existence, making them an attractive option for generating CAR-positive cells (Maddineni, 2022). Studies have demonstrated the effectiveness and specificity of these cells in attacking cancer cells while maintaining a favourable safety profile, as seen in the treatment of CD19-positive lymphoid malignancies (Liu, 2022). The recent FDA approval of CD19-targeted CAR-T cell therapy represents a significant milestone in the development of CAR-positive cell therapies.

METABOLIC REPROGRAMMING USING CYTOKINES

Cytokines play a key role in supporting NK cell function and survival, especially IL-2 and IL-15, and this is important in the field of immunotherapy. These cytokines serve as potent stimulators of NK cell activity, but their systemic administration can sometimes lead to unintended side effects, such as the expansion of regulatory T cells, which can dampen the immune response (Du, 2022). To overcome these challenges, modified versions of these cytokines have been obtained. For example, a modified variant of IL-2 known as "super-2" has been developed that has increased binding affinity for IL-2Rβ (IL-2 receptor beta). This modification helps bypass problems associated with Treg cell interaction (Rosenberg, 1985; Levin, 2012, Du, 2022). Another innovative approach is to create an IL-2 fusion protein that selectively activates cells carrying NKG2D. This targeted activation promotes NK cell expansion without the adverse consequences associated with systemic IL-2 administration (Ghasemi, 2016). In the case of IL-15, early phase clinical trials investigated the use of rIL-15 (recombinant IL-15) in the treatment of refractory solid tumours. However, these studies have shown limited effectiveness, likely due to the inclusion of heavily pre-treated patients and the presence of less cytotoxic NK cells (Miller, 2018; Du, 2021). They also focused on obtaining hetIL-15 (heterodimeric IL-15), which offers extended half-lives and increased bioactivity. HetIL-15 has demonstrated the ability to promote greater persistence and expansion of NK cells and CD8+ T cells, which shows promise in the field of immunotherapy (Bergamaschi, 2021). Moreover, new approaches have emerged, such as N-803, an IL-15 superagonist. N-803 combines the IL-15 mutant with a fusion protein, significantly increasing its biological activity and extending its half-life (Rosario, 2016; Du, 2021). Clinical trials have provided evidence that N-803 when used in combination with immune checkpoint inhibitors or anti-CD20 monoclonal antibodies can increase NK cell cytotoxicity and lead to improved patient survival rates (Margolin, 2018). The goal of these innovative strategies is to unlock the full potential of cytokines while alleviating their limitations, ultimately increasing the effectiveness of NK cell-based therapy in cancer treatment.

BLOCKING KIR AND OTHER RECEPTORS USING MONOGLONAL ANTIBODIES

Inhibitory immunoglobulin-like receptors (KIRs) exert significant influence as potent regulators of NK cell activity, capable of bypassing concurrent activating signals upon interaction with HLA class I ligands. Due to their key role in suppressing NK cell function, inhibitory KIRs are of great interest scientists (Du, 2021). For example, IPH2101, an IgG monoclonal antibody directed against KIR2DL1/2/3 (killer immunoglobulin-like receptor domain long form 1/2/3), has undergone rigorous evaluation in clinical trials as

a stand-alone therapeutic agent. Although it was well tolerated, its ability to significantly increase anticancer potential was limited. Interestingly, its administration led to a reduction in the expression of inhibitory receptors KIR2D (Killer Immunoglobulin-like Receptor 2D) on NK cells (Carlsten, 2016). Of note, when IPH2101 was combined with Lenalidomide in people with relapsed/refractory multiple myeloma, a significant increase in median free survival was observed (Benson, 2015). On the other hand, another anti-KIR antibody, Lirilumab, failed to induce clinically significant responses in two early-stage clinical trials, thus halting its developmental progression (Vey, 2017; Vey, 2018). Additionally, the IPH4102 antibody demonstrated favourable tolerability in a Phase I clinical evaluation in people with relapsed/refractory cutaneous T-cell lymphoma (Bagot, 2019). The study of alternative inhibitory receptors such as CD96 and TIGIT (T cell immunoreceptor with immunoglobulin and ITIM domain) also shows great promise. These receptors are selectively expressed in human NK and T cells and recognize nectin and nectin-like ligands found in various types of cancer cells (Bottino, 2003; Martinet 2015). Notably, TIGIT expression is induced in NK cells upon activation, whereas CD96 is constitutively expressed. Both molecules show strong binding affinity to PVR (poliovirus receptor), although they have different effects on NK cell function. TIGIT interaction with PVR reduces NK cell cytotoxicity by activating DNAM-1 (DNAX-1 accessory molecule), while CD96 downregulates NK cell production of IFN-γ (Bernhardt, 2014; Chan, 2014). Fascinatingly, studies in melanoma and prostate cancer cell lines have provided evidence that administration of antibodies directed against CD96 increases the effectiveness of NK cells in fighting metastasis, primarily by hindering the CD96-CD155 interaction (Blake, 2016). Moreover, various immune checkpoints show potential in this context, including NKG2A and CD94 (Seymour, 2015). These multifaceted approaches highlight the complexity of modulating inhibitory receptors to enhance NK cell function in the field of cancer immunotherapy. Although some strategies show promise in clinical trials, others require further study and optimization.

USE OF BISPECIFIC AND MULTISPECIFIC ANTIBODIES

Bispecific antibodies (BsAbs) have received much attention, especially since the FDA approval of blinatumomab for the treatment of acute myeloid leukaemia (AML) (Labrjin, 2019). These antibodies are valued in the field of cancer therapy due to their characteristic ability to simultaneously bind two different epitopes (Viardot, 2018). Initially, the use of BsAbs was primarily oriented toward redirecting T cells toward tumour cells, an approach that emphasized enhancing the interaction between the extracellular CD3 subunit on T cells and tumour-associated antigens (Valdman, 2020). However, BsAb has also been studied in the context of NK cells. NK cells exhibit strong cytotoxicity, are less susceptible to exhaustion, and their cytotoxic activity does not involve binding to the MHC-Epitope complex (Ordóñez-Reyes, 2022). Work has begun on the development of NKCE (Natural Killer Cell Angers), a subclass of BsAb, with a modified Fc domain (fragment capable of crystallisation) to activate NK cells via NKp46. NKp46 plays a key role in inducing NK cell cytotoxicity, particularly in HLA class I unprotected cells, which is a common occurrence in cancers characterized by reduced HLA class I expression (Demaria, 2021). Growing evidence suggests that treatment with these antibodies can increase tumour infiltration by NK cells, consequently leading to strong anti-tumour responses in animal models (Gauthier, 2019).

Innovative NK cell strategies also include the development of NK cell engagers targeting CD16, in particular FcγRIIIA (Fc receptor gamma IIIA), the CD16A isoform. Currently, most NK cell engagement agents target antigens commonly expressed in hematologic malignancies, including CD19, CD20, CD30, and CD33. This trend is consistent with the trajectory of other immunotherapies such as CAR-T cells and CAR-NK cells (Ellwanger, 2019; Ordóñez-Reyes, 2022). As a pioneering FDA-approved bispecific antibody for the treatment of B-cell malignancies, blinatumomab represents a significant clinical milestone. However, many challenges remain, including issues related to treatment resistance and limited efficacy in solid tumours. In response to these challenges, significant efforts have been made to develop multispecific antibodies (Tapia-Galisteo, 2023). Multispecific antibodies are carefully designed to target activating NK cell receptors in combination with TAA (tumour-associated antigen), marking a promising trajectory in the cancer therapy landscape. The spectrum of NK cell receptor activation has been established in a hierarchical order based on their ability to activate quiescent NK cells, with CD16 > NKp46 > NKG2D dominating in potency (Bryceson, 2006). In the context of acute myeloid leukaemia, an antibody construct targeting CD33/CD16/CD123 was developed, resulting in enhanced NK cell-mediated lysis of primary leukemic cells compared to a trivalent bispecific antibody (Braciak, 2018). Moreover, other triumvirate antibody constructs have been developed targeting CD33 or HLA-DR (human leukocyte antigen-DR) and CD19.

These constructs engage NK cells via an anti-CD16 moiety or use the NKG2D ligand (Vyas, 2016). Additionally, a trispecific antibody format, called aTriFlex, was used to recalibrate NK cell cytotoxicity against two antigens found in multiple myeloma, significantly increasing its selectivity and efficacy (Gantke, 2018). In parallel to these multifaceted formats, IgG-like TsAbs (quadrivalent bispecific antibodies) have been carefully designed using various advanced technologies, including SEEDbody and common light chain technology. These TsAbs have the characteristic ability to simultaneously target multiple antigens such as EGFR (epidermal growth factor receptor), CD16, and PD-L1. This leads to increased binding affinity and increased potency of antibody-dependent cellular cytotoxicity (Pekar, 2020; Bogen, 2021).

Another notable strategy involves dual targeting of NK cell activation receptors. The ANKET (Antibody-based NK cell Engage Therapeutics) platform has achieved significant progress in this field, creating promising NKCEs targeting a diverse set of tumour antigens. These trifunctional NKCEs contain two Fab antibody fragments targeting NKp46 and TAA (CD19, CD20, EGFR), interspersed with an Fc domain to facilitate CD16-mediated ADCC. Preclinical studies have shown that these NKCEs demonstrate remarkable efficacy across a spectrum of cancer types, outperforming results obtained with monoclonal antibody treatment in a Raji B lymphoma model (Gauthier, 2019). Furthermore, in a study focusing on paediatric B-ALL (B-cell acute lymphoblastic leukaemia), trifunctional NKCEs targeting CD19 or CD20, engaging NKp46 or NKp30, demonstrated highly effective NK cell-mediated killing of leukaemia cell lines and primary blasts, in including resistant to the action of NK cells (Colomar-Carando, 2022). Additionally, the TriNKET (Triple-Negative Killer Engager Therapy) platform offers an alternative route to the development of multifunctional NKCEs. A notable agent within this platform, DF1001, is tailored to target HER2 while interacting with CD16 and NKG2D (Myers, 2021). The therapeutic potential of bispecific and multispecific antibodies, especially in the context of NK cell involvement, is a source of hope in the fight against cancer. As research and clinical trials progress, the continued search for multifaceted antibodies offers hope for better treatment outcomes for patients suffering from serious malignancies.

EFFICACY OF MODIFIED NK CELLS IN CANCER TREATMENT – CLINICAL TRIALS

NK cell therapy has shown promising anti-cancer effects in preclinical studies, targeting various malignancies like leukaemia, lymphoma, myeloma, ovarian cancer, and glioblastoma (Daher, 2021).

CAR-NK CELLS

When it comes to clinical trials, as of September 2023, there are more than 30 ongoing clinical trials investigating CAR-NK constructs for the treatment of various hematologic and solid tumour malignancies. These trials are registered on ClinicalTrials.gov. Approximately 70% of these trials are focused on haematological malignancies. Here are some noteworthy examples:

- immunotherapy combination: irradiated PD-L1 CAR-NK cells plus pembrolizumab plus N-803 for subjects with recurrent/metastatic gastric or head and neck cancer (NCT04847466);
- study of anti-PSMA CAR NK Cell (TABP EIC) in metastatic castration-resistant prostate cancer (NCT03692663);
- study of DLL3-CAR-NK cells in the treatment of extensive stage small cell lung cancer (NCT05507593);
- NKX019, intravenous allogeneic chimeric antigen receptor natural killer cells (CAR NK), in adults with B-cell cancers (NCT05020678);
- NKX101, intravenous allogeneic CAR NK cells, in adults with AML or MDS (NCT04623944).

These trials represent a promising avenue of research in the field of immunotherapy, where CAR-NK cells are being investigated for their potential to target and treat a range of cancers.

HERE ARE HIGHLIGHTED THE MOST IMPORTANT CLINICAL STUDIES ASSOCIATED WHITH THIS REVIEW

ACUTE MYELOID LEUKEMIA (AML)

In a significant development in 2018, the commencement of a first-in-human phase I clinical trial introduced CD33-CAR NK cells as a therapeutic modality for patients grappling with relapsed and refractory AML. This pioneering study, which enrolled a trifecta of participants, placed paramount emphasis on evaluating the safety profile of CD33-CAR-NK cells. Notably, the investigation yielded a noteworthy absence of reported adverse events (Tang, 2018; Chu, 2022).

MULTIPLE MYELOMA (MM)

As previously highlighted, a noteworthy clinical trial phase involving IPH2101, when combined with lenalidomide in a cohort of fifteen individuals afflicted by relapsed/refractory multiple myeloma, demonstrated a substantial enhancement in median progression-free survival (Benson, 2015). Additionally, an independent exploration of IPH2101 as a monotherapy among a group of nine patients revealed overall tolerability but limited efficacy in significantly enhancing anti-cancer potential (Carlsten, 2016).

LYMPHOMA

In 2020, a remarkable milestone was achieved in the treatment of high-risk B-cell lymphoma and CD19+ chronic lymphocytic leukaemia (CLL) through the use of allogeneic cord blood-derived CAR-NK cells. This approach was characterized by a favourable safety profile, in particular it did not cause side effects such as cytokine release syndrome, neurotoxicity or GVHD (graft-versus-host disease). Impressively, 73% of patients responded positively to this intervention, achieving complete or partial remission. These responses were rapid and lasted for at least 30 days after infusion (Liu, 2020). Additionally, Bachanova and colleagues presented preliminary clinical results with FT596, an off-the-shelf induced pluripotent stem cell (iPSC)-derived CAR-NK therapy targeting CD19 in the context of B-cell lymphoma. Preliminary observations on the effects of FT596 treatment in one patient indicated a partial response characterized by with a reduction in tumour volume exceeding 50%. However, it is worth noting that several adverse events have been reported, including leukopenia, neutropenia, anaemia, and urinary tract problems (Bachanova, 2020).

SOLID TUMOURS

In the Phase I dose escalation study, the primary objectives were to evaluate the feasibility of large-scale expansion and to assess the safety of administering ex vivo-expanded NK-92 cells as allogeneic cellular immunotherapy to patients with refractory cancer, renal cell carcinoma and melanoma. This study included a cohort of twelve patients, and infusion toxicities associated with NK-92 cell administration were mainly mild. Interestingly, one patient demonstrated durable disease survival four years after NK-92 infusion. Additionally, among the patients, one person with metastatic melanoma showed a modest response during the study period, while another patient had a mixed response (Arai, 2008). Additionally, in a clinical trial, a cohort of fifteen people diagnosed with advanced and refractory cancers, mainly solid tumours and sarcomas, and in some cases leukaemia or lymphoma, received two doses of NK-92 cells given 48 hours apart. Impressively, no adverse events were reported during or after the infusion. Notably, 75% of lung cancer patients showed some form of positive response (Tonn, 2013). In recent developments, ongoing clinical trials are aimed at evaluating the PD-L1 t-haNK system. These cells, derived from NantKwest's proprietary master NK-92 (aNK) cell bank, are genetically engineered to target PD-L1 and produce intracellular IL-2, thereby enhancing their capacity for antibody-targeted cellular cytotoxicity. CD16. Preliminary observations from this study, pending peer review, showed no dose-limiting toxicities in a cohort of six patients diagnosed with locally advanced or metastatic solid tumours. This trial is registered under the identifier (ClinicalTrials.gov: NCT04050709). As previously mentioned, a Phase I clinical trial provided evidence of the utility of recombinant rIL-15 in the treatment of refractory solid tumours in 19 patients. Although no objective responses were observed, several patients experienced disease stabilization (Miller, 2018). Additionally, as previously highlighted, a Phase I clinical trial involving a cohort of 24 patients provided evidence that the use of ALT-803 in combination with immune checkpoint inhibitors or anti-CD20 monoclonal antibodies may increase NK cell cytotoxicity and contribute to better patient survival rates (Margolin, 2018).

CHALLENGES ASSOCIATED WITH MODIFIED NK CELL-BASED THERAPIES

NK cell-based therapy faces several significant challenges. First, ex vivo NK cell expansion represents a significant hurdle, primarily attributed to the inherent difficulties in obtaining sufficient NK cells from a single donor (Chu, 2022). Second, the crucial task of selecting the most appropriate CAR transduction method into NK cells cannot be underestimated. Although viral vectors such as retroviruses are commonly used, their use is associated with potential risks, including insertional mutations and carcinogenesis (Myers, 2021). Third, the complex and multifaceted tumour microenvironment introduces a multitude of complexities into the field of NK therapy (Daher, 2021). Finally, evaluation of preclinical outcomes faces significant obstacles due to the lack of clinically relevant animal models that can faithfully reproduce the intricacies

and interactions in the TME. Unfortunately, the vast majority of studies continue to rely on immunocompromised mice, which, while valuable, do not sufficiently mimic the clinically relevant TME and do not provide a precise assessment of NK cell functionality (Chu, 2022). To optimize NK cell therapeutic strategies, it becomes necessary to obtain a comprehensive understanding of the impact of metabolic markers and signalling pathways on NK cell persistence. Additionally, it is critical to refine manufacturing procedures to ensure consistency and uniformity in NK cell infusions. It is equally important to explore strategies to preserve NK cell persistence without inducing lymphodepletion, especially in the context of co-administration with chemotherapy or radiotherapy (Du, 2021).

DIRECTIONS FOR FURTHER RESEARCH ON THE USE OF MODIFIED NK CELLS IN CANCER THERAPY

The landscape of clinical trials pertaining to NK cell-based immunotherapy encompasses phases I and II, with a predominant focus on haematological malignancies (Wu, 2020). However, NK cells have consistently demonstrated remarkable safety and efficacy profiles in these clinical trials. Whether administered as standalone interventions or in conjunction with other therapeutic modalities, both allogeneic and autologous approaches have exhibited promising outcomes (Laskowski, 2022). Of particular significance is the application of chimeric antigen receptor (CAR)-engineered NK cells, which have proven to be highly effective in targeting tumour-specific antigens and thereby amplifying anti-tumour responses (Zhang, 2022).

Moreover, the development of antibodies meticulously tailored to selectively target inhibitory receptors on NK cells, encompassing KIRs, NKG2A, and TIGIT, has unveiled their potential in enhancing NK cell responses and fortifying the elimination of tumour cells. Several of these antibody candidates are currently undergoing rigorous clinical validation (Du, 2021). Capitalizing on the expansive recognition capabilities intrinsic to NK cells, the integration of NK cell-based therapies into multifaceted immune combination strategies holds substantial promise for further augmenting anti-tumour efficacy. Prospective innovations may involve more intricate genetic modifications aimed at bolstering the longevity and functionality of NK cells while mitigating the risk of unintended side effects (Maddineni, 2022). A forthcoming challenge in the adoption of NK cell-based therapy lies in the need for a more precise characterization of distinct NK cell subsets. This necessitates the identification of specific markers, functional attributes, and regulatory pathways unique to each subgroup. Such insights will facilitate the development of tailored therapeutic approaches for the treatment of tumours that are infiltrated by distinct NK cell populations. Furthermore, to mitigate off-target effects induced by conventional anti-tumour medications within the tumour microenvironment, future clinical trials should incorporate meticulously selected combination therapies. This strategic refinement is pivotal to ensure that treatment effectiveness remains uncompromised (Wu, 2020).

Delving into other subsets of ILCs (Innate Lymphoid Cells) beyond NK cells, such as intraepithelial ILC1-like cells, holds the potential to bolster antitumor responses and broaden the horizons of NK cell therapies in the realm of cancer immunotherapy (Moreno-Nieves, 2021).

CONCLUSIONS

The development and application of genetically modified NK cells with enhanced reactivity hold significant promise in the treatment of therapy-resistant cancers. NK cells exhibit a remarkable ability to target and eliminate cancer cells through various mechanisms, both directly and indirectly. Genetic modifications, such as CAR expression and cytokine supplementation, have shown immense potential in augmenting NK cell cytotoxicity and persistence within the tumour microenvironment. Moreover, the use of monoclonal antibodies targeting inhibitory receptors on NK cells, along with advancements in allogeneic NK cell therapies, offers novel approaches to overcoming immune evasion strategies employed by tumour cells. These strategies broaden the horizons of cancer immunotherapy and hold the potential to improve patient outcomes, particularly in cases where conventional treatments have failed.

While challenges persist, such as optimizing expansion protocols, selecting appropriate sources for NK cells, and improving our understanding of the intricate interactions within the tumour microenvironment, ongoing research and innovation continue to drive the field forward. As we delve into the realm of emerging NK cell subtypes and their potential synergies with other immune therapies, the future of genetically modified NK cell-based treatments for therapy-resistant cancers appears promising.

References

Arai S., Meagher R., Swearingen M., Myint H., Rich E., Martinson J., et al. **Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial**. Cytotherapy. 2010; 10(6): 625-632.

Bachanova V., Cayci Z., Lewis D., Maakaron J.E., Janakiram M., Bartz A., et al. **Clinical activity of FT596, a first-in-class, Multi-Antigen targeted, off-the-shelf, iPSCderived CD19 CAR NK cell therapy in relapsed/refractory B-cell lymphoma**. Blood. 2020; 136: 8-8.

Bagot M., Porcu P., Marie-Cardine A., Battistella M., William B.M., Vermeer M., et al. I**PH4102, a first-in-class anti-KIR3DL2 monoclonal antibody, in patients with relapsed or refractory cutaneous T-cell lymphoma: an international, first-in-human, open-label, phase 1 trial**. Lancet Oncol. 2019; 20(8): 1160-1170.

Bergamaschi C., Stravokefalou V., Stellas D., Karaliota S., Felber, B.K., Pavlakis G.N. **Heterodimeric IL-15 in Cancer Immunotherapy**. Cancers. 2021; 13(4): 837.

Bernhardt G. **TACTILE becomes tangible: CD96 discloses its inhibitory peculiarities**. Nat Immunol. 2014; 15(5): 406-408.

Benson D.M., Cohen A.D., Jagannath S., Munsbrhi N.C., Spitzer G., Hofmeister C.C., et al. **A Phase I Trial of the Anti-KIR Antibody IPH2101 and Lenalidomide in Patients with Relapsed/Refractory Multiple Myeloma. Clinical cancer research.** Clin Cancer Res. 2015; 21(18): 4055-4061.

Blake S.J., Stannard K., Liu J., Allen S., Yong M.C., Mittal D., et al. **Suppression of metastases using a new lymphocyte checkpoint target for cancer immunotherapy**. CancerDiscov. 2016; 6(4): 446-59.

Bogen J.P., Carrara S.C., Fiebig D., Grzeschik J., Hock B., Kolmar H. **Design of a Trispecific Checkpoint Inhibitor and Natural Killer Cell Engager Based on a 2 + 1 Common Light Chain Antibody Architecture**. Frontiers immunol. 2021; 12: 669496.

Bonadonna G., Brusamolino E., Valagussa P., Rossi A., Brugnatelli L., Brambilla C., et al. **Combination chemotherapy as an adjuvant treatment in operable breast cancer**. N Engl J Med. 1976; 294(8): 405-410.

Borrego F., Ulbrecht M., Weiss E.H., Coligan J.E., Brooks A.G. **Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis**. J Exp Med. 1998; 187(5): 813-818.

Bosl G.J., Gluckman R., Geller N.L., Golbey R.B., Whitmore W.F., Jr. Herr H., et al. **VAB-6: an effective chemotherapy regimen for patients with germ-cell tumors**. J Clin Oncol. 1986; 4(10): 1493-1499.

Bottino C., Castriconi R., Pende D., Rivera P., Nanni M., Carnemolla B., et al. **Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule**. J Exp Med. 2003; 198(4): 557-567.

Braciak T.A., Roskopf C.C., Wildenhain S., Fenn N.C., Schiller C.B., Schubert I.A., et al. **Dual-targeting triplebody 33-16-123 (SPM-2) mediates effective redirected lysis of primary blasts from patients with a broad range of AML subtypes in combination with natural killer cells**. Oncoimmunology. 2018; 7(9): e1472195.

Brand A., Singer K., Koehl G.E., Kolitzus, M., Schoenhammer G., Thiel A., et al. **LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells**. Cell Metab. 2016; 24(5): 657-671.

Bryceson Y.T., March M.E., Ljunggren H.G., Long E.O. **Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion**. Blood. 2006; 107: 159-66.

Carlsten M., Korde N., Kotecha R., Reger R., Bor S., Kazandjian D., et al. **Checkpoint Inhibition of KIR2D with the Monoclonal Antibody IPH2101 Induces Contraction and Hyporesponsiveness of NK Cells in Patients with Myeloma**. Clin Cancer Res. 2016; 22(21): 5211-5222.

Carpenito C., Milone M.C., Hassan R., Simonet J.C., Lakhal M., Suhoski M.M., et al. **Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains**. Proc Natl Acad Sci U S A. 2009; 106(9): 3360-3365.

Chan C.J., Martinet L., Gilfillan S., Souza-Fonseca-Guimaraes F., Chow M.T., Town L., et al. **The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions**. Nat Immunol. 2014; 15(5): 431-438.

Chen X., Lu P., Wang D.D., Yang S.J., Wu Y., Shen H.Y., et al. **The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues**. Gene. 2016; 595(2): 221-226.

Chen Y., Liu L., Li J., Du Y., Wang J., Liu J. **Effects of long noncoding RNA (linc-VLDLR) existing in extracellular vesicles on the occurrence and multidrug resistance of esophageal cancer cells**. Pathol Res Pract. 2019; 215(3): 470-477.

Cheng M., Chen Y., Xiao W., Sun R., Tian Z. **NK cell-based immunotherapy for malignant diseases**. Cell Mol Immunol. 2013; 10(3): 230-252.

Cho B.C., Abreu D.R., Hussein M., Cobo M., Patel A. J., Secen N., et al. **Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study**. Lancet. Oncol. 2022; 23(6): 781-792.

Chu J., Gao F., Yan M., Zhao S., Yan Z., Shi B., et al. **Natural killer cells: a promising immunotherapy for cancer**. J Transl Med. 2022; 20(1): 240.

Ciurea S.O., Kongtim P., Soebbing D., Trikha P., Behbehani G., Rondon G., et al. **Decrease post-transplant relapse using donor-derived expanded NK-cells**. Leukemia. 2022; 36(1): 155-164.

Colomar-Carando N., Gauthier L., Merli P., Loiacono F., Canevali P., Falco M., et al. **Exploiting Natural Killer Cell Engagers to Control Pediatric B-cell Precursor Acute Lymphoblastic Leukemia**. Cancer Immunol Res**.** 2022; 10(3): 291-302.

Cong J., Wang X., Zheng X., Wang D., Fu B., Sun R., et al. **Dysfunction of Natural Killer Cells by FBP1-Induced Inhibition of Glycolysis during Lung Cancer Progression**. Cell Metab. 2018; 28(2): 243-255.

Dalle J.H., Menezes J., Wagner E., Blagdon M., Champagne J., Champagne M.A., et al. **Characterization of Cord Blood Natural Killer Cells: Implications for Transplantation and Neonatal Infections**. Pediatr Res. 2005; 57: 649-655.

Daher M., Rezvani K. **Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering**. Curr Opin Immunol. 2018; 51: 146-153.

Daher M., Rezvani K. **Outlook for New CAR-Based Therapies with a Focus on CAR NK Cells: What Lies Beyond CAR-Engineered T Cells in the Race against Cancer**. Cancer Discov. 2021; 11(1): 45-58.

Demaria O., Gauthier L., Debroas G., Vivier E. **Natural killer cell engagers in cancer immunotherapy: Next generation of immuno-oncology treatments**. Eur J Immunol. 2021; 51(8): 1934-1942.

Dolstra H., Roeven M.W.H., Spanholtz J., Hangalapura B.N., Tordoir M., Maas F., et al. **Successful Transfer of Umbilical Cord Blood CD34+ Hematopoietic Stem and Progenitor-derived NK Cells in Older Acute Myeloid Leukemia Patients. Clinical cancer research**. Clin Cancer Res. 2016; 23(15): 4107-4118.

Du N., Guo F., Wang Y., Cui J. **NK Cell Therapy: A Rising Star in Cancer Treatment**. Cancers (Basel). 2021; 13(16): 4129.

Duesberg P., Stindl R., Hehlmann R. **Explaining the high mutation rates of cancer cells to drug and multidrug resistance by chromosome reassortments that are catalyzed by aneuploidy**. Proc Natl Acad Sci U S A. 2000; 97(26): 14295-14300.

Ellwanger K., Reusch U., Fucek I., Wingert S., Ross T., Müller T., et al. **Redirected optimized cell killing (ROCK®): A highly versatile multispecific fit-for-purpose antibody platform for engaging innate immunity**. MAbs. 2019; 11(5): 899-918.

Elshiaty M., Schindler H., Christopoulos P. **Principles and Current Clinical Landscape of Multispecific Antibodies against Cancer**. Int J Mol Sci. 2021; 22(11): 5632.

Emran T.B., Shahriar A., Mahmud A.R., Rahman T., Abir M.H., Siddiquee M.F., et al. **Multidrug Resistance in Cancer: Understanding Molecular Mechanisms, Immunoprevention and Therapeutic Approaches**. Front Oncol. 2022; 12: 891652.

Fu B., Tian Z., Wei H. **Subsets of human natural killer cells and their regulatory effects**. Immunology. 2014; 141(4): 483-489.

Gantke T., Weichel M., Herbrecht C., Reusch U., Ellwanger K., Fucek I., et al. **Trispecific antibodies for CD16A-directed NK cell engagement and dual-targeting of tumor cells. Protein engineering, design & selection**. Protein Eng Des Sel. 2017; 30(9): 673-684.

Gauthier L., Morel A., Anceriz N., Rossi B., Blanchard-Alvarez A., Grondin G., et al. **Multifunctional Natural Killer Cell Engagers Targeting NKp46 Trigger Protective Tumor Immunity**. Cell. 2019; 177(7): 1701-1713.

Ghasemi R., Lazear E., Wang X., Arefanian S., Zheleznyak A., Carreno B.M., et al. **Selective targeting of IL-2 to NKG2D bearing cells for improved immunotherapy**. Nat Commun. 2016; 7: 12878.

Hatjiharissi E., Xu L., Santos D.D., Hunter Z.R., Ciccarelli B.T., Verselis S., et al. **Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism**. Blood. 2007; 110(7): 2561-2564.

Hodgins J.J., Khan S.T., Park M.M., Auer R.C., Ardolino M. **Killers 2.0: NK cell therapies at the forefront of cancer control**. J Clin Invest. 2019; 129(9): 3499-3510.

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(1): 376-83.

Ishikawa T., Okayama T., Sakamoto N., Ideno M., Oka K., Enoki T., et al. **Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer**. Int J Cancer. 2018; 142 :2599-609.

Iwai Y., Ishida M., Tanaka Y., Okazaki T., Honjo T., Minato N. **Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade**. Proc Natl Acad Sci U S A. 2002; 99(19): 12293-12297.

Jensen M.C., Popplewell L., Cooper L.J., DiGiusto D., Kalos M., Ostberg J.R., et al. **Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. Biology of blood and marrow transplantation**. Biol Blood Marrow Transplant. 2010; 16(9): 1245-1256.

Jochems C., Hodge J.W., Fantini M., Fujii R., Morillon Y.M., Greiner J.W., et al. **An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele**. Oncotarget. 2016; 7(52): 86359-86373.

Kang S., Gao X., Zhang L., Yang E., Li Y., Yu L. **The advances and challenges of NK cell-based cancer immunotherapy**. Curr Oncol. 2021; 28: 1077-93.

Kanwal R., Gupta S. **Epigenetic modifications in cancer**. Clin Genet. 2012; 81: 303-311.

Khan M., Arooj S., Wang H. **NK Cell-Based Immune Checkpoint Inhibition**. Front Immunol. 2020; 11: 167.

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

Kruschinski A., Moosmann A., Poschke I., Norell H., Chmielewski M., Seliger B., et al. **Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas**. Proc Natl Acad Sci U S A. 2008; 105(45): 17481-17486.

Kwaśnik P., Lemieszek M.K., Rzeski W. **Possibilities of using NK cells in cancer immunotherapy**. Med Og Nauk Zdr. 2020; 26(1): 8-16.

Labrijn A., Janmaat M., Reichert J., Parren P. **Bispecific Antibodies: A Mechanistic Review of the Pipeline**. Nat. Rev. Drug Discov. 2019; 18: 585-608.

Laskowski T.J., Biederstädt A., Rezvani K. **Natural killer cells in antitumour adoptive cell immunotherapy**. Nat Rev Cancer. 2022; 22: 557-575.

Leach D.R., Krummel M.F., Allison J.P. **Enhancement of antitumor immunity by CTLA-4 blockade**. Science. 1996; 271(5256): 1734-1736.

Levin A.M., Bates D.L., Ring A.M., Krieg C., Lin J.T., Su L., et al. **Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'**. Nature. 2012; 484(7395): 529-533.

Liang B.C. **Effects of hypoxia on drug resistance phenotype and genotype in human glioma cell lines**. J Neurooncol. 1996; 29(2): 149-155.

Liau L.M., Ashkan K., Tran D.D., Campian J.L., Trusheim J. E., Cobbs C.S., et al. **First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma**. J Transl Med. 2018; 16(1): 142.

Liu E., Marin D., Banerjee P., Macapinlac H.A., Thompson P., Basar R., et al. **Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors**. N Engl J Med. 2022; 382(6): 545-553.

Maddineni S., Silberstein J.L., Sunwoo J.B. **Emerging NK cell therapies for cancer and the promise of next generation engineering of iPSC-derived NK cells.** J Immunother Cancer**.** 2022; 10(5): e004693.

Maher J., Brentjens R.J., Gunset G., Rivière I., Sadelain M. **Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor**. Nat Biotechnol. 2002; 20(1): 70-75.

Margolin K., Morishima C., Velcheti V., Miller J.S., Lee S.M., Silk A., et al. **Phase I Trial of ALT-803, A Novel Recombinant IL15 Complex, in Patients with Advanced Solid Tumors**. Clin Cancer Res. 2018; 24(22): 5552–5561.

Martinet L., Smyth MJ. **Balancing natural killer cell activation through paired receptors**. Nat Rev Immunol. 2015; 15(4): 243-254.

Martínez-Lostao L., Anel A., Pardo J. **How Do Cytotoxic Lymphocytes Kill Cancer Cells?** Clin Cancer Res. 2015; 21(22): 5047-5056.

Mesci S., Marakli S., Yazgan B., Yıldırım T. **The effect of ATP-binding cassette (ABC) transporters in human cancers**. J Evid Based Med. 2019; 1(1): 14-19.

Miller J.S., Morishima C., McNeel D.G., Patel M.R., Kohrt H.E.K., Thompson J. A., et al. **A First-in-Human Phase I Study of Subcutaneous Outpatient Recombinant Human IL15 (rhIL15) in Adults with Advanced Solid Tumors**. Clin Cancer Res. 2018; 24(7): 1525-1535.

Moreno-Nieves U.Y., Tay J.K., Saumyaa S., Horowitz N.B., Shin J.H., Mohammad I.A., et al. **Landscape of innate lymphoid cells in human head and neck cancer reveals divergent NK cell states in the tumor microenvironment**. Proc Natl Acad Sci U S A. 2021; 118(28): e2101169118.

Müller T., Uherek C., Maki G., Chow K.U., Schimpf A., Klingemann H.G., et al. **Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcomes NK-resistance of lymphoma and leukemia cells**. Cancer Immunol Immunother. 2008; 57(3): 411-423.

Myers J.A., Miller J.S. **Exploring the NK cell platform for cancer immunotherapy**. Nat Rev Clin Oncol. 2021; 18: 85-100.

Neelapu S.S., Locke F.L., Bartlet, N.L., Lekakis L.J., Miklos D.B., Jacobson C.A., et al. **Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma**. N Engl J Med. 2017; 377(26): 2531-2544.

Nguyen-Pham T.N., Yang D.H., Nguyen T.A., Lim M.S., Hong C.Y., Kim M.H., et al. **Optimal culture conditions for the generation of natural killer cell-induced dendritic cells for cancer immunotherapy**. Cell Mol Immunol. 2012; 9(1): 45-53.

Nisonoff A., Rivers M.M. **Recombination of a mixture of univalent antibody fragments of different specificity**. Arch Biochem Biophys.1961; 93: 460-2.

Ordóñez-Reyes C., Garcia-Robledo J.E., Chamorro D.F., Mosquera A., Sussmann L., Ruiz-Patiño A., et al. **Bispecific Antibodies in Cancer Immunotherapy**. Pharmaceutics. 2022; 14(6): 1243.

Pekar L., Busch M., Valldorf B., Hinz S.C., Toleikis L., Krah S. **Biophysical and biochemical characterization of a VHH-based IgG-like bi- and trispecific antibody platform**. MAbs. 2020; 12(1): 1812210.

Placke T., Salih H.R., Kopp H.G. **GITR ligand provided by thrombopoietic cells inhibits NK cell antitumor activity**. J Immunol. 2012; 189(1): 154-160.

Platonova S., Cherfils-Vicini J., Damotte D., Crozet L., Vieillard V., Validire P., et al. **Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma**. Cancer Res. 2011; 71(16): 5412-5422.

Romee R., Schneider S.E., Leong J.W., Chase J.M., Keppel C.R., Sullivan R.P., et al. **Cytokine activation induces human memory-like NK cells**. Blood. 2012; 120(24): 4751-4760.

Rosario M., Liu B., Kong L., Collins L.I., Schneider S.E., Chen X., et al. **The IL-15-Based ALT-803 Complex Enhances FcγRIIIa-Triggered NK Cell Responses and In Vivo Clearance of B Cell Lymphomas**. Clin Cancer Res. 2016; 22(3): 596-608.

Rosenberg S.A., Lotze M.T., Muul L.M., Leitman S., Chang A.E., Ettinghausen S.E., 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(23): 1485-1492.

Seymour L., Tinker A., Hirte H., Wagtmann N., Dodion P. **Phase I and dose ranging, phase II studies with IPH2201, a humanized monoclonal antibody targeting HLA-E receptor CD94/NKG2A**. Ann Oncol. 2015; 26: ii3.

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(3): 301-6.

Shapiro R.M., Birch G.C., Hu G., Vergara Cadavid J., Nikiforow S., Baginska J., et al. **Expansion, persistence, and efficacy of donor memory-like NK cells infused for posttransplant relapse**. J Clin Invest. 2022; 132(11): e154334.

Sharma P., Hu-Lieskovan S., Wargo J.A., Ribas A. **Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy**. Cell. 2017; 168(4): 707-723.

Smyth M.J., Crowe N.Y., Pellicci D.G., Kyparissoudis K., Kelly J.M., Takeda K., et al. **Sequential production of interferon-gamma by NK1.1(+) T cells and natural killer cells is essential for the antimetastatic effect of alpha-galactosylceramide**. Blood. 2002; 99(4): 1259-1266.

Tang X., Yang L., Li Z., Nalin A.P., Dai H., Xu T., et al. **First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia**. Am J Cancer Res. 2018; 8(6): 1083-1089.

Tapia-Galisteo A., Compte M., Álvarez-Vallina L., Sanz, L. **When three is not a crowd: trispecific antibodies for enhanced cancer immunotherapy**. Theranostics. 2023; 13(3): 1028-1041.

Tonn T., Schwabe D., Klingemann H.G., Becker S., Esser R., Koehl U., et al. **Treatment of patients with advanced cancer with the natural killer cell line NK-92**. Cytotherapy. 2013; 15(12): 1563-1570.

Töpfer K., Cartellieri M., Michen S., Wiedemuth R., Müller N., Lindemann D., et al. **DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy**. J Immunol. 2015; 194(7): 3201-3212.

van Vuuren R.J., Botes M., Jurgens T., Joubert A.M., van den Bout I. **Novel sulphamoylated 2-methoxy estradiol derivatives inhibit breast cancer migration by disrupting microtubule turnover and organization**. Cancer Cell Int. 2019; 19: 1.

Vasan N., Baselga J., Hyman D.M. **A view on drug resistance in cancer.** Nature. 2019; 575(7782): 299-309.

Vey N., Karlin L., Sadot-Lebouvier S., Broussais F., Berton-Rigaud D., Rey J., et al. **A phase 1 study of lirilumab (antibody against killer immunoglobulin-like receptor antibody KIR2D; IPH2102) in patients with solid tumors and hematologic malignancies**. Oncotarget. 2018; 9(25): 17675-17688.

Viardot A., Bargou R. **Bispecific Antibodies in Haematological Malignancies**. Cancer Treat. Rev. 2018; 65: 87-95.

Vitale M., Cantoni C., Pietra G., Mingari M.C., Moretta L. **Effect of tumor cells and tumor microenvironment on NK-cell function.** Eur J Immunol. 2014; 44(6): 1582-1592.

Vivier E., Tomasello E., Baratin M., Walzer T., Ugolini S. **Functions of natural killer cells**. Nat Immunol. 2008; 9(5): 503-510.

Vyas M., Schneider A.C., Shatnyeva O., Reiners K.S., Tawadros S., Kloess S., et al. **Mono- and dual-targeting triplebodies activate natural killer cells and have anti-tumor activity in vitro and in vivo against chronic lymphocytic leukemia**. Oncoimmunolog. 2016; 5(9): e1211220.

Waldhauer I., Goehlsdorf D., Gieseke F., Weinschenk T., Wittenbrink M., Ludwig A., et al. **Tumor-associated MICA is shed by ADAM proteases**. Cancer Res. 2008; 68(15): 6368-6376.

Waldman A.D., Fritz J.M., Lenardo M.J. **A guide to cancer immunotherapy: from T cell basic science to clinical practice**. Nat Rev Immunol. 2020; 20: 651-668.

Wu J., Gao F.X., Wang C., Qin M., Han F., Xu T., et al. **IL-6 and IL-8 secreted by tumour cells impair the function of NK cells via the STAT3 pathway in oesophageal squamous cell carcinoma**. J Exp Clin Cancer Res. 2018; 38(1): 321.

Wu S.Y., Fu T., Jiang Y.Z., Shao, Z.M. **Natural killer cells in cancer biology and therapy**. Mol Cancer. 2020; 19(1): 120.

Xie G., Dong H., Liang Y., Ham J.D., Rizwan R., Chen J. **CAR-NK cells: A promising cellular immunotherapy for cancer**. EBioMedicine. 2020; 59: 102975.

Yu J., Freud A.G., Caligiuri M.A. **Location and cellular stages of natural killer cell development**. Trends Immunol. 2013; 34(12): 573-582.

Yu J., Wei M., Becknell B., Trotta R., Liu S., Boyd Z., et al. **Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells**. Immunity. 2006; 24(5): 575-590.

Zhang L., Meng Y., Feng X., Han Z. **CAR-NK cells for cancer immunotherapy: from bench to bedside**. Biomark Res. 2022; 10: 12.