Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(8): 284
https://doi.org/10.31083/j.fbl2908284

Editorial

New Insights into the Potential Role of Chimeric Activating Receptors-Engineered Natural Killer Cells to Fight Cancer

Loredana Cifaldi[1,*], Laura Masuelli[2], Roberto Bei[1,*]

[1]Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy
[2]Department of Experimental Medicine, University of Rome "Sapienza", 00161 Rome, Italy
*Correspondence: cifaldi@med.uniroma2.it (Loredana Cifaldi); bei@med.uniroma2.it (Roberto Bei)
Academic Editor: Graham Pawelec
Submitted: 20 May 2024 Revised: 11 July 2024 Accepted: 30 July 2024 Published: 16 August 2024

The treatment of adult and pediatric solid tumors remains a major challenge for oncologists. Considerable attention has focused on the adoptive transfer of *ex vivo* expanded and activated natural killer (NK) cells, defined as an excellent "off-the-shelf" product for novel cell-based anticancer therapeutic strategies [1].

Notably, NK cells are cytotoxic lymphocytes that participate in innate immune responses and recognize virus-infected and transformed cells without prior specific sensitization, exceptional specificity, or acquisition of long-term memory.

NK cell-mediated recognition and lysis of cancer cells are strongly dependent on the tumor cell surface expression of ligands recognized by NK cell-activating receptors [1,2]. The ligands for Natural-Killer receptor group 2, member D (NKG2D), are cellular stress-inducible major histocompatibility complex (MHC)-I-related proteins such as MICA, MICB, and six members of the UL16-binding protein (ULBP) family. The NKG2D receptor consists of a homodimer of two disulfide-associated transmembrane proteins that lack the ability to signal via intracellular domains. However, the adaptor protein DAP10 ensures signaling activation after NKG2D binding in humans [3]. The ligands for DNAX accessory molecule-1 (DNAM-1), such as the poliovirus receptor (PVR; nectin-like molecule, CD155) and Nectin-2 (CD112), are highly expressed in solid tumor cells and weakly expressed in normal tissue cells. Notably, inhibitory receptors such as T-cell immunoglobulin and ITIM domain (TIGIT), CD96 (TACTILE; T-cell activation, increased late expression) and PVRIG compete with DNAM-1 for binding to PVR and Nectin-2 ligands (PVR is recognized by TIGIT, and CD96 and Nectin-2 are recognized by TIGIT and PVRIG) [4]. Therefore, the antitumor efficacy of NK cells depends on their phenotype, which is governed by the expression of activating/inhibitory receptors, thus indicating the activated/exhausted status of the cells [4]. On the other hand, defective expression of NK cell-activating receptors has been reported in cancer patients [5], thus supporting the idea that rescue of NK cell-mediated signaling constitutes a rationale for the development of new NK cell-based immunotherapies.

Current good manufacturing practice (GMP) for the adoptive transfer of NK cells involve the expansion of NK cells with high activation and low exhaustion and with increased trafficking and killing performance [2]. The results of several clinical trials have demonstrated that NK cell-based immunotherapy in combination with cytokines, monoclonal antibodies (mAbs), and immune checkpoint inhibitors is an effective and safe anticancer treatment strategy (ClinicalTrials.gov and [2]). Moreover, we recently reported that a low dose of polyphenols improves NK cell functions *in vitro* [6]. In addition, the use of NK cells armed with chimeric antigen receptors (CARs), which recognize specific molecules expressed on the surface of cancer cells, has shown numerous advantages by ensuring the recognition and eradication of several types of cancer cells [7].

The biological rationale that led to the development of engineered NK cells was based on a wide range of limitations previously reported from the clinical use of CAR-T cells, that require stringent haploidentical mismatch conditions, which are difficult to apply to a wide range of cancer patients [8]. In contrast, NK cells can be used therapeutically in allogeneic settings with a major safety, since, unlike T cells, they showed a low risk of proliferation and fewer side effects [9]. The use of CAR-T cells showed limitations associated with antigen escape, poor tumor infiltration and trafficking, and high toxicity leading to the risk of graft-versus-host disease (GvHD) and cytokine release syndrome [8], pathological conditions that have never been reported after adoptive transfer of allogeneic NK cells (except for a few cases of cotransfusion with hematopoietic stem cells (HSCs), which are subsequently responsible for the development of GvHD) [9]. Different sources of NK cells have been explored, from established cell lines to allogeneic and alloreactive primary cells isolated from peripheral and umbilical cord blood or from induced pluripotent stem cells (iPSC), thus circumventing ethical issues that effectively limit the use of human embryonic stem cells (hESC), whose use remains restricted in some countries. Moreover, improved *ex vivo* amplification and transfection methods have made it possible to obtain more stable and efficient CAR-NK cells [1]. However, limits of *in vivo* cell persistence, transduction efficiency and infil-

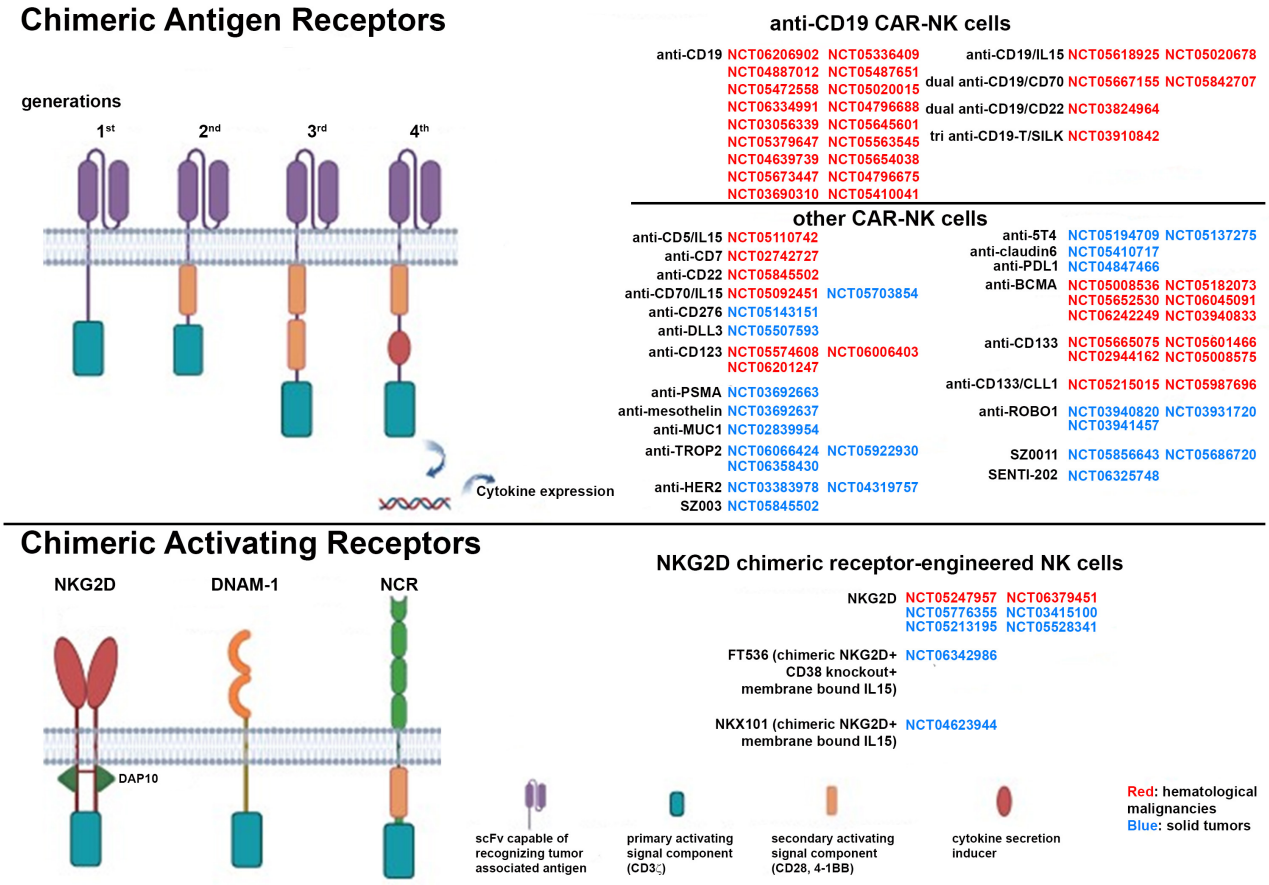

Fig. 1. Schematic model of chimeric antigen receptors (CARs) and chimeric-activating receptors-engineered Natural Killer (NK) cells and their clinical applications. Upper panel: Description of chimeric antigen receptors (CARs)-engineered NK cells and their clinical applications. Lower panel: Description of chimeric-activating receptors-engineered NK cells and their clinical applications. The ClinicalTrials.gov identification numbers for each clinical study are reported. scFv, single-chain fragment variable; NKG2D, Natural Killer receptor group 2, member D; DNAM-1, DNAX-accessory molecule-1; NCR, natural cytotoxic receptor.

tration capacity into the tumor microenvironment (TME) have not yet been completely overcome [2]. TME is composed of immunosuppressive cells that negatively regulate NK cells activity; their neutralization by monoclonal antibodies, in association with immune checkpoint inhibitors, should therefore support CAR-NK cell-mediated antitumor efficacy [2]. CARs expressed by NK cells are engineered membrane fusion proteins consisting of an extracellular domain (scFv; single-chain fragment variable) that targets tumor-associated and spacer/transmembrane domains that are linked to an intracellular region containing the primary activator (for example, the CD3ζ chain) and costimulatory signals (for example, 4-1BB and CD28). In contrast, chimeric activating receptors are composed of an extracellular region displaying NKG2D, DNAM-1, and natural cytotoxicity receptors (NCRs), such as NKp30, NKp44, and NKp46, which identify ligands that are specifically overexpressed in tumors or virus-infected cells. Furthermore, similar to CARs, chimeric activating receptors contain an intracellular domain that includes T-cell intracellular or costimulatory signaling units [1,10] (Fig. 1).

NK cells have been engineered with many different CARs containing a scFv, and these cells have been evaluated in 65 clinical trials to date (ClinicalTrials.gov, Fig. 1). Anti-CD19 CAR-NK cells were the first to be developed with supporting data from 24 clinical trials [11]. Other CAR-NK cells targeting CD5, CD7, CD22, CD33, CD70, CD123, CD276, DLL3, 5T4, Claudin6, PD-L1, BCMA, PSMA, mesothelin, TROP2, ErbB2/HER2, ROBO1 and MUC1 have been evaluated in 41 clinical trials to date. In contrast, NK cells engineered with chimeric NKG2D-activating receptors have been evaluated in only 8 clinical trials [1] (ClinicalTrials.gov, Fig. 1). In addition, the need to overcome the toxicity that often results from targeting molecules expressed not only by tumor cells but also by normal cells, necessitates the search for less toxic, more efficient and tumor-specific chimeric molecules, such as those represented by chimeric-activating receptors, for use in arming NK cells.

The use of NK cells engineered with the chimeric activating receptor NKG2D has been extended to several types of cancers (Fig. 1). NK cells expressing the chimeric ac-

tivating receptor NKG2D were engineered to express full-length NKG2D in frame with the CD3 ζ chain as a first-generation product and were then engineered with costimulatory molecules such as DAP10 and 4-1BB as second- and third-generation products [1]. The NK-92 cell line was replaced by *ex vivo* expanded and activated primary NK cells as the source of NK cells [1]. Furthermore, lentiviral transduction allowed to obtain more efficient and stable NKG2D-CAR-NK cells [1].

Therefore, NK cells engineered with chimeric NKG2D have rapidly gained prominence as one of the most promising types of nontoxic chimeric molecule-engineered NK cells with activity against tumors expressing ligands recognized by NKG2D. The successful use of NK cells engineered with chimeric NKG2D is also due to their potential to restore the often compromised immune response [2]. Indeed, chimeric NKG2D receptor-engineered NK cells have been reported to bind ligands expressed by tumor-infiltrating myeloid-derived suppressor cells (MDSCs) in the TME, thus neutralizing the immunosuppressive functions of these cells [12].

The use of NKG2D chimeric activating receptor-engineered NK cells, based on the high expression of ligands recognized by NKG2D on both hematological and solid tumor cells, has also broadened their application prospects for the treatment of solid tumors, as compared with hematological malignancies (27 for solid tumors compared with 38 for Acute Myeloid Leukemia (AML), Multiple Myeloma (MM) and B-lymphoma; Fig. 1). On the basis of this need and considering that many solid tumor cells exhibit high expression of ligands recognized mainly by DNAM-1, such as PVR and Nectin-2, we recently demonstrated the effective cytotoxicity of DNAM-1 chimeric activating receptor-engineered NK cells in neuroblastoma cell lines [10,13,14]. We demonstrated the cytotoxic potential of primary human NK cells engineered with chimeric DNAM-1-CD3ζ, which was further increased by immunomodulation with Nutlin-3a targeting MDM2 [14]. The overexpression of a chimeric form of DNAM-1 led to direct competition with inhibitory receptors such as TIGIT, CD96, and PVRIG for the binding of PVR and Nectin-2 [4], thus conferring an advantage over the physiological expression of DNAM-1. However, further efforts to develop stable methods of transfection for engineering human primary NK cells with chimeric DNAM-1 receptors are required for the translation of this approach into clinical practice. Thus, the use of drugs to antagonize MDM2 and restore p53 function might increase the expression of ligands for NK cell-activating receptors [14], thus further supporting the activities of DNAM-1 chimeric activating receptor-engineered NK cells.

Very recently, NK cells engineered with NCRs such as NKp30, NKp46 and especially NKp44 depending on DAP12, conjugated to an extracellular anti-HER2 scFv, were reported to augment NK cell activities, such as tumor lysis and cytokine production, against ovarian cancer cells [15].

Overall, NK cells engineered with chimeric activating receptors have shown promising results, as reported in preclinical studies and clinical trials. However, further efforts are needed to generate engineered NK cells with greater antitumor efficacy and less toxicity. In addition, improved methods should be employed to make engineered NK cells more stable, easier to apply clinically, and easier to cryopreserve for immediate use against a broad spectrum of tumors, mainly solid tumors.

Author Contributions

LC, ML, RB wrote the editorial. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We gratefully acknowledge the assistance in Fig. 1 preparation from Dr. Raffaele Carrano recipient of the Tor Vergata PhD program in Tissue Engineering and Remodeling Biotechnologies for Body Functions.

Funding

This research was funded by Ministero dell'Università e della Ricerca, PRIN 2022 grants CUP: E53D23001190006 to Loredana Cifaldi.

Conflict of Interest

The authors declare no conflict of interest. Given Roberto Bei's role as the Editorial Board Member, he had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Graham Pawelec.

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