Effectiveness and Comparison of CAR-NK in Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a deadly cancer of the plasma cells, which disrupts antibody production and overall health. Commonly used treatments for MM include chemotherapy, radiation therapy, and a less used CAR-T therapy. Although these therapies are mostly effective, debilitating and severe side effects can emerge. CAR-NK therapy poses a promising treatment for patients with MM, as the CAR-NK cytokine profile allows for lower side effects compared to CAR-T and still shows strong anti-tumor action. This review evaluates the mechanism and function of CAR-NK receptors and their efficacy against multiple myeloma compared to other therapies.

I. Introduction

1.1: Innate Immunity

The innate immune system is an important part of the body for survival. Due to the extremely fast doubling time of many pathogens like bacteria and viruses, a fast immune response known as the innate immune system is needed. Unlike the adaptive immune system, the innate immune system is nonspecific. Instead of recognizing a specific antigen via B or T cell receptors, cells of the innate immune system recognize classes of pathogens which allow them to be activated quickly. Innate immunity is a very diverse system consisting of leukocytes, innate lymphocytes, antimicrobial peptides, and complement. [1]

1.2: NK Lymphocytes

NK (Natural Killer cells) effector lymphocytes of the innate immune system which limit microbial growth and tumor growth. NK cells are large granular lymphocytes that derive from the common lymphoid progenitor, similar to B and T cells. Similar to cytotoxic T lymphocytes, NK cells eliminate infected cells and tumors in their early stages, but unlike cytotoxic T lymphocytes, NK cells can eliminate abnormal cells in the absence of MHC-1 or antibodies, allowing for a much faster immune reaction. [24] NK lymphocytes generally CD56 positive and CD3 negative (CD56+, CD3-). They possess both activating and inhibiting receptors, which allow NK cells to have a wide variety of functions. [26]

Activating Receptors

NK lymphocytes possess activating receptors including:

- Ly49, a homodimer which binds to MHC I to distinguish self from non-self.
    - In addition, if NK cells do not receive an MHC I signal, they will be activated. This is known as the *missing self hypothesis.* [30]
- CD16, important in ADCC, which allows NK cells to recognize IgG bound to pathogens. [17]
- NCRs, natural cytotoxicity receptors, which are type 1 transmembrane receptors that mediate NK killing and release IFNγ. They commonly bind to diverse ligands expressed on tumors or viral infected cells. [13,23]
- TLRs, Toll-like Receptors, which allow NK cells to recognize a variety of molecules that belong to pathogens. [33]
- NKG2 (CD159), which binds to nonclassical MHC-1 such as Qa1b and HLA-E which activates NK cytotoxicity. [10]

Inhibiting Receptors

NK lymphocytes possess inhibiting receptors including:

- KIRs, killer cell immunoglobulin-like receptors, regulate killing of NK cells by interacting with MHC I. [25]
- CD94, can stimulate or inhibit NK cell activity, and can dimerize with NKG2 to become an inhibitor. [10]
- Ly49 also has inhibitory activity. [30]

Tumor Surveillance

NK lymphocytes are extremely important to prevent the progression of cancer. While T cells require a specific antigen binding, NK cells can directly kill many cells without needing antigenic peptides or surface adhesion molecules. NK cells produce a number of cytokines, including TNF$\boldsymbol{\alpha}$, IL-10, and IFNγ. TNF$\boldsymbol{\alpha}$ is a proinflammatory cytokine and IL-10 is an immunosuppressive one. [26] NK cells also express Fc receptors, which allows them to bind to the Fc region of IgG antibodies, allowing ADCC to happen. [34,38]

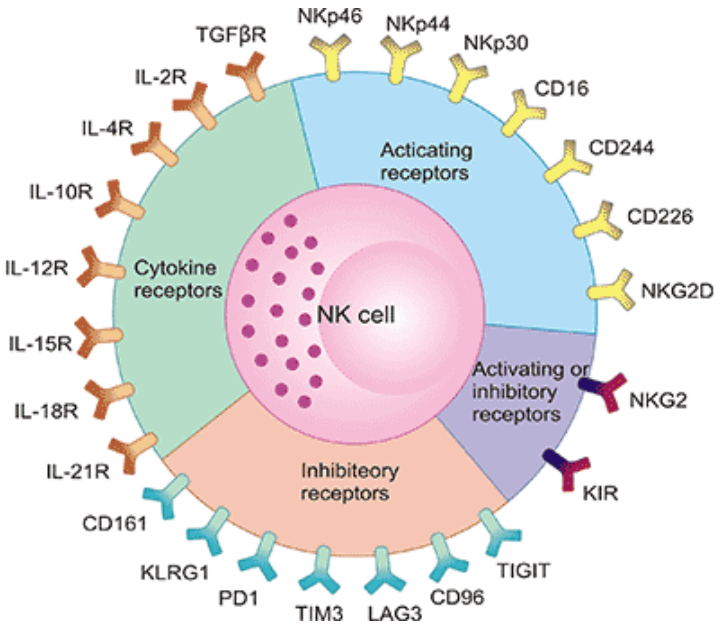

Figure 1. *NK cell receptors. Figure adapted from* [39]

1.3: Multiple Myeloma

Multiple myeloma (MM) is a deadly cancer of the plasma cells. Plasma cells are critical in producing functional antibodies for immunity, but in MM they are cancerous and produce nonfunctional proteins in addition to crowding out healthy cells. [19] In 2020, an estimated 117,077 people died from multiple myeloma. It is estimated that 12,590 people will die from the disease in 2023. The relative 5-year survival rate is about 58%. [18]

Types of Multiple Myeloma

There are 8 general types of multiple myeloma: [37]

- *Light chain myeloma*. Patients with light chain myeloma cannot produce functional antibodies which causes them to deposit in the kidneys. About 20% of MM patients have LCM.
- *Non-secretory myeloma.* Patients with non-secretory myeloma do not produce enough M proteins or light chains to show on tests. Antibody production is extremely low.
- *Solitary plasmacytoma,* a localized tumor that stems from cancerous plasma cells. MM is likely for those with solitary plasmacytomas.
- *Extramedullary plasmacytoma.* Tumors grow in soft tissue bone marrow. About 30% of people with extramedullary plasmacytoma will develop MM.
- *Monoclonal Gammopathy of Undetermined Significance.* Patients with MGUS have higher levels of M proteins and are asymptomatic, but may progress to active myeloma.
- *Smoldering Multiple Myeloma,* an asymptomatic type of multiple myeloma that can progress into active myeloma.

- *IgD Myeloma,* a rare type that affects 1%-2% of myeloma patients. The signs and symptoms are the same as the other types.
- *IgE Myeloma,* the rarest type of multiple myeloma. It tends to be more aggressive and can cause leukemia.

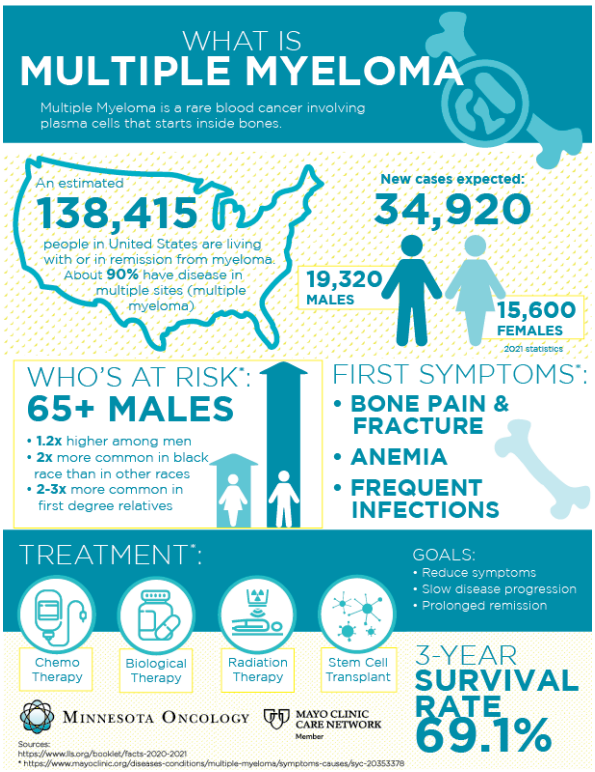

Figure 2. *Multiple myeloma statistics. Figure adapted from* [41]

II. CAR-NK Molecular Interactions

2.1: Chimeric Antigen Receptor

A chimeric antigen receptor is a receptor protein that allows an immune cell to recognize any antigen engineered. They are *chimeric* because they combine both antigen recognition and cell activation in one receptor. CARs are extremely advantageous because they allow the immune cell to kill once recognizing an antigen, independent of MHC restrictions. [4,32]

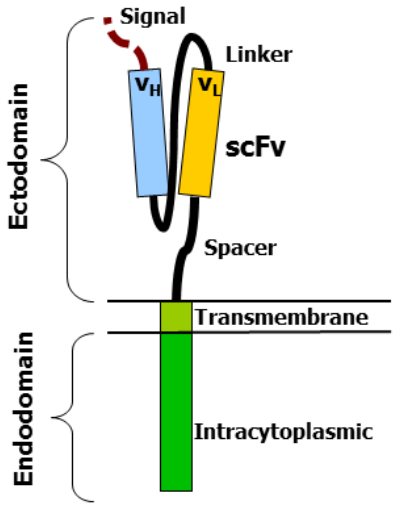

Figure 3. *Chimeric Antigen Receptor. Figure adapted from* [20]

Antigen Recognition Domain

CARs are typically created by combining a receptor complex and a monoclonal antibody (mAb). The mAb will recognize an antigen that the immune cell would not usually recognize. The variable region of the heavy and light chain is taken from the mAb (single chain variable fragment, or scFv) and combined with the receptor complex and joined by a linker. The mAb to derive an scFv from is chosen in advance to recognize a desired antigen. However, not all CARs are restricted to using scFvs. [8]

Hinge Region

The hinge region, or spacer, sits between the antigen recognition domain and transmembrane domain.These hinges can be derived from a wide variety of immune molecules, but ideally, a spacer should allow the receptor to be flexible and promote antigen binding. However, the specific mechanism is still unknown. Flexibility in the hinge region has shown to affect CAR function in CAR-T lymphocytes. [8] Zhang et al. has shown that while increasing the CAR hinge flexibility by using IgG instead of CD28 alone causes an increase of pro-cytokines and better recognition of antigens, reducing the flexibility by removing two consecutive glycine residues allows for better tumor control. Pro-inflammatory cytokines such as TNF***α*** and IL-6 were reduced and prevented CAR-T overreaction. [42]

Transmembrane Domain

The transmembrane domain allows the CAR to be anchored stably to the immune cell. It consists of a hydrophobic alpha helix that spans the plasma membrane and binds the

extracellular domain with the intracellular domain. CD28 has been shown to create a very stable highly expressed receptor. [15]

Intracellular Signaling Domain

The intracellular domain is important for the immune cell to begin its cytotoxic action. They seek to mimic classical lymphocyte activation as closely as possible. For example, T cell activation relies on immunoreceptor tyrosine-based activation motifs (ITAMs). Thus, stimulatory domains such as CD3ζ are employed. Further generations of CAR-T use a "Frankenstein" approach by introducing proteins from more stimulatory domains onto the CAR. [22]

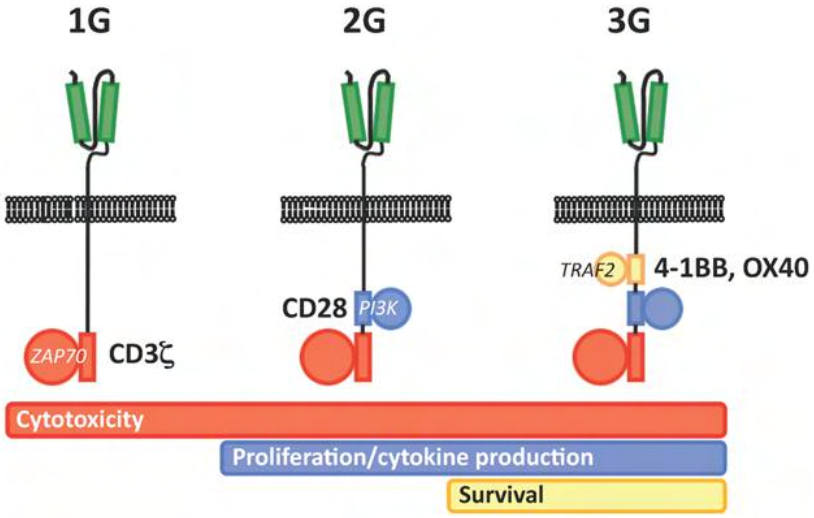

Figure 4. *Different generations of CAR-T intracellular domains. Figure adapted from* [6]

2.2: CAR-NK Immunotherapy

CAR-NK immunotherapy occurs in the following stages:

1. Acquiring NK cells
2. CAR-NK engineering
3. Culturing of NK cells
4. Body Preparation
5. Infusion of NK cells
6. Monitoring patient

Acquiring NK cells

NK cells for CAR engineering can be derived from a variety of sources.

Apheresis is a process where blood is drawn from the body to extract NK lymphocytes. Typically patients will receive citrate to reduce clotting. Leukocytes and stem cells are extracted with centrifugation while the rest of the blood is returned via IV. [28]

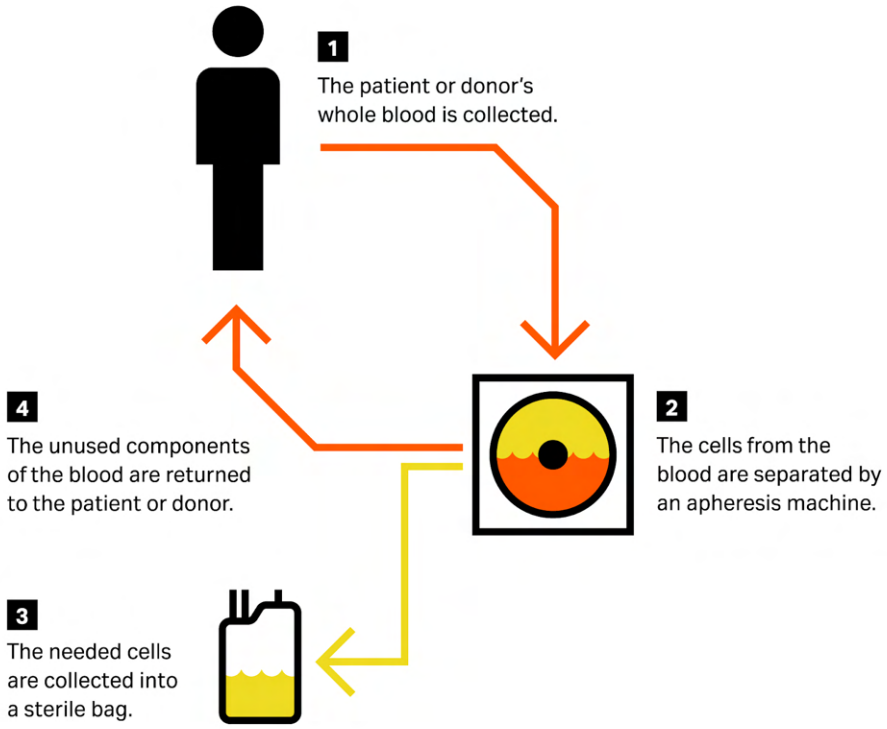

Figure 5. *Apheresis process. Figure adapted from* [3]

Other sources include umbilical cord blood, the placenta, memory like NK cells, and stem cell-derived NK cells.

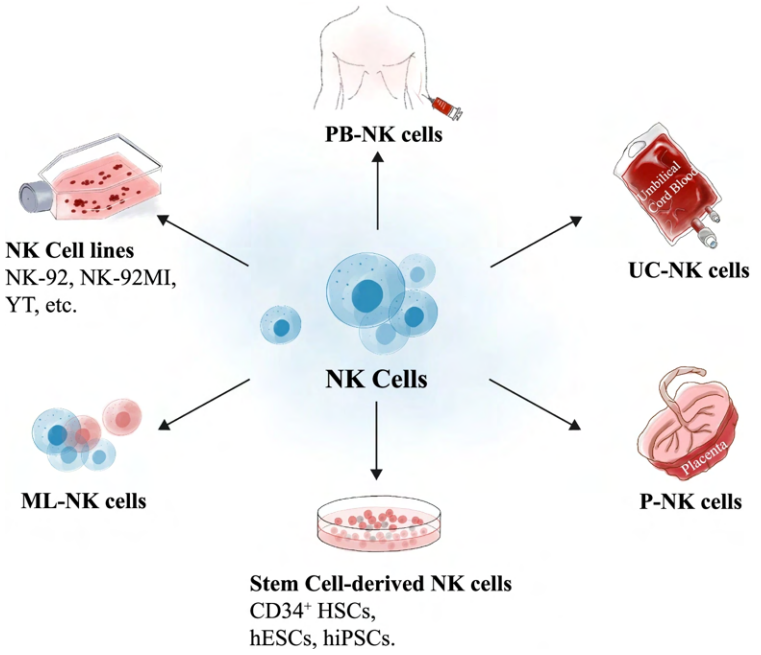

Figure 6. *Sources of NK cells. Figure adapted from* [43]

CAR-NK Receptor Structure

The CAR-NK receptor is very similar to the CAR-T receptors, in that similar extracellular, transmembrane, and intracellular domains are present in both cell types.

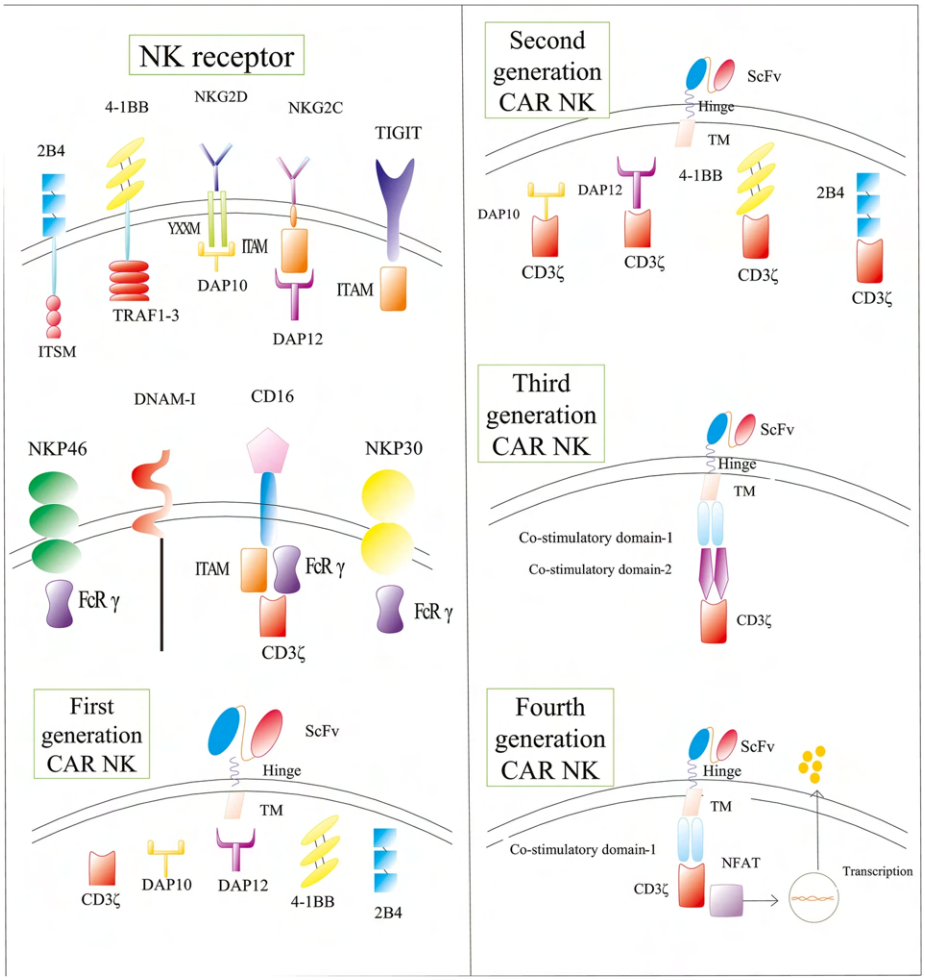

Figure 7. *CAR-NK receptor generations. Figure adapted from* [9]

In this model, generation 1 CAR-NKs have a signaling domain consisting of only CD3ζ or DAP12. DAP12 appears to be the best out of CD3ζ, DAP10, and DAP12 in activating NK cells. Second generation CAR-NKs express CD28, 4-1BB, and 2B4 in conjunction with the primary signaling domain. This allows the cell to form an intracellular signaling motif. Third generation CAR-NKs also express CD134 and NKG2D to further increase cytotoxicity and cytokine production. Due to NKG2D's NK mechanisms, a construct where NKG2D as the ectodomain to link DAP10 and CD3ζ was developed. Fourth generation CARs (TRUCKs) can express cytokines to further enhance tumor killing with the aid of signaling domain NFAT. [9] CAR-NKs can be engineered via viral vectors carrying the CAR gene. Newer technologies involving CRISPR-NKs have also been researched. [21]

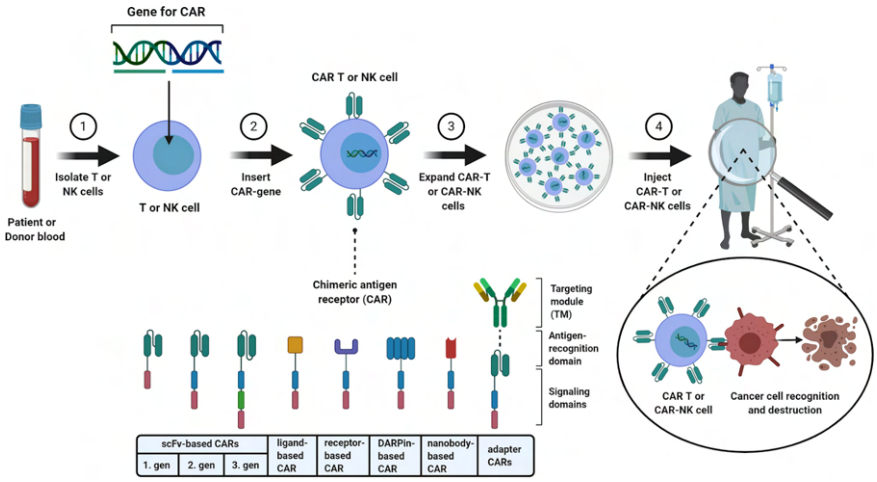

Figure 8. *CAR treatment schema. Figure adapted from* [2]

Body Preparation

To prepare the body for CAR-NK therapy, the patient commonly undergoes chemotherapy. This is to lower the number of other immune cells to increase the CAR-NK killing capacity. Some patients may also undergo radiation therapy to irradiate the bone marrow.

Culturing NK cells

NK cells can then be cultured in the lab to grow millions for the cancer patient. This process can take weeks, and can also be analyzed with flow cytometry to see the number of each type of NK lymphocyte.

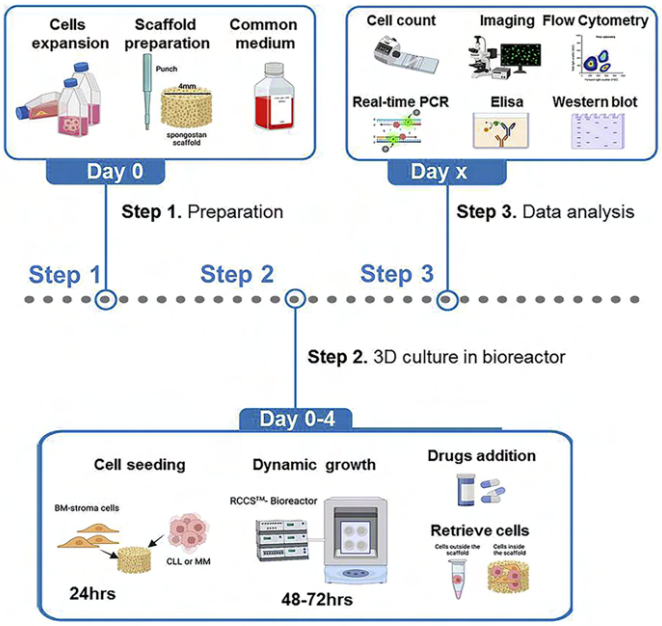

Figure 9. *CAR engineering process. Figure adapted from* [29]

Infusion of NK cells

Similar to a blood transfusion, the new CAR-NK cells are infused through a central line. The patient is then monitored.

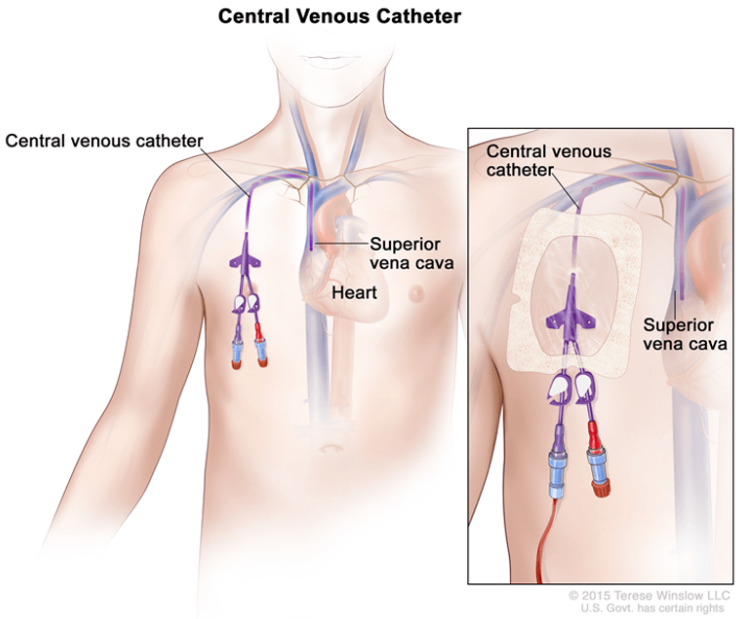

Figure 10. *Central Line. Figure adapted from* [27]

2.3: CAR-NK Effects

The chimeric antigen receptor on CAR-NK cells can be engineered to target antigens found on various cancers.

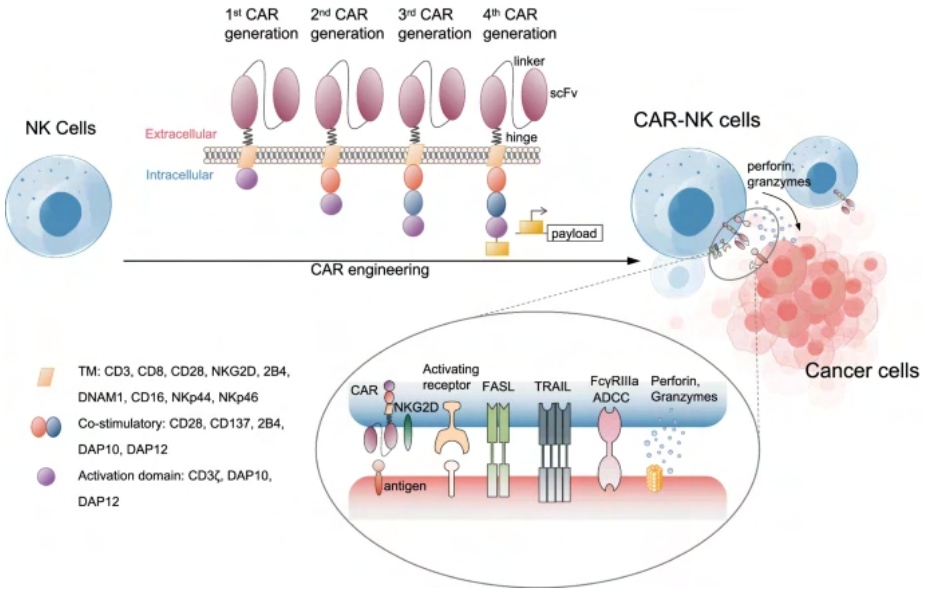

Figure 11. *CAR-NK function. Figure adapted from* [43]

CAR-NKs have the special ability to directly kill cells expressing a particular antigen without MHC restrictions. With their intracellular signaling pathways, CAR-NKs can efficiently destroy tumor cells.

Several receptor ligand complexes are involved in this process:

- CAR and NKG2D, which recognize the antigen.
- FASL and FcγRIIIa, which can activate cytolytic pathways through ITAMs.
- TRAIL, TNF-related apoptosis inducing ligands, cytokines that induce apoptosis through cell surface death receptors. [36]

CAR-NKs will secrete perforins and granzymes to rapidly destroy the cancerous cell.

In addition, CAR-NK was seen to have minimal side effects. [40]

III. CAR-NK Cancer Efficacy

3.1: Effectiveness of Other Treatments

Chemotherapy

Chemotherapy essentially uses powerful cytotoxic drugs to kill cancerous cells. Indeed, a wide diversity of chemotherapy drugs for MM allows for effective remission in many individuals. However, side effects are a noticeable concern.

Results of Recent Phase III Randomized Studies in Newly Diagnosed Myeloma

Trial	Regimen	No. of patients	Overall response rate (%)	CR plus VGPR (%)	Progression-free survival (Median in months)	P value for progression free survival	Overall survival (Median in months)*	P value for overall survival
Durie et al (S0777)[42]	Rd	229	72	32	31	0.002	64	0.025
	VRd	242	82	43	43		75	
Attal et al (IFM 2009)[43]	VRd	350	97	77	36	<0.001	NR; 82% at 4 years	0.87
	VRd-ASCT	350	98	88	50		NR; 81% at 4 years	
Facon et al (MAIA)[87]	Rd	369	81	53	32	<0.001	NR	N/A
	DRd	368	93	79	NR; 71% at 30 months		NR	
Moreau et al (CASSIOPEIA)[88]	VTd	542	90	78	NR; 85% at 18 months	<0.001	NR; 90% at 30 months	P<0.05
	Dara-VTd	543	90	83	NR; 93% at 18 months		NR; 96% at 30 months	
Facon et al (TOURMALINE MM2)[89]	Rd	354	80	48	22	0.073	NR; 52% at 5 years	0.99
	IRd	351	82	63	35		NR; 52% at 5 years	
Kumar et al (ENDURANCE)[90]	VRd	542	85	65	34	0.74	84 at 3 years	0.92
	KRd	545	87	74	35		86 at 3 years	

*Estimated from survival curves when not reported

Figure 12. *Chemotherapy effectiveness in multiple myeloma. Abbreviations: Rd, lenalidomide plus dexamethasone; VRd, bortezomib, lenalidomide plus dexamethasone; DRd, daratumumab, lenalidomide, dexamethasone; VTd, bortezomib, thalidomide, dexamethasone; Dara-VTd, daratumumab, bortezomib, thalidomide, dexamethasone; IRd, ixazomib, lenalidomide, dexamethasone; KRd, carfilzomib, lenalidomide, dexamethasone; ASCT, autologous stem cell transplantation; N/A, not available; CR, complete response; VGPR, very good partial response. Figure adapted from* [31]

Radiation Therapy

Radiation therapy is largely ineffective against multiple myeloma alone, but helps patients ease pain and may be used in conjunction with chemotherapy. [7]

CAR-T

CAR-T is a close relative of CAR-NK therapy. Instead of using NK cells for expressing CARs, T lymphocytes are used. CAR-T has been called a “breakthrough” by the FDA and has been seen to show complete remission in 50% of ALL patients and have also been repurposed for autoimmune diseases.

CAR-T lymphocytes engineered to target BCMA (which is exclusively expressed on plasma cells) were engineered by NCI investigators and showed that while there was a high response rate, cytokine release syndrome was also seen. [5]

Summary of major BCMA CAR T-cell trials

Trial	Dose Range	Response Rate	VGPR or better	PFS	CRS any grade (grade 3-4)	Neurotoxicity any grade
Bb2121 (n=33)	50-800 million cells	85%	72%	11.8 months	76% (6%)	42%
JCARH125 (n=44)	50-450 million cells	82%	48%	NA	80% (9%)	25%
LCAR-B38M (n=57)	0.07 to 2.1 million cells/kg	88%	73%	15 months	90% (7%)	2%
P-BCMA-101 (n=19)	50-1143 million cells	63%	22%	9.5 months	10% (0%)	5%

Figure 13. *CAR-T trials. Figure adapted from* [35]

3.2: CAR-NK Effectiveness

A preclinical study from 2021 by *Leivas et al.* showed that antitumor activity of NKG2D-CAR *in vitro* (86.6% ± 13.9%) was considerably higher than CD45RA- T lymphocytes (16.7% ± 13.6%).
.
Multiple myeloma cells were injected into a NSG mouse model to evaluate CAR effectiveness *in vivo*. At day 4 the mice were injected with CAR-NK or non transduced NK cells. After 14 days, it was reported that CAR-NK showed higher antitumor efficiency. After 42 days, only mice receiving CAR-NK remained free from disease.

It was found that even with CAR transduction CD45RA- T lymphocytes were ineffective in eliminating MM *in vitro.* Using CAR-NKG2D NKAE was most effective both *in vivo* and *in vitro* models. [16]

3.3: Analysis of NK

CAR-NK has been shown to effectively kill multiple myeloma cells with its CAR and its CAR-independent NK mediated killing.

Advantages over CAR-T

CAR-NK is a superior form of adoptive cell therapy that does not require autologous immune cells because of their alternative cytokine profiles. T cells can cause graft-versus-host disease (GVHD) when used from a different individual, but NK cells can actually suppress GVHD while maintaining all antitumor activity. NKs can also come from preexisting cell lines which simplifies NK acquisition. CAR-T acquisition can be cumbersome and make many patients ineligible for its therapy. [11,12]

CAR-NK has also been shown to have lower neurotoxicity and cytokine release syndrome, which is extremely common in CAR-T therapy. CAR-NKs do not have an increase of inflammatory cytokines and instead release different profiles such as GM-CSF. [22]

As a generally safer, more accessible, and diverse adoptive cell therapy, with more studies and clinical trials CAR-NK can be a widely available off the shelf cancer immunotherapy. [43]

Properties	CAR-T	CAR-NK
Low risk of GVHD		✓
High tumor-killing potential	✓	✓✓
Low risk of Cytokine release syndrome		✓
High graft-versus-tumor (GVT) potential		✓
Low cost off-the-shelf cancer immunotherapy	✓	✓✓
Sources of harvestation	✓	✓✓

Figure 14. *Comparing CAR-T and CAR-NK. Figure adapted from* [14]

3.4: Limitations

Travel to Tumors

Homing to tumor sites is governed by a complex network of chemokines and cellular interactions. NK cell homing to tumors has been controversial and prompted improvements including adding chemokine receptors such as CCR7 and overexpressing CXCR3. [14]

Low Persistence

NK cell durability is an observed problem within CAR-NK therapy. Although CAR-NK therapy may be safer, the efficacy may be lowered. Exogenous cytokines can increase proliferation but also increase undesired side effects like inhibitory immune cells. Fourth generation CARs with transgenes coding for cytokines are under development. [14]

Immunosuppression

The tumor microenvironment (TME) contains immunosuppressive chemicals which may hinder the response of CAR-NK cells. These immunosuppressive chemicals include TGF-β, IDO, and PGE2. Researchers are working toward engineering NK cells to be resistant to these immunosuppressive chemicals and improve antitumor activity. [14]

Conclusion

CAR-NK is a promising new therapy that improves on previously approved adoptive cell transfer therapies. Engineering the NK cell with a CAR allows it to recognize new antigens in addition to its CAR-independent antitumor capacities. Due to the nature and cytokine profile of the NK cell, CAR-NK therapy also overcomes the previously found CRS and neurotoxicity with CAR-T therapy. Multiple myeloma studies with CAR-NK found its remarkable efficacy to eliminate tumors and minimize side effects, and its potential to become an off-the-shelf product. Progress and advancement in the cancer immunotherapy fields have led to new therapies and ideas. With increases in clinical trials and investigations, CAR-NK will see progress and improvements in the near future.

References

1. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Innate Immunity. In *Molecular Biology of the Cell. 4th edition*. Garland Science. https://www.ncbi.nlm.nih.gov/books/NBK26846/
2. Albinger, N., Hartmann, J., & Ullrich, E. (2021). Current status and perspective of CAR-T and CAR-NK cell therapy trials in Germany. *Gene Therapy*, *28*(9), 513–527. https://doi.org/10.1038/s41434-021-00246-w
3. *Apheresis variability control*. (n.d.). Cytiva. Retrieved October 29, 2023, from https://www.cytivalifesciences.com/en/us/solutions/cell-therapy/knowledge-center/resources/apheresis-variability-control
4. *CAR T cell principles*. (2018, June 28). Carl DeSelm Lab. https://deselmlab.wustl.edu/research/car-t-cell-principles/
5. Carpenter, R. O., Evbuomwan, M. O., Pittaluga, S., Rose, J. J., Raffeld, M., Yang, S., Gress, R. E., Hakim, F. T., & Kochenderfer, J. N. (2013). B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *19*(8), 2048–2060. https://doi.org/10.1158/1078-0432.CCR-12-2422
6. Casucci, M., & Bondanza, A. (2011). Suicide Gene Therapy to Increase the Safety of Chimeric Antigen Receptor-Redirected T Lymphocytes. *Journal of Cancer*, *2*, 378–382. https://doi.org/10.7150/jca.2.378

7. Contributors, W. E. (n.d.). *Radiation Therapy for Treating Multiple Myeloma*. WebMD. Retrieved October 29, 2023, from https://www.webmd.com/cancer/multiple-myeloma/multiple-myeloma-radiation
8. Dotti, G., Gottschalk, S., Savoldo, B., & Brenner, M. K. (2014). Design and Development of Therapies using Chimeric Antigen Receptor-Expressing T cells. *Immunological Reviews*, *257*(1), 10.1111/imr.12131. https://doi.org/10.1111/imr.12131
9. Elahi, R., Heidary, A. H., Hadiloo, K., & Esmaeilzadeh, A. (2021). Chimeric Antigen Receptor-Engineered Natural Killer (CAR NK) Cells in Cancer Treatment; Recent Advances and Future Prospects. *Stem Cell Reviews and Reports*, *17*(6), 2081. https://doi.org/10.1007/s12015-021-10246-3
10. Gunturi, A., Berg, R. E., & Forman, J. (2004). The role of CD94/NKG2 in innate and adaptive immunity. *Immunologic Research*, *30*(1), 29–34. https://doi.org/10.1385/IR:30:1:029
11. Habib, S., Tariq, S. M., & Tariq, M. (2019). Chimeric Antigen Receptor-Natural Killer Cells: The Future of Cancer Immunotherapy. *The Ochsner Journal*, *19*(3), 186–187. https://doi.org/10.31486/toj.19.0033
12. Hu, Y., Tian, Z., & Zhang, C. (2018). Chimeric antigen receptor (CAR)-transduced natural killer cells in tumor immunotherapy. *Acta Pharmacologica Sinica*, *39*(2), Article 2. https://doi.org/10.1038/aps.2017.125
13. Hudspeth, K., Silva-Santos, B., & Mavilio, D. (2013). Natural Cytotoxicity Receptors: Broader Expression Patterns and Functions in Innate and Adaptive Immune Cells. *Frontiers in Immunology*, *4*, 69. https://doi.org/10.3389/fimmu.2013.00069

14. Khawar, M. B., & Sun, H. (2021). CAR-NK Cells: From Natural Basis to Design for Kill. *Frontiers in Immunology*, *12*. https://www.frontiersin.org/articles/10.3389/fimmu.2021.707542
15. Leddon, S. A., Fettis, M. M., Abramo, K., Kelly, R., Oleksyn, D., & Miller, J. (2020). The CD28 Transmembrane Domain Contains an Essential Dimerization Motif. *Frontiers in Immunology*, *11*, 1519. https://doi.org/10.3389/fimmu.2020.01519
16. Leivas, A., Rio, P., Mateos, R., Paciello, M. L., Garcia-Ortiz, A., Fernandez, L., Perez-Martinez, A., Lee, D. A., Powell, D. J., Valeri, A., & Martinez-Lopez, J. (2018). NKG2D-CAR Transduced Primary Natural Killer Cells Efficiently Target Multiple Myeloma Cells. *Blood*, *132*, 590. https://doi.org/10.1182/blood-2018-99-114522
17. Mandelboim, O., Malik, P., Davis, D. M., Jo, C. H., Boyson, J. E., & Strominger, J. L. (1999). Human CD16 as a lysis receptor mediating direct natural killer cell cytotoxicity. *Proceedings of the National Academy of Sciences*, *96*(10), 5640–5644. https://doi.org/10.1073/pnas.96.10.5640
18. *Multiple Myeloma—Statistics*. (2012, June 25). Cancer.Net. https://www.cancer.net/cancer-types/multiple-myeloma/statistics
19. *Multiple myeloma—Symptoms and causes—Mayo Clinic*. (n.d.). Retrieved October 29, 2023, from https://www.mayoclinic.org/diseases-conditions/multiple-myeloma/symptoms-causes/syc-20353378
20. Mxpule. (2011). *English: Chimeric antigen receptor*. Own work. https://commons.wikimedia.org/wiki/File:CAR_cartoon.png

21. Naeimi Kararoudi, M., Likhite, S., Elmas, E., Yamamoto, K., Schwartz, M., Sorathia, K., De Souza Fernandes Pereira, M., Sezgin, Y., Devine, R. D., Lyberger, J. M., Behbehani, G. K., Chakravarti, N., Moriarity, B. S., Meyer, K., & Lee, D. A. (2022). Optimization and validation of CAR transduction into human primary NK cells using CRISPR and AAV. *Cell Reports Methods*, *2*(6), 100236. https://doi.org/10.1016/j.crmeth.2022.100236
22. Pan, K., Farrukh, H., Chittepu, V. C. S. R., Xu, H., Pan, C., & Zhu, Z. (2022). CAR race to cancer immunotherapy: From CAR T, CAR NK to CAR macrophage therapy. *Journal of Experimental & Clinical Cancer Research*, *41*(1), 119. https://doi.org/10.1186/s13046-022-02327-z
23. Paul, S., & Lal, G. (2017). The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. *Frontiers in Immunology*, *8*, 1124. https://doi.org/10.3389/fimmu.2017.01124
24. Pegram, H. J., Andrews, D. M., Smyth, M. J., Darcy, P. K., & Kershaw, M. H. (2011). Activating and inhibitory receptors of natural killer cells. *Immunology and Cell Biology*, *89*(2), 216–224. https://doi.org/10.1038/icb.2010.78
25. Pende, D., Falco, M., Vitale, M., Cantoni, C., Vitale, C., Munari, E., Bertaina, A., Moretta, F., Del Zotto, G., Pietra, G., Mingari, M. C., Locatelli, F., & Moretta, L. (2019). Killer Ig-Like Receptors (KIRs): Their Role in NK Cell Modulation and Developments Leading to Their Clinical Exploitation. *Frontiers in Immunology*, *10*. https://www.frontiersin.org/articles/10.3389/fimmu.2019.01179
26. Pfefferle, A., Jacobs, B., Haroun-Izquierdo, A., Kveberg, L., Sohlberg, E., & Malmberg, K.-J. (2020). Deciphering Natural Killer Cell Homeostasis. *Frontiers in Immunology*, *11*, 812. https://doi.org/10.3389/fimmu.2020.00812

27. Philadelphia, T. C. H. of. (2014, March 30). *Tunneled Central Line (Tunneled Central Venous Catheter)* [Text]. The Children's Hospital of Philadelphia. https://www.chop.edu/treatments/tunneled-central-line

28. Philadelphia, T. C. H. of. (2016, January 21). *T Cell Collection by Apheresis* [Text]. The Children's Hospital of Philadelphia. https://www.chop.edu/services/t-cell-collection-apheresis

29. *Protocol for generation of 3D bone marrow surrogate microenvironments in a rotary cell culture system*. (n.d.). Retrieved October 29, 2023, from https://star-protocols.cell.com/protocols/1888

30. Rahim, M. M. A., Tu, M. M., Mahmoud, A. B., Wight, A., Abou-Samra, E., Lima, P. D. A., & Makrigiannis, A. P. (2014). Ly49 Receptors: Innate and Adaptive Immune Paradigms. *Frontiers in Immunology*, *5*, 145. https://doi.org/10.3389/fimmu.2014.00145

31. Rajkumar, S. V. (2022). Multiple Myeloma: 2022 update on Diagnosis, Risk-stratification and Management. *American Journal of Hematology*, *97*(8), 1086–1107. https://doi.org/10.1002/ajh.26590

32. Sadelain, M., Brentjens, R., & Riviere, I. (2013). The basic principles of chimeric antigen receptor (CAR) design. *Cancer Discovery*, *3*(4), 388–398. https://doi.org/10.1158/2159-8290.CD-12-0548

33. Sameer, A. S., & Nissar, S. (2021). Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. *BioMed Research International*, *2021*, 1157023. https://doi.org/10.1155/2021/1157023

34. Sanseviero, E. (2019). NK Cell-Fc Receptors Advance Tumor Immunotherapy. *Journal of Clinical Medicine*, *8*(10), 1667. https://doi.org/10.3390/jcm8101667

35. Shah, U. A., & Mailankody, S. (2020). CAR T and CAR NK cells in multiple myeloma: Expanding the targets. *Best Practice & Research. Clinical Haematology*, *33*(1), 101141. https://doi.org/10.1016/j.beha.2020.101141
36. Thorburn, A. (2007). Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Pathway Signaling. *Journal of Thoracic Oncology*, *2*(6), 461–465. https://doi.org/10.1097/JTO.0b013e31805fea64
37. *Types of multiple myeloma | Canadian Cancer Society*. (n.d.). Retrieved October 29, 2023, from https://cancer.ca/en/cancer-information/cancer-types/multiple-myeloma/what-is-multiple-myeloma/types-of-multiple-myeloma
38. Vivier, E., Tomasello, E., Baratin, M., Walzer, T., & Ugolini, S. (2008). Functions of natural killer cells. *Nature Immunology*, *9*(5), Article 5. https://doi.org/10.1038/ni1582
39. *What are NK (natural killer cell) Cell Receptors*. (n.d.). CUSABIO. Retrieved October 29, 2023, from https://www.cusabio.com/receptor/NK-Cell-Receptor.html
40. *What is CAR NK therapy? | MD Anderson Cancer Center*. (n.d.). Retrieved October 29, 2023, from https://www.mdanderson.org/cancerwise/car-nk-therapy-offers-new-treatment-option-for-blood-cancers.h00-159379578.html
41. *What You Need to Know About Multiple Myeloma*. (n.d.). Retrieved October 29, 2023, from https://mnoncology.com/resources/blog/what-you-need-to-know-about-multiple-myeloma
42. Zhang, A., Sun, Y., Du, J., Dong, Y., Pang, H., Ma, L., Si, S., Zhang, Z., He, M., Yue, Y., Zhang, X., Zhao, W., Pi, J., Chang, M., Wang, Q., & Zhang, Y. (2021). Reducing Hinge

Flexibility of CAR-T Cells Prolongs Survival In Vivo With Low Cytokines Release. *Frontiers in Immunology*, *12*, 724211. https://doi.org/10.3389/fimmu.2021.724211

43. Zhang, L., Meng, Y., Feng, X., & Han, Z. (2022). CAR-NK cells for cancer immunotherapy: From bench to bedside. *Biomarker Research*, *10*(1), 12. https://doi.org/10.1186/s40364-022-00364-6