Natural Killer Cell Therapy

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NK cells

- NK cells are part of the innate immune system, providing the first line of defense against pathogens and cancer cells.
- Considering the critical role of NK cells in defense against tumor growth and metastasis, NK cell-based immunotherapies, including the adoptive transfer of NK cells, have been widely explored.
- NK cells for adoptive immunotherapy can be generated from a myriad of autologous or allogeneic sources, including PB, BM-derived hematopoietic progenitor cells, human embryonic stem cells (hESCs), umbilical CB, NK cell lines (such as NK-92) or memory-like NK cells,

  each with its advantages and disadvantages.

The function of NK cells

The function of NK cells is multifaceted. They possess potent cytotoxic properties while at the same time functioning as cytokine-producing cells.

NK-cell activation is mediated through different mechanisms, including an interplay between the signals from
activating [NK group 2 member D (NKG2D), NKp46, NKp33] and inhibitory receptors (NKG2A),
through direct CD16A signaling, which triggers antibody-dependent cell-mediated cytotoxicity (ADCC),
and via various cytokines such as type I interferon (INF), interleukin (IL)-2, IL-12, IL-15 and IL-18.

The function of NK cells

NK cells can also receive signals through toll-like receptors (TLRs) on their surface, recognizing pathogen-associated molecular patterns (PAMPs) expressed by injured cells.

NK cells kill their targets by releasing lytic granules such as perforin and granzymes, as well as by induction of death signals through the expression of death receptors [Fas ligand (FasL)/FasR,
TNF-related apoptosis-inducing ligand/Receptor (TRAIL/TRAIL-R)].

Besides their known cytotoxicity, NK cells are a major source of cytokines and chemokines, such as type 1 cytokines, INF-c, TNF-a, GM-CSF, and others.

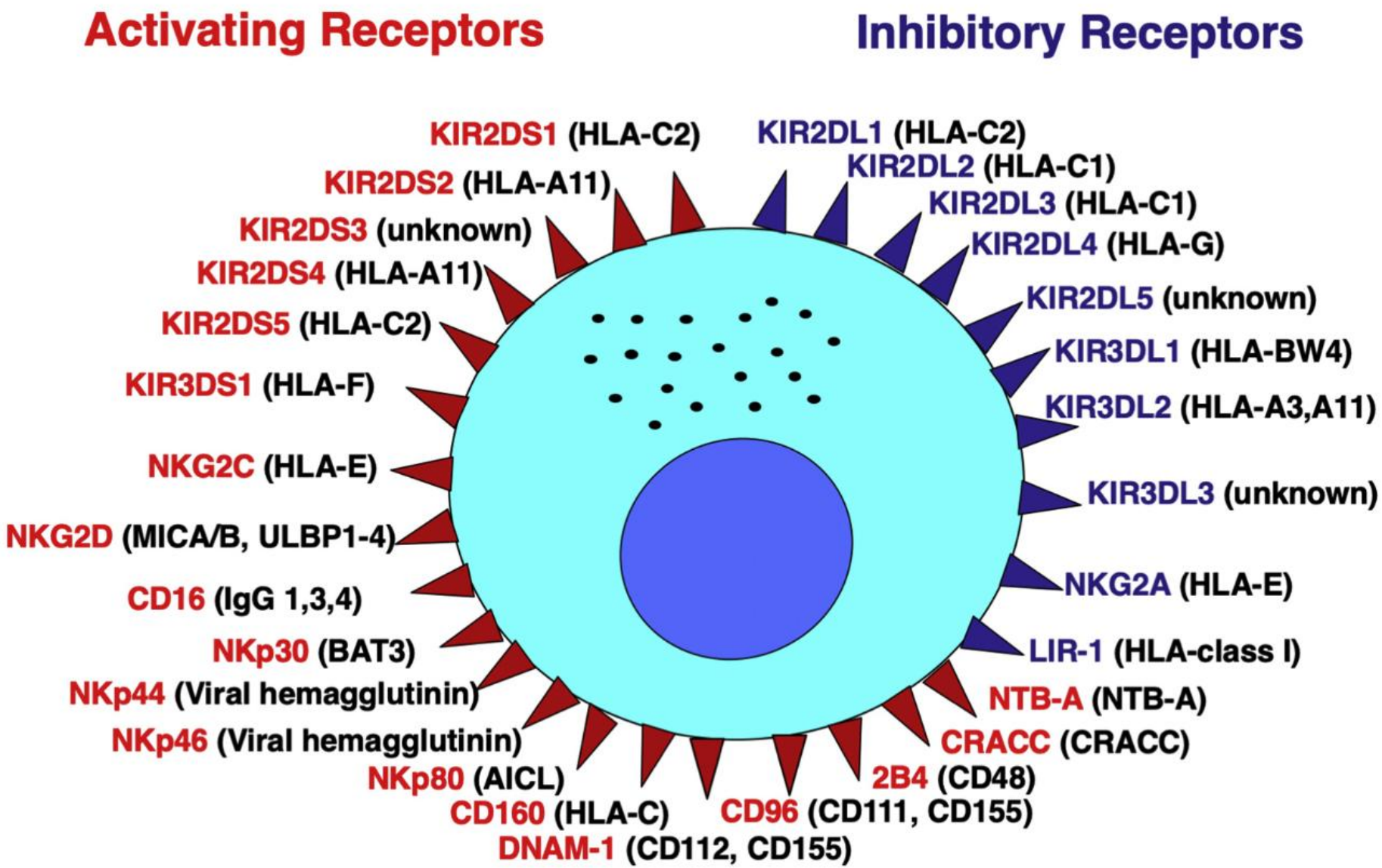


Fig. 1. Activating and inhibitory receptors on NK cells and their ligands.

The function of NK cells

- Cytotoxic NK cells predominantly target cells with downregulated type I major histocompatibility complex (MHC-I), normally expressed by healthy body cells. Downregulation of MHC-I is a common mechanism by which cancer cells and virus-infected cells evade recognition by cytotoxic T lymphocytes (CTLs) through their T-cell receptors (‘missing self’) .

- This characteristic feature of NK cell recognition of target cells in contrast to T cells provides a strategy to overcome tolerance in cancer and leukemia patients.

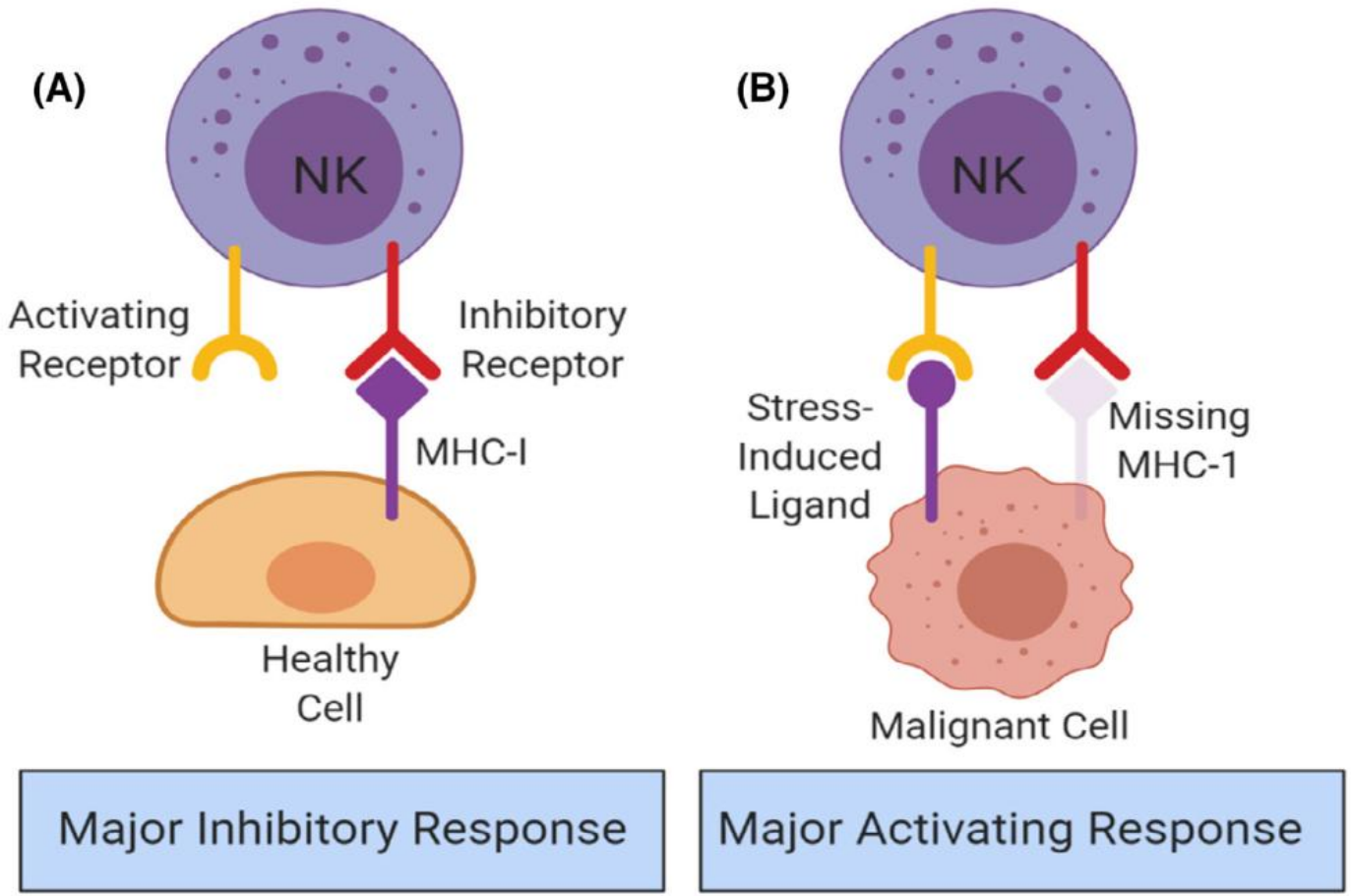


Fig 1. Regulation of natural killer (NK) cell response according to the 'missing self' and 'altered self' models. (A) The presence of major histocompatibility complex (MHC)-I, which are ligands for inhibitory NK-cell receptors, and the lack of stress-induced ligands on the surface of healthy cells, lead to a major inhibitory signal of NK cells. (B) The presence of stress-induced ligands for the activating NK-cell receptors and the downregulation of MHC-I by tumour cells lead to a major activating signal of NK cells.

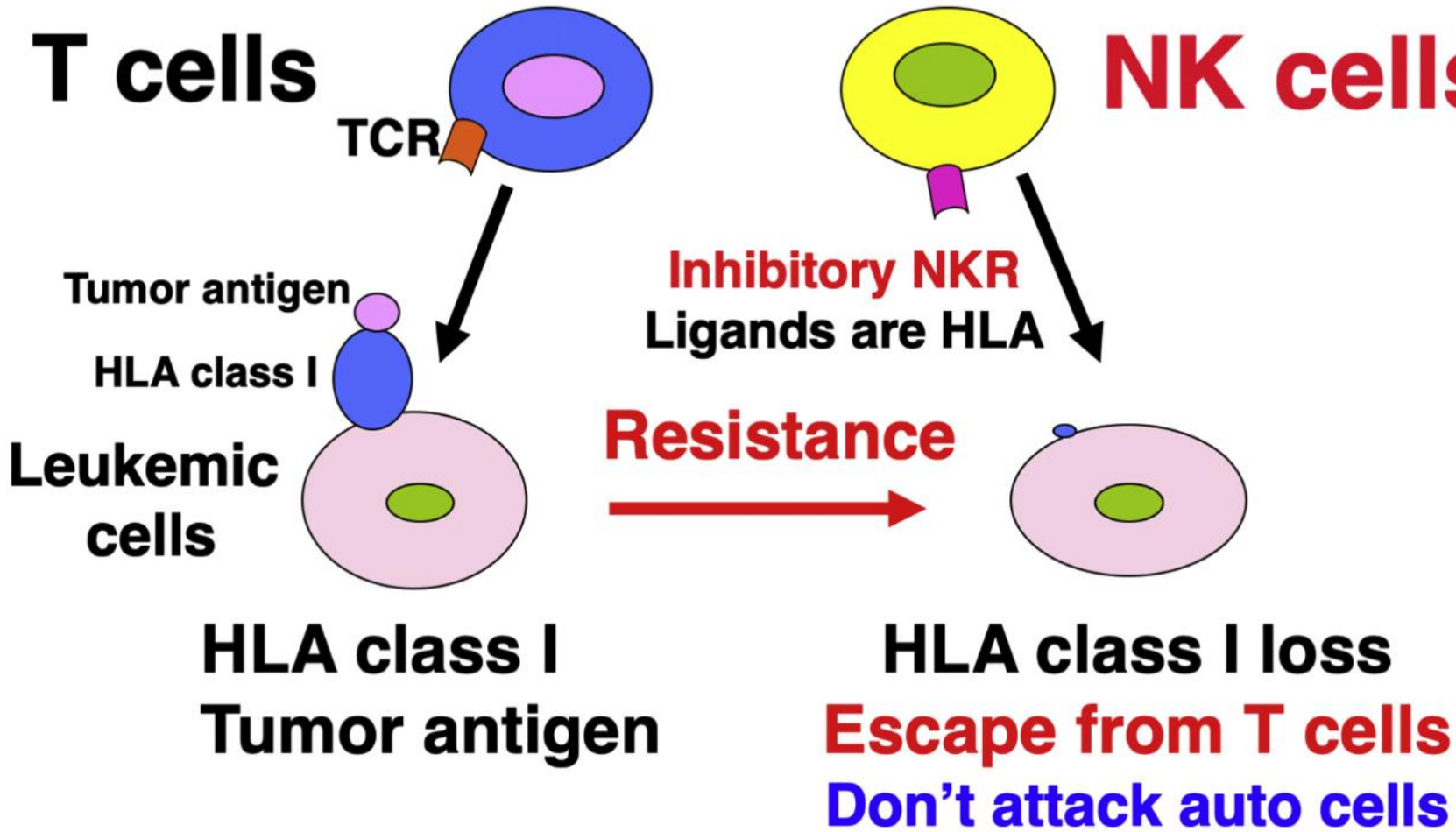


Fig. 2. Target recognition of T cells and NK cells.
Loss of or decreased surface HLA class I expression leads to resistance to T cells and then leads to increased susceptibility to NK cells.

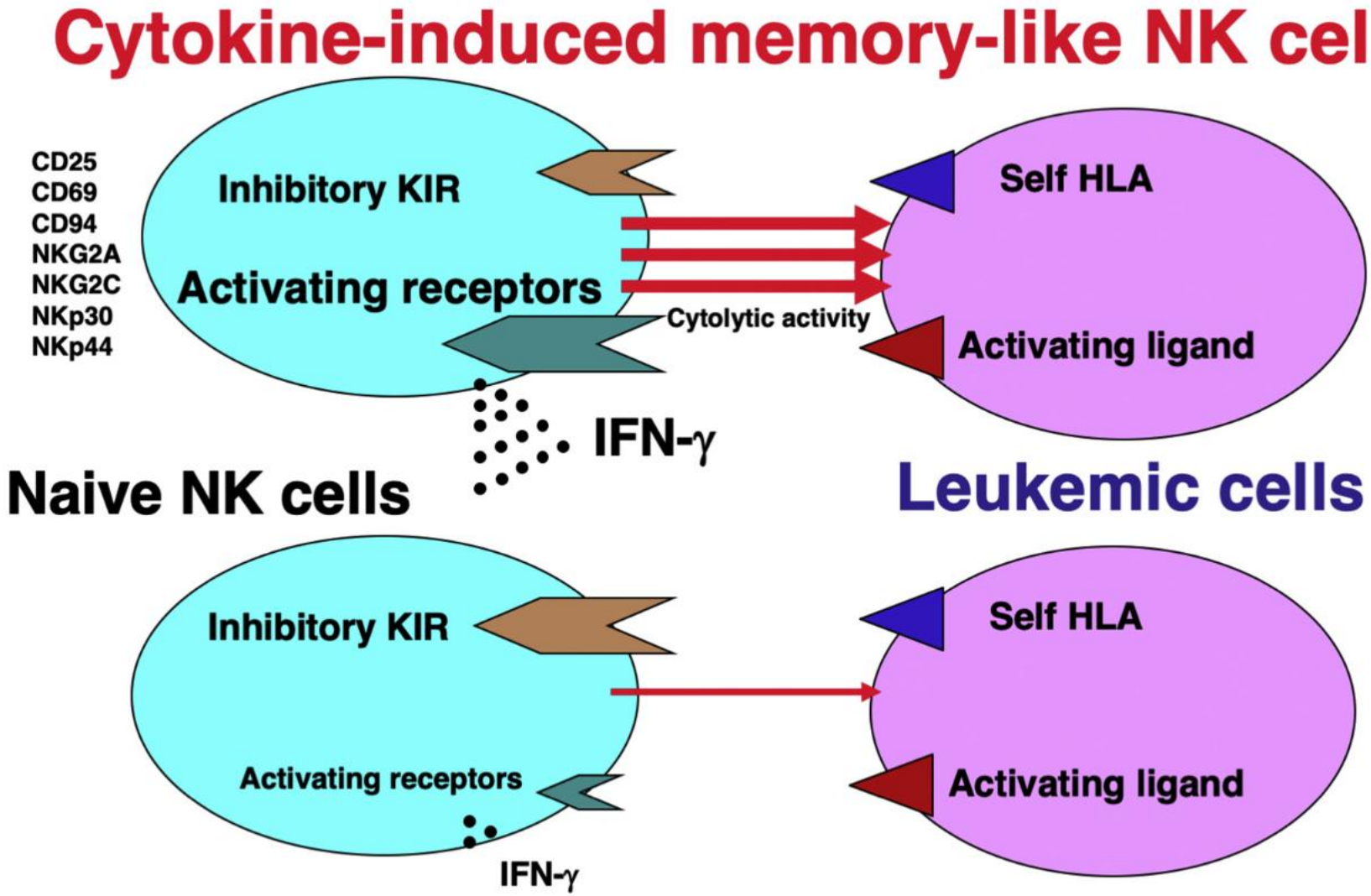
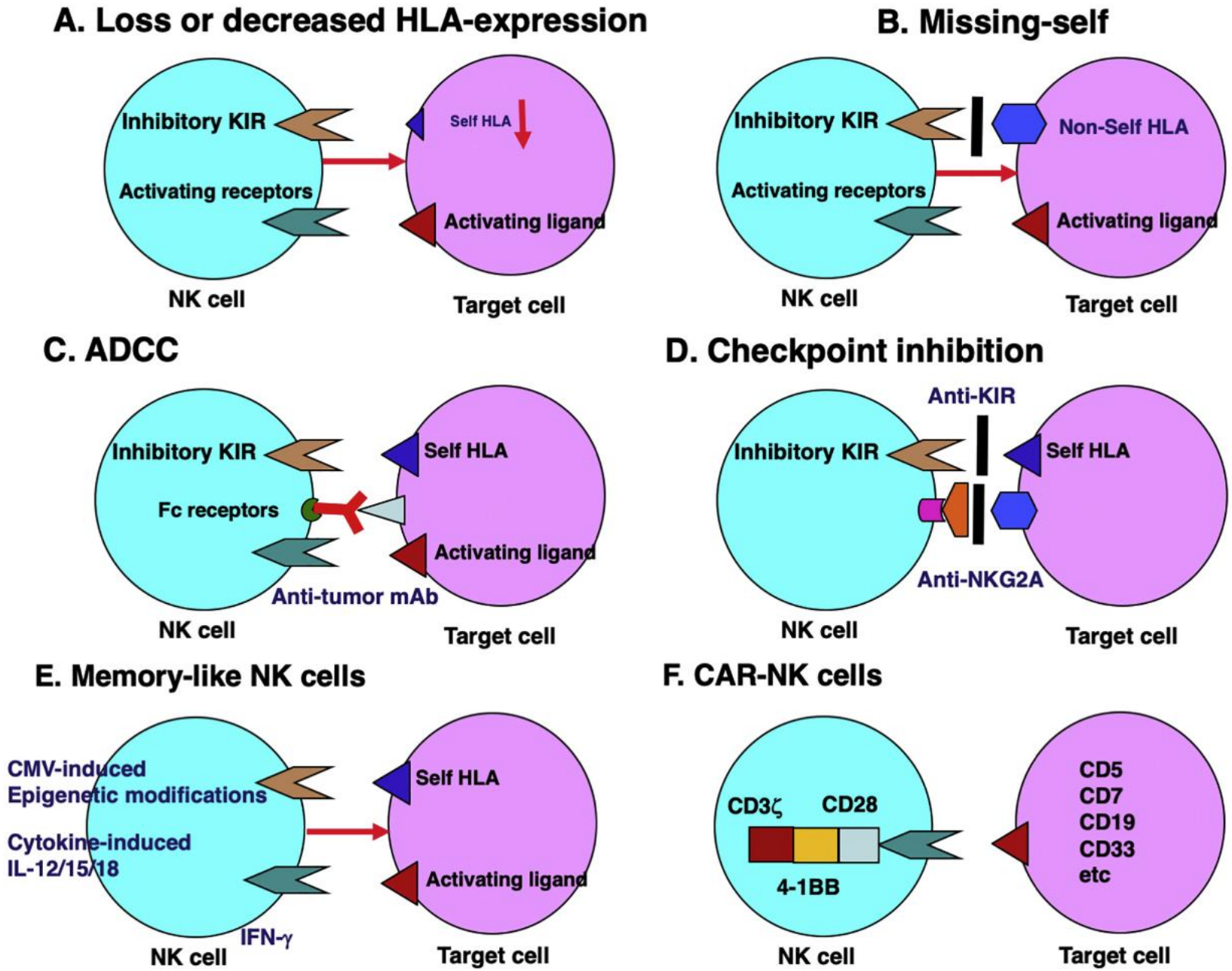


Fig. 3. Cytokine-induced memory-like NK cells.
Cytokine-induced memory-like (CIML) NK cells are generated by combined preactivation with cytokines such as IL-12, IL-15 and IL-18, and they exhibit enhanced functions for several weeks after initial preactivation. CIML-NK cells have increased expression levels of activating receptors and increased production of IFN-γ.


A. Loss or decreased HLA-expression
Inhibitory KIR
Activating receptors
Self HLA
Activating ligand
NK cell
Target cell
B. Missing-self
Inhibitory KIR
Activating receptors
Non-Self HLA
Activating ligand
NK cell
Target cell
C. ADCC
Inhibitory KIR
Fc receptors
Self HLA
Activating ligand
Anti-tumor mAb
NK cell
Target cell
D. Checkpoint inhibition
Anti-KIR
Inhibitory KIR
Self HLA
Anti-NKG2A
NK cell
Target cell
E. Memory-like NK cells
CMV-induced
Epigenetic modifications
Cytokine-induced
IL-12/15/18
Self HLA
Activating ligand
IFN-γ
NK cell
Target cell
F. CAR-NK cells
CD3ζ
CD28
4-1BB
CD5
CD7
CD19
CD33
etc
NK cell
Target cell

Enhancement of NK cells' activities

- NK cells can be expanded using cytokines in vitro in autologous and allogeneic settings as adoptive immunotherapy for hematological malignancies and solid cancers.
- Also, the use of checkpoint receptor blockade and bi- and tri-specific killer engagers (BiKEs and TriKEs) may be beneficial for NK cell-mediated anti-tumor immunotherapy.
- Methods to enhance the activities of NK cells using an anti-KIR monoclonal antibody and chimeric antigen receptor (CAR)-engineered NK cells (CAR-NK cel ls) have recently been developed.

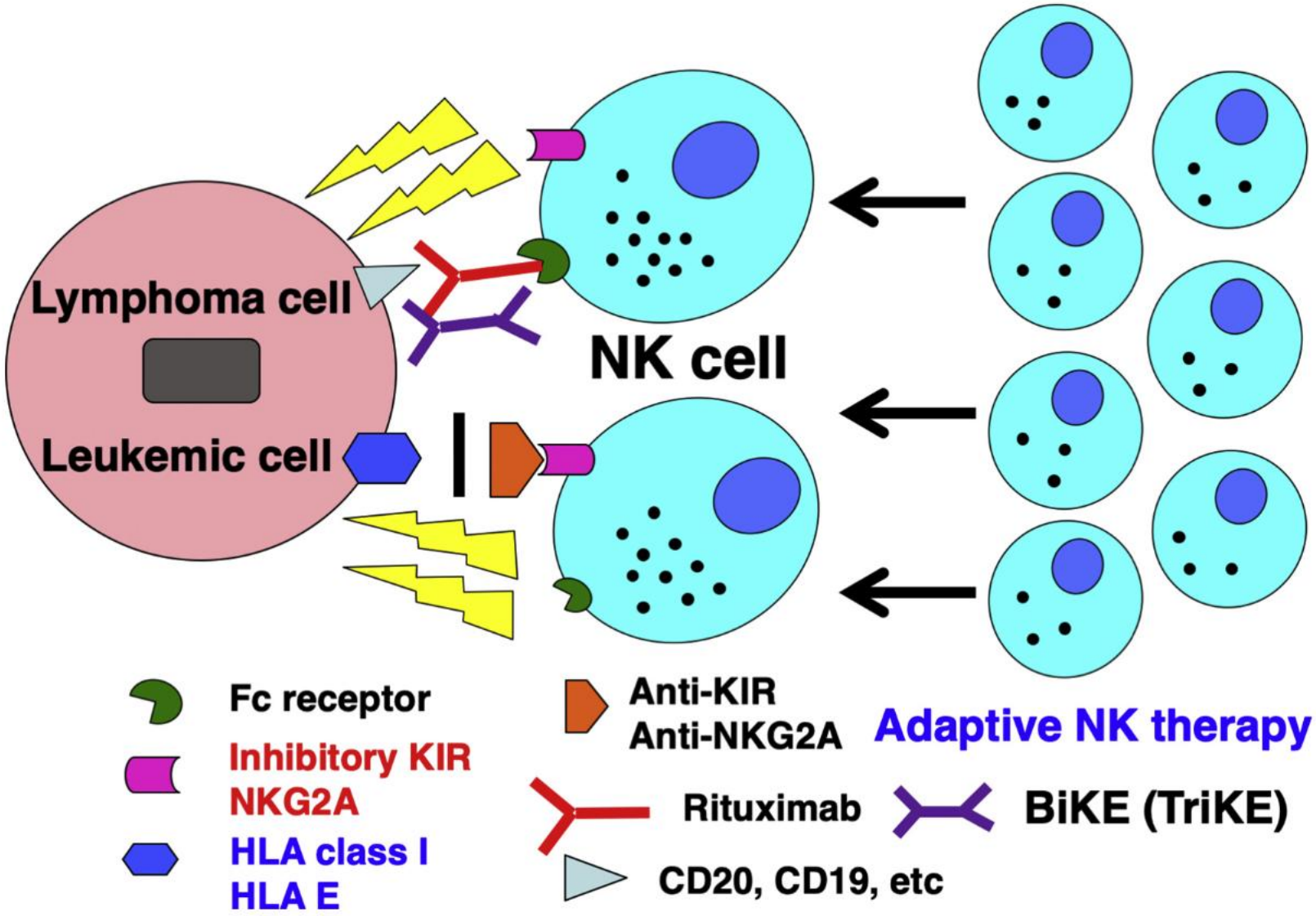


Fig. 6. Adoptive NK cell therapy.
Multidisciplinary strategies using NK cells with anti-tumor antibodies, checkpoint inhibitors and BiKEs and TriKEs may be a promising way to overcome intractable hematological malignancies.

Table 1
Adoptive NK cell therapy using ex vivo expanded NK cells.

Reference	Method	Donor	Disease
Passweg et al. (2006) [117]	CD3 depletion followed by CD56 enrichment	Haploidentical	AML(4), CML(1)
Yoon et al. (2010) [118]	CD34 positive selection from PBSC 2.22 × 10^6/kg (0.29–5.66) Stem cell factor, FLT3 ligand, Hydrocortisone IL-15, IL-21 culture for 3–6 weeks Donor NK cell infusion 6–7 weeks after PBSCT	HLA mismatch	12 acute leukemia 2 MDS
Parkhurst et al. (2011) [119]	CD3 depleted PBMC from leukapheresis Irradiated auto PBSC as feeder cell IL-2, OKT-3 culture for 21 days Cy/Flu	Autologous	7 metastatic melanoma 1 renal carcinoma
Choi et al. (2014) [120]	CD3 depleted PBMC from leukapheresis IL-15, IL-21 culture for 13–20 days NK cell infusion 2 and 3 week after Haploidentical PBSCT with Bu/Flu/ATG Escalating dose 0.2, 0.5, 1.0, > 1.0 x10^8/kg, A median total dose 2.0 x10^8/kg	Haploidentical	41 hematological malignancies
Shah et al. (2015) [121]	CD3 depletion followed by CD56 enrichment cultured with IL-15R/4-1BBL-APC	Matched sibling/ unrelated	9 sarcoma
Choi et al. (2016) [122]	CD3 depleted leukapheresis IL-15, IL-21 and hydrocortisone For 2–3 weeks	Haploidentical	51 AML
Boyiadzis et al. (2017) [123]	NK-92 human cell line IL-2	Non-transplantation off-the-self	7 AML
Ciurea et al. (2017) [124]	CD3-depleted PBMC Membrane-bound IL-21	Haploidentical	8 AML, 4 CML 1 CML with Dose/Purity -2, +7, +28
Tanaka et al. (2019) [23]	PBMC IL-2, IL-15, OKT3 Tacrolimus, dalteparin sodium NK cell combined with rituximab-containing chemotherapy	Autologous	4 FL, 5 DLBCL

Table 1
Adoptive NK cell therapy using ex vivo expanded NK cells.

Reference	Method	Donor	Dose/Purity	Adverse effect	Result
Passweg et al. (2006) [117]	CD3 depletion followed by CD56 enrichment	Haploidentical	NK cell 1.7–30 × 10^8 5–20 folds	No GVHD	3/5 patients alive and CR 18–36M after NK-DLI
Yoon et al. (2010) [118]	CD34 positive selection from PBSC 2.22 × 10^6/kg (0.29–5.66) Stem cell factor, FLT3 ligand, Hydrocortisone IL-15, IL-21 culture for 3–6 weeks Donor NK cell infusion 6–7 weeks after PBSCT	HLA mismatch	9.28 × 10^6/kg (0.33–24.5) CD3 1.0%(0–2.6)	1 aGVHD 5 cGVHD	4/14 alive and well 16.2–21.6 months
Parkhurst et al. (2011) [119]	CD3 depleted PBMC from leukapheresis Irradiated auto PBSC as feeder cell IL-2, OKT-3 culture for 21 days Cy/Flu	Autologous	4.7 × 10^{10} (± 2.1) 96 ± 2%	One transient Shortness of breath	No clinical response
Choi et al. (2014) [120]	CD3 depleted PBMC from leukapheresis IL-15, IL-21 culture for 13–20 days NK cell infusion 2 and 3 week after Haploidentical PBSCT with Bu/Flu/ATG Escalating dose 0.2, 0.5, 1.0, > 1.0 x10^8/kg, A median total dose 2.0 x10^8/kg	Haploidentical	$CD56^+CD122^+$ 91–95 (49–99)% 3.7(0.8–70) fold increase CD3 1.3–2.8%	No acute toxicity	Significant reduction of leukemia progression 46% vs 74%(historical)
Shah et al. (2015) [121]	CD3 depletion followed by CD56 enrichment cultured with IL-15R/4-1BBL-APC	Matched sibling/ unrelated	NK cell 1, 10 × 10^5/kg CD3 cell 1.1–2.0 × 10^4/kg	5/9 aGVHD 3 grade 4	2 patients CR 4/9 patients alive 12.5–27.4M
Choi et al. (2016) [122]	CD3 depleted leukapheresis IL-15, IL-21 and hydrocortisone For 2–3 weeks	Haploidentical	After haplo HSCT on day 6, 9, 13, 20 0.5, 0.5, 1.0 2.0 × 10^8/kg	grade 2 to 4 aGVHD 28% cGVHD 30% fever 73%	CR at 1M 57% 3Y leukemia progression 75%
Boyiadzis et al. (2017) [123]	NK-92 human cell line IL-2	Non-transplantatio off-the-self	1 × 10^9/m^2 (3) 3 × 10^9/m^2 (3)	No dose limiting toxicities	1 blast reduction 2 stable disease
Ciurea et al. (2017) [124]	CD3-depleted PBMC Membrane-bound IL-21	Haploidentical	1 × 10^5 to 1 × 10^8/kg	aGVHD grade 1–2 54%	11 of 13 patients alive in CR median 14.7M
Tanaka et al. (2019) [23]	PBMC IL-2, IL-15, OKT3 Tacrolimus, dalteparin sodium NK cell combined with rituximab-containing chemotherapy	Autologous	1 × 10^6, 3 × 10^6, 10 × 106/kg	No severe non- hematological	7 of 9 patients maintained CR a median duration of 44 months (6–56M)

A strong NK cell-mediated graft-versus-leukemia effect in haploidentical HSCT is important for curing high-risk leukemia.
NK cells have an important role in the graft-versus-leukemia/tumor (GVL/T) effect after allogeneic stem cell transplantation.
GVL/T effectors may be operational in patients who have undergone HLA-mismatched hematopoietic cell transplantation based on the rule of NKR incompatibility.
Ruggeri et al. reported exciting clinical results after HLA-haploidentical transplantations with KIR ligand incompatibility in the GVH direction for AML patients. Also, donor allogeneic NK cells attacked host antigen-presenting cells (APC), resulting in the suppression of GVHD.

Limitations of NK-cell immunotherapy

A major consideration for the successful clinical application of NK-cell immunotherapy is their lack of persistence after adoptive transfer in the absence of cytokine support.

While this could help reduce long-term adverse effects and toxicity, it may also significantly reduce their clinical efficacy. Indeed, many studies have shown that the *in vivo* persistence and proliferation of NK cells after adoptive transfer may predict clinical response.

Thus, enhancing NK-cell persistence is the subject of active research by many groups.

Limitations of NK-cell immunotherapy

Several strategies have been explored, including the administration of exogenous cytokines such as IL-2 or IL-15.
This approach, while enhancing the in vivo expansion and proliferation of NK cells, is associated with systemic toxicity, including capillary leak syndrome following IL-2 infusion and neutropenia after IL-15 infusion.

In addition, IL-2 induces the expansion of regulatory T cells (Tregs), which can suppress the expansion, persistence, and anti-tumor activity of NK cells.

Limitations of NK-cell immunotherapy

Another strategy that could help with the persistence of NK cells after adoptive infusion involves the administration of lymphodepleting agents such as cyclophosphamide and fludarabine before NK-cell transfer. It caused:
eliminating the recipient lymphocytes to allow for NK cells to expand and proliferate,
eliminating immunosuppressive elements such as Tregs and MDSCs,
inducing expression of co-stimulatory molecules and down-regulating immunosuppressive molecules such as indoleamine 2,3-dioxygenase (IDO) in tumor cells.

However, even this approach does not fully protect against early loss
of response, which could be due to competition between the donor NK cells and the recipient tumor-related immunosuppression.

CAR NK-cell therapy

- Chimeric antigen receptor (CAR) T cells are a rapidly emerging form of cancer treatment and have resulted in remarkable responses in refractory lymphoid malignancies.
- However, CAR-T cells have several drawbacks, including high manufacturing costs and serious toxicities such as cytokine release syndrome (CRS) and neurotoxicity.
- These challenges require investigating novel, universal, efficient, and safe cell therapy products. Natural killer (NK) cells do not carry the risk of graft-versus-host disease (GvHD) and therefore offer the potential for an off-the-shelf cellular product that could be readily available for immediate clinical use.

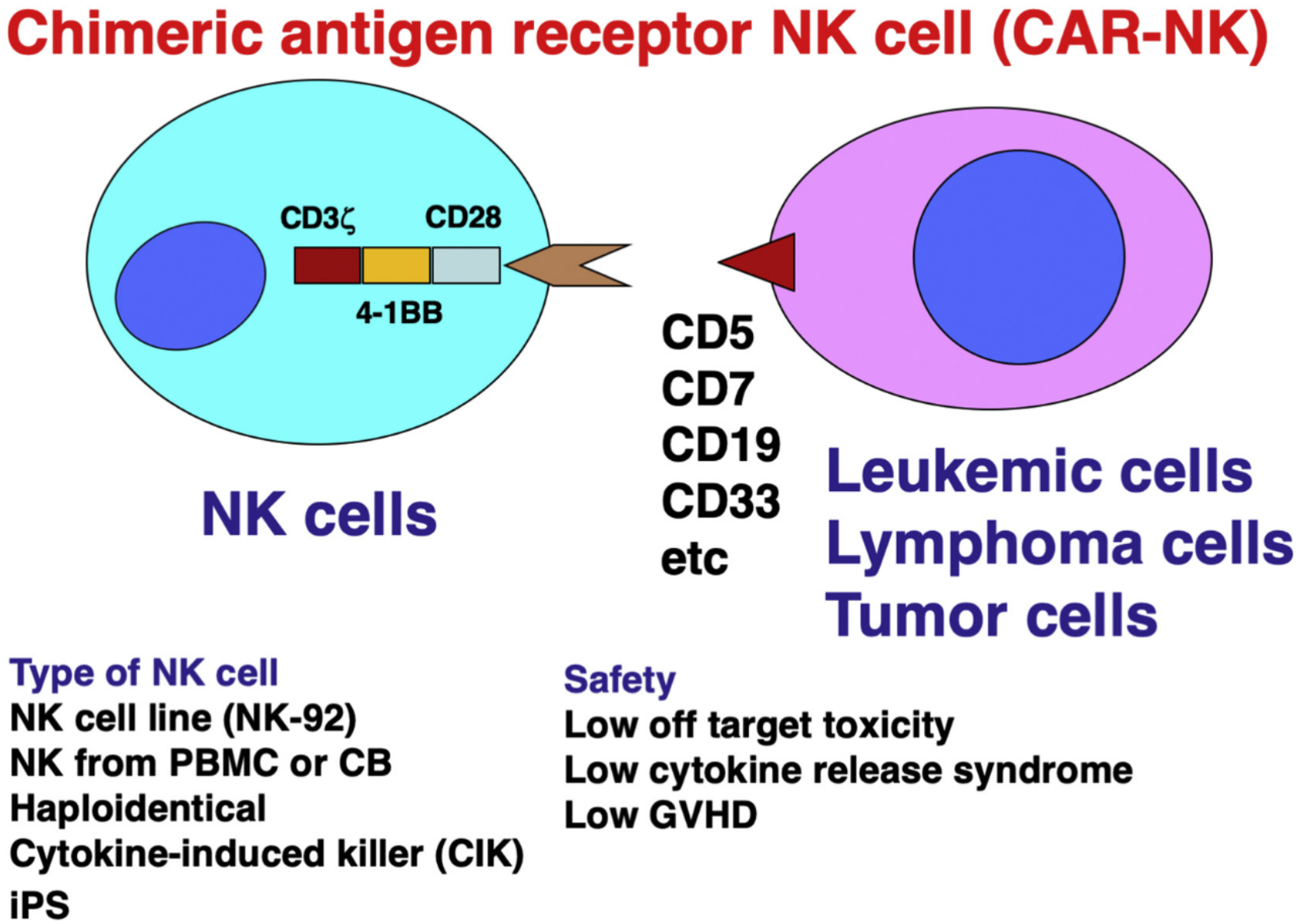


Fig. 4. Chimeric antigen receptor NK cells (CAR-NK cells).
CAR-expressing NK (CAR-NK) cells may be beneficial in terms of less side effects and more universal access.

CAR NK-cell therapy

- CARs are genetically engineered transmembrane receptors with two main functions: (i) specific antigen recognition independent of MHC restriction and (ii) activation of the modified cells through signal transduction.
- NK cells are attractive candidates for CAR engineering because of their short lifespan, nearly 2 weeks in vivo, and their different cytokine profiles that may reduce the risk of toxicity after adoptive transfer.
- Other advantages of NK cells are related to their intrinsic capacity to recognize and target tumor cells through their own receptors, which allows them to kill cancer cells even when antigen escape mechanisms such as CAR target antigen downregulation evolve.

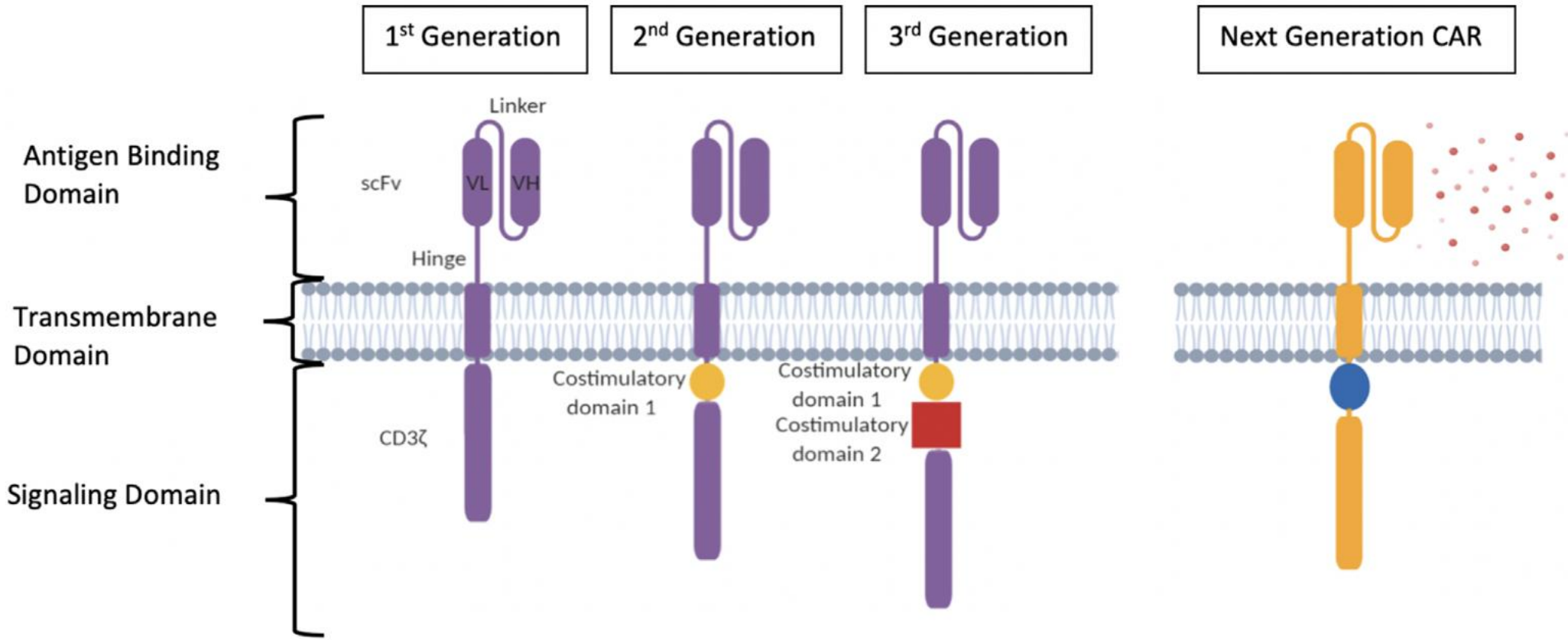
CAR NK-cell therapy for AML

The application of CAR therapy in AML remains challenging. Although none of the ongoing clinical trials of CAR T-cell therapy in AML have yet yielded mature data, the clinical responses reported to date have been at best modest.

Major barriers to the success of CAR therapy in AML include shared expression of antigen on AML cells and normal HSCs (such as CD123), thus increasing the risk of marrow aplasia, and heterogeneous expression on progenitors or absence of target antigen on blasts (e.g. CD33), resulting in leukemia escape.

CAR NK-cell therapy

- AML cells are highly susceptible to NK cell-mediated killing as they express many of the ligands recognized by NK-cell activating receptors, such as MHC chain-related antigens (MICA/B) and the UL16-binding proteins (ULBPs) recognized by NKG2D, and CD155 (recognized by DNAM).
- Thus, CAR-NK cells may overcome challenges related to antigen escape and tumor heterogeneity through their innate ability to recognize and target AML cells.
- In addition, their short lifespan may limit the extent and length of cytopenia when targeting antigens also expressed on normal HSCs. Thus, efforts to develop CAR-NK cells against AML are underway.
- In a recent study, primary human NK cells transduced with a self-inactivating alpha-retroviral vector incorporating anti-CD123 and dual co-stimulation with CD28 and 4-1BB were reported to efficiently kill AML cell lines and primary AML blasts in vitro.


1st Generation
2nd Generation
3rd Generation
Next Generation CAR
Antigen Binding Domain
Transmembrane Domain
Signaling Domain
Linker
scFv
VL
VH
Hinge
CD3ζ
Costimulatory domain 1
Costimulatory domain 1
Costimulatory domain 2

CAR NK-cell therapy

- Preclinical studies with CAR-NK cells initially investigated the safety and efficacy of anti-CD19 and anti-CD20 CAR-NK cells against B-cell malignancies, although results with first-generation CARs were modest.
- The addition of a co-stimulatory domain to the CAR construct resulted in significant improvement in the efficacy of CAR-NK cells.
- Chuet al. genetically modified PB NK cells from healthy donors to express anti-CD20.4-1BB.CD3f CAR using mRNA nucleofection. This approach resulted in 67% CAR expression and significant in vitro and in vivo activity against Burkitt lymphoma in preclinical models.

Clinical trial identifier	Start date	Phase	NK-cell source	Construct	Disease	Target
Multiple myeloma						
NCT03940833	May 2019	I/II	NK-92 cell line	N/A	Multiple myeloma	BCMA
B-lymphoid malignancies						
NCT03579927	October 2019	I/II	CB	CAR.CD19-CD28-zeta-2A-iCasp9-IL-15	B-cell lymphoma	CD19
NCT03692767	March 2019	Early Phase I	N/A	N/A	B-cell lymphoma	CD22
NCT03690310	March 2019	Early Phase I	N/A	N/A	B-cell lymphoma	CD19
NCT03824964	February 2019	Early Phase I	N/A	N/A	B-cell lymphoma	CD19/CD22
NCT03056339	June 2017	I/II	CB	CAR.CD19-CD28-zeta-2A-iCasp9-IL-15	B-cell lymphoma	CD19
NCT02892695	September 2016	I/II	NK-92	CAR.CD19-TCRζ-CD28-4-1BB	Leukaemia and lymphoma	CD19
NCT01974479	September 2013	Phase I	Haploidentical donor	CAR.CD19-BB-zeta	B-ALL	CD19
NCT00995137	October 2009	I	Donor	CAR.CD19-BB-zeta	B-ALL	CD19
AML						
NCT02944162	October 2016	I/II	NK-92 cell line	CAR.CD33-CD28-CD137-CD3ζ	AML	CD33
T-cell leukaemia and lymphoma/AML						
NCT02742727	March 2016	I/II	NK-92 cell line	CAR.CD7-TCRζ-CD28-4-1BB	CD7 positive leukaemia and lymphoma	CD7

THANKS