Enhancing Neuroblastoma Immunotherapies by Engaging iNKT and NK Cells

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Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor in children and accounts for ~15% of childhood cancer deaths
NB arises from developing sympathetic nervous system
Most primary tumors localize in the abdomen, the adrenal gland, or lumbar sympathetic ganglia.
Amplification in tumor cells of MYCN, the major oncogenic driver, patients' age over 18 months, and the presence at diagnosis of a metastatic disease (stage IV, M) identify NB at high risk of treatment failure.
Conventional therapies did not significantly improve the overall survival of these patients.
NB is stratified into risk groups on the basis of clinical and molecular features using the International Neuroblastoma Risk Group (INRG) classification with the high-risk group being the most prevalent

Introduction

High risk neuroblastoma (HRNB) is treated with a combination of conventional chemotherapy, surgical resection, autologous stem-cell transplant, radiation, and immunotherapy.

Despite the extensive treatment regimen, HRNB still carries a 5-year overall survival (OS) of ~50%, and these treatments have significant late adverse effects including hearing loss, cognitive deficits, endocrinopathies, and ovarian failure

As such, there is a critical need for more tolerable and effective treatments in this group.

Immunotherapy

Immunotherapy with dinutuximab, a monoclonal antibody against GD2 (a disialoganglioside that is highly expressed on neuroblasts), has been incorporated into the recommended treatment regimen for HRNB after FDA-approval in 2015, and significantly improved 2-year event-free-survival (EFS)

Additionally, the addition of dinutuximab to irinotecan and temozolomide in the relapsed or refractory NB setting demonstrated dramatic clinical activity.

In those treated with the dinutuximab-containing regimen, 9 out of 17 patients (53%) had a disease response vs. 1 out of 18 patients (6%) in a comparator arm that included the same chemotherapy but without dinutuximab

These studies demonstrate that dinutuximab immunotherapy has significant clinical utility in both minimal and high disease burden contexts.

However, despite the improved 2-year event-free-survival (EFS) in the minimal residual disease setting and substantial response in the bulk disease setting, 5-year overall survival (OS) in HRNB remains ~50%

Improving the long-term efficacy of these promising immunotherapies in HRNB therefore remains an important unsolved challenge.

Introduction

Use of chimeric antigen receptor (CAR) T cells and checkpoint inhibitors have shown success in the treatment of other malignancies, these immunotherapies have not yet demonstrated similar efficacy in HRNB or other pediatric solid tumors

The lack of response in HRNB is thought to be due to multiple factors including an immunosuppressive tumor microenvironment (TME), low expression of MHC-I, and MHC-II antigens on neuroblasts leading to immune evasion, low mutational burden in NB with a paucity of neoantigens, and diminished T cell persistence (for CAR T cell therapies)

Alternative immunotherapies that can overcome these barriers are therefore being sought.

Introduction

Interestingly, the presence of invariant natural killer T cells (iNKTs), and natural killer cells (NKs) is associated with improved prognosis of patients with NB as well as other malignancies

iNKTs and NKs inhibit tumor-associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs), kill cancer stem cells (CSCs), and tumor cells that have downregulated their MHC antigens, and robustly secrete cytokines to recruit additional immune effectors.

These features allow NKs and iNKTs to decrease tumor immunosuppression and overcome immune evasion in the HRNB TME NK and iNKT therapies may prove useful in both standalone and combination treatments for NB and other pediatric solid tumors.

BIOLOGY OF INKT CELLS

Overview and Relevance in Human Cancer

Natural Killer T cells (NKTs)

Innate-like lymphocytes that make up about 1% of total lymphocytes in the human liver, and also have residence in the spleen and bone marrow.

They bridge the innate and adaptive immune systems, helping to coordinate robust responses to malignant or infected cells, and have demonstrated importance in tumor immunosurveillance

NKTs share features with both NKs and T cells, and are divided into type I, or invariant NKTs (iNKTs), that express a conserved T cell receptor (TCR)made up of an a chain composed of Va24 and Ja18 segments paired with a b chain composed of the Vb11 segment.

Conversely, type II NKTs express polyclonal TCRs, similar to conventional CD4+ and CD8+ T cells.

These invariant TCRs allow for the recognition of glycolipid antigens (GAgs) presented by a non-polymorphic and conserved MHC class 1-like protein called CD1d.

iNKTs

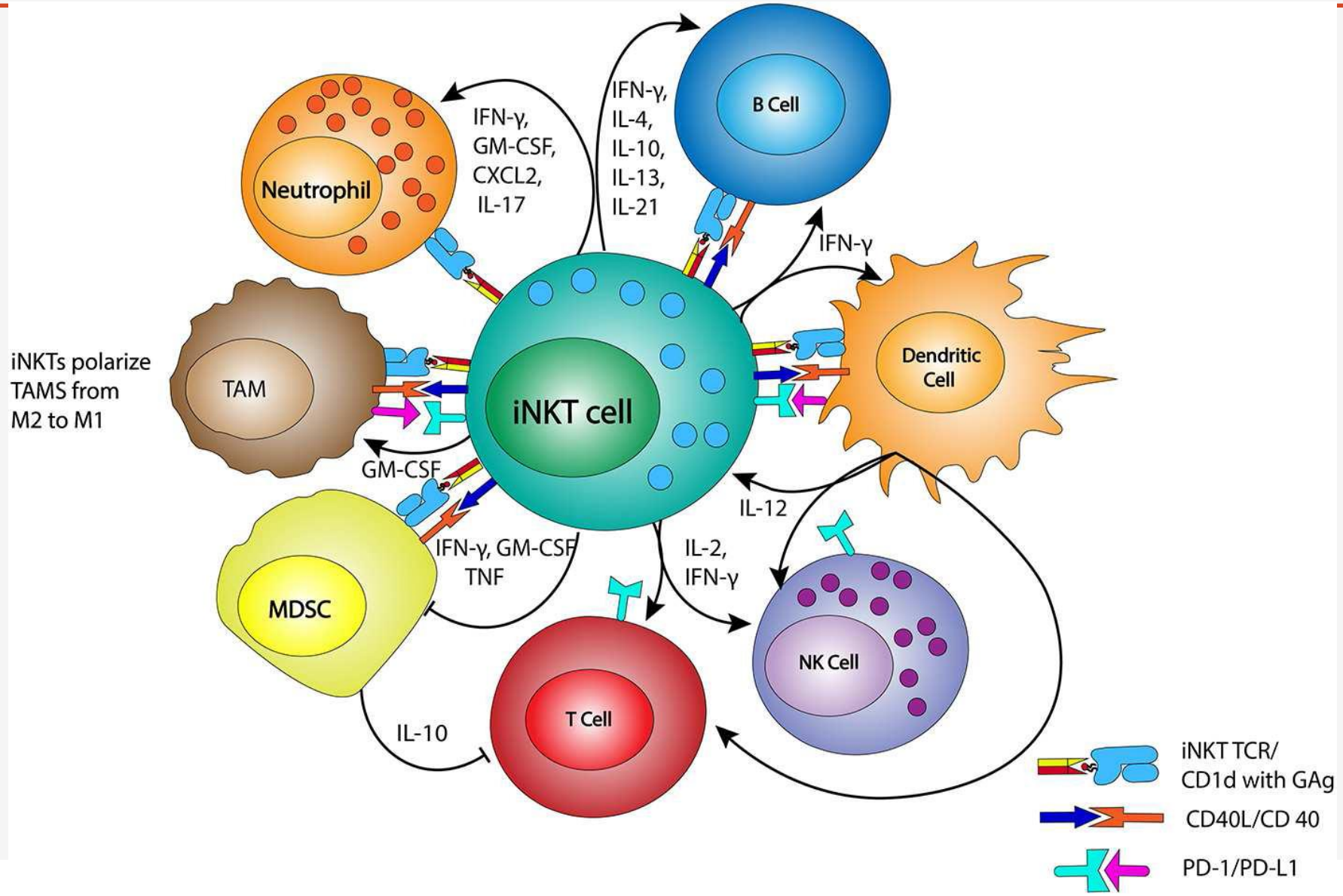
The frequency of iNKTs within a tumor, or in circulation, has been associated with improved survival and reduced progression in various malignancies including prostate cancer, medulloblastoma, melanoma, multiple myeloma, colon cancer, lung cancer, breast cancer, head and neck squamous cell carcinomas (HNSCC), and NB

Lack of function in iNKTs is associated with advanced cancers and worse prognosis in patients with multiple myeloma, myelodysplastic syndrome, and prostate cancer

NB patients at the time of diagnosis, a high frequency of iNKTs in NB tumors was found to be associated with improved survival and lower stage NB

When iNKTs are activated, they release large amounts of cytokines that mature, recruit, and activate other immune effector cells.

iNKTs and interactions with immune effectors



Anergy and Tumor Microenvironment Immunosuppression of iNKT Cells

Anergy, a state in which iNKTs fail to produce cytokines,or proliferate after stimulation

Similar to conventional T cells, an additional barrier can be imposed by proteins expressed on neuroblasts, TAMs, MDSCs, and regulatory T cells (TREG).

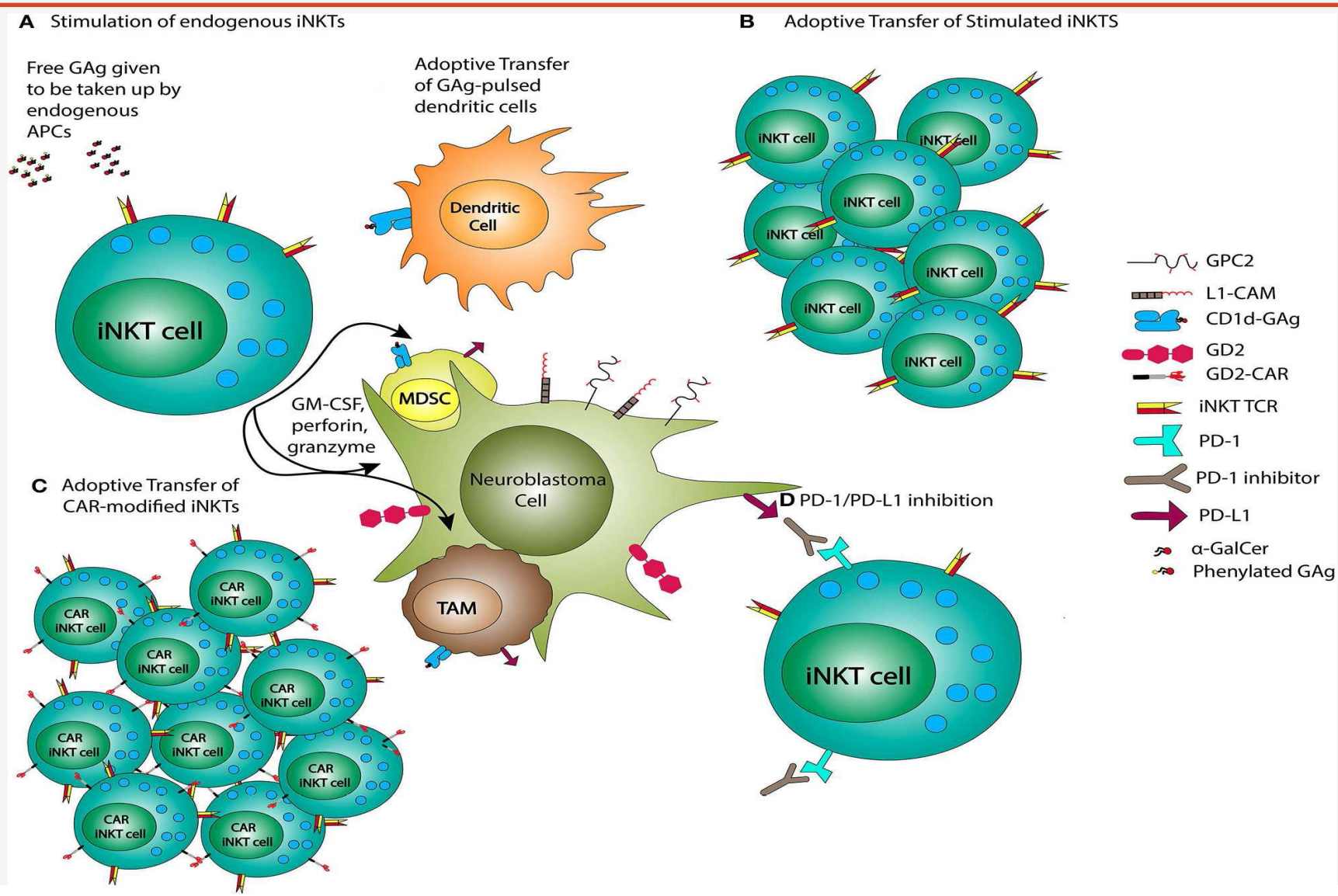
Proteins such as programmed cell death ligand-1 (PD-L1) binds to PD1 on iNKTs and other immune effectors to inhibit their cytotoxic function

Aside from expression of checkpoint ligands such as PD-L1, neuroblasts, and co-opted immune cells also release TGF-b1, IL-4, IL-6, IL-10, IL-13, adenosine, and prostaglandin E-2 to suppress infiltrating immune cells

This immunosuppressive milieu can bias iNKTs toward Th2 cytokine release, thereby skewing the TME in an immunosuppressive direction.

Immunosuppressive effects of checkpoint receptor expression have been targeted by antibody mediated blockade of PD-1/PD-L1 interactions; this blockade restored IFN-g release and augmented anti-tumor activity of iNKTs iNKTs are associated with favorable prognosis in various human malignancies, likely due to their ability to secrete pro-inflammatory cytokines and culling and/or reprogramming of immunosuppressive and tumor-growth promoting cells in the TME.

iNKT-based NB treatments



INKT CELL-BASED TREATMENTS OF NEUROBLASTOMA

GAg Stimulation of iNKT Cells

Adoptive Transfer of iNKT Cells

CAR-iNKT Cells

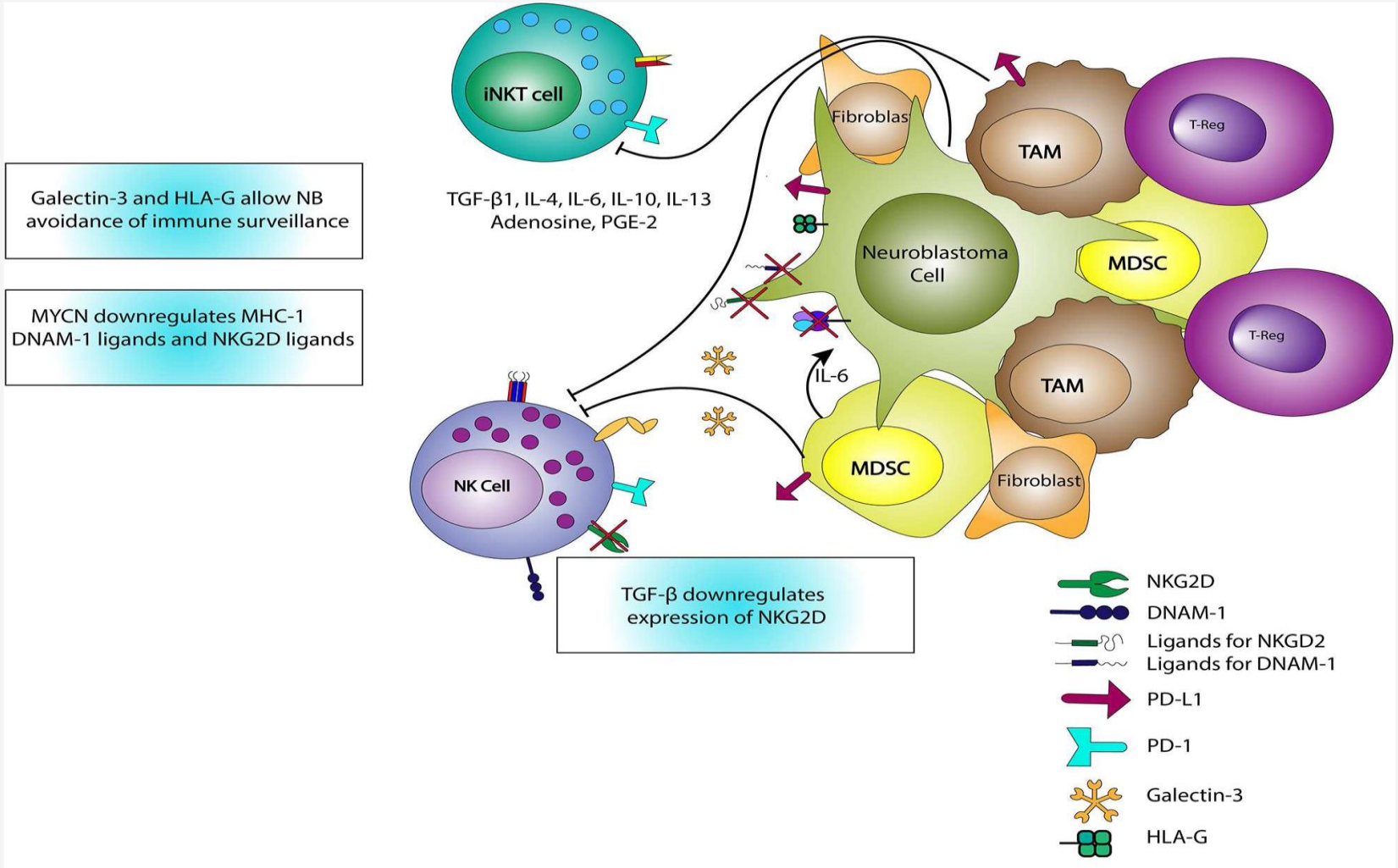
BIOLOGY OF NK CELLS

NK Activation and Activity in Neuroblastoma

NK Receptors and Influence on Anti-tumor Activity

Neuroblastoma TME and NK Cells

Interactions of iNKTs, NKs, and the TME



NK CELLS IN THE TREATMENT OF NEUROBLASTOMA

Soluble Factors Activating NK Cells in Neuroblastoma

NK Adoptive Cellular Therapy

CAR-NK Cells

NK-based treatments of neuroblastoma

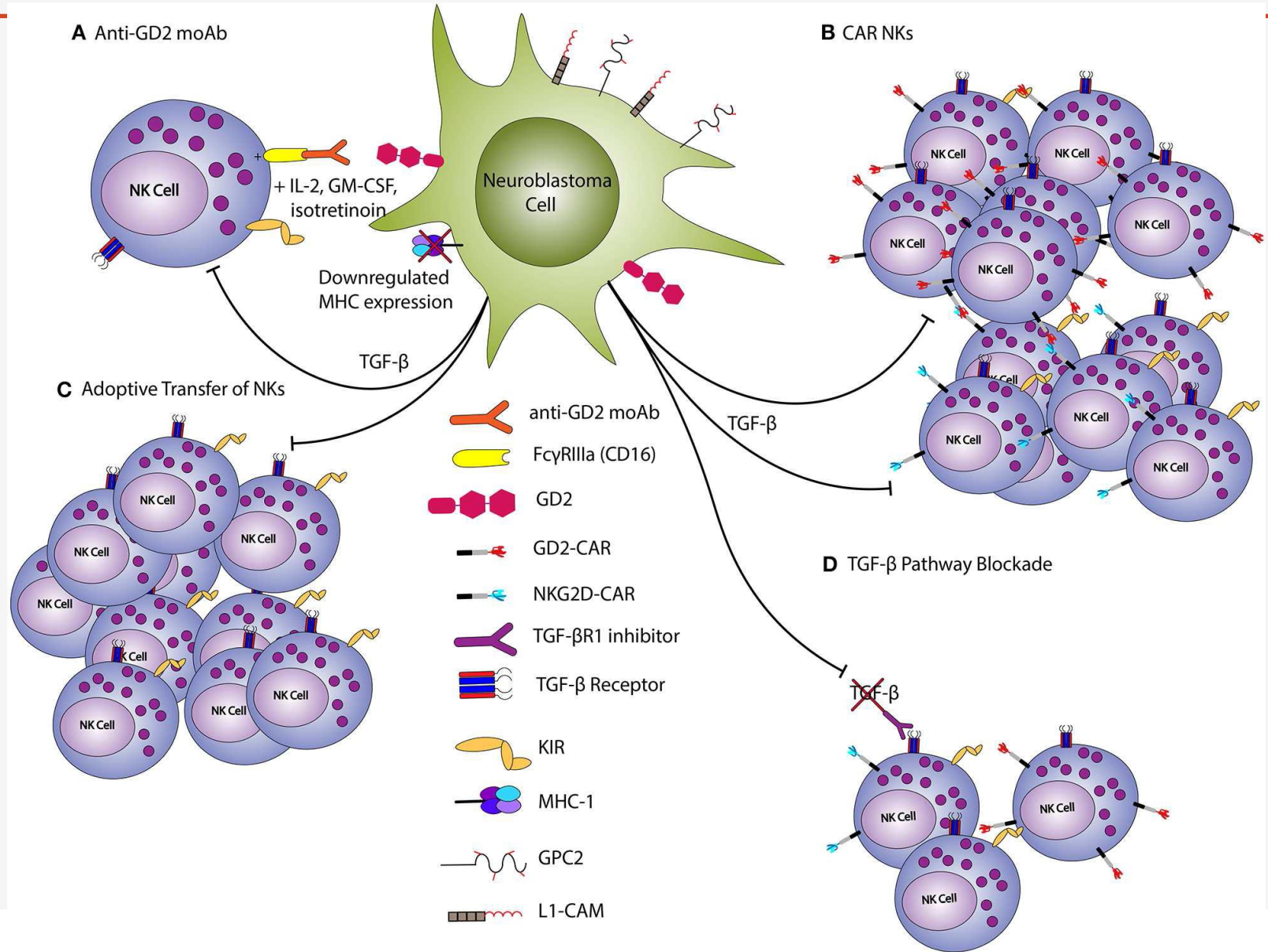


TABLE 1 | Ongoing clinical trials using NK cell-based therapies for neuroblastoma.

Title	Status	Phase	NCT number	Sponsor	Study start date	Anticipated completion date
Haploidentical stem cell transplantation and NK cell therapy in patients with high-risk solid tumors	Active, not recruiting	2	01807486	Samsung medical center	05/2013	6/2019
Phase II STIR trial: haploidentical transplant and donor NK cells for solid tumors	Recruiting	2	02100891	Monica thakar medical college of wisconsin	03/2014	12/2021
Immunotherapy of neuroblastoma patients using a combination of anti-GD2 and NK cells	Recruiting	1,2	03242603	National university hospital, singapore	10/2017	8/2020
NK cell infusions with irinotecan, temozolomide, and dinutuximab	Not yet recruiting	1,2	04211675	Nationwide children's hospital	4/2020	12/2023
Immunotherapy of relapsed refractory neuroblastoma with expanded NK cells	Recruiting	1	02573896	New approaches to neuroblastoma therapy consortium	11/2018	8/2022
Humanized anti-GD2 antibody Hu3F8 and allogeneic NK cells for high-risk neuroblastoma	Recruiting	1	02650648	Memorial sloan kettering cancer center	01/2016	1/2021
Treatment of relapsed or refractory neuroblastoma with expanded haploidentical NK cells and Hu14.18-IL2	Recruiting	1	03209869	University of wisconsin	3/2018	9/2021

CHALLENGES FOR NK CELL-BASED THERAPIES

Despite the promise of NK-based therapies, several challenges still exist.

These include limited in vivo proliferation and persistence of CAR NKs in vivo, and the immunosuppressive TME of solid tumors.

TME of solid tumors can express appreciable levels of TGFb1 as well as checkpoint ligands (e.g., PD-L1); as such, the use of TGFbR1 or PD-1 inhibitors may enhance NK efficacy

Future studies to refine and optimize CAR-NK manufacturing may allow for translation of the promising in vitro and in vivo results into clinically improved outcomes for NB patients, as such optimization and experimental refinement have done for CAR-T therapeutics.

SUMMARY

iNKTs and NKs have unique and complementary features that hold promise in the treatment of NB and other solid tumors.

In particular, the abilities of NKs and iNKTs to kill TAMs and MDSCs, mature dendritic cells, and robustly release proinflammatory cytokines to recruit and activate conventional T cells, make these cells powerful tools in the armamentarium for NB therapy.

The ability of these cells to break down the barriers that have previously limited CAR T cell therapies in solid tumors (limited T cell persistence and potency, inability to kill tumors without target TAA or MHC-I expression, and an immunosuppressive TME), and promising pre-clinical data suggest great potential for iNKT and NK therapies in NB.

The potential for therapeutic synergy with CAR T cells and checkpoint inhibitors is an exciting area of future study.

Summary

Progress in the understanding of NB cell biology will allow a more accurate stratification of patients, thus reducing toxic side effects of aggressive therapy in low-risk patients.

High-risk patients who currently have a dismal prognosis could benefit from multidisciplinary therapeutic protocols that include novel NK cell-based immunotherapeutic strategies.

The latter will take advantage of our knowledge about the presence/absence of NB-associated ligands interacting with activating/inhibitory receptors expressed by NK cells.

Moreover, it should also take into account the multiple immunomodulatory strategies set up by NB and various immune cell types to impair the recruitment and activation of NK cells in the tumor microenvironment.

The encouraging results emerged from haploidentical hematopoietic cell transplantation in pediatric hematological malignancies, might strongly motivate are-evaluation of transplant approaches in the therapy of high-risk NB patients.