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Natural killer cell therapies for non-oncological diseases: A narrative review

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ABSTRACT
Natural killer (NK) cells represent a critical component of the innate immune system, contributing to the surveillance and elimination of infected or aberrant cells. While, extensively studied as in the case of cancer immunotherapy as they hold potential to recognize cancer cells without prior exposure, their potential therapeutic applications extend beyond oncology to encompass a spectrum of non-oncological diseases. This review discusses the evolving landscape of NK cell therapies for non-oncological diseases, focusing on their roles in infection, chronic inflammatory conditions, and autoimmune disorders. Further, this paper delves into the intricate interplay between NK cells and immune checkpoints such as T cell immunoreceptor with Ig and ITIM domains (TIGIT), T cell Ig – and mucin-domain-containing molecule-3 (TIM-3), and lymphocyte activation gene 3 (LAG3), elucidating their influence on NK cell functionality and their implications for disease pathogenesis. Additionally, the discussion highlights the emerging paradigm of chimeric antigen receptor natural killer (CAR-NK) cells as a promising avenue for targeted therapy in diseases such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human immunodeficiency virus (HIV), and autoimmune disorders. By synthesizing findings from diverse studies, it underscores the therapeutic potential of NK cell-based interventions in non-oncological diseases. Furthermore, it encompasses the need for further research to elucidate the mechanisms underlying NK cell function in these contexts, optimize therapeutic strategies, and translate these advancements into clinical practice.
Keywords: Natural killer cell, Immunotherapy, Immune checkpoints, CAR-NK

1. INTRODUCTION

Natural killer (NK) cells are descendants of bone marrow $CD34^+$ hematopoietic stem cells (HSCs) as they are derived from oligopotent common lymphoid progenitors (CLPs), which are also the precursors of the T and B lymphocytes. In the derivation pathways of common lymphoid progenitors (CLPs), two branches diverge into common innate lymphoid progenitors (CILPs) and common helper innate lymphoid progenitors (CHILPs). While, the innate lymphoid cells (ILCs) are derived from CHILPS, NK cell precursors (NKPs) originate from CILPs in order to eventually become mature NK cells. Even though, immune cells derived from lymphoid lineage are responsible for adaptive immune responses, NK cells are critical contributors to innate immune functions as the term "innate lymphoid" implies and functionally fill the gap between the adaptive and immune responses [1, 2]. Although, the location of differentiation of NK cells is not well defined because of the distribution of HSCs in different sites of the human body, NK cells can be present in primary or secondary lymphoid organs. They may be found as tissue-resident or circulatory as they constitute 5-20% of all peripheral blood lymphocytes following the numbers of T and B lymphocytes which are abundantly found in the blood [3-5]. The classification of NK cells is generally identified via the CD56 marker and according to their functionality and phenotypic features they can be summarized as cytotoxic ($CD56^{dim}$) which also express CD16 (Fc gamma receptor III – FcγIII) that is responsible for antibody-dependent cellular cytotoxicity (ADCC) and often

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described as $CD56^{dim}CD16^{bright}$ cell population that maintains cytotoxicity via perforin and/or granzyme secretion through degranulation, regulatory ($CD56^{bright}CD16^{-}$) which is mainly distinguished by cytokine secretion and responsible in establishing cell to cell communication, and the NK cell subset that is commonly seen after the persistent viral infections also known as tolerant NK cells which are mainly characterized as $CD56^{bright}CD27^{-}CD11b^{-}$ and responsible in tolerating self-antigens [6-9].

The immune effector functions of NK cells are diverse but well-coordinated to maintain homeostasis in the human body through the inhibitory and activation receptors, the influence of these receptors on NK cell functions are comprehensively discussed by researchers as they gain interests [10]. Like other innate immune cells, NK cells act as a first line of defense by mediating cytotoxicity or producing critical cytokines such as interferon γ (IFN-γ), tumor necrosis factor-α (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), lymphotoxin and interleukin 8 (IL-8) which all can be grouped as type 1 cytokines . They also produce chemo-attractants such as CXCL1 (C-X-C motif chemokine ligand 1) and CCL5 (C-C motif ligand 5) to resolve inflammation [11, 12]. One of the remarkable features that belong to NK cells is they execute their function through the "missing-self recognition". In which implies the downregulation of major histocompatibility complex I (MHC-1) molecules, without the requirement of previous antigen exposure but rather surveillance of MHC-I molecules on the surfaces of suspected cell [13]. The cancerous or infected cells with the virus eventually have diminished MHC-I expression. MHC-I downregulation are detected via inhibitory receptors of NK cells such as killer-cell immunoglobulin-like receptors (KIRs). Then, NK cells can exert their function as producing cytokines and mediating cytotoxicity towards suspected cells. This way, tolerance to self-antigens via MHC-I discrimination and spontaneous reactivity towards suspected cells can be maintained [14].

There is a vast number of works in the literature on utilizing NK cells for therapeutic purposes for cancer but in this review, the relationship between NK cell therapies and their possible applications in the eradication of immune-related diseases rather than cancer such as infection, chronic inflammatory diseases, and autoimmune diseases will be discussed. Patients with non-oncological diseases can also benefit from NK cell therapies such as monoclonal-based or cell-based (Figure 1). However, all these options are now available for oncological diseases, studies that cover these techniques are on the way of development and expected to be on the shelf products such as treatment in autoimmune diseases or viral infections, etc. (Table I).

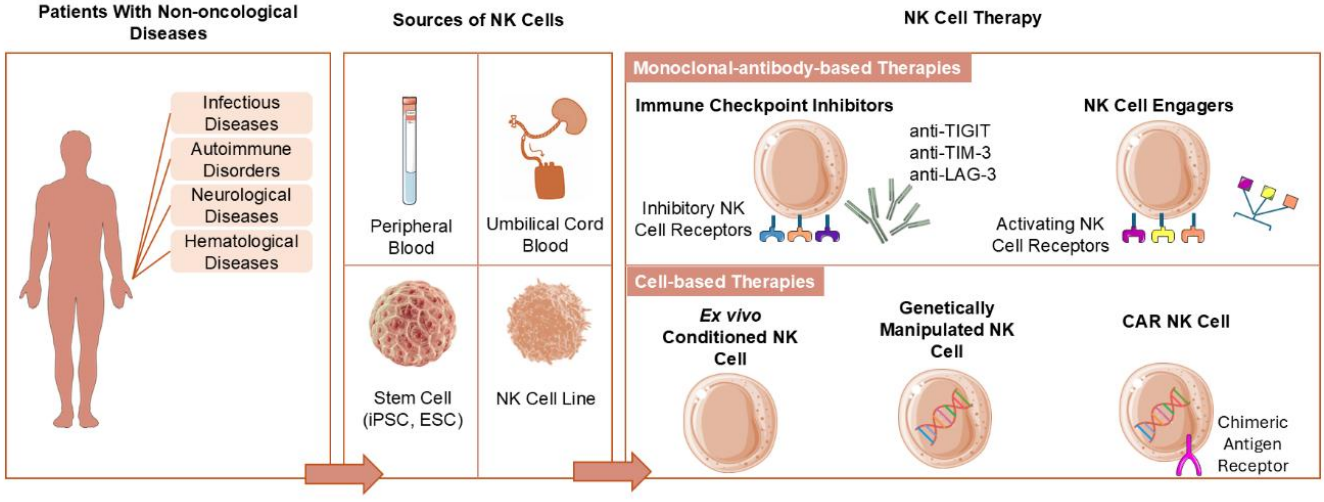

Figure 1. *Methodological summary of Natural Killer (NK) cell therapy.*

iPSC: induced pluripotent stem cells, ESC: embriyonic stem cells, TIGIT: T cell immunoreceptor with immunoglobulin and ITIM domain, TIM-3: T-cell immunoglobulin and mucin domain-3, LAG-3: Lymphocyte Activation Gene 3, CAR: Chimeric Antigen Receptor.

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Table I. *Clinical Trials. The table reports registered clinical trials on https://clinicaltrials.gov focused on NK cell therapies on non-oncological diseases.*

NCT Number	Title	Status	Conditions	Interventions	Number of subjects	Sponsor/Collaborators	Phase
			INFECTIOUS DISEASES				
			Coronavirus disease (COVID-19) or Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection				
NCT04324996	A Phase I/II Study Of Universal Off-The-Shelf NKG2D-ACE2 CAR-NK Cells Secreting IL15 Superagonist And GM-CSF-Neutralizing Scfv For Therapy Of COVID-19	Unknown	COVID-19	Biological: NK cells,IL15-NK cells,NKG2D CAR-NK cells,ACE2 CAR-NK cells,NKG2D-ACE2 CAR-NK cells	90	Chongqing Sidemu Biotech Zhejiang Qixin Biotech	Phase I/II
NCT04578210	A Phase I/II Dose-Escalation Multi-Center Study To Evaluate The Safety Of Infusion Of Natural Killer Cells Or Memory T Cells As Adoptive Therapy In Coronavirus Pneumonia And/Or Lymphopenia (RELEASE)	Completed	COVID-19	Biological: T memory cells and NK cells	84	Instituto de Investigación Hospital Universitario La Paz / Universidad Autonoma de Madrid, Universidad Miguel Hernandez de Elche, Biocruces Bizkaia Health Research Institute, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana	Phase I/II
NCT04344548	Phase I / II Clinical Study Of Immunotherapy Based On Adoptive Cell Transfer As A Therapeutic Alternative For Patients With COVID-19 In Colombia	Withdrawn	COVID-19	Biological: Allogeneic NK transfer	0	Universidad Nacional de Colombia / Fundación Salud de los Andes	Phase I/II
NCT04900454	A Phase 1 Study Of DVX201, An Allogeneic Natural Killer (NK) Cell Therapy In Subjects Hospitalized For COVID-19	Completed	COVID-19 Pneumonia	Biological: DVX201	12	Coeptis Therapeutics / Fred Hutchinson Cancer Center	Phase I
NCT04797975	A Phase ½a Multicenter Randomized Double-Blind Placebo-Controlled Trial Of Off-The-Shelf Natural Killer Cells (KDS-1000) As Immunotherapy For Adult Patients With Mild To Moderate COVID-19 Symptoms At Risk For Complications	Withdrawn	COVID-19	Biological: KDS-1000 Other: Placebo	0	Kiadis Pharma	Phase I/II
NCT04634370	Phase I Clinical Trial On Natural Killer Cells For COVID-19	Unknown Status	COVID-19	Biological: Natural Killer Cells infusion	24	Hospital de Clinicas de Porto Alegre	Phase I
NCT04363346	Study Of FT516 Safety And Feasibility For The Treatment Of Coronavirus Disease 2019 (COVID-19) In Hospitalized Patients With Hypoxia	Completed	COVID-19	Drug: FT516	5	Masonic Cancer Center, University of Minnesota	Phase I
NCT04280224	Clinical Investigation Of Natural Killer Cells Treatment In Pneumonia Patients Infected With 2019 Novel Coronavirus	Completed	Novel Coronavirus Pneumonia	Biological: NK Cells	2	Xinxiang medical university / First Affiliated Hospital of Xinjiang Medical University	Phase I
NCT04365101	A Phase I/II Study Of Human Placental Hematopoietic Stem Cell Derived Natural Killer Cells (CYNK-001) For The Treatment Of Adults With COVID-19	Unknown Status	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Biological: CYNK-001	86	Celularity Incorporated / Access to Advanced Health Institute (AAHI), Lung Biotechnology PBC, California Institute for Regenerative Medicine (CIRM)	Phase I/II

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NCT Number	Title	Status	Conditions	Interventions	Number of subjects	Sponsor/Collaborators	Phase
			Cytomegalovirus (CMV) Infection				
NCT04320303	CMV Infection And Immune Intervention After Haploidentical Hematopoietic Stem Cell Transplantation	Unknown Status	CMV Viremia, Transplantation Infection	Biological: expanded NK cells	30	Peking University People's Hospital	Not Applicable
			Human Immunodeficiency Virus (HIV) Infection				
NCT02191098	Proof Of Principle Study Of Pulse Dosing Of IL-15 To Deplete The Reservoir İn HIV Infected People On Optimized ART With Undetectable Plasma HIV RNA	Completed	HIV, Stable ART	Drug: ALT-803	10	University of Minnesota	Phase I
NCT03346499	Adoptive Transfer Of Haploidentical Natural Killer Cells And IL-2 In Human Immunodeficiency Virus (HIV)	Completed	Human Immunodeficiency Virus (HIV)	Biological: NK cells and IL-2	4	University of Minnesota	Phase I
NCT03899480	Adoptive Transfer Of Haploidentical Natural Killer Cells And IL-15 Super Agonist ALT-803 In Human Immunodeficiency Virus (HIV)	Completed	Human Immunodeficiency Virus (HIV)	Biological: Haploidentical Natural Killer (NK) Cells	9	University of Minnesota	Phase I
NCT05700630	MT2022-06: Phase I Study Of FT538 Monotherapy And In Combination With Vorinostat For The Treatment Of Persistent Low-Level HIV Viremia	Withdrawn	Human Immunodeficiency Virus (HIV)	Biological: FT538 Drug: Vorinostat	0	Masonic Cancer Center, University of Minnesota	Phase I
NCT05243381	Inflammation, NK Cells, Antisense Protein And Exosomes, And Correlation With Immune Response During HIV Infection (INKASE)	Unknown Status	Human immunodeficiency virus (HIV)	Biological: 20 ml blood test	60	University Hospital, Montpellier	Not Applicable
			AUTOIMMUNE DISORDERS				
NCT06464679	An Exploratory Clinical Study Of The Safety And Efficacy Of CD19 Chimeric Antigen Receptor NK Cell Injection For The Treatment Of Relapsed/Refractory Autoimmune Diseases	Recruiting	Autoimmune Diseases	Drug: CD19 CAR NK cells	72	Changhai Hospital / Rui Therapeutics	Phase I
NCT06318533	An Exploratory Clinical Study Of The Safety And Efficacy Of CD19 Chimeric Antigen Receptor NK Cell Injections For The Treatment Of Relapsed/Refractory B-Cell Related Autoimmune Diseases	Recruiting	Autoimmune Diseases	Drug: anti-CD19 CAR NK cells	15	Yanru Wang / Rui Therapeutics Co., Ltd	Early Phase I
NCT06208280	An Exploratory Clinical Study To Evaluate The Safety And Tolerability Of F01 İn The Treatment Of Autoimmune Diseases	Recruiting	Autoimmune Diseases	Drug: After preconditioning with Fludarabine and Cyclophosphamide, F01 will be evaluated.	20	RenJi Hospital / Shanghai Simnova Biotechnology Co.,Ltd.	Phase I
NCT06581562	Open-Label Single-Center Study To Evaluate The Safety And Efficacy Of Combining Rituximab And AB-101 In B-Cell Associated Autoimmune Diseases.	Recruiting	Rheumatoid Arthritis, Pemphigus Vulgaris, Granulomatosis With Polyangiitis, Systemic Lupus Erythematosus	Drug: AB-101 Drug: Rituximab Drug: Cyclophosphamide Drug: Fludarabine	30	Artiva Biotherapeutics, Inc.	Phase I

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NCT Number	Title	Status	Conditions	Interventions	Number of subjects	Sponsor/Collaborators	Phase
NCT06614270	Anti-CD19 CAR-NK Cells In Refractory/ Relapsed Autoimmune Diseases	Not yet recruiting	Systemic Sclerosis (SSc), ANCA Associated Vasculitis (AAV), Idiopathic Inflammatory Myopathy (IMM), Sjogren Syndrome, Antipospholipid Syndrome	Drug: anti-CD19 CAR-NK cells	15	Second Affiliated Hospital, School of Medicine, Zhejiang University	Not Applicable
			Systemic Lupus Erthymatosus (SLE)				
NCT06421701	A Clinical Study Of Anti-CD19 CAR-NK Cells In The Treatment Of Refractory/Relapsed Systemic Lupus Erythematosus	Recruiting	Systemic Lupus Erythematosus (SLE)	Drug: anti-CD19 CAR-NK cells	10	Second Affiliated Hospital, School of Medicine, Zhejiang University	Phase I
NCT06010472	An Exploratory Clinical Study Of The Safety And Efficacy Of Anti-CD19 Chimeric Antigen Receptor (CAR) Nature Killer Cells (KN5501) In The Treatment Of Moderate To Severe Refractory Systemic Lupus Erythematosus (SLE)	Recruiting	Systemic Lupus Erythematosus (SLE)	Drug: anti-CD19 CAR NK cells (KN5501)	12	Changhai Hospital / Rui Therapeutics Co., Ltd	Early Phase I
NCT06255028	The Calipso-1 Study: A Study Of CNTY-101, A CD19-Targeted CAR Ink Cell Product, In Participants With Refractory B Cell-Mediated Autoimmune Diseasesthe Calipso-1 Study: A Study Of CNTY-101, A CD19-Targeted CAR Ink Cell Product, In Participants With Refractory B Cell-Mediated Autoimmune Diseases	Recruiting	Systemic Lupus Erythematosus (SLE), Lupus Nephritis	Biological: CNTY-101 Biological: IL-2 Drug: Lymphodepleting Chemotherapy	30	Century Therapeutics, Inc.	Phase I
NCT06518668	A Phase 1 Study Of NKX019, A CD19 Chimeric Antigen Receptor Natural Killer (CAR NK) Cell Therapy, In Subjects With Systemic Lupus Erythematosus	Recruiting	Systemic Lupus Erythematosus	Biological: NKX019 Drug: Cyclophosphamide LD	6	Columbia University	Phase I
NCT06468683	A Phase I Clinical Study To Evaluate The Safety, Pharmacokinetics, And Preliminary Efficacy Of F01 İn Patients With Moderate-To-Severe Refractory Systemic Lupus Erythematosus	Not yet recruiting	Lupus Erythematosis	Biological: After preconditioning with chemotherapy, F01 will be evaluated.	50	Shanghai Simnova Biotechnology Co.,Ltd.	Phase I
NCT06377228	A Phase 1b, Open-Label, Multicenter Study To Evaluate The Safety And Efficacy Of TAK-007, An Allogeneic Anti-CD19 Chimeric Antigen Receptor Natural Killer Cell (CD19 CAR-NK) Therapy, In Adult Subjects With Refractory Lupus Nephritis	Not Yet Recruiting	Refractory Lupus Nephritis	Biological: TAK-007 Drug: Chemotherapy Agents	20	Takeda	Phase I
NCT06265220	A Phase 1 Study To Evaluate The Efficacy And Safety Of AB-101, An Allogeneic Cord Blood – Derived NK-Cell Therapy In Combination With B-Cell Depleting Mab In Patients Who Failed Treatment For Class III Or IV Lupus Nephritis Or Other Forms Of Refractory Systemic Lupus Erythematosus	Recruiting	Lupus Nephritis (WHO class III and IV), Refractory Systemic Lupus Erythematosus	Drug: AB-101 Drug: Cyclophosphamide Drug: Fludarabine Drug: Rituximab Drug: Obinutuzumab	51	Artiva Biotherapeutics, Inc.	Phase I

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NCT Number	Title	Status	Conditions	Interventions	Number of subjects	Sponsor/Collaborators	Phase
NCT06557265	A Phase 1 Study Of NKX019, A CD19 Chimeric Antigen Receptor Natural Killer (CAR NK) Cell Therapy, In Subjects With Autoimmune Disease	Recruiting	Lupus Nephritis, Glomerulonephritis	Drug: NKX019 Drug: Cyclophosphamide	21	Nkarta, Inc.	Phase I
			Rheumatoid Arthritis				
NCT06613490	An Exploratory Clinical Study Of The Safety And Efficacy Of CD19 Chimeric Antigen Receptor NK Cells For The Treatment Of Refractory Antisynthetase Antibody Syndrome And Rheumatoid Arthritis	Not yet recruiting	Antisynthetase Syndrome, Rheumatoid Arthritis	Drug: anti CD19 CAR NK cells	24	The First Affiliated Hospital with Nanjing Medical University / Rui Therapeutics Co., Ltd	Early Phase I
			Psoriasis				
NCT03894579	Phase 1, Open-Label, Safety Study Of Escalating Doses Of Ex Vivo Expanded, Autologous Natural Killer Cells In Subjects With Plaque Psoriasis	Completed	Plaque Psoriasis	Biological: Study Product: SNK01	9	NKGen Biotech, Inc.	Phase I
			NEUROLOGICAL DISORDERS				
			Alzheimer's Disease				
NCT06189963	A Phase I/Iia, Study To Evaluate The Safety, Tolerability And Exploratory Efficacy Of SNK01 In Participants With Moderate Alzheimer's Disease	Recruiting	Moderate Alzheimer's Disease	Biological: SNK01 Other: Placebo	36	NKGen Biotech, Inc.	Phase I/II
NCT04678453	Single Center, Open Label, Phase 1 Study To Evaluate The Safety, Tolerability And Exploratory Efficacy Of SNK01 In Subjects With Alzheimer's Disease (AD)	Terminated	Alzheimer's Disease, Neuro-Degenerative Disease	Biological: SNK01	10	NKGen Biotech, Inc.	Phase I
			HEMATOLOGICAL DISORDERS				
NCT06337474	An Exploratory Clinical Study Of The Safety And Efficacy Of CD19 Chimeric Antigen Receptor NK Cell Injections For The Treatment Of Refractory Primary Immune Thrombocytopenia	Not yet recruiting	Thrombocytopenia Alloimmune	Drug: anti-CD19 CAR NK cells (KN5501)	9	Changzhou No.2 People's Hospital / Rui Therapeutics Co., Ltd	Early Phase I
			OTHER CONDITIONS				
NCT03948828	Clinical Study On The Treatment Of Endometriosis By Combining With The Pathogenesis Of Endometriosis And The Application Characteristics Of NK Cells	Unknown	Endometriosis	Biological: Autologous NK cell therapy Drug: GnRHa combained with reverse addition therapy	60	Shenzhen People's Hospital	Phase I
NCT06469190	An Exploratory Clinical Study Of The Safety And Efficacy Of Anti-CD19 Chimeric Antigen Receptor NK Cell Injections (KN5501) In The Treatment Of Relapsed/Refractory Immune Nephropathy	Recruiting	Nephropathy	Drug: KN5501	36	Changhai Hospital / Rui Therapeutics Co., Ltd	Early Phase I
NCT03792997	A Therapeutic Protocol İn Previous Failed ART Patients With High Total NK Cells: A Randomized Controlled Trial	Completed	Unexplained Infertility	Combination Product: combination therapy for high peripheral NK cells	100	Benha University / Hawaa Fertility Center	Phase II

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2. NK CELL THERAPIES

NK CELL THERAPIES AS EVALUATING IMMUNE CHECKPOINTS IN NON-ONCOLOGICAL DISEASES

This section aims to describe immune checkpoints and their roles in NK cell functionality, even though the number of therapeutic interventions of these immune checkpoints and usage of immune checkpoint inhibition in a manner of treating non-oncological disease as NK cell therapy is low, we tried to underline the physiological importance and possible drug targets which may be evaluated in the future.

T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT)

When compared to those of healthy volunteers NK cells isolated from ulcerative colitis (UC) patients were shown to have high TIGIT-expressing. A study conducted by Fuchs et al., demonstrated that inhibiting TIGIT led to a slight but valuable change in the activation of NK cell effector function. This finding was confirmed using an experimental model in which NK cells collected from both UC patients and healthy donors were incubated with the MHC-I deficient K562 cell line. The K562 cell line stimulates NK cell activation via KIRs [15]. In a study that evaluated the relationship between NK cells and *Candida albicans (C. albicans)* infection through TIGIT, it was shown that *C. albicans* uses Agglutinin-like Sequences (Als) adhesion proteins to evade immune surveillance by binding to TIGIT on NK cells. In addition, the NK cells either TIGIT-expressing or non-expressing which are further separated as groups that are treated by anti-TIGIT antibodies or not and possible effector functions of these NK cell lines such as cytotoxicity through IFN-γ production on *C. albicans* and possible reduction of *C. albicans* colonies which were depicted as colony formation unit (CFU) were assessed. Then, the molecular interaction between TIGIT and *C. albicans* Als proteins was illustrated by fluorescent probes. Overall, it was shown that different strains of C. albicans and their mutations in the Als protein's binding region to TIGIT on NK cells affect NK cell functions by limiting them through TIGIT inhibition. This finding underscores the importance of this mechanism in controlling C. albicans infection. Control is achieved through immune checkpoint inhibition rather than antibiotics. Antibiotics treatments are generally prone to resistance [16]. Investigation of TIGIT on NK cells needs to be extensively studied in the context of non-oncological disorders which holds the possibility to change the course of the diseases by augmenting NK cell function via inhibition of TIGIT, which eventually may lead to resolution in inflammation caused by pathogens or inflammation inducers.

T cell Ig – and mucin-domain-containing molecule-3 (TIM-3)

As an inhibitory receptor of NK cells, TIM-3 is found to be accompanied in the medical history of people with hepatitis B virus (HBV) infection. With the study consisting of hepatitis B envelope antigen (HBeAg) positive (HBeAg+) or negative (HBeAg-), and hepatitis B virus (HBV) related hepatocellular carcinoma (HBV-HCC) patients, it is shown that the TIM-3 expression in NK cells was elevated in the HBeAg+ and HBV-HCC in compared to seronegative patients. It is also postulated that the presence of TIM-3 in HBeAg+ patients can be an obstacle in the way of conversion from seropositive to seronegative (seroconversion) in serum [17]. In another study that assessed the relationship between the SARS-CoV-2 infection and NK cell function, it was shown that there was no significant difference in the TIM-3 expression in the NK cells cultured with $A549^{ACE2/TMPRSS2}$ airway epithelial cells and 3D *ex vivo* model of human airway epithelium infected with SARS-CoV-2 [18]. For the investigation of the relationship between the HIV latency as well as the formation of reservoir and NK cells, within the scope of the TIM-3 inhibition, it is found that the NK cell functionality onto the viral replication of HIV is diminished. Still, it is thought that it may not affect the cytotoxicity mediated through CD8+ cytotoxic T cells. In contrast, both $CD4^+$ helper T cells and NK cells co-cultured with cytotoxic T cells, even though it was associated with a decrease in the concentration of p24 antigen [19]. In a study that done by Kared et al., when human cytomegalovirus (HCMV) and HIV infections are assessed in the context of TIM-3 and adaptive NK cells, separately, TIM-3 may induce apoptosis in NK cells when the HCMV infection occurs regardless of their maturation status while NK cells loss their capacity to synthesize cytokines which further lead to chronic inflammation of HIV [20]. Moreover, in a study published by Devulder et al., the authors postulated that when peripherical blood mononuclear cells (PBMC) incubated with the rhinovirus A9 (RV-A9) which is responsible for the progression of asthmatic response, effector functions of NK such as NK stimulation, secretion of its granular content, IFN-γ production, and subsequently cytotoxic activity are lesser compared to unstimulated NK which are all estimated to be related to elevated levels of TIM-3 expression found on the NK cells [21]. By utilizing TIM-3-deficient ($TIM\text{-}3^{-/-}$) pregnant mice to clarify the relationship of *Toxoplasma gondii (T. gondii)* with the function of decidual NK (dNK) cells which are localized in the uterine tissue and subsequent pregnancy abnormalities, Li et al. highlighted that the inhibition of TIM-3 is associated with the poor prognosis in the pregnant mice after *T. gondii* infection. It is thought to be associated with dysregulation of dNK receptors, and abnormal secretion of both granular contents and cytokines throughout Phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) and Janus kinase/signal transducers and activators of transcription (JAK-STAT) biochemical pathways [22]. However, these are some of the studies that briefly summarize the TIM-3 and NK cell axis, the underlying mechanisms that they cover are the possible NK cell therapy strategies to balance pathological and physiological events in non-oncological diseases.

Lymphocyte-activation gene (LAG3)

Expression of LAG3 in NK cells was associated with an exhaustion profile that may be characterized by diminished secretory activity (such as IFN-γ and TNF-α), secretion of cytotoxic granules because of obstacles in the degranulation

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and by a regulatory profile as secreting immunosuppressive cytokine, IL-10 [23]. A prospective study that comprised 86 patients infected with COVID-19 and compared them with patients with non-COVID respiratory infections and healthy volunteers in the context of hospitalization aimed to reveal a distinct immunological profile that involves cells that are effective in the eradication of viral infections such as NK cells and the regulatory factors which they are influenced with. In this study, it was demonstrated that severe to moderate levels of patients with COVID-19 immunological profile were presented with significantly upregulated levels of both $LAG3^+$ activated and exhausted NK cells, making it possible drug target for coping with the COVID-19 related hospitalization [24]. Furthermore, a longitudinal study that investigated the opportunistic viral infection that may occur after taking immunosuppressive drugs among patients with a history of haploidentical stem cell transplantation, especially HCMV infection which can be seen in more than 35% of the transplantation patients. In this study, NK cells are evaluated in both HCMV-reinfected and non-infected patients, according to their LAG3 expression, it was shown that reinfected patients are characterized by an exhausted NK cell profile which is also associated with upregulated LAG3 levels and down-regulated IFN-γ production and it is postulated that even though LAG3 inhibition done in NK cell taken from reinfected patients, IFN-γ levels cannot reach the same level as non-infected patients' IFN-γ levels [25]. By using the humanized MISTRG-6-15 mice model, Sungur et al., created a model for assessing human NK cell profiles in HIV-1 injected MISTRG-6-15 mice to detect LAG3 levels on NK cells and showed that after 14 days there was an increase in the levels of LAG3 and down-regulation of cytotoxic cytokines such as granzyme B (GZMB), TNF-α and IFN-γ. In addition to that, antiretroviral therapy (ART) for 8 weeks, after a 4-week injection of HIV-1 to the mice, has been shown to moderately down-regulated the levels of LAG3 which can be seen as a new therapeutic intervention as augmenting NK cell function within the context of HIV-1 infections [26]. According to a longitudinal study done by Ty et al., which constitute Ugandan children as a cohort who are prone to a frequent malarial infection are shown to have increased LAG3 expression in mature NK cells which may be characterized by diminished cytokine production, although, the main characteristics assessed through the identification of NK cells in malarial infection, there should be shreds of evidence about the influence of malaria trained NK cells on the comorbidities and their possible targeted therapeutic intervention must consider their influence [27]. Overall, LAG3 should be extensively explored in the context of NK cells and other pathological mechanisms in non-oncological diseases which may hold a new therapeutic revenue.

NK-CELL-BASED THERAPIES IN NON-ONCOLOGICAL DISEASES

Chimeric antigen receptor natural killer cells (CAR-NK)

A study conducted by Lu et al., evaluated the effectiveness of CAR-NK in combating the SARS-CoV-2 infection by utilizing umbilical cord blood NK cells. The CAR-NK cells were engineered to be efficient in promoting bioavailability and specific targeting antigens associated with SARS-CoV-2 as transduced to have soluble IL-15 and angiotensin-converting enzyme (ACE) receptor to induce binding through Spike (S) protein of SARS-CoV-2, respectively. However, these mACE2-CAR-sIL15 NK cells were approved for having promising results for eradication of SARS-CoV-2 in the transgenic *in vivo* setting, a clinical trial should be done to better understand the effectiveness of this therapy especially for the patients with severe SARS-CoV-2 pathology [28]. In addition, another study that utilized CAR-NK cells to eradicate SARS-CoV-2 by targeting conversed domains of S protein which may also provide recognition of other variants, was confirmed with the design of CAR-NK bearing scFV domain of S309 which is a neutralizing antibody. These S309-CAR-NK cells were proved to have higher effector functions even compared to other CAR constructs as elevated levels of TNF-α and IFN-γ when they recognize the SARS-CoV-2 infected A549 cell line which makes S309-CAR-NK cells efficient in eradicating infection and these results imply that there should be further studies to confirm clinical efficiency [29]. In another study published by Lim et al., it was aimed to specifically target glycoprotein 160 (gp160) which is a glycoprotein expressed by HIV-1 via designing CAR-NK as well as to provide intracellular signaling, the CD28 transmembrane and intracellular domain are inserted into the genetic construct of the CAR lentiviral vector with the CD3ζ domain. However, there is a vast number of distinctive genetic variations among HIV-1 subtypes which makes direct gp160 targeting infeasible, to cope with this problem, researchers developed a CAR construct with an anti-2,4-dinitrophenyl (anti-DNP) domain that uses the principle of binding DNP-containing antibody which provides inducement to bind to target gp160 and its subtypes A and C shown by the co-culture the CAR-NKs with gp160 expressing cell lines transduced from HEK293T cell line. In addition, effective cytotoxicity against $CD4^+$ T cells infected by HIV-1 was also demonstrated which makes the usage of anti-DNP CAR-NK cells for the HIV-1 treatment regimen a promising approach [30]. The utilization of the NK-92 cell line in a study has resulted in the creation of programmed cell death 1 (PD-1) targeting CAR-NK cells via transduction of NK-92 to programmed cell death ligand 1 (PD-L1)-bearing cells. These CAR-NK cells have been specifically designed to selectively suppress T follicular helper (T_{FH}) cells, which express high levels of PD-1 relative to other lymphocytes, through degranulation. This approach can be promising for eradicating T_{FH}-related diseases such as autoimmune and disorders that can be characterized by excessive T_{FH} activation [31]. Altogether, CAR-NK holds immense promises in addition to oncological diseases, augmenting NK cell effector function via

CAR constructs for eradicating viral infections or autoimmune diseases paving the way for novel therapeutic approaches with potentially fewer side effects than traditional treatments.

Conclusions and Future Directions

Natural Killer cells, integral components of the innate immune system, are emerging as potent therapeutic modalities beyond oncology, particularly in non-oncological diseases. This review synthesizes current insights into NK cell therapies, emphasizing their pivotal roles in combating infections, managing chronic inflammatory conditions, and potentially ameliorating autoimmune disorders. NK cells perform diverse functions crucial for immune surveillance and response, including direct cytotoxicity against infected or aberrant cells, secretion of key cytokines for immune regulation, and modulation of immune checkpoints such as TIGIT, TIM-3, and LAG3. Targeting these checkpoints holds promise for fine-tuning NK cell activity in a spectrum of diseases, from viral infections like SARS-CoV-2 to chronic ailments such as HIV. The advent of CAR – NK cell therapy represents a transformative approach in precision medicine, demonstrating efficacy in targeting specific pathogens and diseases. Despite these advancements, the clinical translation of NK cell therapies necessitates overcoming challenges such as optimizing manufacturing processes, enhancing cell persistence and efficacy, and ensuring safety profiles across diverse patient populations. Future research directions should prioritize elucidating the intricate mechanisms governing NK cell biology, understanding disease-specific variations in immune checkpoint interactions, and leveraging genetic modification and CAR technologies to tailor therapies for individual patients. Rigorous clinical validation through well-designed trials is imperative to establish the safety, efficacy, and long-term benefits of NK cell therapies across diverse disease contexts. Furthermore, exploring synergies with existing treatments and addressing ethical and regulatory considerations are critical for maximizing the therapeutic potential of NK cell-based interventions. In summary, NK cell therapies represent a promising frontier in immunotherapy, poised to reshape the landscape of non-oncological disease treatment through ongoing innovation and collaborative efforts across scientific, clinical, and regulatory domains.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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REFERENCES

[1] Murin CD. Considerations of antibody geometric constraints on NK cell antibody dependent cellular cytotoxicity. Front Immunol 2020;11:1635. doi: 10.3389/fimmu.2020.01635.

[2] An Z, Flores-Borja F, Irshad S, Deng J, Ng T. Pleiotropic role and bidirectional immunomodulation of innate lymphoid cells in cancer. Front Immunol 2020;10:3111. doi: 10.3389/fimmu.2019.03111.

[3] Montazersaheb S, Fathi E, Farahzadi R. Cytokines and signaling pathways involved in differentiation potential of hematopoietic stem cells towards natural killer cells. Tissue Cell 2021;69:101501. doi: 10.1016/j.tice.2021.101501.

[4] Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. Front Immunol 2018;9:1869. doi: 10.3389/fimmu.2018.01869.

[5] Roma S, Carpen L, Raveane A, Bertolini F. The dual role of innate lymphoid and natural killer cells in cancer. From phenotype to single-cell transcriptomics, functions and clinical uses. Cancers (Basel) 2021;13:5042. doi: 10.3390/cancers13205042.

[6] Roe K. Immunoregulatory natural killer cells. Clin Chim Acta 2024;546:117896. doi: 10.1016/j.cca.2024.117896.

[7] Letafati A, Ardekani OS, Naderisemiromi N, et al. Unraveling the dynamic mechanisms of natural killer cells in viral infections: insights and implications. Virol J 2024;21:18. doi: 10.1186/s12985.024.02287-0.

[8] Fu B, Tian Z, Wei H. Subsets of human natural killer cells and their regulatory effects. Immunology 2014;141:483-9. doi: 10.1111/imm.12224.

[9] Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN-γ-secreting and IFN-γ-nonsecreting NK cells. Eur J Immunol 2002;32:879-84. doi: 10.1002/1521-4141(200203)32:3<879::AID-IMMU879>3.0.CO;2-2.

[10] Page A, Chuvin N, Valladeau-Guilemond J, Depil S. Development of NK cell-based cancer immunotherapies through receptor engineering. Cell Mol Immunol 2024;21:673-87. doi: 10.1038/s41423.024.01145-x.

[11] He S, Su L, Hu H, et al. Immunoregulatory functions and therapeutic potential of natural killer cell-derived extracellular vesicles in chronic diseases. Front Immunol 2023;14:1328094. doi: 10.3389/fimmu.2023.132.8094.

[12] Vivier E, Rebuffet L, Narni-Mancinelli E, Cornen S, Igarashi RY, Fantin VR. Natural killer cell therapies. Nature. 2024;626(8018):711-27. doi: 10.1038/s41586.023.06945-1.

[13] Forbes C, Nierkens S, Cornel AM. Thymic NK-cells and their potential in cancer immunotherapy. Immunotargets Ther 2024;13:35-47. doi: 10.2147/ITT.S441639.

[14] Rascle P, Woolley G, Jost S, Manickam C, Reeves RK. NK cell education: physiological and pathological influences. Front Immunol. 2023;14:1087155. doi: 10.3389/fimmu.2023.108.7155.

[15] Fuchs S, Sawas N, Staedler N, et al. High-dimensional single-cell proteomics analysis identifies immune checkpoint

signatures and therapeutic targets in ulcerative colitis. Eur J Immunol 2019;49:462-75. doi: 10.1002/eji.201847862.

[16] Charpak-Amikam Y, Lapidus T, Isaacson B, et al. Candida albicans evades NK cell elimination via binding of Agglutinin-Like Sequence proteins to the checkpoint receptor TIGIT. Nat Commun 2022;13:30087. doi: 10.1038/s41467.022.30087-z.

[17] Huang X, Mo Q, Fu T, Liu Y, Diao B. STAT1 is associated with NK cell dysfunction by downregulating NKG2D transcription in chronic HBV-infected patients. Immunobiology 2022;227:152272. doi: 10.1016/j.imbio.2022.152272.

[18] Lenart M, Gorecka M, Bochenej M, et al. SARS-CoV-2 infection impairs NK cell functions via activation of the LLT1-CD161 axis. Front Immunol 2023;14:1123155. doi: 10.3389/fimmu.2023.112.3155.

[19] Sanz M, Madrid-Elena N, Serrano-Villar S, Vallejo A, Gutiérrez C, Moreno S. Effect of the use of Galectin-9 and blockade of the TIM-3 receptor in the latent cellular reservoir of HIV-1. J Virol 2021;95:e022014-20. doi: 10.1128/JVI.02214-20

[20] Kared H, Martelli S, Tan SV, et al. Adaptive NKG2C+CD57+ natural killer cell and Tim-3 expression during viral infections. Front Immunol 2018;9:686. doi: 10.3389/fimmu.2018.00686.

[21] Devulder J, Chenivesse C, Ledroit V, et al. Aberrant anti-viral response of natural killer cells in severe asthma. Eur Respir J 2020;55:1902422. doi: 10.1183/13993.003.02422-2018.

[22] Li T, Cui L, Xu X, et al. The role of Tim-3 on dNK cells dysfunction during abnormal pregnancy with Toxoplasma gondii infection. Front Cell Infect Microbiol 2021;11:587150. doi: 10.3389/fcimb.2021.587150.

[23] Esen F, Deniz G, Aktas EC. PD-1, CTLA-4, LAG-3, and TIGIT: the roles of immune checkpoint receptors on the regulation of human NK cell phenotype and functions. Immunol Lett 2021;240:15-23. doi: 10.1016/j.imlet.2021.09.009.

[24] Uranga-Murillo I, Morte E, Hidalgo S, et al. Integrated analysis of circulating immune cellular and soluble mediators reveals specific COVID-19 signatures at hospital admission with utility for prediction of clinical outcomes. Theranostics 2022;12:290-306. doi: 10.7150/THNO.63463.

[25] Zaghi E, Calvi M, Puccio S, et al. Single-cell profiling identifies impaired adaptive NK cells expanded after HCMV reactivation in haploidentical HSCT. J Clin Invest 2021;6:e146973. doi: 10.1172/jci.insight.146973

[26] Kim JT, Zack JA. A humanized mouse model to study NK cell biology during HIV infection. J Clin Invest 2022;132:e165620. doi: 10.1172/jci/insight165620.

[27] Ty M, Sun S, Callaway PC, et al. Malaria-driven expansion of adaptive-like functional CD56-negative NK cells correlates with clinical immunity to malaria. Sci Transl Med 202315:eadd9012. doi: 10.1126/scitranslmed.add9012

[28] Lu T, Ma R, Dong W, et al. Off-the-shelf CAR natural killer cells secreting IL-15 target spike in treating COVID-19. Nat Commun 2022;13:2576. doi: 10.1038/s41467.022.30216-8.

[29] Ma MT, Badeti S, Chen C-H, et al. CAR-NK cells effectively target SARS-CoV-2-spike-expressing cell lines in vitro. Front Immunol 2021;12:652223. doi: 10.3389/fimmu.2021.652223.

[30] Lim RM, Rong L, Zhen A, Xie J. A universal CAR-NK cell targeting various epitopes of HIV-1 gp160. ACS Chem Biol 2020;15:2299-310. doi: 10.1021/acschembio.0c00537.

[31] Reighard SD, Cranert S A, Rangel K M, et al. Therapeutic targeting of follicular T cells with chimeric antigen receptor-expressing natural killer cells. Cell Rep Med 2020;1:100003 doi: 10.3389/fimmu.2021.652223.