TIGIT Engagement Impairs Antiviral Effector Functions of $CD8^+$ T Cells from People Living with HIV

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ABSTRACT

A persistent latent reservoir in long-lived $CD4^+$ T-cells is the main obstacle to eradicating HIV-1 infection. Chronic HIV-1 infection functionally alters $CD8^+$ T cells through upregulation of immune checkpoint receptors (ICs) such as T cell immunoreceptor with Ig and ITIM domains (TIGIT). Expression of ICs can result in impaired cytolytic activity and failure to suppress viral replication. Therefore, blocking IC receptors could be an adjunct therapeutic approach targeting the HIV reservoir in eradication strategies.

This study employed TIGIT engagement and blockade on $CD8^+$ T cells from people living with HIV(PLWH) to test how TIGIT expression affects T cell function. We tested TIGIT engagement on $CD8^+$ T cells from PLWH in non-specific redirected cytotoxicity assays and tested the impact of TIGIT blockade on HIV antigen-specific $CD8^+$ T cells.

For PLWH with circulating $CD8^+$ T cell cytotoxicity >10% in redirected killing assays, TIGIT engagement reduced cytotoxicity in 8/14 cases, showing that TIGIT engagement impairs killing by $CD8^+$ T cells from some PLWH. About 20% of subjects tested by ELISpot had strong interferon-gamma (IFN)-γ responses against HIV Gag and/or Nef peptides (>1000 spot-forming units/10^6 peripheral blood mononuclear cells). Stimulation of HIV-specific $CD8^+$ T cells with peptides in the presence of TIGIT-blocking mAb increased $CD8^+$ T cell degranulation and IFN-γ production in certain individuals. Thus, generalized and HIV specific effector functions of $CD8^+$ T cells from a subset of PLWH are inhibited by TIGIT expression.

These data show that TIGIT blockade can improve antiviral effector cell function in certain PLWH. Identifying features of the subset of responsive $CD8^+$ T cells will help

direct blockade therapy to those PLWH most likely to benefit.

Keywords: HIV-1, CD8, T cell, TIGIT, TIGIT blockade, immune checkpoint, IFN-γ , CD107A

General Summary

HIV-1 infection is challenging to eradicate because the virus hides in a group of long-lived immune cells called $CD4^+$ T-cells. This hidden reservoir of the virus is difficult to target. Chronic HIV-1 infection also affects another type of immune cell, the $CD8^+$ T-cell, which normally helps control infections. In people living with HIV (PLWH), these $CD8^+$ T-cells can become less effective due to the increased presence of certain molecules on their surface, such as TIGIT. These molecules can hinder the cells' ability to kill infected cells and control the virus.

In our study, we found that the presence of TIGIT on $CD8^+$ T-cells reduced their ability to fight the virus in some PLWH. Blocking TIGIT improved the function of these immune cells in certain individuals, suggesting that targeting TIGIT could be a new way to treat HIV.

Our results underscore the inhibitory role of TIGIT in regulating T cell activity and its potential as a therapeutic target in HIV infection. Further analysis revealed not all individuals responded the same way to TIGIT blockade, emphasizing the need to identify predictive markers for treatment effectiveness. Additionally, phenotyping responsive T cells highlighted the complex nature of immune responses and the importance of personalized approaches to immunotherapy.

Overall, our findings provide insights into the potential of TIGIT blockade as a therapeutic intervention in HIV infection and underscore the importance of personalized treatment approaches.

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Co-authorship statement

All individuals listed below have influenced the work of this thesis manuscript and have obtained authorship.

Nazanin Ghasemi was principally responsible for conducting all experiments and the preparation of this thesis.

Dr. Michael Grant served as the supervisor for this project, playing a crucial role in the study design, revision process, and the final approval of the thesis.

Kim Burt as a registered nurse undertook blood sample collections and obtained informed consent from study participants.

Danielle Ings, Kathleen Fifield and Nazanin Ghasemi processed blood samples.

Dr. Kayla Holder provided invaluable guidance on how to conduct experiments and their development.

Furthermore, Stephanie Tucker, medical laboratory technologist at Memorial University of Newfoundland provided instruction and teaching on how to work with Beckman Cytoflex Flow cytometer.

Conflict of interest

I declare that there are no conflicts of interest. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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°C	degrees Celsius
%	percentage
α	alpha
Ab	antibody
ADCC	antibody-dependent cell-mediated cytotoxicity
AIDS	acquired immunodeficiency syndrome
cART	combination antiretroviral therapy
β	beta
BSA	bovine serum albumin
CD	cluster of differentiation
CMV	cytomegalovirus
Cr	Chromium
CTLA-4	cytotoxic T lymphocyte-associated protein 4
DC	dendritic cell
DNAM-1	DNAX accessory molecule-1
DMEM	Dulbecco's modified Eagles medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid

EDTA	ethylenediaminetetraacetic acid
E:T	effector to target ratio
Fc	fragment crystallizable region
FcγRI	CD64, Fc-gamma receptor I
FcRII	CD32, Fc-gamma receptor II
FcγRIII	CD16, Fc-gamma receptor III
FcR	Fc receptor
FCS	fetal calf serum
γ	gamma
g	gram(s)
g	gravitational force
h	hours
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
HLA	human histocompatibility-linked leukocyte antigen
IC	isotype control
IF	immunofluorescence
IFN	interferon

Ig	immunoglobulin
IL	interleukin
ILC	innate lymphoid cell
ITAM	immunoreceptor tyrosine-based activating motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
k	kilo
L	litre
LAG-3	lymphocyte-activation gene 3
LILR	leukocyte immunoglobulin-like receptor
M	molar
mAb	monoclonal antibody
MHC	major histocompatibility complex
Min	minute
mL	millilitre
MMP	matrix metalloproteinase
n	nano
NaCl	sodium chloride
$Na_2{}^{51}CrO_4$	radiolabelled sodium chromate
NFκB	nuclear factor kappa light chain enhancer of activated B

NK	natural killer
NKG	natural killer group
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PD-1	programmed cell death protein 1
pH	potential of hydrogen
pDC	plasmacytoid DC
PLWH	people living with HIV
PVR	poliovirus receptor
RPMI	Roswell Park Memorial Institute medium
SAP	SLAM-associated protein
SD	standard deviation
SEM	standard error of the mean
SIV	simian immunodeficiency virus
STAT	signal transducer and activator of transcription
Syk	spleen tyrosine kinase
TACTILE	T cell-activated increased late expression
T-bet	T-box transcription factor
TCR	T cell receptor

TFH	follicular T helper cell
TIGIT	T cell immunoreceptor with Ig and ITIM domains
Tim3	T cell immunoglobulin mucin-3
TLR	toll-like receptor
TNF-α	tumour necrosis factor alpha
µ	micro
µL	microlitre
UV	ultraviolet
Vf	final volume
Vpr	viral protein R
Vpu	viral protein U

1. Introduction and Overview

1.1 HIV-1 Infection

Human immunodeficiency virus-1 (HIV-1) is a retrovirus belonging to the Lentivirus family [1]. The origin of HIV can be traced back to the late 19th to early 20th century in Central and West Africa. It is believed to have emerged as a result of the cross-species transmission of simian immunodeficiency viruses (SIVs) from chimpanzees to humans. HIV-1 and HIV-2 emerged from zoonotic transfer of viruses infecting lower primates. HIV-1 is the predominant and more virulent strain, responsible for the majority of global HIV infections, while HIV-2 is less pathogenic and endemic in Western Africa [2,3].

Infection with HIV in the absence of treatment leads to acquired immunodeficiency syndrome (AIDS) [1]. Thirty-nine million people globally were reported living with HIV in 2022 [4]. Over the 20th century, HIV rapidly spread across the globe, primarily through sexual contact, contaminated blood products, and from mother to child during gestation, childbirth or breastfeeding [3]. A better understanding of the virus allowed development of antiretroviral therapies (ARTs), reduction of HIV-1 disease progression to AIDS and introduction of a range of prevention strategies.

1.2 HIV-1 genome

The HIV-1 genome is a single-stranded RNA molecule that encodes major structural and non-structural proteins for virus replication and survival. From the 5′ to 3′ end of the genome, three major genes, *gag*, *pol* and *env*, code for structural proteins (gag), essential for the assembly of new viral particles (Matrix, Capsid, Nucleocapsid, p6), enzymes (pol) necessary for processing structural proteins, converting RNA into DNA

and integration of the proviral DNA into the host's genome (Protease, Reverse transcriptase (RT), Integrase) and envelope proteins (env), essential for entry of the virus to target cells (GP120, GP41), respectively. In addition to the structural proteins, the HIV genome codes several regulatory proteins (Tat, Rev) and accessory proteins (Vif, Vpr, Vpu/Vpx, Nef), Tat (transactivator protein) and Rev (RNA splicing-regulator) are necessary for the regulation of HIV-1 gene expression. *Tat* gene encodes a protein that enhances the transcription of viral genes by binding to the transactivation response region (TAR) on viral RNA. Tat promotes efficient viral replication by activating the elongation phase of transcription. *Rev* gene encodes a protein that regulates the expression of viral proteins by facilitating the export of unspliced and partially spliced viral RNAs from the nucleus to the cytoplasm. This process ensures the production of structural and enzymatic proteins necessary for virion assembly. The other regulatory proteins Nef (negative regulating factor), Vif (viral infectivity factor), Vpr (virus protein r) and Vpu (virus protein unique) have an impact on viral replication, virus budding from the host cell's membrane and pathogenesis (Figure 1.2.1) [5- 7].

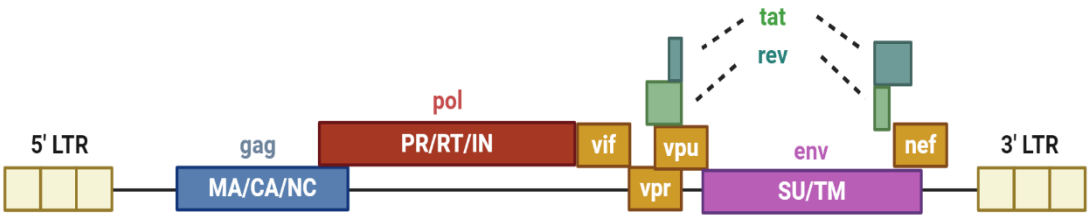


Figure 1.2.1: HIV-1 genome organization. HIV-1 encodes Gag, Pol, and Env polyproteins. *Gag* encodes structural proteins matrix (MA), capsid (CA), and nucleocapsid (NC), *pol* encodes the viral enzymes protease (PR), reverse-transcriptase (RT), and integrase (IN), and *env* encodes the surface (SU) and transmembrane (TM) envelope glycoprotein GP160, which is processed into the receptor-binding GP120 and transmembrane GP41. In addition to the *gag*, *pol*, and *env* genes that are typical of all retroviruses, there are two regulatory (*tat* and *rev*) and four accessory (*vif*, *vpr*, *vpu*, and *nef*) gene. Image created with BioRender.com.

1.3 HIV life cycle

HIV has the ability to establish long-lasting cellular reservoirs within the human body. These reservoirs are primarily composed of infected immune cells that allow the virus to persist even in the presence of ART, making HIV a challenging virus to eliminate completely. HIV primarily infects and replicates within $CD4^+$ T cells. Its two major cellular reservoirs are latently infected resting $CD4^+$ T cells and macrophages. While ART can effectively suppress viral replication, the virus can persist in a latent, non-replicating state in a small population of $CD4^+$ T cells. These latently infected cells serve as a long-term reservoir, capable of reactivating and producing the virus if ART is interrupted or if other activation signals occur [8-10].

Entry into Host Cells:

The initial step in the HIV life cycle involves the interactions between envelope (Env) proteins (gp120 subunit of the HIV-1 envelope), the host cell CD4 receptor, and additional coreceptors (CCR5 or the CXC chemokine receptor CXCR4), followed by gp41 subunit of HIV-1 envelope-mediated fusion with the cellular membrane. Subsequently, the capsid disintegrates in the host cell cytoplasm, and viral RNA is released. (Figure1.3.1) [11].

Reverse Transcription and Integration:

Once inside the host cell, viral RNA is released, and HIV reverse transcriptase transcribes the viral RNA into double-stranded DNA. The viral DNA is transported to the nucleus, along with associated proteins, forms the pre-integration complex (PIC), and migrates to the host cell nucleus, where it integrates into the host's DNA. In the

nucleus, the viral DNA is integrated into the host genome by the viral enzyme integrase, where it persists as a provirus (Figure1.3.1) [12].

Transcription and Translation:

Upon integration into the host genome, the proviral DNA can undergo transcription. The integrated HIV DNA transcribes new copies of HIV RNA using the host's transcription machinery. The mRNA acts as a template for synthesis of HIV proteins and these proteins are cleaved by viral protease into functional viral proteins, including structural proteins (Gag, Pol, and Env) and regulatory proteins (Tat and Rev) (Figure1.3.1).

Assembly and Budding:

The newly synthesized viral proteins migrate to the cell membrane, where viral RNA and structural proteins assemble at the plasma membrane lipid rafts. The assembly process involves the incorporation of viral RNA into the assembling virion, followed by budding through the host cell membrane. During budding, the viral protease cleaves the polyproteins to produce mature virions. The budding process involves acquisition of the host cell membrane by the virions resulting in the incorporation of viral envelope glycoproteins into assembling virions as they bud from the cell surface. Finally, the virus is released from the cell (Figure1.3.1) [11-14].

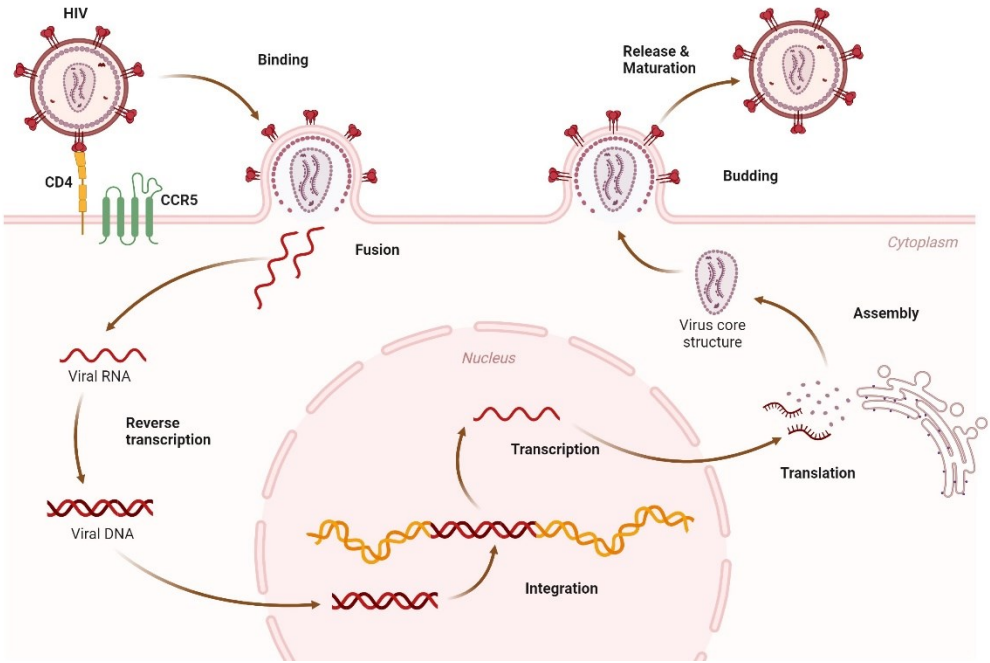


Figure 1.3.1: The life cycle of the HIV-1 virus. The early stage involves the virus binding to host cell receptors, leading to fusion and release of the viral RNA into the cell. Inside the host cell, HIV reverse transcriptase converts the viral RNA into DNA. Once in the nucleus, integration of the viral genome occurs. Following integration, viral genes are transcribed and translated to protein, which leads to assembly, budding and release of immature virions. Maturation is the final step in the HIV-1 lifecycle, during which the viral protease cleaves the polyproteins into individual functional proteins. Image was modified with BioRender.com.

1.4 HIV-1 and the Immune system

1.4.1 HIV-1 and Innate Immunity

The innate immune system acts as the first line of defense against infection and is activated rapidly after virus exposure [15]. It is activated through Pattern Recognition Receptors (PRRs) that recognize common features of pathogens. Toll-like receptors (TLRs) are one of the main PRRs that sense Pathogen-Associated Molecular Patterns (PAMPs) of viral products to initiate cell-intrinsic innate immune responses and direct antiviral defenses. TLRs can detect double-stranded RNA (dsRNA) and structured single-stranded RNA (ssRNA), as well as certain viral proteins [15-17]. TLR7/8 recognize HIV-1 ssRNA and induce the production of several cytokines, including type I interferons (IFNs) [18, 19]. Interaction of IFNs with their receptors on the surface of cells in turn induces expression of interferon-stimulated genes (ISGs) [16], which are important for limiting viral replication [20]. Plasmacytoid dendritic cells (pDCs) and macrophages are important cells in acute or early HIV-1 infection for reducing HIV-1 replication [21, 22]. TLR7 and TLR8, are expressed in pDCs and macrophages and can detect HIV-1 RNA and viral proteins. Activation of TLR7/8 in pDCs triggers the release of type I IFN cytokines, such as IFN-α and secretion of other cytokines, including tumor necrosis factor (TNF)-α, interferon inducible protein 10 (IP-10), interleukin (IL)-18, IL-10 and finally type II IFN (Figure 1.4.1) [20,23]. Secretion of these cytokines is associated with activation of the HIV-specific adaptive immune response and innate immune cells like natural killer (NK) cells [23, 24]. In addition, type I IFNs regulate development of immune cells like $CD4^+$ and $CD8^+$ T cells by inhibiting apoptosis [25,26]. Although, HIV can interfere with the signaling pathways initiated by TLRs on

innate immune cells. By doing so, it can modulate the production of cytokines and interferons that are essential for an effective antiviral response [20,23].

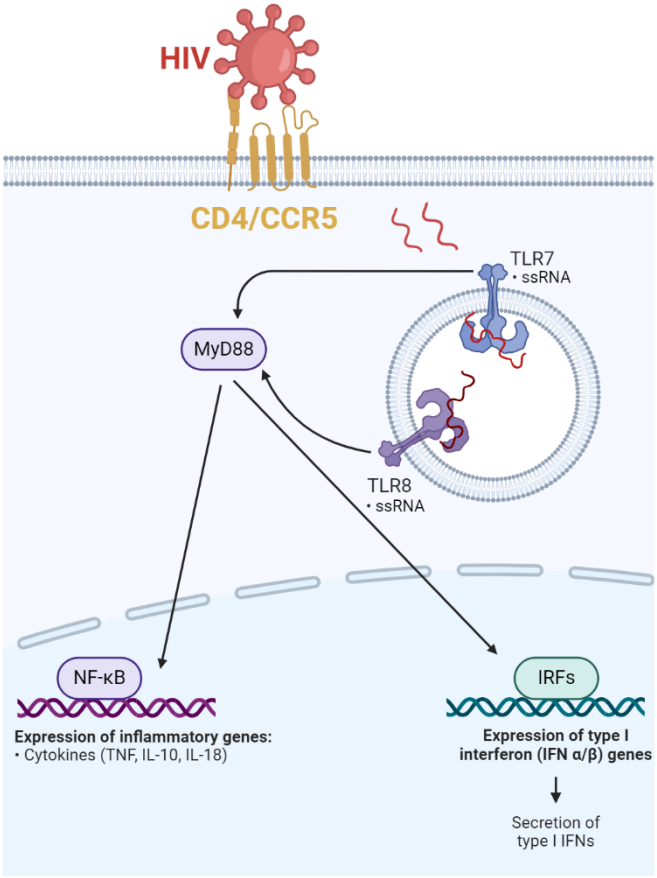


Figure 1.4.1: The Role of TLRs in HIV-1 Infection. When HIV is endocytosed, viral RNA is recognized by TLR-7/8 inside endosomes, leading to a signaling cascade through the adaptor protein MyD88. This activates transcription factors such as NF-κB and IRFs, which stimulate the expression of inflammatory cytokines (TNF, IL-10, IL-18) and type I interferon. Image created with BioRender.com.

NK cells produce pro-inflammatory cytokines to increase the responses of innate and adaptive immune cells and can directly lyse infected cells by release of secretory lysosomes containing perforin and granzymes. Perforin forms pores in the target cell membrane, leading to osmotic shock and granzyme A and B facilitate the cleavage of caspases that leads to programmed cell death of the target cell [27-29]. NK cells can recognize HIV-infected $CD4^+$ T cells based on reduced expression of major histocompatibility complex class I (MHC-I) molecules on the surface of infected cells. MICA and MICB are ligands for activating NK cell receptors such as NK group 2D (NKG2D) receptor and they are upregulated on HIV- infected cells. The expression of these NKG2D ligands can activate NK cells and trigger their cytotoxicity [30]. Therefore, NK cells can kill infected $CD4^+$ T cells. Also, NK cells can recognize and kill HIV-infected cells that have been opsonized (coated with antibodies) by the humoral immune response through antibody-dependent cell-mediated cytotoxicity (ADCC) [31,32]. NK cells have receptors for the Fc portion of some subclasses of IgG antibody that bind to antibodies on infected cell surfaces and trigger the NK cells to eliminate the sensitized and opsonized infected cells [33].

Some studies have found that increased NK cell activity is associated with resistance to HIV disease progression [34, 35], but as HIV infection progresses, it can impair NK cell function through downregulating activating receptors and upregulating inhibitory receptors. This helps the virus evade the immune system (Figure 1.4.2) [27, 28].

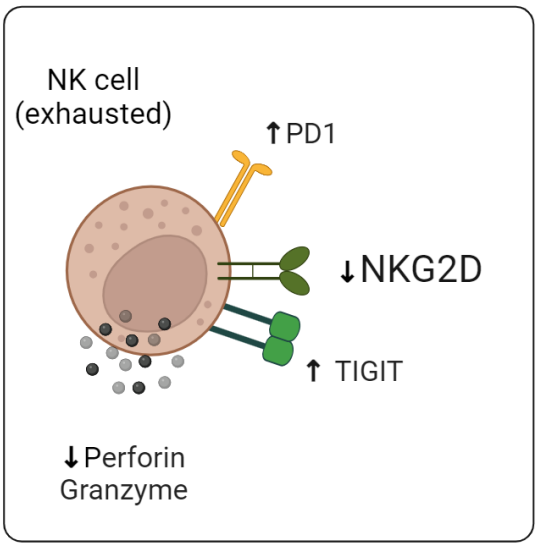


Figure 1.4.2: Natural killer cell activity with HIV progression. The figure illustrates the altered expression of key receptors of NK cell that significantly impact NK cell functionality. The increased expression of inhibitory receptors such as PD-1 and TIGIT and downregulation or impaired function of NKG2D, decreases NK cell efficacy in eliminating infected cells. Image created with BioRender.com.

1.4.2 HIV-1 and Adaptive Immunity

The adaptive immune system can recognize and specifically respond to antigens, such as HIV. The adaptive immune response involves activation and differentiation of T cells and B cells, which results in effector cells and memory cells.

1.5 HIV-1 and B cells

HIV infection has a profound impact on the immune system, including disruption of B cell function. B cells are responsible for producing antibodies and contributing to the adaptive immune response. HIV can affect B cells in different ways, leading to changes in the distribution and function of different B cell subsets**.** In healthy individuals, B cells are mostly resting naive and memory B cells, whereas in HIV-infected individuals, the majority are exhausted B cells. This is indicated by increased expression of inhibitory receptors, which make them less capable of performing their normal functions. There is also an increase in immature transitional B cells and plasmablasts, which are progenitors of the cells responsible for producing and secreting antibodies [36, 37]. HIV can cause generalized B cell hyperactivation, which resulting in polyclonal hypergammaglobulinemia. These antibodies are not HIV-specific and therefore, not highly effective against HIV. They often have no or limited neutralizing activity, and the virus can rapidly mutate, to escape recognition by neutralizing antibodies that are made [38, 39]. HIV disrupts B cell populations by reducing expression of CD19 and CD21, which are important for B cell survival and function, and by increasing expression of activation markers like CD38 [42], CD69 and CD86 [40,43,44]. Increased expression of activation markers is associated with activation-induced apoptosis and an increased frequency of B-cell malignancies (Figure 1.5.1) [40, 41].

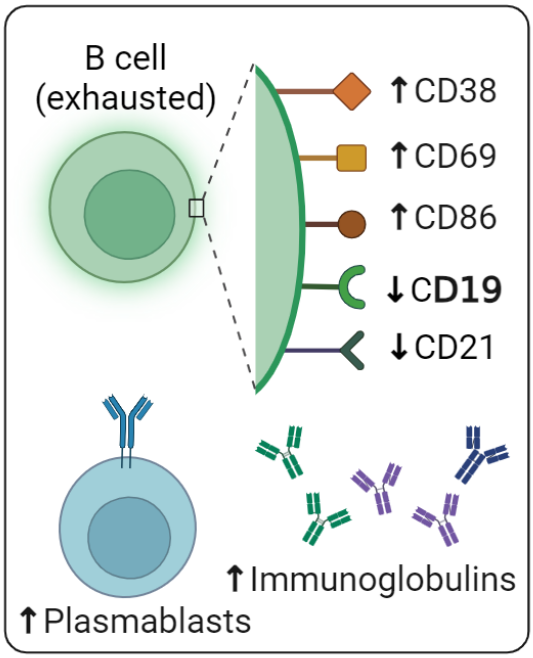


Figure 1.5.1: B cell activity with HIV progression. Prolonged exposure to antigens during HIV infections leads to impaired B cell functions. The upregulation of markers such as CD38, CD69, and CD86 compromise the B cell's ability to make an effective and sustained antibody response, reducing overall immune defense against infection. Image created with BioRender.com.

1.6 HIV-1 and T cells

HIV infects and destroys T helper cells, which are essential for activating other immune cells and aids in antibody production and specialization. It does so by using gp120 protein on its surface, which binds to CD4 or helper T cells [42, 43]. Also, HIV-specific memory $CD4^+$ T cells in infected individuals have more HIV viral DNA than other memory $CD4^+$ T cells, indicating that HIV-specific $CD4^+$ T cells are preferentially targeted by HIV [44]. So, as HIV disease progresses, the number of $CD4^+$ T cells decreases. Even with lifelong administration of ART, the maintenance of a latent viral reservoir compartment, especially in follicular helper $CD4^+$ T cells (TFH), is the main obstacle in their treatment. If ART is interrupted, HIV-1 reactivates and produces replication-competent viruses [45, 46]. In the early phase of HIV infection, TFH cells express high levels of the inhibitory receptor PD-1. High expression of PD-1 may limit the immune response and facilitate viral persistence by inhibition of T cell receptor (TCR) signaling and CD28 co-stimulation and inhibition of T cell recruitment into the lymph node follicle [47, 48]. During disease progression, regulatory T cells (Treg) expand, which also weakens HIV-specific responses and contributes to viral persistence [49, 50]. HIV is associated with defective HIV- specific cytotoxic T cell responses, which are usually responsible for lysing the cell harboring viral infection and thus, preventing viral replication. In HIV-infected patients, $CD8^+$ T-cells are altered by higher expression of exhaustion markers and reduced cytotoxic ability, reflected in a low degranulation ability, low surface expression of CD107a and low expression levels of granzyme B and perforin [51, 52]. Some studies showed that HIV-specific $CD8^+$ T cells in elite controllers had greater ability to express perforin and granzymes [53] that might relate to better effector activity against HIV and control of HIV replication. HIV-

specific $CD8^+$ T cells of elite controllers, compared with HIV-1 progressors, also have greater ability to proliferate and secrete IFN-γ and IL-2 [54, 55]. The high mutation rate of HIV in addition to its ability to down-regulate MHC-I expression, helps the virus to escape from $CD8^+$ T-cell recognition [56]. In chronic infection, T cells experience elevated levels of immune checkpoint inhibitors (ICIs) including programmed cell death receptor 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), lymphocyte activation gene protein (LAG3), T-cell immunoglobulin domain and mucin domain-containing protein 3 (TIM3) and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), which reduce T cells' effector function (Figure 1.6.1) [57, 58].

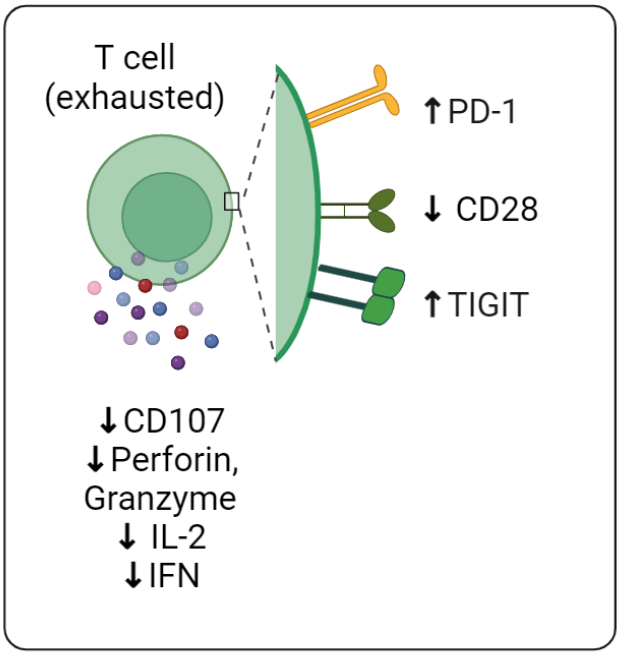


Figure 1.6.1: T cell activity with HIV progression. Persistent antigen exposure in HIV infection leads to T cells expressing high levels of inhibitory receptors such as PD-1 and TIGIT. This results in a diminished capacity for T cells to secrete cytokines, and effectively eliminate infected cells. Image created with BioRender.com.

1.6.1 $CD8^+$ T cells

$CD8^+$ T-cells play an essential role in the control of HIV replication by decreasing viremia in infected persons. The HIV-specific $CD8^+$ T cell response can contribute to reducing the number of HIV-infected cells and the HIV reservoir. $CD8^+$ T cells secrete cytokines, such as TNF-α and IFN-γ, with antiviral effects, and they release cytotoxic granules carrying perforin and granzymes. Perforin forms a pore in the membrane of the target cell, allowing the granzymes to enter. Granzymes are serine proteases that cleave proteins inside the cell, inhibiting the production of viral proteins and inducing apoptosis of the target cell by activating caspases. For T-cell activation, three signals are needed that involve the interaction of T-cells with antigen-presenting cells (APCs) and the recognition of specific antigens by the T-cell receptor (TCR) on the surface of T-cells. T-cells require a number of secondary signals to become activated. CD28 is the primary costimulatory receptor through binding to its ligands, CD80 and CD86. Cytotoxic T-cells also receive signals from other co-stimulatory molecules such as CD70 and 4-1BB (CD137) on APCs. The final signal is provided by cytokines, which ensure proper differentiation of the activated T-cells [59-61]. As previously mentioned, HIV infection leads to dysfunction of $CD8^+$ T-cells, which cannot be restored by ART. CD8 impairment by elevation of ICIs [62], is an effective way for HIV to escape from the cytotoxic function of $CD8^+$ T cells. Upregulation of inhibitory receptors can weaken T-cell activation and cause an exhaustion state, which is a mechanism aiding virus escape [63,64]. Also, with a high mutation rate, HIV is highly variable, which allows the virus to escape the immune system, particularly cytotoxic T cells ($CD8^+$ T cells) and neutralizing antibodies [39].

1.7 Receptors

1.7.1 Activating T cell receptors

When activating receptors on cytotoxic cells bind to their targets, they initiate a signal through the phosphorylation of specific protein structures known as immunoreceptor tyrosine-based activation motifs (ITAMs). This signaling triggers the cytotoxic cells to attack by releasing cytotoxins onto the surface of target cells. The tyrosine residues within these motifs become phosphorylated by Src family kinases following interaction of the receptor molecules with their ligands. Phosphorylated ITAMs serve as docking sites for other proteins containing a SH2 domain, inducing a signaling cascade mediated by Syk family kinases, either Syk or ZAP-70. This cascade ultimately activates the cell [65, 66]. Additionally, the interaction of the CD28 molecule on T cells with its ligands CD80 or CD86, which are expressed on APCs provides a major co-stimulatory signal. This co-stimulation, along with other co-stimulatory molecules, promotes communication between T cells and APCs and enhances T cell activation. One marker on $CD8^+$ T cells which promotes co-stimulation is an immunoglobulin-like superfamily adhesion molecule called DNAX accessory molecule 1 (DNAM-1; also known as CD226). The poliovirus receptor (PVR or CD155 or Necl-5) and PVRL2 (CD112 or nectin-2) have been identified as ligands for DNAM-1 [67]. DNAM-1 contains an immunoreceptor tyrosine tail (ITT)-like motif in its cytoplasmic region. Upon tyrosine phosphorylation of DNAM-1, the ITT-like motif enables binding of DNAM-1 to the cytosolic adaptor Grb2, which leads to cytotoxicity and cytokine production by T cells [67,71]. Thus, interaction of DNAM- 1 with its ligands supports $CD8^+$ T cell-mediated cytotoxicity and cytokine secretion [68]. DNAM-1 shares its ligands with an inhibitory marker called T-cell immunoreceptor with immunoglobulin and immunoreceptor

tyrosine-based inhibitory motif (ITIM) domain (TIGIT). After interaction with its ligand, TIGIT inhibits activation of the T cell, antagonizing activation of costimulatory DNAM-1 and the proliferation, cytokine production and cytotoxic activity of effector lymphocytes [69, 70].

1.7.2 Inhibitory T cell receptors

Expression of inhibitory receptors, also called immune checkpoint inhibitors (ICIs), depends on T cell status, and is frequently associated with "T cell exhaustion". These receptors including PD-1, CTLA-4, LAG3, TIM3 and TIGIT, transduce inhibitory signals upon engagement with their ligands. Signal transduction happens through phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIM), which in turn recruit either Src homology (SH) 2 domain-containing protein tyrosine phosphatases SHP1 and SHP2 or the inositol phosphatases SHIP1 and SHIP2 to mediate negative signaling. This pathway interferes with activating receptors to inhibit downstream activating pathways to regulate immune function [71-73]. Under normal conditions, ICI upregulation at the end of acute infection can modulate the immune system to preserve self-tolerance, but HIV infection can impair functions of effector T-cells by upregulating of expression of inhibitory molecules on T cells to escape the $CD8^+$ T cell response [74-77]. In this state, exhausted $CD8^+$ T cells lose their cytotoxic capacity and have reduced ability to produce IFN-γ and other cytokines [78-80].

1.7.3 TIGIT

Despite ART, expression of the inhibitory receptor TIGIT is broadly dysregulated on $CD8^+$ T cells in HIV infection [81, 82]. TIGIT contains both canonical ITIM and ITT-like motifs in its cytoplasmic region, but the ITT-like motif was shown to play a major role in TIGIT signaling [83, 84]. TIGIT has multiple ligands, including PVR (Necl-5 or

CD155) with high affinity and, nectin-2 (CD112), nectin-3 (CD113), and nectin-4 (PVRL4) with lesser affinities (Figure1.7.1). TIGIT shares its ligands with DNAM-1, but binds with higher affinity than DNAM-1 [85-87]. Upon ligand interaction, the cytoplasmic tail of TIGIT is phosphorylated and binds to cytosolic adaptor growth factor receptor-bound protein 2 (Grb2), recruiting Src homology 2 (SH2)-containing inositol phosphatase-1 (SHIP-1), to block phosphoinositide 3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling cascades [84, 88]. The domination of TIGIT over DNAM-1 is in favor of inhibition over effector cell costimulation. TIGIT also controls T cell activation by interfering with DNAM-1 homodimerization via forming a heterodimer with DNAM-1 in cis [89]. Therefore, TIGIT interaction with its ligands reduces T cell activation, proliferation and effector functions.

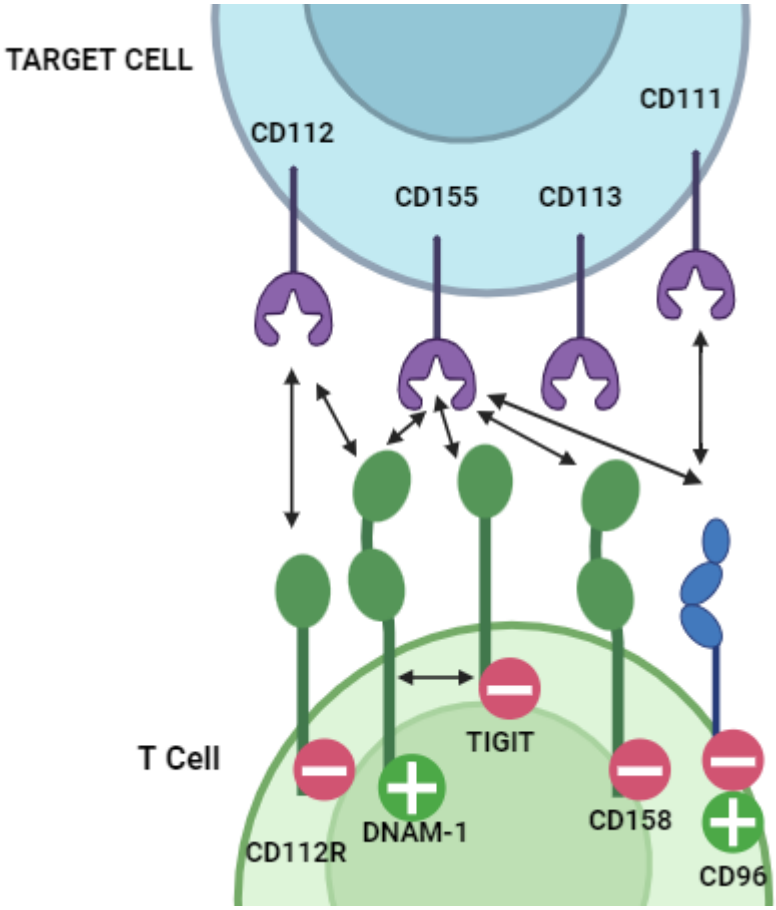


Figure 1.7.1: TIGIT/DNAM-1 axis. TIGIT, DNAM-1, CD112R, CD158 and CD96 are expressed on activated T cells. Their respective ligands, CD155, CD112, CD113, and CD111 are expressed on target cells (APCs). DNAM-1 interacts with both CD155 and CD112 to counter inhibition and delivers an activating signal, but binds with lower affinity than TIGIT. TIGIT, CD158, and CD112R which contain ITIM motifs in their cytoplasmic tail trigger inhibitory signals to cells. Whether CD96 elicits a positive or negative signal in human T cells remains to be demonstrated. Image created with BioRender.com.

1.8 HIV-1 Immunotherapy Strategies

1.8.1 Checkpoint Inhibitors Enhance Effector Responses

Antiretroviral therapy is the treatment of choice for HIV. Antiretroviral drugs are classified according to the step they inhibit in the viral life cycle. They cannot cure HIV and, therefore, treatment is lifelong. It can reduce the viral load in plasma by controlling virus replication, increase the number of $CD4^+$ T cells, slow progression to AIDS, reduce risk of transmission and help people with HIV live longer [90, 91]. There is a need to identify better strategies to cure HIV infection and eliminate the need for lifelong treatment. Since in HIV infection, expression of inhibitory molecules is increased and is associated with immune exhaustion, one treatment strategy is administration of checkpoint inhibitors to reinvigorate immune effector functions. Some studies in cancer and chronic viral infection have shown that blocking inhibitory receptors with mAb augments tumor-directed T-cell responses and restores the cytolytic activity of $CD8^+$ T against infected or transformed cells [92-97].

1.8.2 TIGIT blockade

TIGIT is an inhibitory receptor expressed on activated T-cells, memory T-cells, Tregs, NK cells and Tfh cells. Expression of TIGIT is upregulated on $CD8^+$ T cells and NK cells in HIV infection, despite early ART and almost all HIV specific $CD8^+$ T cells from PWH express TIGIT [99]. Compared with $TIGIT^-$ $CD8^+$ T cells, $TIGIT^+$ $CD8^+$ T cells showed weaker antiviral responses in PLWH [98,99]. TIGIT blockade using therapeutic mAb has been shown to invigorate $CD8^+$ T cells through upregulation of IFN-γ responses and CD107a expression (Figure 1.8.1) [81,82,89]. Therefore, blocking TIGIT could be part of therapeutic approaches targeting the HIV reservoir in eradication strategies.

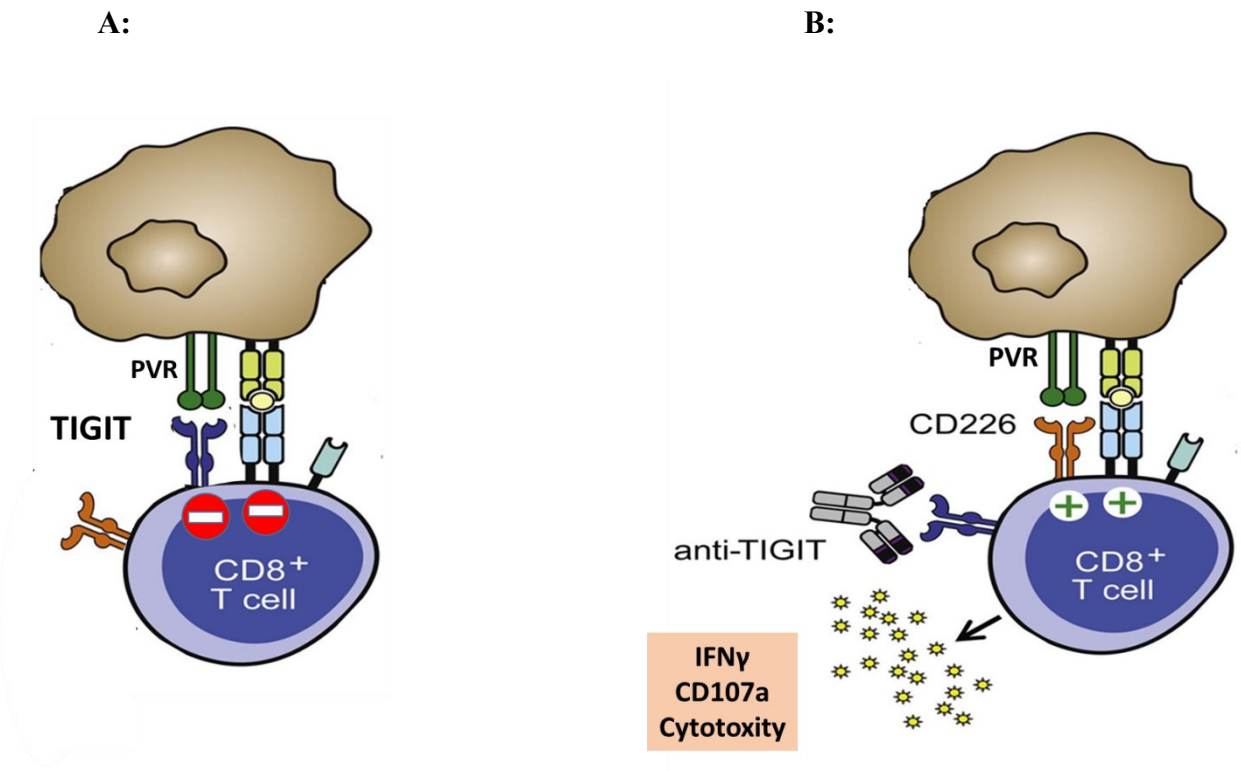


Figure 1.8.1: Impact of TIGIT and TIGIT blockade on antiviral activity. (A) TIGIT activation through binding its ligand PVR inhibits $CD8^+$ T cell-dependent responses. (B) Blocking TIGIT increases antiviral activity against HIV-1 by increasing $CD8^+$ T cell cytotoxicity function, upregulation of IFN-γ production and degranulation. Image was modified from Johnston RJ, et al (2014).

1.9 Rationale and Hypothesis

As detailed previously, the immune checkpoint receptor TIGIT plays a significant role in modulating immune responses, particularly through its inhibitory effects on $CD8^+$ T cells and NK cells. In the context of HIV infection, the expression and engagement of TIGIT are important as they can contribute to the immune exhaustion observed in PLWH. The increased expression of TIGIT on $CD8^+$ T cells in PLWH compared to HIV-negative controls suggests a potential mechanism contributing to HIV's evasion of the immune system.

Blockade of TIGIT can be a strategy to enhance the cytotoxic function of T cells, for promoting clearance of HIV infection. By inhibiting TIGIT, it may be possible to reinvigorate exhausted T cells, thereby improving their ability to target and eliminate HIV-infected cells. This approach is supported by studies showing the effectiveness of immune checkpoint inhibitors in treating certain cancers [94,96,97].

With this knowledge, the hypothesis underlying this study is that blocking TIGIT will enhance the cytotoxic response of HIV-specific $CD8^+$ T cells in PLWH. This hypothesis is based on several key observations and study components:

Comparison of TIGIT expression on PLWH and controls and understanding of how TIGIT might be contributing to immune dysfunction in HIV infection.

Assessing the impact of TIGIT engagement on non-specific T cell cytotoxicity TIGIT, then evaluating the effect of TIGIT blockade on HIV-specific $CD8^+$ T cell response to antigen-specific stimulation. And finally, characterizing the profiles of PLWH whose $CD8^+$ T cells are responsive to TIGIT blockade to identify potential markers predicting responsiveness to this treatment.

2. Materials and Methods:

2.1 Sample collection and processing:

This study involved one hundred and nine human participants in total and ethical approval for recruitment of human participants was obtained from the Health Research Ethics Authority of Newfoundland and Labrador, Canada (HREB #2017.220). Informed consent for participation was obtained by the nurse at the Newfoundland and Labrador Provincial Immunodeficiency Clinic (St. John's, NL, Canada).

Peripheral blood was collected by forearm venipuncture into sterile vacutainer tubes containing Acid Citric Dextrose (ACD) (BD, USA, Catalog No.02-684-29) anticoagulant solution. Human peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient separation using Ficoll-Paque PLUS density gradient media (Cytiva, Sweden, Catalog No.36-101-6383). Briefly, whole blood drawn in an ACD tube was centrifuged at 500*g* for 10 minutes (Beckman Coulter: Allegra X-12 R centrifuge). After this step, the upper plasma layer was removed with a sterile pipette, aliquoted into 1.5 mL microcentrifuge tubes and stored at -80°C until use. The lower cellular layer was diluted with phosphate-buffered saline (see Table 2.1) to the original whole blood volume, carefully underlaid with Ficoll-Paque and centrifuged at 400*g* for 30 min at room temperature. The interface layer containing mononuclear cells was harvested, washed with PBS containing 2% fetal calf/bovine serum (FCS, Hyclone, USA, Catalog No.SH3008704HI) and centrifuged for 10 min at 300*g*. The supernatant was discarded, and pellet resuspended in 10 mL 2% FCS in PBS. After centrifugation, PBMC were resuspended in lymphocyte medium (Roswell Park Memorial Institute (RPMI)-1640, Gibco, Catalog No.11875093) supplemented with 10% FCS (Hyclone, USA), 200 IU/mL penicillin/streptomycin (Gibco, Catalog

No.10378016), 1% L-glutamine (Gibco, Catalog No. 25030164), 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Gibco, Catalog No.15630130) and 0.1% ß-mercaptoethanol (Sigma-Aldrich, Catalog No. 516732).

Table 2.1 Solution Preparation

Solution	Ingredients
PBS	8 g of NaCl 0.2 g of KCl 1.44 g of Na2HPO4 0.24 g of KH2PO4 1 L dH20 (Adjust pH with 1N HCl or 1N NaOH to 7.2 - 7.4)
Flow Cytometry Buffer	5 mM or 2.08 g EDTA 0.5% 5 mm or 1.04 g EDTA or 5 mL FCS 0.2% or 2 g Sodium azide (NaA3) 1 L PBS (Adjust pH with 1N HCl or 1N NaOH to 7.0-7.2)
Kit Buffer (Inside Stain Kit, Miltenyi Biotec)	0.5% or 0.25 g Bovine Serum Albumin (BSA) 2 mM or 0.04 g EDTA 50 mL PBS (Adjust pH with 1N HCl or 1N NaOH to 7.0-7.2)

2.2 Cryopreservation of PBMC

If fresh PBMC were not used immediately, they were cryopreserved until use. In this case, cells were resuspended in freezing medium (FCS containing 10% dimethyl sulfoxide (DMSO,Sigma-Aldrich, Catalog No. D8418), then aliquoted at up to 2 x 10^7 PBMC/mL, stored overnight at -80°C in a Mr. Frosty container, then transferred into liquid nitrogen until needed.

2.3 Recovery of PBMC

When PBMC were recovered for experiments, the frozen vial was removed from liquid nitrogen, and thawed quickly in a 37ºC water bath. The cells were transferred to a 15 mL centrifuge tube with 10 mL lymphocyte medium, centrifuged at 450*g* for 5 minutes, resuspended in fresh lymphocyte medium, incubated at 37°C with 5% CO_2 overnight and counted before use.

2.4 Redirected cytotoxicity assays

Non-specific anti-CD3 triggered cytotoxicity was measured in Chromium-51 (^{51}Cr) release assays in twenty PLWH. We used FcR-expressing P815 murine mastocytoma cells (ATCC® TIB-64™) as targets for cytotoxic T cells in PBMC. P815 were propagated in lymphocyte medium (RPMI-1640, Gibco, Catalog No.11875093) supplemented with 10% FCS (Hyclone, USA), 200 IU/mL penicillin/streptomycin (Gibco, Catalog No.10378016), 1% L-glutamine (Gibco, Catalog No. 25030164), 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Gibco, Catalog No.15630130) and 0.1% ß-mercaptoethanol (ß -ME or 2-ME, Sigma-Aldrich, Catalog No. 516732) at 37°C, 5% CO2. P815 cells are non-adherent and were passaged twice weekly by splitting 1/10. Approximately 5 x 10^5 target cells were labeled in a small volume (less than 500 µL) with 100 µCi $Na_2{}^{51}CrO_4$ for 90 minutes at 37˚C in 5% CO_2.

Then, the labeled cells were washed 4 times with PBS containing 1% FCS and resuspended in lymphocyte medium at 1 x 10^5 target cells/mL. Effector cells were added to wells at an effector to target (E: T) ratio of 50:1. An IgG1 isotype control (Clone: 11711, R&D, Catalog No. MAB002) or anti-TIGIT (Clone: MBSA43, Thermo Fisher Scientific, Catalog No. 16-9500-82) were added to PBMCs to a concentration of 5 µg/mL and incubated 30 min. Then, 5 x 10^3 P815 were added per well in 50 µL lymphocyte medium. We also added anti-CD3 mAb OKT3 to 0.125 µg/mL to trigger circulating cytotoxic T cells (CTL). The final volume per well was 295 µL. Controls for spontaneous release (targets alone in lymphocyte medium) and maximum release were included in the plate. For maximum release target cells were incubated with 1 N hydrochloric acid (HCL) to completely lyse target cells. The plate was incubated 5 hours at 37°C, 5% CO_2. Following incubation, 125 µL of supernatant was transferred to kimble glass tubes (ThermoFisher Scientific, Catalog No. TT9800) containing 50 µL bleach. Release of ^{51}Cr was measured using the Wallac 1480 Gamma Counter (New life Scientific INC) and specific lysis of P815 was calculated using the following formula:

Percent specific lysis = [(Experimental Release – Spontaneous Release)/(Maximum Release – Spontaneous Release)] x 100.

2.5 Peptide preparation for antigen-specific T cell stimulation

To measure antigen-specific T cell activation, PBMC were stimulated with pools of overlapping synthetic peptides spanning HIV Gag and/or Nef proteins. The HIV-1 consensus clade B Nef peptide pool consisting of 15mers with 11 amino acid (aa) overlap and the HIV-1 consensus clade B Gag peptide pool consisting of 15mers with 11 aa overlap were provided by the National Institutes of Health (NIH) HIV Reagent

Program. All were diluted to 100 µg/mL each individual peptide with serum free RPMI and stored at -80 °C until use.

2.6 ELISpot assays

The enzyme-linked immunospot (ELISpot) assay was used to screen for strong interferon-gamma (IFN)-γ responses against HIV Gag and HIV Nef peptides in one hundred and nine PLWH. Pre-coated anti-IFN-γ antibody plates (ImmunoSpot® CTL, Cleveland, USA) were washed one time with 150 µL PBS. HIV peptides for a 1 µg/mL final concentration, along with negative controls (DMSO) and anti-CD3 (clone: OKT3-eBioscience, Catalog No.16-0037-81) at 1 µg/mL final concentration as a positive control were prepared and 100 µL/well added to plate. Then PBMCs as cytokine-secreting cells were added in duplicate at 2 x 10^5/well and incubated overnight at 37°C with 5% CO_2 in a final volume of 200 µL. The next day, plates were washed two times with 200 µL/well PBS and then two times with 200 µL/well 0.05% Tween-PBS. Anti-human IFN-γ detection antibody was diluted in diluent B **(**ImmunoSpot® Kit, CTL), filtered through a 0.1 µm filter, and 80 µL was added to each well. Following two hours of incubation at room temperature, the plate was washed three times with 200 µL/well 0.05% Tween-PBS. Then, 80 µL/well of tertiary solution (SA-HRP) in diluent B was added and incubated for 30 minutes at room temperature. Then, 80 µL substrate solution (Blue developer solution) was added to each well after two washes with 0.05% Tween-PBS and two washes with distilled water. The reaction was stopped by gently washing the plate with tap water. Plates were air-dried face down for two to twenty-four hours in the laminar flow hood. Finally, the frequency of spot forming cells (SFUs)/ million PBMC was calculated after scanning the plates to enumerate the number of spots/well using a CTL-Immunospot Analyzer (CTL, Cleveland, USA).

2.7 HIV-specific $CD8^+$ T cell assessment

To evaluate responses of HIV-specific $CD8^+$ T cells and the role of TIGIT in regulating these responses, PBMC were stimulated with HIV peptides in the presence of TIGIT-blocking mAb or an IgG1 isotype control in forty PLWH . 4 x 10^6 PBMC in 1 mL lymphocyte medium, were placed in polystyrene tubes (BD) for non-stimulated and stimulated conditions. An anti-TIGIT fluorescence-conjugated antibody (see Table 2.2) was added to one tube for 20 minutes to identify TIGIT-expressing T cells. Then unlabeled TIGIT- blocking mAb or IgG1 isotype control were added to cells, at a final concentration of 5 μg/mL. These cells were pre-incubated for 30 minutes and then Gag and Nef peptide pools were added to a final concentration of 1 μg/mL each individual peptide with an equivalent amount of DMSO added to a duplicate tube as vehicle control. Cells were stimulated for 5 hours in an incubator at 37°C with 5% CO_2 with 5 μg/mL brefeldin A, 5 μg/mL monensin and labeled anti-CD107a fluorescence-conjugated antibody (see Table 2.2). After stimulation, the cells were washed and stained for analysis by surface and intracellular flow cytometry.

2.8 Assessment of Trogocytosis

To evaluate Trogocytosis, mediated through Fcγ receptors (FcγR) on monocytes in the presence of TIGIT mAbs, we measured the level of TIGIT on $CD8^+$ T cells and monocyte after 5 hours incubation and compared the effect of anti-TIGIT to the IC. Firstly, 2 x 10^6 PBMC in 1 mL lymphocyte medium were divided in two polystyrene tubes (BD, Catalog No. 352058) and an anti-TIGIT fluorescence-conjugated antibody (see Table 2.2) or isotype control was added and they were incubated for 5 hours in an incubator at 37°C with 5% CO_2. After incubation, the cells were washed and stained for analysis by surface flow cytometry for TIGIT expression.

2.9 Flow cytometry

2.9.1 Cell surface protein expression

CD3, CD8, CD107a and TIGIT surface expression was assessed by flow cytometry. At a final concentration of 1 x 10^6 cells/mL, PBMC were labeled with anti-CD3, anti-CD8, anti-CD107a, and anti-TIGIT fluorescence-conjugated antibodies (see Table 2.2) for 30 minutes on ice in the dark. Following incubation, the cells were washed with flow cytometry buffer (Table 2.1) and fixed by adding 200 µL of 2% paraformaldehyde (PFA) to the pellet for 20 minutes. Then cells were washed, centrifuged and resuspended in flow cytometry buffer. Samples were kept at 4°C until measuring surface expression using a Beckman Cytoflex flow cytometer (Beckman Coulter, USA). Analysis was done with Kaluza Flow Cytometry Software.

2.9.2 Intracellular protein expression

Following staining cell surface antigens, PBMC were washed by adding 3 mL of flow cytometry buffer (Table 2.1) and centrifuged at 300*g* for 10 minutes. In order to stain for interferon gamma (IFNγ) and T Cell Factor 1 (TCF1/TCF7), the cells were resuspended in 250 µL Inside Fix (Miltenyi Biotec) along with 250 µL kit buffer (see Table 2.1) and incubated for 20 minutes in the dark at room temperature. Cells were washed by adding 1 mL of buffer and centrifuged at 300*g* for 5 minutes. 100 µL Inside Perm (Miltenyi Biotec) was added to the cell pellet, followed by the appropriate volume of anti-IFNγ fluorescence-conjugated antibody (see Table 2.2) for 10 minutes in the dark at room temperature. Finally, the sample was washed once with 1 mL Inside Perm and once with 1 mL of kit buffer, then kept in the dark at 4°C until acquisition on a Beckman Cytoflex flow cytometer (Beckman Coulter, USA). Analysis was done with Kaluza Flow Cytometry Software after data acquisition.

Table 2.2. Fluorochrome-conjugated antibodies used for flow cytometry

Antibody	**Conjugated Fluorochrome**	**(µg) Added per 1 x 10^6 PBMC**	**Supplier and Clone**
Anti-Human CD3	VioGreen	0.25	Miltenyi Biotec (Clone: REA613, Catalog No.130-113-142)
Anti-Human CD8	PerCP	0.25	BioLegend (Clone: H1T8a, Catalog No.300922)
Anti-Human CD107a (LAMP-1)	Brilliant Violet 421	0.25	BioLegend (Clone: H4A3, Catalog No.328626)
Anti-Human TIGIT	Alexa Fluor 647	0.125	eBioscience (Clone: MBSA43, Catalog No. 51-9500-42)
Anti-Human IFNγ	PE	0.125	eBioscience (Clone: 4S.B3, Catalog No.12-7319-42)
Anti-Human TCF1/TCF7	Alexa Fluor 700	0.25	Cell Signaling Technology (Clone: C63D9, Catalog No. 90904S)

Anti-Human CD45 RA	Brilliant Violet 421	0.25	BD Biosceince (Clone: HI30, 563879)
Anti-Human KLRG1	APC Vio-770	0.25	Miltenyi Biotec (Clone: REA261, Catalog No. 130-120-423)

3. Results

3.1: Comparison of TIGIT expression on $CD8^+$ T cells of PLWH and HIV-negative controls

3.1.1: Rationale

TIGIT (T cell immunoglobulin and ITIM domain) is a negative modulator of T-cell function that is expressed on the surface of some T cells. It acts through interaction with PVR as its ligand to reduce T cell function. Despite antiretroviral therapy (ART), which suppresses HIV replication below detectable levels, the frequency of $TIGIT^+CD8^+$ T cells is significantly higher in persons living with HIV (PLWH) versus uninfected controls [100-104].

Upregulation of inhibitory receptors such as TIGIT on $CD8^+$ T cells can impair the ability of CTL to eliminate HIV-infected target cells [101, 102, 104]. To address the main objective of our study, we first needed to confirm higher TIGIT expression on $CD8^+$ T cells from PLWH in our study cohort compared to HIV-seronegative persons. The overall aim is to test whether blocking TIGIT can rescue $CD8^+$ T cell function and thereby invigorate antiviral T cell responses.

3.1.2: TIGIT levels on $CD8^+$ T cells from PLWH and HIV-seronegative persons

TIGIT expression on $CD8^+$ T cells was assessed by flow cytometry following extracellular staining with anti-CD3, -CD8 and -TIGIT (Figure 3.1.1.A). We observed a significant increase in the frequency of $TIGIT^+$ $CD8^+$ T cells in PLWH compared to HIV-negative controls, so the TIGIT expression status of our cohort is consistent with the findings of other studies (Figure 3.1.1.B).

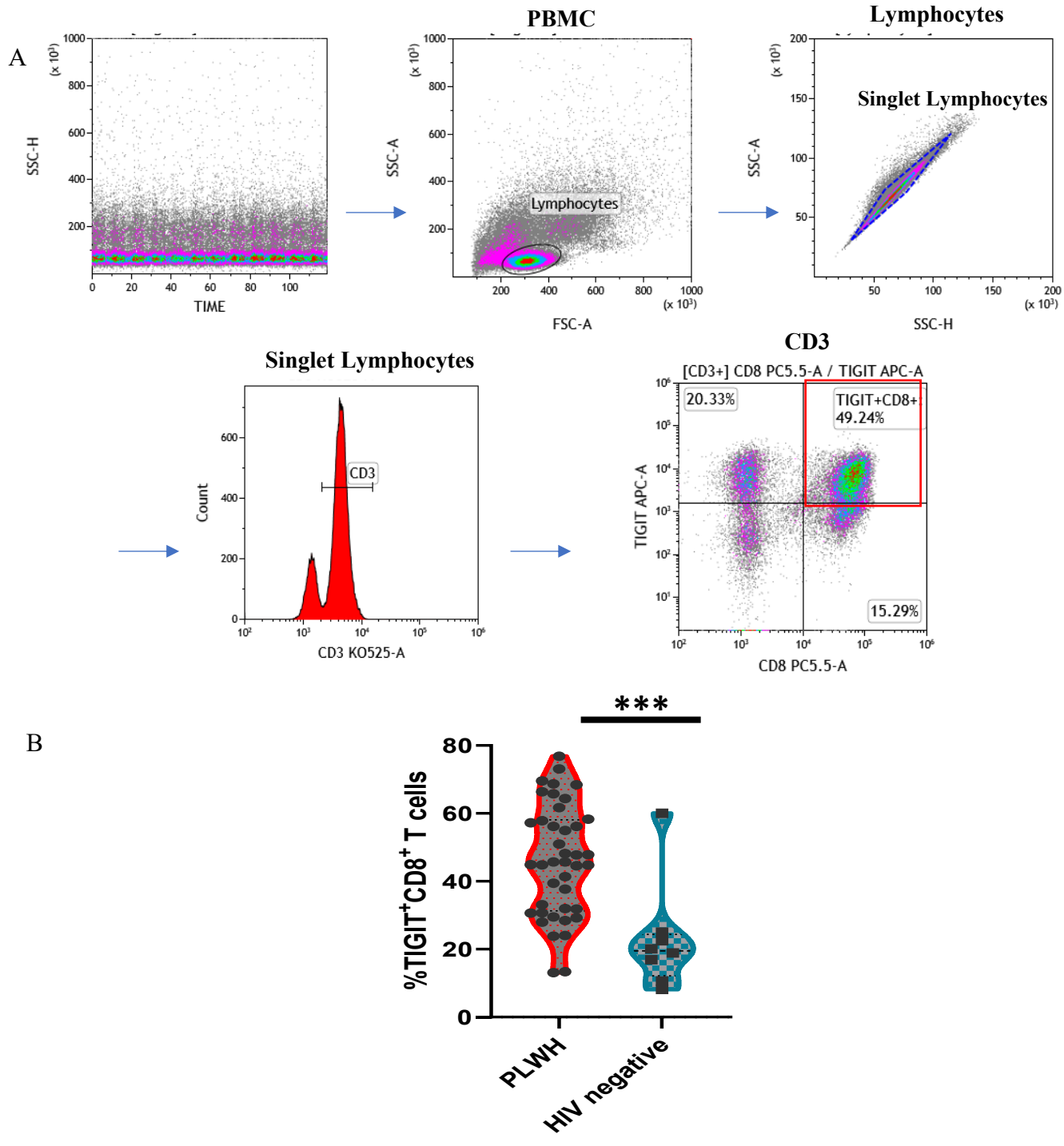


Figure 3.1.1: Comparison of TIGIT expression on CD8$^+$ T cells from PLWH and HIV-negative controls. (A) Gating strategy for flow cytometry is shown. Quality was assessed by side scatter versus time. Singlet lymphocytes were gated by scatter characteristics and doublet exclusion. T cells were identified as CD3$^+$ lymphocytes, and

the percentage of $TIGIT^+$ $CD8^+$ T cells was determined. The red square represents the percentage of $CD8^+$ T cells expressing TIGIT. (B) PBMC were stained with anti-CD3, -CD8 and -TIGIT fluorescence-conjugated antibodies for analysis by flow cytometry. The percentage of $TIGIT^+$ $CD8^+$ T cells in PLWH is higher than in HIV-negative individuals, shown here in a comparison of n = 41 PLWH and n = 7 HIV-1 negative individuals. The non-parametric Mann Whitney test was used for statistical analysis as data were not normally distributed. ***$p < 0.001$.

3.2: Assessing the impact of TIGIT engagement on T cell cytotoxicity

3.2.1: Rationale

There is limited information available regarding the effect of TIGIT engagement on human T cell cytotoxicity. Therefore, we used anti-TIGIT antibody and the murine Fcγ receptor-expressing mastocytoma cell line P815 to measure the impact of TIGIT engagement on cytotoxicity. Circulating T cells within PBMC were added to P815 cells as effector cells at an E:T ratio of 50:1, in 5 hr ^{51}Cr-release assays [105, 106]. To trigger the cytotoxicity of circulating T cells, anti-CD3 mAb OKT3 was used (Figure 3.2.1). In this system the Fc receptor on P815 cells cross-links TIGIT via anti-TIGIT antibody to mimic PVR engagement to test for inhibition of cytotoxicity. If a subject's baseline PBMC-mediated cytotoxicity against P815 target cells was more than 10% in this assay, they were included in the study of TIGIT mediated inhibition of cytotoxicity.

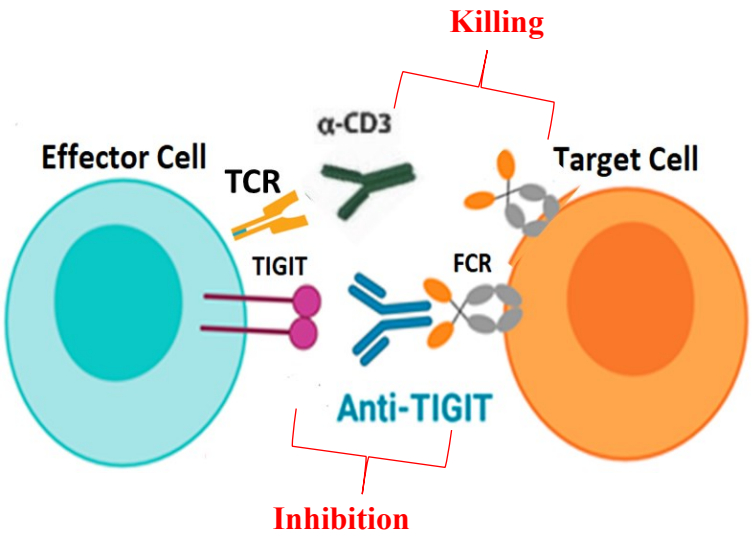


Figure 3.2.1: Method of TIGIT cross-linking on T cells triggered to lyse P815 cells using anti-CD3. The effect of TIGIT cross-linking is measured with an IgG1 isotype control for comparison.

3.2.2: Effect of TIGIT engagement on T cell cytotoxicity against Fc receptor expressing P815 cells

We tested TIGIT engagement on $CD8^+$ T cells from PLWH in non-specific redirected cytotoxicity assays using monoclonal anti-TIGIT antibody. In this test, PBMC as effector cells were pretreated with either anti-TIGIT or IgG1 isotype control, then anti-CD3 mediated lysis of P815 targets was measured and compared. As anti-CD3 concentrations of 0.125 and 1 μg/mL showed the same maximal percentage of lysis, 0.125 μg/mL was chosen as an optimal anti-CD3 concentration for this assay (Figure 3.2.2). A 5 μg/mL concentration of anti-TIGIT showed maximum inhibition of cytolysis (Figure 3.2.3) and was chosen for further studies.

The percentage of killing or cytotoxicity was calculated based on the amount of ^{51}Cr released. Engagement of TIGIT decreased T cell activity against P815 cells more than 10% in 14 of 20 cases compared to the isotype control (Figure 3.2.4), showing that TIGIT engagement impairs killing by $CD8^+$ T cells from some PLWH. These experiments established that engaging TIGIT can inhibit T cell cytotoxicity in PLWH.

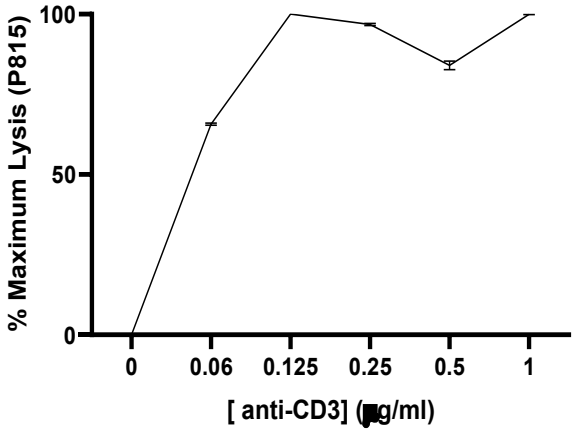


Figure 3.2.2: Titration of anti-CD3 triggered lysis of P815 cells. The lowest amount of anti-CD3 required for maximal lysis was determined to identify the minimal amount of antibody for triggering T cells. Percent specific lysis of P815 cells mediated by anti-CD3 was assessed prior to measuring the effect of TIGIT engagement on T cell cytotoxicity.

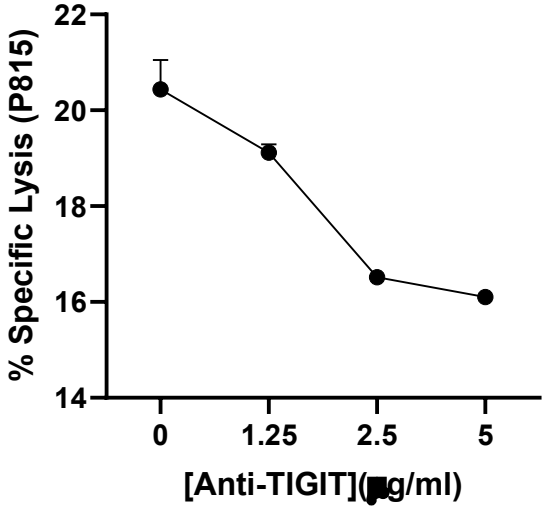


Figure 3.2.3: Titration of anti-TIGIT concentration to inhibit anti-CD3- triggered lysis of P815 cells. T cells were triggered to lyse P815 cells using anti-CD3, and the effect of T cell TIGIT cross-linking on P815 lysis was measured using IgG1 isotype control (not depicted) or anti-TIGIT.

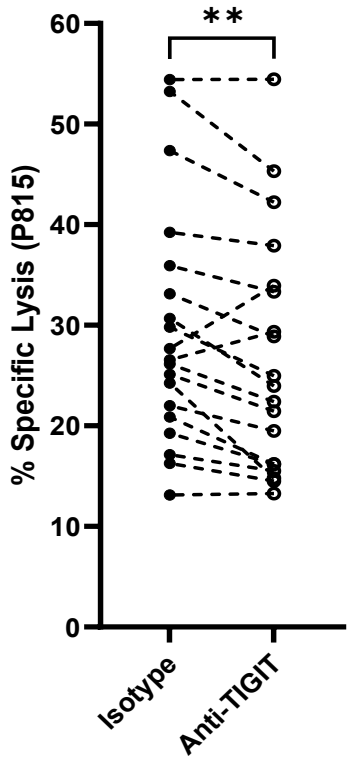


Figure 3.2.4: TIGIT engagement impairs killing by CD8$^+$ T cells from some PLWH. Targets were pretreated with anti-TIGIT or IgG1 and were incubated with PBMC from PLWH at an E: T ratio of 50:1 in the presence of anti-CD3. In 70% of subjects tested, T cell TIGIT cross-linking inhibited T cell cytotoxicity by more than 10%. As data were not normally distributed, the non-parametric Mann Whitney test was used to test for a significant effect of TIGIT engagement on cytotoxicity. **p < 0. 01.

3.3: Identification of subjects with strong HIV-specific T cell responses by ELISpot

3.3.1: Rationale

In order to assess the effect of TIGIT blockade on Ag-specific T cell responses, the response must be robust enough to be readily measurable by cytokine flow cytometry (CFC), therefore, we first screened the frequency of HIV-specific T cells in different PLWH by ELISpot assay [107]. The CFC assay would be less likely to detect small changes associated with TIGIT blockade if the response was small [108]. Therefore, an IFN-γ- ELISpot assay was done first to find responders with HIV–1–specific T cell responses large enough to be readily detectable by flow cytometry.

3.3.2: Identifying individuals with strong T cell responses against HIV Gag/Nef peptides using ELISpot

The ELISpot results are expressed in conventional units of SFC/10^6 PBMC. For the ELISpot assay, responses of more than 1000 SFU/10^6 PBMC were considered the minimum to be selected for follow-up studies by flow cytometry. We evaluated responses from 109 individuals to HIV Gag and Nef peptide pools. Of 109 subjects, 23 had >1000 IFN-γ producing T cells/10^6 PBMC in response to either Gag or Nef peptide stimulation and we chose these responders against Gag and/or Nef for flow cytometry analysis.

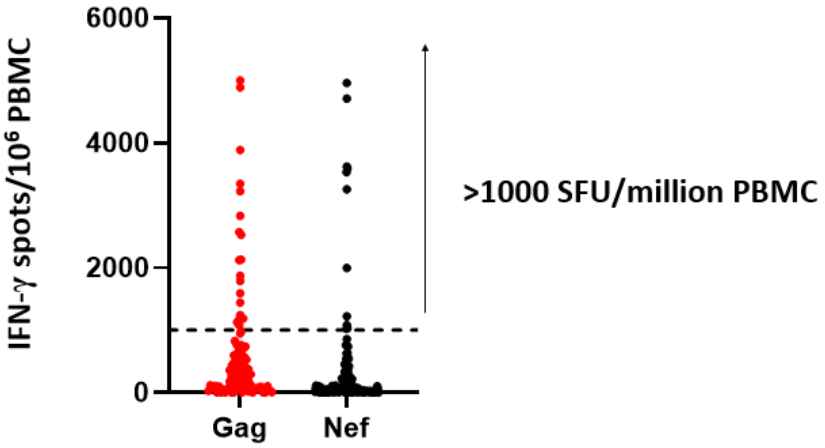


Figure 3.3.1: Measurement of HIV-specific T cell responses in PLWH by ELISpot. The dotted line in the graph indicates a threshold (1000 SFU/10^6 PBMC) for the size of T cell responses suitable for further study by flow cytometry. Twenty-three subjects had >1000 IFN-γ producing T cells/10^6 PBMC in response to either Gag or Nef peptide stimulation.

3.4: Impact of TIGIT blockade on HIV-specific $CD8^+$ T cells

3.4.1: Rationale

While HIV-specific $CD8^+$ T-cells can control the virus during HIV infection, their cytotoxic function decreases with disease progression. Exhausted $CD8^+$ T cells with augmented expression of inhibitory markers are less capable of exerting an effective antiviral response [109, 110]. TIGIT is an inhibitory receptor expressed on T cells and NK cells and its expression increases on $CD8^+$ T cells in parallel with HIV-1 disease progression [101-103, 111].Variability in individual responses to immune checkpoint blockade must be addressed in evaluating the impact of TIGIT blockade. The reported percentage of responders is low, around 15% to 25% [112]. Therefore, characterizing those most likely to respond to immune checkpoint blockade can be beneficial. Blocking TIGIT may invigorate antiviral T cell responses in HV-specific $CD8^+$ T cells by enhancing cytokine production and degranulation [113-115].

3.4.2: Effect of TIGIT blockade on $CD8^+$ T cell TIGIT expression

Interrupting TIGIT signaling by mAb blockade can rescue $CD8^+$ T cell antiviral activity. Based on ELISpot results, to test the effect of TIGIT blockade in persons with strong responses, PBMCs were divided into two conditions: one condition of treatment with IgG1 and one condition of treatment with anti-TIGIT monoclonal antibody. PBMC, in the condition of treatment with anti-TIGIT mAb, were first labelled with 0.25 μg per 10^6 PBMC anti-human TIGIT (Alexa Fluor 647, MBSA43) to indicate TIGIT expression. Then, we treated them with anti-TIGIT or IgG1. We found that certain individuals (11/40 subjects) had a lack of TIGIT downregulation in response to TIGIT blockade (Figure 3.4.1- left), while the majority (29/40 subjects) showed a loss of TIGIT expression on $CD8^+$ T cells after TIGIT blockade (Figure 3.4.1- right).

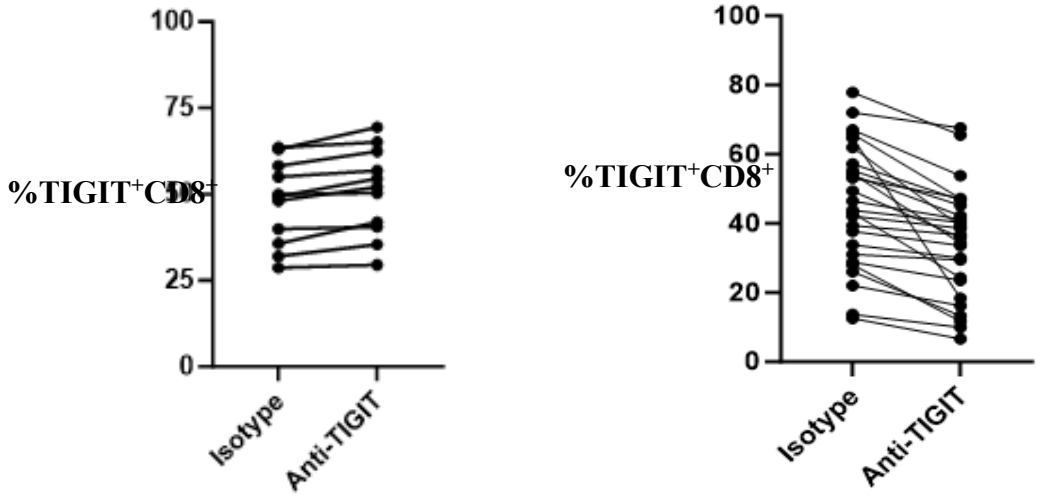


Figure 3.4.1: Impact of TIGIT blockade on levels of TIGIT expression on CD8$^+$ T cells. Persons with strong responses were treated with TIGIT mAb or IgG1.TIGIT expression on HIV-specific CD8$^+$ T cells was assessed after treatment. Eleven individuals did not show loss of TIGIT expression after blocking TIGIT by mAb (left), while twenty-nine individuals responded to TIGIT blockade with loss of TIGIT expression on CD8$^+$ T cells (right).

3.4.3: Impact of TIGIT blockade on HIV-specific $CD8^+$ T cell IFN-γ production and degranulation

We compared the effects of anti-TIGIT antibody with isotype, on the frequency of $CD8^+$ T cells producing IFN-γ or degranulating in response to either Gag or Nef peptide stimulation. The differences were not statistically significant overall, but the subjects who did not respond by downregulation of TIGIT in response to TIGIT blockade showed that their IFN-γ production decreased (Figure 3.4.2). Also, within the PLWH who responded well by loss of TIGIT following TIGIT blockade, 6 individuals showed improvement in IFN-γ and degranulation of CD107a (Figure 3.4.3).

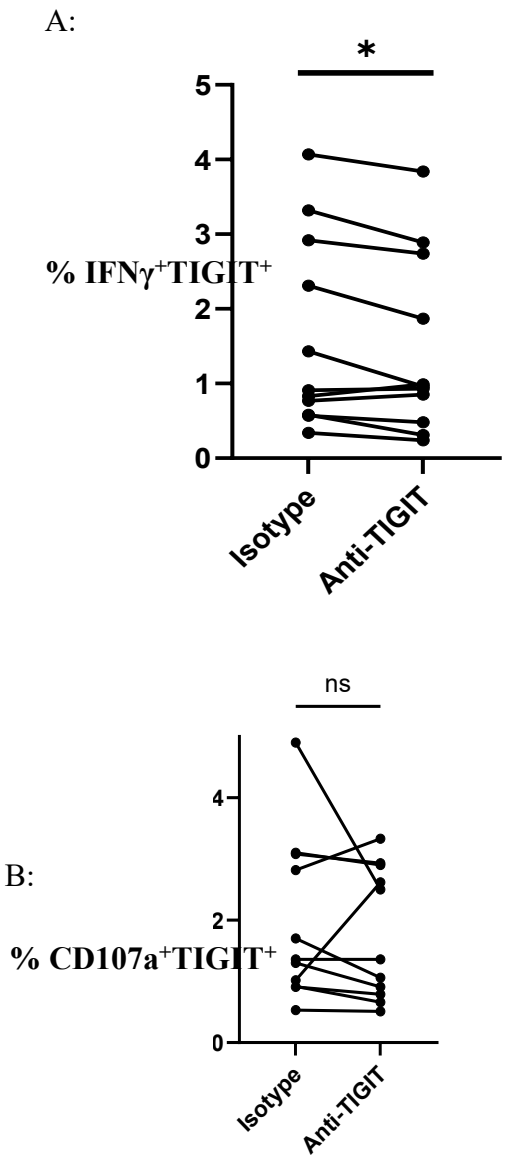
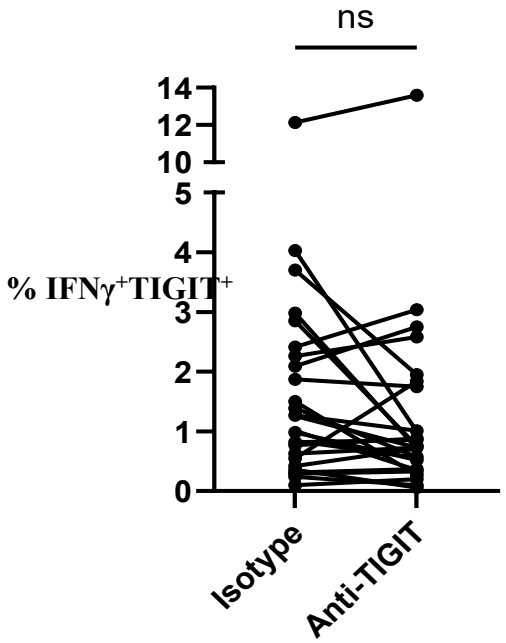


Figure 3.4.2: Effect of TIGIT blockade on IFN-γ production and CD107a expression by HIV-specific T cells from non responders to TIGIT blockade in terms of loss of TIGIT expression (Refer to left graph of Figure 3-4-1). (A) Effect of TIGIT blockade on IFN-γ production by HIV-specific T cells from non responders to TIGIT blockade in terms of TIGIT loss. The subjects who did not show TIGIT loss after TIGIT blockade showed downregulated IFN-γ production after blocking TIGIT with mAb compared to the isotype control. (B) Effect of TIGIT blockade on CD107a expression of HIV-specific T cells from non responders to TIGIT blockade in terms of TIGIT loss. The non-parametric Mann Whitney test was used for statistical analysis as data were not normally distributed. *p < 0. 05.

A:



B:

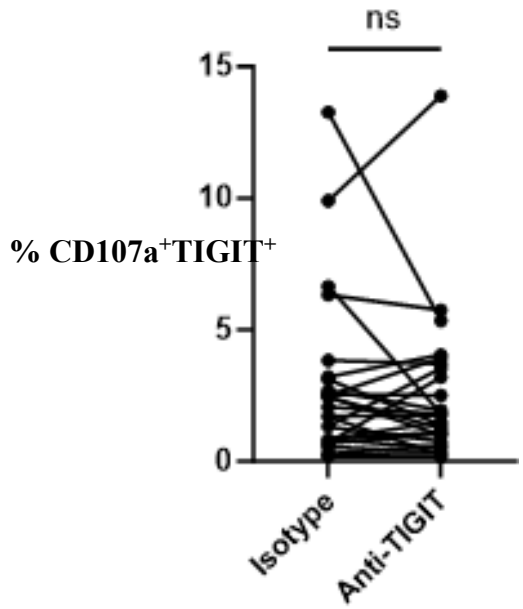


Figure 3.4.3: Effect of TIGIT blockade on IFN-γ production and CD107a expression of HIV-specific T cells in responders to TIGIT blockade in terms of loss of TIGIT expression (Refer to right graph of Figure 3-4-1). (A) Effect of TIGIT blockade on IFN-γ production of HIV-specific T cells in responders to TIGIT blockade in terms of TIGIT loss. The subjects who responded to TIGIT blockade by loss of TIGIT expression did not show significant difference in IFN-γ production compared to

treatment with isotype control overall. However, 6 individuals showed effector cell reinvigoration after TIGIT blockade. (B) Effect of TIGIT blockade on CD107a degranulation of HIV-specific T cells in responders to TIGIT blockade. The result was not significant for the entire group of subjects who responded to TIGIT blockade by loss of TIGIT expression, but 6 individuals showed an increase in effector cell function after TIGIT blockade. The non-parametric Mann Whitney test was used for statistical analysis.

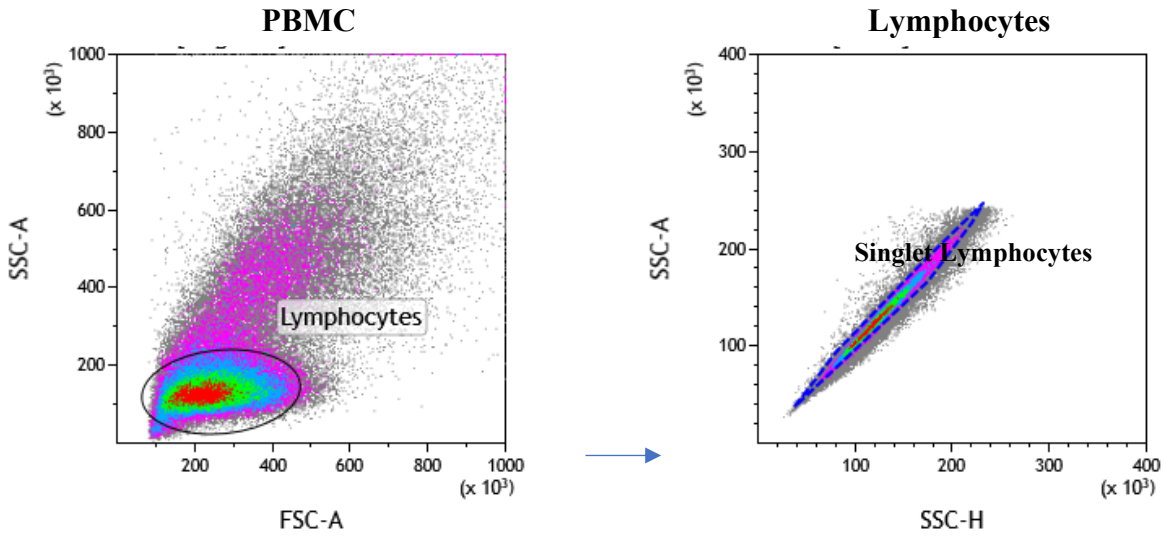
3.5: Characterization of PLWH with $CD8^+$ T cells responsive to TIGIT blockade

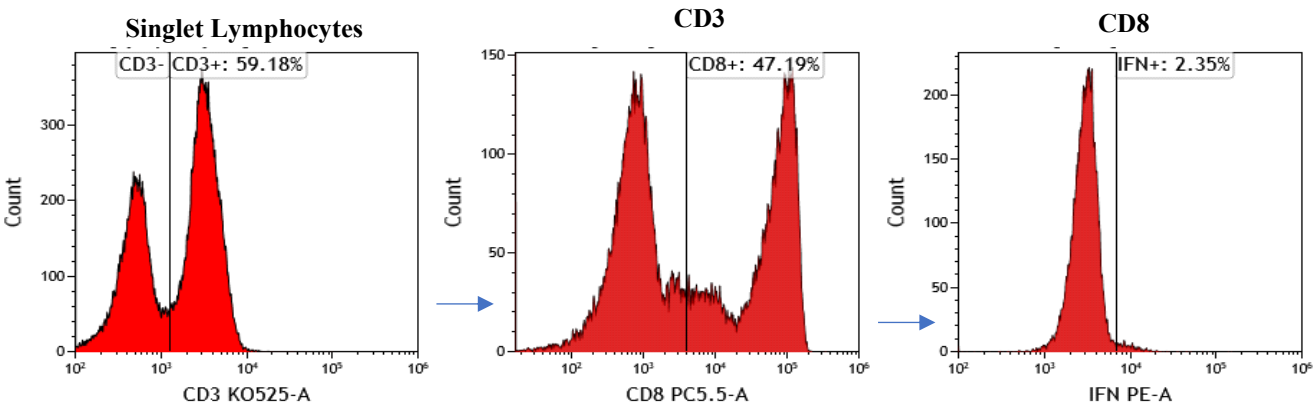
3.5.1: Rationale

Since one possible factor contributing to the failure of the immune system to control HIV infection is antigen-specific $CD8^+$ T cell exhaustion by upregulation of inhibitory markers and impaired effector functions [116, 117], characterization of $CD8^+$ T cells from PLWH responding to TIGIT blockade can be beneficial. T cell factor 1 (TCF-1) TCF-1 is highly expressed in naive, central memory, and stem cell memory $CD8^+$ T cells [118,119]. Some studies showed that TCF-1 expression is elevated in HIV-specific $CD8^+$ T cells of elite controllers (rare group of PLWH who can naturally control the virus without the need for ART) compared to non-controllers **(**who cannot control HIV replication without ART) [120,121]. Also, killer cell lectin-like receptor subfamily G member (KLRG1) plays an inhibitory role in human NK cells and T cells [122, 123] and was identified to be upregulated in HIV-1 specific exhausted $CD8^+$ T cells [124], suggesting that KLRG1 might contribute to regulation of HIV infection. Furthermore, CD45RA is expressed on naïve and effector T cells and its expression is lost when these cells become activated and transition to effector or memory T cells [125,126]. While CD45RA expression can provide valuable information about the state of the immune system in PLWH, the relationship between CD45RA expression and the reduction of latent viral reservoir during ART is complex [127, 128]. Therefore, characterization of $CD8^+$ T cells responsive to TIGIT blockade can help in identifying those PLWH most likely to benefit from TIGIT blockade.

3.5.2: Features of the subset of IFN-γ^+ $CD8^+$ T cells responsive to TIGIT blockade

We carried out phenotyping of responsive T cells after TIGIT blockade or with isotype control treatment, following to either Gag or Nef peptide stimulation regarding the frequency of TCF-1, KLRG1 and CD45RA on IFN-γ^+ $CD8^+$ T cells. We identified features of $CD8^+$ T cells from 6 individuals who responded to TIGIT blockade by loss of TIGIT expression and increased effector cell function.


PBMC
Lymphocytes
SSC-A
FSC-A
Lymphocytes
SSC-H
Singlet Lymphocytes


Singlet Lymphocytes
CD3
CD8
CD3- CD3+: 59.18%
CD8+: 47.19%
IFN+: 2.35%
Count
CD3 KO525-A
CD8 PC5.5-A
IFN PE-A

IFN-γ^{+} CD8^{+} T

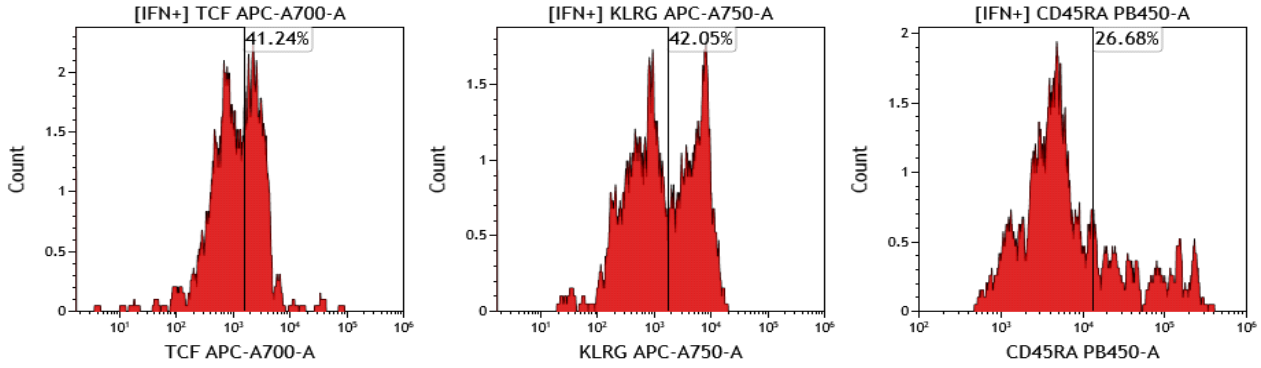
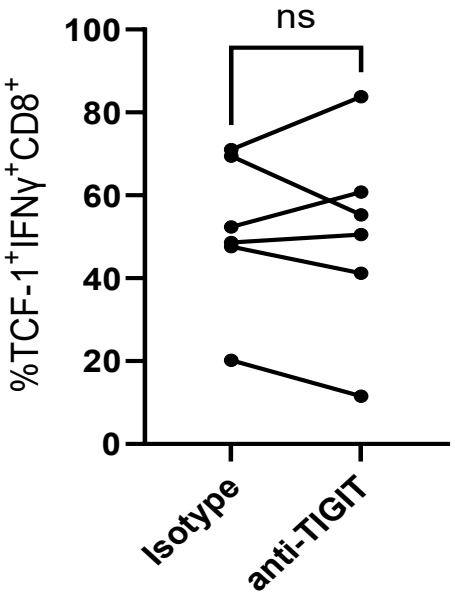
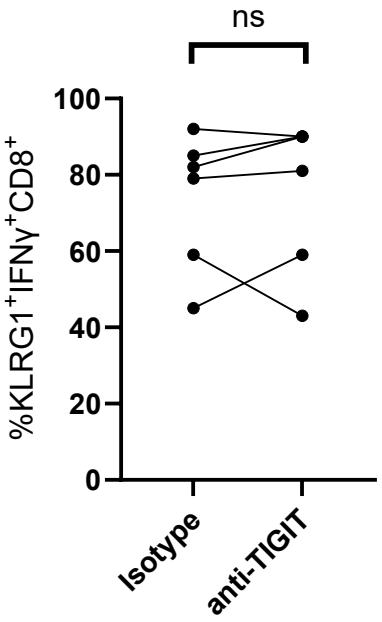

[IFN+] TCF APC-A700-A
41.24%
[IFN+] KLRG APC-A750-A
42.05%
[IFN+] CD45RA PB450-A
26.68%
Count
TCF APC-A700-A
KLRG APC-A750-A
CD45RA PB450-A

Figure 3.5.1: Gating strategy for characterization of the subset of IFN-γ^+ $CD8^+$ T cells after TIGIT blockade compared to isotype control treatment is shown. Lymphocytes were gated by scatter characteristics and doublet exclusion. T cells were identified as $CD3^+$ lymphocytes, and the percentage of IFN-γ^+ $CD8^+$ T cells, TCF-1^+, $KLRG1^+$ and $CD45RA^+$ in the population of IFN-γ^+ $CD8^+$ was determined.

A:


ns
100
80
60
40
20
0
%TCF-1+IFNγ+CD8+
Isotype
anti-TIGIT

B:


ns
100
80
60
40
20
0
%KLRG1+IFNγ+CD8+
Isotype
anti-TIGIT

C:

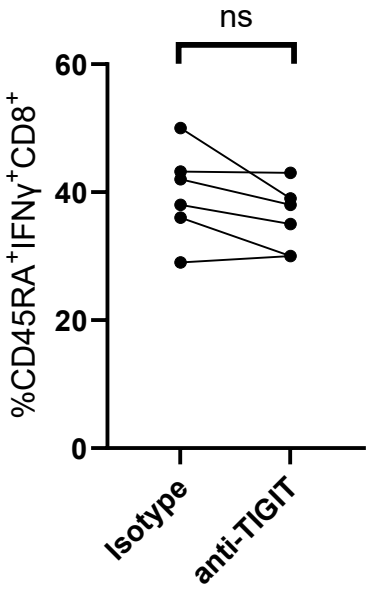
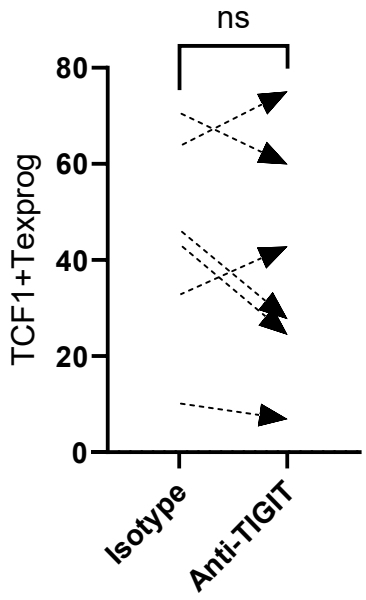


Figure 3.5.2: Characterization of the subset of responsive CD8$^+$ T cells. TCF-1 (A), KLRG1 (B), and CD45RA expression (C) on the subset of CD8$^+$ T cells producing IFN-γ$^+$ with and without TIGIT blockade. There was no significant difference based on any of the markers tested. N=6 and the paired *t*-test was used for statistical analysis.

3.5.3: Characterization of exhausted T cells in the subset of $CD8^+$ T cells responsive to TIGIT blockade

Exhausted $CD8^+$ T cells (Tex) can be categorized into two subpopulations of Tex; progenitor exhausted T cells (Tex^{prog}) and terminally exhausted T cells (Tex^{term}). They are distinguished by the expression of TCF1, which plays a fundamental role in T cell development. $CD8^+TIGIT^+TCF1^+$ ($TCF1^+Tex^{prog}$) are stem-like cells and can be differentiated into $CD8^+TIGIT^+TCF1^-$ ($TCF1^-Tex^{term}$) [129]. The frequency of Tex^{prog} and Tex^{term} was assessed to test the effect of TIGIT blockade or isotype in responder groups, but the results were not statistically significant (Figure 3.5.3).

A:



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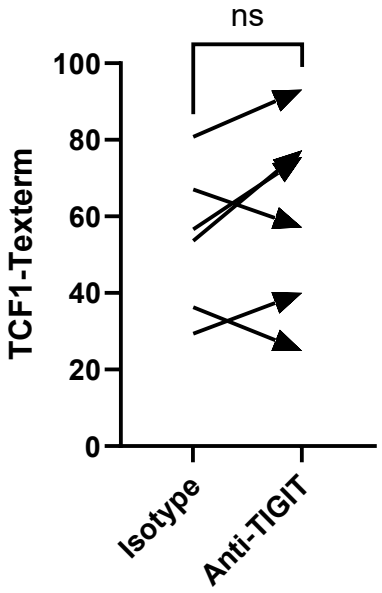

Figure 3.5.3: Distribution of exhausted CD8$^+$ T cell (Tex) subpopulations. Flow cytometry results showed that TCF1$^+$Tex^{prog} (A) and TCF1$^-$Tex^{term} (B) in responders to TIGIT blockade changed, but their differences were not statistically significant. The paired *t*-test was used for statistical analysis.

3.6: Evaluation of Trogocytosis between monocytes and TIGIT-expressing T cells

3.6.1: Rationale

Trogocytosis, mediated through Fcγ receptors (FcγR) on cells such as monocytes, can lead to removal of cell surface molecules from Ab-targeted T cells and internalization of the Ab-bound molecules in phagocytic cells [130-132].

A previous study showed that anti-TIGIT mAb can induce trogocytosis of TIGIT through FcγR+ cells [133]. Therefore, by gating on monocytes in PBMCs and evaluating the anti-TIGIT-mAb fluorescence level on them, we determined whether anti-TIGIT antibody treatment caused any uptake of TIGIT by monocytes. It is plausible that trogocytosis may contribute to the loss of TIGIT expression after TIGIT blockade.

3.6.2: Relationship between monocyte population and frequency of cells coated with fluorescent anti-TIGIT mAb

To investigate whether monocytes can remove TIGIT from cell surface of $CD8^+$ T cells, we measured changes in the level of TIGIT expression on $CD8^+$ T cells and the monocyte population in the presence of anti-TIGIT mAbs (Figure 3.6.1-A) and the baseline varies from 5 to 25% (Figure 3.6.1-B).

As shown in Figure 3.6.1- B, antibody-bound TIGIT from T cell surface was removed, in 6 subjects who showed good responses to TIGIT blockade. In these cases, the percentage of monocytes positive for anti-TIGIT mAb fluorescence increased from 5% after TGIT blockade compared to isotype control treatment (Figure 3.6.1-B).

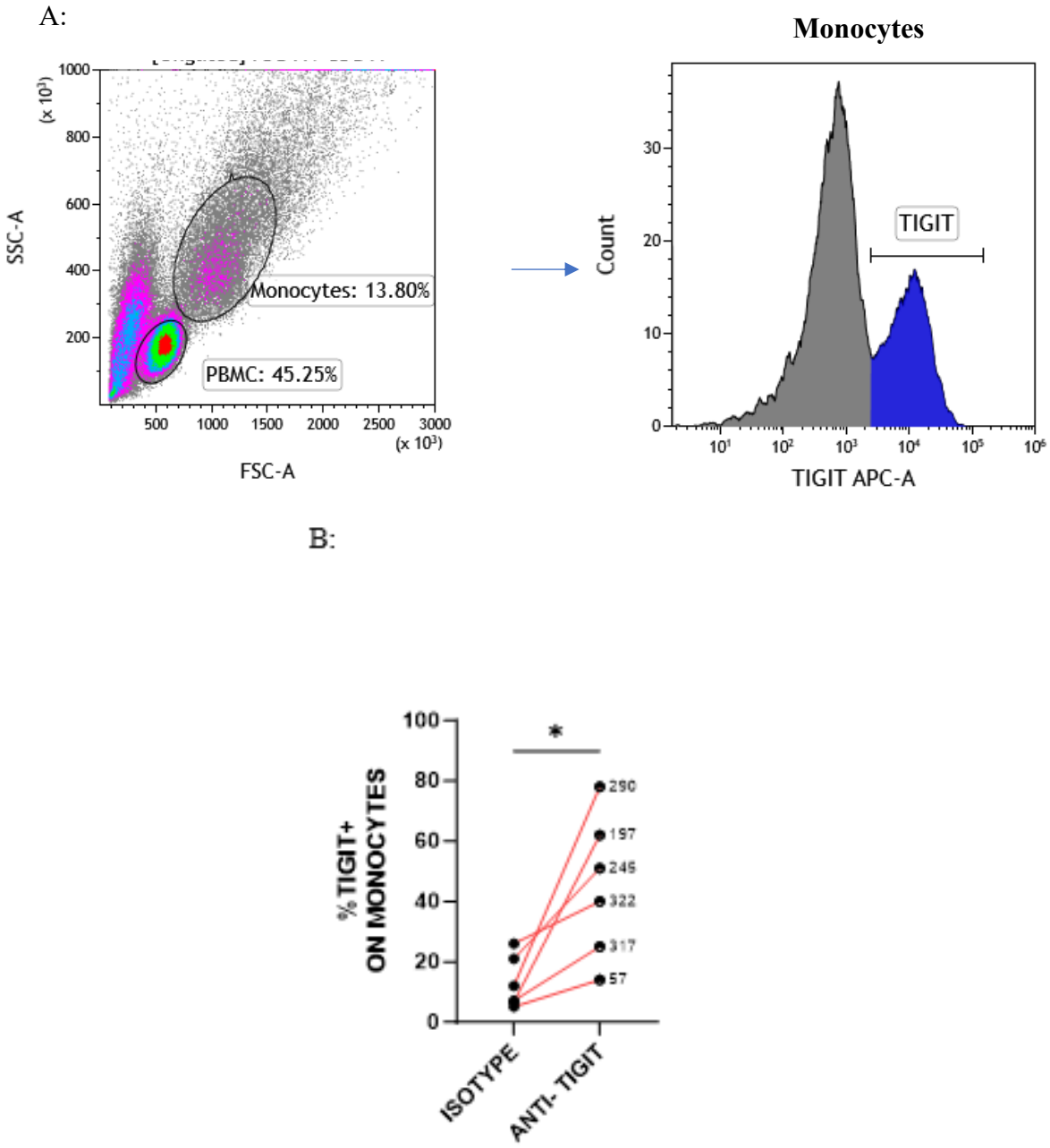

Figure 3.6.1: Monocyte-mediated trogocytosis of TIGIT. (A) Gating strategy for flow cytometry is shown. Monocytes were gated by scatter characteristics and the percentage of TIGIT^{+} monocytes was determined. (B) The frequency of anti-TIGIT mAb fluorescence increased after TIGIT blockade on monocytes of subjects with good responses to TIIGIT blockade compared to isotype control. After TGIT blockade, the percentage of monocytes positive for anti-TIGIT mAb fluorescence increased compared to isotype control treatment. The paired *t*-test was used for statistical analysis. *p < 0. 05.

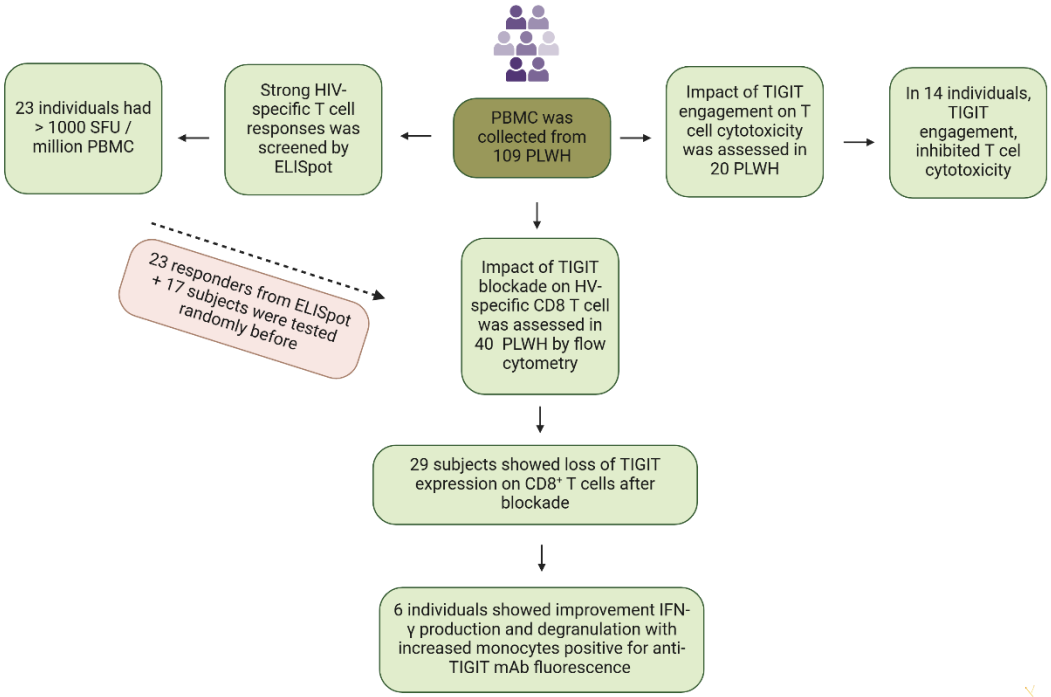

Figure 3.6.2: Schematic representation of our study. Our findings provide insights into the potential of TIGIT blockade as a therapeutic intervention in HIV infection.

4. Discussion

$CD8^+$ T cells play a key role in antigen-specific immune responses. During HIV infection, these cells recognize viral antigenic peptides presented on major histocompatibility complex-I (MHC-I) molecules via their T-cell receptors (TCRs). Upon encountering these antigens, naive $CD8^+$ T cells become activated and differentiated into effector cells, primed to exert their antiviral functions. HIV has evolved numerous strategies to evade $CD8^+$ T cell-mediated immune responses. These include rapid mutation of viral epitopes, downregulation of MHC-I molecules on infected cells, and expression of viral proteins that interfere with antigen processing and presentation pathways. Despite the presence of a large pool of HIV-specific $CD8^+$ T cells, the virus persists due to various factors, including viral reservoirs, immune evasion mechanisms, and immune dysfunction. Also, prolonged exposure to high levels of viral antigens leads to the functional exhaustion of $CD8^+$ T cells. The increasing levels of inhibitory receptors including PD-1, CTLA-4, Tim-3, and TIGIT on $CD8^+$ T cells in HIV infection represent a key aspect of immune dysregulation and T cell exhaustion observed in PLWH. The state of exhaustion is mainly characterized by sequential loss of T cell effector functions such as reduction of IFN-γ production, which is the main concern in HIV immunotherapy. Therefore, additional studies will be critical for developing new approaches to target inhibitory receptors or their ligands in order to effectively boost immunity against HIV.

Among these inhibitory receptors, TIGIT is a recently identified member of the immunoglobulin receptor superfamily, which plays a role in immune system regulation.

However, little is known about the blockade of TIGIT on T cells to regulate immune system function during HIV infection.

In our study we aimed to target TIGIT to assess whether there is improvement on anti viral effector cells such as $CD8^+$ T cells. Our first aim was to investigate and compare expression levels on $CD8^+$ T cells of HIV-infected and HIV-negative individuals in our study cohorts. Previous studies have shown that TIGIT is expressed on activated T cells, memory T cells, regulatory T (Treg) cell subsets, NK cells, and follicular helper T (Tfh) cells, all of which have an important role in HIV infection [98, 99]. Despite the advent of ART, which effectively suppresses viral replication, PLWH continue to exhibit dysregulated immune responses characterized by the expansion of $TIGIT^+$ $CD8^+$ T cells. Our results revealed significant expansion of $TIGIT^+$ $CD8^+$ T cells in PLWH despite ART, compared to HIV-negative controls, indicating that the $CD8^+$ T cells of PLWH have progressed towards an exhausted phenotype with increased expression of inhibitory receptors. This is consistent with previous studies showing that T cell exhaustion is a characteristic feature of chronic viral infections and the level of TIGIT on $CD8^+$ T cells is higher in PLWH than in uninfected individuals [81, 82, 134]. Moreover, the persistence of elevated TIGIT expression on $CD8^+$ T cells despite ART underscores the challenges in restoring immune function in PLWH. Even with effective viral suppression, the immune system remains dysregulated, with $CD8^+$ T cells exhibiting signs of exhaustion and impaired antiviral responses. This suggests that adjunctive immunotherapeutic approaches targeting inhibitory receptors like TIGIT may be necessary to overcome immune dysfunction and enhance immune control of HIV infection.

Understanding the impact of TIGIT engagement on T cell cytotoxicity in PLWH can highlight one mechanism potentially contributing to immune dysfunction in HIV infection. Elevated TIGIT expression on $CD8^+$ T cells, as observed in previous findings, may be one of the factors responsible for an impaired immune system in PLWH, therefore, we investigated the effect of TIGIT engagement on $CD8^+$ T cells from PLWH in non-specific redirected cytotoxicity assays.

We used ^{51}Cr-release assays to investigate the influence of TIGIT engagement on T cell cytotoxicity by adding either anti-TIGIT or isotype control to the assay and comparing the level of cytolysis. Engaging P815 cell cross linking of TIGIT by anti-TIGIT mAb effectively decreased T cell cytotoxicity. The findings revealed that TIGIT engagement decreased T cell killing of P815 target cells in the majority of cases. Our results underscore the inhibitory role of TIGIT in regulating T cell activity, including cytotoxicity, and the therapeutic potential of TIGIT as a target in the context of HIV infection. These results highlight the potential therapeutic implications of targeting TIGIT in HIV-infected individuals. By blocking TIGIT-mediated inhibition, it may be possible to enhance $CD8^+$ T cell cytotoxicity and improve immune responses against HIV-infected cells. Thus, TIGIT emerges as a promising immunotherapeutic target for restoring immune function and controlling viral replication in PLWH.

Since the ELISpot assay is a well-established method for enumerating antigen-specific T cells, we used this assay to measure T cell responses to HIV-Gag and Nef peptide pools. Not all PLWH have robust T cell responses against HIV peptides, therefore, ELISpot was used to assist us in screening a larger number of individuals more efficiently. We screened 109 subjects for their T cell responses to HIV peptides and found 23 of them had strong enough HIV-1 specific T cell responses to be readily

followed by flow cytometry for detailed analysis. This means that although around 78 percent of PLWH subjects did not have strong enough responses, 22 percent of them had strong enough HIV-1 specific T cell responses for analysis by flowcytometry. Strong HIV-1 specific T cell responses may be a good marker for identifying responders to TIGIT blockade, although we identified few responders in this group of 23 individuals.

Considering the higher prevalence of $TIGIT^+$ T cells in PLWH than in the HIV-seronegative group, there is a likelihood that blocking the TIGIT signaling pathway may partially restore T cell function. The next objective aimed to evaluate the effects of interfering with the TIGIT pathways to restore HIV- specific T cell effector functions. TIGIT blockade may increase T cell cytotoxicity and degranulation in response to HIV-1-peptides [82, 135, 136]. We investigated the potential of interrupting TIGIT signaling through monoclonal antibody (mAb) blockade to rescue $CD8^+$ T cell antiviral activity. Interestingly, results revealed a heterogeneous response to TIGIT blockade among the subjects. While a significant proportion of subjects (29/40) exhibited some loss of TIGIT expression in response to the blockade, others (11/40) did not. This result underscores the complex nature of immune responses and illustrates that not all individuals will respond equally to this therapeutic strategy. Therefore, understanding the factors that determine responder status is important, as it may help identify predictive markers for the effectiveness of TIGIT blockade.

In the next step, we evaluated the impact of anti-TIGIT antibody treatment on $CD8^+$ T cell function regarding interferon-gamma (IFN-γ) production and degranulation (CD107 a expression) in response to Gag or Nef peptide stimulation in groups of people that did or did not show loss of TIGIT expression after TIGIT blockade. While the study

did not show statistically significant results on the overall population, it is important to consider that subjects who did not respond by downregulation of TIGIT in response to TIGIT blockade demonstrated less IFN-γ production. This observation suggests that in certain cases, TIGIT blockade may not effectively restore T cell effector function, highlighting the presence of non-responders. Conversely, among those who did respond to TIGIT blockade by loss of TIGIT expression, improvements were seen in both IFN-γ production and degranulation of CD107a in a subset of individuals. These findings emphasize the heterogeneity in responses to TIGIT blockade, indicating that for some individuals, this approach can enhance T cell effector functions, while for others, it may have no effect. This lack of overall significance may reflect the complexity and variability in individual immune responses. Overall, this study provides evidence that TIGIT blockade may improve $CD8^+$ T cell function and restore existing anti-HIV CD8 T cell effector functions to target HIV-infected cells in some PLWH.

Future research should aim to elucidate the factors that influence individual responsiveness to TIGIT blockade, such as baseline TIGIT expression, immune microenvironment or other genetic and environmental factors. Since only 6/29 individuals showing loss of TIGIT expression after TGIT blockade had improvements in both IFN-γ production and degranulation of CD107a, better markers of responsiveness are required. This knowledge will be crucial for identifying predictive markers that can guide the selection of patients most likely to benefit from TIGIT blockade and for refining the design of clinical trials.

This study sheds light on the complex and variable nature of responsiveness to TIGIT blockade. While not significant overall, the observed differences in individual responses underscore the importance of personalized approaches to immunotherapy. The results

provide a foundation for further research and optimization of TIGIT blockade as a potential immunomodulatory strategy, highlighting its potential benefits and the need for interventions based on individual profiles.

Phenotyping responsive T cells after TIGIT blockade could identify features of $CD8^+$ T cells from the individuals who respond to TIGIT blockade by downregulation of TIGIT expression and increased effector cell function. However, the effect of TIGIT blockade may vary among individuals, and further research is needed to fully understand the role of TIGIT in T cell exhaustion and to explore its potential as a therapeutic target. We focused on TCF-1, KLRG1, and CD45RA to investigate the differentiation and functional state of $CD8^+$ T cells responding to peptide stimulation and TIGIT blockade.

TCF-1, a transcription factor associated with memory T cells, was investigated to assess the potential for TIGIT blockade to selectively affect long-lasting, memory-like T cell populations. An increase in TCF-1 expression in responding cells could indicate a phenotype associated with responsiveness to TIGIT blockade.

KLRG1, an effector cell marker, is associated with short-lived, terminally differentiated effector T cells. The low expression of KLRG1 on IFN-γ^+ $CD8^+$ T cells after TIGIT blockade, could suggest that TIGIT blockade is associated with responsiveness within a less terminally differentiated effector phenotype, potentially associated with T cell longevity and sustained cytotoxic activity. However, our results do not prove that and we could not find any clear relation between the percentage of cells expressing IFN-γ and KLRG1 with TIGIT blockade compared to the isotype control.

CD45RA is a marker of naive T cells, and it is re-expressed on some memory T cells. The study has examined CD45RA expression to explore whether phenotyping responding cells can give us better understanding the mechanism of responsiveness to TIGIT blockade.

Our results did not show a significant relationship between the expression of TCF-1, KLRG1 or CD45RA and responsiveness of $CD8^+$ T cells. Of note, no correlation was observed between TIGIT expression and any of these markers. Among the six responders to TIGIT blockade, for subject 197, level of KLRG1 expression on $CD8^+$ T cells expressing IFN-γ decreased after blockade, suggesting potential modulation of the immune response in this particular case (Figure 3-5-2). KLRG1 is associated with effector or terminally differentiated T cells, and a reduction in its expression on responsive cells, might suggest a less differentiated memory-like state.

The expression of CD45RA on responsive $CD8^+$ T cells was steady, suggesting a consistent level of this marker after TIGIT blockade compared to isotype controls. The observed variations within the group of six individuals (Figure 3-5-2) shed light on the impact of TIGIT blockade on TCF-1 expression in responsive $CD8^+$ T cells. Notably, in two subjects, 317 and 322, blockade of TIGIT resulted in apparent selection of IFN-γ^+ TCF-1^+ cells. Enhancement in TCF-1^+ on IFN-γ^+ $CD8^+$ T cells following TIGIT blockade implies a positive modulation of T cell responses in TCF-1^+ cells from these subjects. The observed variability among individuals may indicate that the effects of TIGIT inhibition on TCF-1^+ T cell responses are subject-specific, highlighting the complex nature of immune responses. Further exploration is warranted to elucidate the underlying mechanisms and determine whether this observed enhancement in TCF-1^+

cells is associated with improved overall T cell function. However, additional research and larger-scale studies are essential to validate and generalize these findings.

Next, we tried to characterize $CD8^+TIGIT^+TCF1^+$ ($TCF1^+Tex^{prog}$) and $CD8^+TIGIT^+TCF1^-$ ($TCF1^-Tex^{term}$) in responder groups. Understanding the heterogeneity within the exhausted $CD8^+$ T cells is essential for immune checkpoint blockade therapy efficacy, as it can have implications for immunotherapeutic interventions for reinvigorating these two subsets, but the results were not statistically significant. Including additional markers like CD45RO (a memory T cell marker), PD-1 and considering proportion of Tregs can be considered for future experiments.

An earlier study demonstrated that anti-TIGIT monoclonal antibodies can induce trogocytosis of TIGIT through FcγR-expressing cells. This finding suggests that the treatment with anti-TIGIT antibodies may lead to the internalization of TIGIT from T cells by monocytes [133]. As TIGIT is an immune checkpoint receptor that plays a crucial role in regulating T cell function, its removal from the T cell surface could have positive implications for T cell activation and immune responses. To gain better insight into the impact of anti-TIGIT antibody treatment, we gated on monocytes within PBMC and measured TIGIT levels on these cells to determine whether anti-TIGIT antibody treatment resulted in changes in the frequency of TIGIT expression on monocytes and to find out why downregulation of TIGIT was observed in the responder group during anti-TIGIT treatment. Our results showed that individuals who lost TIGIT expression after TIGIT blockade had an increased percentages of monocytes positive for anti-TIGIT mAb fluorescence. This might indicate that the process of trogocytosis is more active in individuals most likely benefit from TIGIT blockade. Trogocytosis is a process where immune cells extract antigens from the surface of other cells. This process can

be initiated by the engagement of FcRs. CD64 (FcγRI) and CD16 (FcγRIII) in the context of trogocytosis show disparate affinities, so specific comparison of CD64 and CD16 can be addressed in future research. Also, the higher percentage of TIGIT and FcγR expression can be related to FcγR-mediated immune cell activation and restoring immune stimulation [137, 138].

4.1: Study Limitations

As with most research involving human subjects, there were a number limitations to our study. The participants in our study were recruited through the Newfoundland and Labrador Provincial Immunodeficiency Clinic, and were receiving ART to treat HIV infection. This may introduce selection bias and limit the representativeness of our study population. Variation in demographic characteristics, genetic background, and clinical status among individuals with HIV infection could affect the observed outcomes and limit the extrapolation of results to broader populations of PLWH. While ART is highly effective in suppressing viral replication and restoring $CD4^+$ T cell counts, it may also influence immune responses and T cell function. It can decrease viral replication, resulting in weaker anti-HIV T cell responses. Additionally, longitudinal studies are needed to assess the dynamic changes in TIGIT expression and T cell function over time.

Furthermore, our study focused primarily on peripheral blood samples, which may not fully capture the complexity of immune responses occurring within lymphoid tissues as they are central to the pathology of HIV[139]. Future studies incorporating tissue-based analyses, such as lymph nodes, may provide a more comprehensive understanding of TIGIT expression and its impact on T cell function in HIV infection.

Another limitation issue was selecting the best target for T cells in a cytotoxicity assay. While P815 was the cell line used, the best option would be B cells. However, they do not have PVR as a ligand for TIGIT and we can not transfect B cells of all people to express PVR. With a better physiologic target for T cells, we could see the effect of more physiologically realistic engagement between TIGIT and its physiological ligand.

4.2: Future Directions

Given the inherent heterogeneity in immune responses, studies on epigenetic mechanisms and its impact on immune cells that direct cellular differentiation and function could be informative [140]. Epigenetic modifications, such as DNA methylation, histone modifications, and chromatin remodeling, can impact the accessibility of regulatory regions within the genome, thereby influencing gene expression levels. There are some studies that showed expression of negative checkpoint receptors is controlled by epigenetic regulation which can be related to T cell function [140-142]. Understanding how epigenetic regulation influences the expression of negative checkpoint receptors, such as TIGIT, could provide valuable insights into T cell dysfunction and potential therapeutic strategies. While TIGIT is upregulated on T cells during HIV infection, the genome-wide epigenetic states of $TIGIT^+$ $CD8^+$ T cells can be studied as well, especially in the group that responded better to TIGIT blockade by $CD8^+$ T cell reinvigoration. Epigenetic markers associated with restoring T cell function could serve as prognostic indicators for identifying individuals who are most likely to benefit from this therapeutic approach.

Considering the relationship between HIV-1 infection and the expression of poliovirus receptor (PVR) and TIGIT on $CD4^+$ T cells could be helpful in future studies due to its potential implications for the maintenance of the viral reservoir. Recent evidence

suggests that HIV-1 infection induces upregulation of PVR expression on $CD4^+$ T cells, providing a mechanism by which the virus may interact with its host cells and modulate immune responses [115]. The latent reservoir, consisting of quiescent $CD4^+$ T cells harboring integrated proviral DNA, represents a major barrier to viral eradication and cure efforts. This reservoir is concentrated in $CD4^+$ T cells expressing PVR and TIGIT. The co-expression of PVR and TIGIT on $CD4^+$ T cells may contribute to the maintenance of latency by promoting T cell energy and intractable viral latency. Engagement of TIGIT by its ligands, including PVR, leads to the suppression of T cell activation and effector function, thereby facilitating viral persistence and evasion of immune surveillance. Understanding the interplay between HIV-1 infection, PVR expression, and TIGIT signaling on $CD4^+$ T cells is crucial for developing targeted strategies to eliminate the latent reservoir and achieve a functional cure for HIV/AIDS. Therapeutic interventions aimed at disrupting the PVR-TIGIT axis or reversing T cell exhaustion could potentially reactivate latent virus and render infected cells susceptible to immune-mediated clearance. Future research should focus on elucidating the molecular mechanisms underlying the regulation of PVR and TIGIT expression in the context of HIV-1 infection, as well as exploring the therapeutic potential of targeting these pathways to eradicate the latent reservoir in PLWH.

Another aspect of using checkpoint inhibitors for consideration is whether to use them singly or in combination. To limit T cell responses, after activation, T cells upregulate inhibitory receptors such as CTLA-4 and PD-1 [94, 100]. TIGIT has high expression on NK cells and $CD8^+$ T cells, which increases the potential for TIGIT blockade to have an impact on effector cells in chronic infections such as HIV-1 [81, 89, 99, 136]. Using mAbs such as anti-PD-1 or anti-TIGIT can help shock $CD4^+$ T cells that harbor latent

HIV-1, into activation and shift latent HIV-1 into expressing HIV-1 antigens which enable antiviral effector cells to recognize them. However, some studies suggest that dual blockade of TIGIT and PD-1 or CTLA-4, compared to single blockade is more effective in cancer immunotherapy and some infectious diseases [143-145]. So, dual blockade such as combination of anti-PD-1 and anti-TIGIT could be a better therapeutic strategy for targeting the HIV reservoir by enhancing proliferation, cytokine production and cytotoxicity of $CD8^+$ T cells. While PD-1 primarily inhibits T cell effector function and proliferation, TIGIT regulates T cell activation and cytokine production [82, 89]. By simultaneously blocking both pathways, a dual blockade strategy may exert synergistic effects in restoring T cell function and enhancing antiviral immune responses potentially leading to more comprehensive and effective reinvigoration of antiviral immune responses to target the reservoir. Also, transcriptomic analysis identifies distinct gene expression profiles associated with dual blockade therapy, including upregulation of genes involved in immune activation and effector function [124]. While preclinical studies have shown promise on HIV-specific immunity [146, 147], the translation of dual blockade therapy into clinical practice poses several challenges. These include potential toxicity from excessive immune activation, the need for careful patient selection, and the optimal dosing and combination strategies. Additionally, long-term safety and efficacy data are needed to assess the durability of immune responses and the impact on viral reservoirs.

In future studies, we can employ different methods such as RNA sequencing (RNA-seq). RNA-seq enables the high-throughput analysis of gene expression in $CD8^+$ T cells from HIV-infected individuals, allowing us to identify transcriptional signatures associated with TIGIT expression and T cell exhaustion. Comparative transcriptomic

analyses between TIGIT$^+$ and TIGIT$^-$ CD8$^+$ T cell subsets can reveal dysregulated pathways, signaling networks, and gene expression programs underlying T cell dysfunction during HIV infection. RNA-seq facilitates the discovery of regulatory pathways and molecular mechanisms governing TIGIT expression and function in HIV-infected CD8$^+$ T cells. By integrating RNA-seq data with functional and pathway analyses, we can delineate the upstream regulators, epigenetic modifiers, and transcriptional regulators involved in TIGIT-mediated immune regulation and T cell exhaustion. This method can help to identify predictive biomarkers of immune cells response and disease progression in HIV-infected individuals. By correlating gene expression profiles with therapeutic responses, we can develop prognostic biomarkers to guide personalized treatment approaches to individual people.

Understanding the role of T cell immunometabolism in T cell reinvigoration is another future direction to be addressed. T cell responses are intricately linked to metabolic reprogramming, as these cells undergo dynamic changes in metabolism to meet the energetic demands associated with activation, proliferation, and effector functions. During antiviral responses, T cells experience metabolic shifts aimed at optimizing their function and survival. They may increase their uptake of glucose and amino acids, and ramp up certain metabolic pathways to meet the increased energy and biosynthetic demands. One well-studied metabolic pathway is glycolysis, which provides the necessary energy and biosynthetic precursors for rapidly proliferating effector T cells. However, recent studies have highlighted the importance of other metabolic pathways, including fatty acid oxidation (FAO), in regulating T cell function. Negative checkpoint receptors, such as TIGIT, plays a crucial role in modulating T cell metabolism. Engagement of these receptors by their ligands results in downstream signaling

cascades that impact metabolic pathways within T cells. There are some studies indicating that engagement of negative checkpoint receptors results in T cell metabolic changes, such as inhibition of glycolysis and increased fatty acid oxidation [148-151]. T cells utilize glycolysis during differentiation to effectors, so TIGIT blockade may improve effector cell differentiation and function. Future studies should focus on further dissecting the molecular mechanisms underlying T cell immunometabolism and its regulation by negative checkpoint receptors, such as modulating glycolysis or FAO, in combination with checkpoint blockade therapies for enhancing T cell function and controlling viral replication in HIV-infected individuals.

In summary, the findings from our research showed that TIGIT blockade can improve antiviral effector cell function in certain PLWH. In this context, this improvement suggests that a blockade of TIGIT could be an effective treatment strategy. Specific characteristics of a subset of $CD8^+$ T cells that respond positively to TIGIT blockade must be identified in order to optimize the efficacy of this treatment. Understanding these characteristics will facilitate a more effective design of blockade therapy, directing it towards those PLWH who are most likely to experience benefits from this treatment. By doing so, we can improve therapeutic outcomes in HIV treatment.

5. References

[1] **A. Lever, H. Gottlinger, W. Haseltine, and J. Sodroski, "Identification of a sequence required for efficient packaging of human immunodeficiency virus type 1 RNA into virions," *J Virol*, vol. 63, no. 9, pp. 4085–4087, Sep. 1989, doi: 10.1128/jvi.63.9.4085-4087.1989.**

[2] **F. Gao *et al.*, "Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes," *Nature*, vol. 397, no. 6718, pp. 436–441, Feb. 1999, doi: 10.1038/17130.**

[3] **P. M. Sharp and B. H. Hahn, "The evolution of HIV-1 and the origin of AIDS," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1552, pp. 2487–2494, Aug. 2010, doi: 10.1098/rstb.2010.0031.**

[4] **The Joint United Nations Programme on HIV/AIDS (UNAIDS), "Global HIV & AIDS statistics — Fact sheet." [Online]. Available: https://www.unaids.org/en/resources/fact-sheet**

[5] **G. Li *et al.*, "An integrated map of HIV genome-wide variation from a population perspective," *Retrovirology*, vol. 12, no. 1, p. 18, Dec. 2015, doi: 10.1186/s12977-015-0148-6.**

[6] **P. Ramdas, A. K. Sahu, T. Mishra, V. Bhardwaj, and A. Chande, "From Entry to Egress: Strategic Exploitation of the Cellular Processes by HIV-1," *Front Microbiol*, vol. 11, Dec. 2020, doi: 10.3389/fmicb.2020.559792.**

[7] **K. Lu, X. Heng, and M. F. Summers, "Structural Determinants and Mechanism of HIV-1 Genome Packaging," *J Mol Biol*, vol. 410, no. 4, pp. 609–633, Jul. 2011, doi: 10.1016/j.jmb.2011.04.029.**

[8] **S. Pallikkuth *et al.*, "Peripheral T Follicular Helper Cells Are the Major HIV Reservoir within Central Memory CD4 T Cells in Peripheral Blood from Chronically HIV-Infected Individuals on Combination Antiretroviral Therapy," *J Virol*, vol. 90, no. 6, pp. 2718–2728, Mar. 2016, doi: 10.1128/JVI.02883-15.**

[9] **J. Embretson *et al.*, "Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS," *Nature*, vol. 362, no. 6418, pp. 359–362, Mar. 1993, doi: 10.1038/362359a0.**

[10] **C. F. Perno, V. Svicher, D. Schols, M. Pollicita, J. Balzarini, and S. Aquaro, "Therapeutic strategies towards HIV-1 infection in macrophages," *Antiviral Res*, vol. 71, no. 2–3, pp. 293–300, Sep. 2006, doi: 10.1016/j.antiviral.2006.05.015.**

[11] **E. A. Berger, P. M. Murphy, and J. M. Farber, "CHEMOKINE RECEPTORS AS HIV-1 CORECEPTORS: Roles in Viral Entry, Tropism, and Disease," *Annu Rev Immunol*, vol. 17, no. 1, pp. 657–700, Apr. 1999, doi: 10.1146/annurev.immunol.17.1.657.**

[12] **A. Engelman and P. Cherepanov, "The structural biology of HIV-1: mechanistic and therapeutic insights," *Nat Rev Microbiol*, vol. 10, no. 4, pp. 279–290, Apr. 2012, doi: 10.1038/nrmicro2747.**

[13] E. J. Arts and D. J. Hazuda, "HIV-1 Antiretroviral Drug Therapy," *Cold Spring Harb Perspect Med*, vol. 2, no. 4, pp. a007161–a007161, Apr. 2012, doi: 10.1101/cshperspect.a007161.

[14] B. Lee, M. Sharron, L. J. Montaner, D. Weissman, and R. W. Doms, "Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages," *Proceedings of the National Academy of Sciences*, vol. 96, no. 9, pp. 5215–5220, Apr. 1999, doi: 10.1073/pnas.96.9.5215.

[15] P. Borrow, "Innate immunity in acute HIV-1 infection.," *Curr Opin HIV AIDS*, vol. 6, no. 5, pp. 353–63, Sep. 2011, doi: 10.1097/COH.0b013e3283495996.

[16] S. R. El-Zayat, H. Sibaii, and F. A. Mannaa, "Toll-like receptors activation, signaling, and targeting: an overview," *Bull Natl Res Cent*, vol. 43, no. 1, p. 187, Dec. 2019, doi: 10.1186/s42269-019-0227-2.

[17] T. Kawasaki and T. Kawai, "Toll-Like Receptor Signaling Pathways," *Front Immunol*, vol. 5, Sep. 2014, doi: 10.3389/fimmu.2014.00461.

[18] S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa, "Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA," *Science (1979)*, vol. 303, no. 5663, pp. 1529–1531, Mar. 2004, doi: 10.1126/science.1093616.

[19] N. Kadowaki *et al.*, "Subsets of Human Dendritic Cell Precursors Express Different Toll-like Receptors and Respond to Different Microbial

Antigens," *J Exp Med*, vol. 194, no. 6, pp. 863–870, Sep. 2001, doi: 10.1084/jem.194.6.863.

[20] A. R. Stacey *et al.*, "Induction of a Striking Systemic Cytokine Cascade prior to Peak Viremia in Acute Human Immunodeficiency Virus Type 1 Infection, in Contrast to More Modest and Delayed Responses in Acute Hepatitis B and C Virus Infections," *J Virol*, vol. 83, no. 8, pp. 3719–3733, Apr. 2009, doi: 10.1128/JVI.01844-08.

[21] O. M. Diop *et al.*, "Plasmacytoid Dendritic Cell Dynamics and Alpha Interferon Production during Simian Immunodeficiency Virus Infection with a Nonpathogenic Outcome," *J Virol*, vol. 82, no. 11, pp. 5145–5152, Jun. 2008, doi: 10.1128/JVI.02433-07.

[22] B. Malleret *et al.*, "Primary infection with simian immunodeficiency virus: plasmacytoid dendritic cell homing to lymph nodes, type I interferon, and immune suppression," *Blood*, vol. 112, no. 12, pp. 4598–4608, Dec. 2008, doi: 10.1182/blood-2008-06-162651.

[23] P. J. Norris, B. L. Pappalardo, B. Custer, G. Spotts, F. M. Hecht, and M. P. Busch, "Elevations in IL-10, TNF- α , and IFN- γ from the Earliest Point of HIV Type 1 Infection," *AIDS Res Hum Retroviruses*, vol. 22, no. 8, pp. 757–762, Aug. 2006, doi: 10.1089/aid.2006.22.757.

[24] C. Tomescu, J. Chehimi, V. C. Maino, and L. J. Montaner, "NK Cell Lysis of HIV-1-Infected Autologous CD4 Primary T Cells: Requirement for IFN-Mediated NK Activation by Plasmacytoid Dendritic Cells," *The*

Journal of Immunology, vol. 179, no. 4, pp. 2097–2104, Aug. 2007, doi: 10.4049/jimmunol.179.4.2097.

[25] L. Adalid-Peralta _et al._, "Stimulation of the primary anti-HIV antibody response by IFN-α in patients with acute HIV-1 infection," _J Leukoc Biol_, vol. 83, no. 4, pp. 1060–1067, Apr. 2008, doi: 10.1189/jlb.1007675.

[26] B. Rodriguez _et al._, "Interferon-alpha differentially rescues CD4 and CD8 T cells from apoptosis in HIV infection.," _AIDS_, vol. 20, no. 10, pp. 1379–89, Jun. 2006, doi: 10.1097/01.aids.0000233571.51899.ab.

[27] E. Scully and G. Alter, "NK Cells in HIV Disease," _Curr HIV/AIDS Rep_, vol. 13, no. 2, pp. 85–94, Apr. 2016, doi: 10.1007/s11904-016-0310-3.

[28] L. Flórez-Álvarez, J. C. Hernandez, and W. Zapata, "NK Cells in HIV-1 Infection: From Basic Science to Vaccine Strategies," _Front Immunol_, vol. 9, Oct. 2018, doi: 10.3389/fimmu.2018.02290.

[29] X. Cao _et al._, "Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance.," _Immunity_, vol. 27, no. 4, pp. 635–46, Oct. 2007, doi: 10.1016/j.immuni.2007.08.014.

[30] A. M. Abel, C. Yang, M. S. Thakar, and S. Malarkannan, "Natural Killer Cells: Development, Maturation, and Clinical Utilization," _Front Immunol_, vol. 9, Aug. 2018, doi: 10.3389/fimmu.2018.01869.

[31] K. S. Campbell and J. Hasegawa, "Natural killer cell biology: an update and future directions.," _J Allergy Clin Immunol_, vol. 132, no. 3, pp. 536–544, Sep. 2013, doi: 10.1016/j.jaci.2013.07.006.

[32] F. Li and S. Liu, "Focusing on NK cells and ADCC: A promising immunotherapy approach in targeted therapy for HER2-positive breast cancer," *Front Immunol*, vol. 13, Dec. 2022, doi: 10.3389/fimmu.2022.1083462.

[33] S. Duan and S. Liu, "Targeting NK Cells for HIV-1 Treatment and Reservoir Clearance," *Front Immunol*, vol. 13, Mar. 2022, doi: 10.3389/fimmu.2022.842746.

[34] L. Flórez-Álvarez, J. C. Hernandez, and W. Zapata, "NK Cells in HIV-1 Infection: From Basic Science to Vaccine Strategies," *Front Immunol*, vol. 9, Oct. 2018, doi: 10.3389/fimmu.2018.02290.

[35] A. S. Fauci, D. Mavilio, and S. Kottilil, "NK cells in HIV infection: Paradigm for protection or targets for ambush," *Nat Rev Immunol*, vol. 5, no. 11, pp. 835–843, Nov. 2005, doi: 10.1038/nri1711.

[36] S. Moir and A. S. Fauci, "B-cell responses to HIV infection," *Immunol Rev*, vol. 275, no. 1, pp. 33–48, Jan. 2017, doi: 10.1111/imr.12502.

[37] S. Moir and A. S. Fauci, "B cells in HIV infection and disease," *Nat Rev Immunol*, vol. 9, no. 4, pp. 235–245, Apr. 2009, doi: 10.1038/nri2524.

[38] S. Moir *et al.*, "Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily.," *J Exp Med*, vol. 200, no. 7, pp. 587–99, Oct. 2004.

[39] **S. Moir *et al.*, "HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals.," *Proc Natl Acad Sci U S A*, vol. 98, no. 18, pp. 10362–7, Aug. 2001, doi: 10.1073/pnas.181347898.**

[40] **S. Moir and A. S. Fauci, "Insights into B cells and HIV-specific B-cell responses in HIV-infected individuals," *Immunol Rev*, vol. 254, no. 1, pp. 207–224, Jul. 2013, doi: 10.1111/imr.12067.**

[41] **H. Longwe, S. Gordon, R. Malamba, and N. French, "Characterising B cell numbers and memory B cells in HIV infected and uninfected Malawian adults," *BMC Infect Dis*, vol. 10, no. 1, p. 280, Dec. 2010, doi: 10.1186/1471-2334-10-280.**

[42] **D. S. Goodsell, "Illustrations of the HIV life cycle," *The Future of HIV-1 Therapeutics*, pp. 243–252, 2015.**

[43] **E. Fanales-Belasio, M. Raimondo, B. Suligoi, and S. Buttò, "HIV virology and pathogenetic mechanisms of infection: a brief overview," *Ann Ist Super Sanita*, vol. 46, pp. 5–14, 2010.**

[44] **D. C. Douek *et al.*, "HIV preferentially infects HIV-specific CD4+ T cells," *Nature*, vol. 417, no. 6884, pp. 95–98, May 2002, doi: 10.1038/417095a.**

[45] **M. Perreau *et al.*, "Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production," *Journal of Experimental Medicine*, vol. 210, no. 1, pp. 143–156, Jan. 2013, doi: 10.1084/jem.20121932.**

[46] J. S. Hale and R. Ahmed, "Memory T Follicular Helper CD4 T Cells," *Front Immunol*, vol. 6, Feb. 2015, doi: 10.3389/fimmu.2015.00016.

[47] K. L. Good-Jacobson, C. G. Szumilas, L. Chen, A. H. Sharpe, M. M. Tomayko, and M. J. Shlomchik, "PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells," *Nat Immunol*, vol. 11, no. 6, pp. 535–542, Jun. 2010, doi: 10.1038/ni.1877.

[48] R. Banga *et al.*, "PD-1+ and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals," *Nat Med*, vol. 22, no. 7, pp. 754–761, Jul. 2016, doi: 10.1038/nm.4113.

[49] M. F. Chevalier and L. Weiss, "The split personality of regulatory T cells in HIV infection," *Blood*, vol. 121, no. 1, pp. 29–37, Jan. 2013, doi: 10.1182/blood-2012-07-409755.

[50] A. Bandera *et al.*, "CD4+ T cell depletion, immune activation and increased production of regulatory T cells in the thymus of HIV-infected individuals.," *PLoS One*, vol. 5, no. 5, p. e10788, May 2010, doi: 10.1371/journal.pone.0010788.

[51] D. Zhang *et al.*, "Most antiviral CD8 T cells during chronic viral infection do not express high levels of perforin and are not directly cytotoxic," *Blood*, vol. 101, no. 1, pp. 226–235, Jan. 2003, doi: 10.1182/blood-2002-03-0791.

[52] F. Perdomo-Celis, P. A. Velilla, N. A. Taborda, and M. T. Rugeles, "An altered cytotoxic program of CD8+ T-cells in HIV-infected patients despite

HAART-induced viral suppression," *PLoS One*, vol. 14, no. 1, p. e0210540, Jan. 2019, doi: 10.1371/journal.pone.0210540.

[53] A. R. Hersperger *et al.*, "Increased HIV-specific CD8+ T-cell cytotoxic potential in HIV elite controllers is associated with T-bet expression," *Blood*, vol. 117, no. 14, pp. 3799–3808, Apr. 2011, doi: 10.1182/blood-2010-12-322727.

[54] D. R. Collins, G. D. Gaiha, and B. D. Walker, "CD8+ T cells in HIV control, cure and prevention," *Nat Rev Immunol*, vol. 20, no. 8, pp. 471–482, Aug. 2020, doi: 10.1038/s41577-020-0274-9.

[55] P. Adams, G. Iserentant, J.-Y. Servais, L. Vandekerckhove, G. Vanham, and C. Seguin-Devaux, "Cytotoxic CD8+ T Cells Expressing CXCR5 Are Detectable in HIV-1 Elite Controllers After Prolonged In Vitro Peptide Stimulation," *Front Immunol*, vol. 11, Feb. 2021, doi: 10.3389/fimmu.2020.622343.

[56] N. Gulzar and K. Copeland, "CD8+ T-Cells: Function and Response to HIV Infection," *Curr HIV Res*, vol. 2, no. 1, pp. 23–37, Jan. 2004, doi: 10.2174/1570162043485077.

[57] J. Attanasio and E. J. Wherry, "Costimulatory and Coinhibitory Receptor Pathways in Infectious Disease," *Immunity*, vol. 44, no. 5, pp. 1052–1068, May 2016, doi: 10.1016/j.immuni.2016.04.022.

[58] E. J. Wherry and M. Kurachi, "Molecular and cellular insights into T cell exhaustion," *Nat Rev Immunol*, vol. 15, no. 8, pp. 486–499, Aug. 2015, doi: 10.1038/nri3862.

[59] S. J. Keppler, K. Rosenits, T. Koegl, S. Vucikuja, and P. Aichele, "Signal 3 Cytokines as Modulators of Primary Immune Responses during Infections: The Interplay of Type I IFN and IL-12 in CD8 T Cell Responses," *PLoS One*, vol. 7, no. 7, p. e40865, Jul. 2012, doi: 10.1371/journal.pone.0040865.

[60] J. E. Smith-Garvin, G. A. Koretzky, and M. S. Jordan, "T Cell Activation," *Annu Rev Immunol*, vol. 27, no. 1, pp. 591–619, Apr. 2009, doi: 10.1146/annurev.immunol.021908.132706.

[61] J. A. O. J. P. S. A. S. P. P. J. J. Kuby, *Kuby immunology*. New York : W.H. Freeman. , 2013.

[62] A. Pathni *et al.*, "Cytotoxic T Lymphocyte Activation Signals Modulate Cytoskeletal Dynamics and Mechanical Force Generation," *Front Immunol*, vol. 13, Mar. 2022, doi: 10.3389/fimmu.2022.779888.

[63] E. J. Wherry and M. Kurachi, "Molecular and cellular insights into T cell exhaustion," *Nat Rev Immunol*, vol. 15, no. 8, pp. 486–499, Aug. 2015, doi: 10.1038/nri3862.

[64] J. Attanasio and E. J. Wherry, "Costimulatory and Coinhibitory Receptor Pathways in Infectious Disease," *Immunity*, vol. 44, no. 5, pp. 1052–1068, May 2016, doi: 10.1016/j.immuni.2016.04.022.

[65] O. Dushek, J. Goyette, and P. A. van der Merwe, "Non-catalytic tyrosine-phosphorylated receptors," *Immunol Rev*, vol. 250, no. 1, pp. 258–276, Nov. 2012, doi: 10.1111/imr.12008.

[66] J.-R. Hwang, Y. Byeon, D. Kim, and S.-G. Park, "Recent insights of T cell receptor-mediated signaling pathways for T cell activation and development," *Exp Mol Med*, vol. 52, no. 5, pp. 750–761, May 2020, doi: 10.1038/s12276-020-0435-8.

[67] A. Shibuya, S. Tahara-Hanaoka, and K. Shibuya, "DNAM-1 (CD226): A Two-Sword Fencer for Innate and Adaptive Immunity," *Curr Med Chem Anti Inflamm Anti Allergy Agents*, vol. 4, no. 1, pp. 53–58, Feb. 2005, doi: 10.2174/1568014053005390.

[68] S. Gilfillan *et al.*, "DNAM-1 promotes activation of cytotoxic lymphocytes by nonprofessional antigen-presenting cells and tumors," *J Exp Med*, vol. 205, no. 13, pp. 2965–2973, Dec. 2008, doi: 10.1084/jem.20081752.

[69] B. Sanchez-Correa *et al.*, "DNAM-1 and the TIGIT/PVRIG/TACTILE Axis: Novel Immune Checkpoints for Natural Killer Cell-Based Cancer Immunotherapy.," *Cancers (Basel)*, vol. 11, no. 6, Jun. 2019, doi: 10.3390/cancers11060877.

[70] A. C. Anderson, N. Joller, and V. K. Kuchroo, "Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation.," *Immunity*, vol. 44, no. 5, pp. 989–1004, May 2016, doi: 10.1016/j.immuni.2016.05.001.

[71] M. Reth, "Antigen receptor tail clue.," *Nature*, vol. 338, no. 6214, pp. 383–4, Mar. 1989, doi: 10.1038/338383b0.

[72] D. N. Burshtyn *et al.*, "Recruitment of tyrosine phosphatase HCP by the killer cell inhibitor receptor.," *Immunity*, vol. 4, no. 1, pp. 77–85, Jan. 1996, doi: 10.1016/s1074-7613(00)80300-3.

[73] L. L. Lanier, "Up on the tightrope: natural killer cell activation and inhibition.," *Nat Immunol*, vol. 9, no. 5, pp. 495–502, May 2008, doi: 10.1038/ni1581.

[74] W. Cao, V. Mehraj, D. E. Kaufmann, T. Li, and J.-P. Routy, "Elevation and persistence of CD8 T-cells in HIV infection: the Achilles heel in the ART era," *J Int AIDS Soc*, vol. 19, no. 1, p. 20697, Jan. 2016, doi: 10.7448/IAS.19.1.20697.

[75] M. Hoffmann *et al.*, "Exhaustion of Activated CD8 T Cells Predicts Disease Progression in Primary HIV-1 Infection," *PLoS Pathog*, vol. 12, no. 7, p. e1005661, Jul. 2016, doi: 10.1371/journal.ppat.1005661.

[76] C. Fenwick *et al.*, "T-cell exhaustion in HIV infection," *Immunol Rev*, vol. 292, no. 1, pp. 149–163, Nov. 2019, doi: 10.1111/imr.12823.

[77] A. Varela-Rohena *et al.*, "Control of HIV-1 immune escape by CD8 T cells expressing enhanced T-cell receptor," *Nat Med*, vol. 14, no. 12, pp. 1390–1395, Dec. 2008, doi: 10.1038/nm.1779.

[78] M. J. Fuller and A. J. Zajac, "Ablation of CD8 and CD4 T Cell Responses by High Viral Loads," *The Journal of Immunology*, vol. 170, no. 1, pp. 477–486, Jan. 2003, doi: 10.4049/jimmunol.170.1.477.

[79] R. M. Welsh, "Assessing Cd8 T Cell Number and Dysfunction in the Presence of Antigen," *J Exp Med*, vol. 193, no. 5, pp. F19–F22, Mar. 2001, doi: 10.1084/jem.193.5.F19.

[80] E. J. Wherry, J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed, "Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment.," *J Virol*, vol. 77, no. 8, pp. 4911–27, Apr. 2003, doi: 10.1128/jvi.77.8.4911-4927.2003.

[81] J. Tauriainen *et al.*, "Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals," *Sci Rep*, vol. 7, no. 1, p. 40354, Jan. 2017, doi: 10.1038/srep40354.

[82] G. M. Chew *et al.*, "TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection," *PLoS Pathog*, vol. 12, no. 1, p. e1005349, Jan. 2016, doi: 10.1371/journal.ppat.1005349.

[83] A. Shibuya and K. Shibuya, "DNAM-1 versus TIGIT: competitive roles in tumor immunity and inflammatory responses," *Int Immunol*, vol. 33, no. 12, pp. 687–692, Nov. 2021, doi: 10.1093/intimm/dxab085.

[84] **L. Martinet and M. J. Smyth, "Regulation of Immune Cell Functions through Nectin and Nectin-Like Receptors," in *Encyclopedia of Immunobiology*, Elsevier, 2016, pp. 404–414. doi: 10.1016/B978-0-12-374279-7.02010-5.**

[85] **J. Yeo, M. Ko, D.-H. Lee, Y. Park, and H. Jin, "TIGIT/CD226 Axis Regulates Anti-Tumor Immunity," *Pharmaceuticals*, vol. 14, no. 3, p. 200, Feb. 2021, doi: 10.3390/ph14030200.**

[86] **H. Harjunpää and C. Guillerey, "TIGIT as an emerging immune checkpoint," *Clin Exp Immunol*, vol. 200, no. 2, pp. 108–119, Apr. 2020, doi: 10.1111/cei.13407.**

[87] **B. Sanchez-Correa *et al.*, "DNAM-1 and the TIGIT/PVRIG/TACTILE Axis: Novel Immune Checkpoints for Natural Killer Cell-Based Cancer Immunotherapy," *Cancers (Basel)*, vol. 11, no. 6, p. 877, Jun. 2019, doi: 10.3390/cancers11060877.**

[88] **S. Liu *et al.*, "Recruitment of Grb2 and SHIP1 by the ITT-like motif of TIGIT suppresses granule polarization and cytotoxicity of NK cells," *Cell Death Differ*, vol. 20, no. 3, pp. 456–464, Mar. 2013, doi: 10.1038/cdd.2012.141.**

[89] **R. J. Johnston *et al.*, "The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function.," *Cancer Cell*, vol. 26, no. 6, pp. 923–937, Dec. 2014, doi: 10.1016/j.ccell.2014.10.018.**

[90] **M. Battegay, R. Nüesch, B. Hirschel, and G. R. Kaufmann, "Immunological recovery and antiretroviral therapy in HIV-1 infection," *Lancet Infect Dis*, vol. 6, no. 5, pp. 280–287, May 2006, doi: 10.1016/S1473-3099(06)70463-7.**

[91] **L. Palmisano and S. Vella, "A brief history of antiretroviral therapy of HIV infection: success and challenges.," *Ann Ist Super Sanita*, vol. 47, no. 1, pp. 44–8, 2011, doi: 10.4415/ANN_11_01_10.**

[92] **E. P. Scully *et al.*, "Inconsistent HIV reservoir dynamics and immune responses following anti-PD-1 therapy in cancer patients with HIV infection," *Annals of Oncology*, vol. 29, no. 10, pp. 2141–2142, Oct. 2018, doi: 10.1093/annonc/mdy259.**

[93] **S. Bari *et al.*, "Outcomes of Programmed Cell Death Protein 1 (PD-1) and Programmed Death-Ligand 1(PD-L1) Inhibitor Therapy in HIV Patients with Advanced Cancer," *J Oncol*, vol. 2019, pp. 1–5, Jan. 2019, doi: 10.1155/2019/2989048.**

[94] **M. R. Cook and C. Kim, "Safety and Efficacy of Immune Checkpoint Inhibitor Therapy in Patients With HIV Infection and Advanced-Stage Cancer: A Systematic Review.," *JAMA Oncol*, vol. 5, no. 7, pp. 1049–1054, Jul. 2019, doi: 10.1001/jamaoncol.2018.6737.**

[95] **E. Colston *et al.*, "An open-label, multiple ascending dose study of the anti-CTLA-4 antibody ipilimumab in viremic HIV patients," *PLoS One*, vol. 13, no. 6, p. e0198158, Jun. 2018, doi: 10.1371/journal.pone.0198158.**

[96] J. D. Wolchok *et al.*, "Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma.," *N Engl J Med*, vol. 377, no. 14, pp. 1345–1356, Oct. 2017, doi: 10.1056/NEJMoa1709684.

[97] T. S. Uldrick *et al.*, "Assessment of the Safety of Pembrolizumab in Patients With HIV and Advanced Cancer—A Phase 1 Study," *JAMA Oncol*, vol. 5, no. 9, p. 1332, Sep. 2019, doi: 10.1001/jamaoncol.2019.2244.

[98] C. Gubser, C. Chiu, S. R. Lewin, and T. A. Rasmussen, "Immune checkpoint blockade in HIV," *EBioMedicine*, vol. 76, p. 103840, Feb. 2022, doi: 10.1016/j.ebiom.2022.103840.

[99] K. A. Holder and M. D. Grant, "TIGIT Blockade: A Multipronged Approach to Target the HIV Reservoir.," *Front Cell Infect Microbiol*, vol. 10, p. 175, 2020, doi: 10.3389/fcimb.2020.00175.

[100] Y. Sun and J. Xue, "Expression Profile and Biological Role of Immune Checkpoints in Disease Progression of HIV/SIV Infection," *Viruses*, vol. 14, no. 3, p. 581, Mar. 2022, doi: 10.3390/v14030581.

[101] G. M. Chew *et al.*, "TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection," *PLoS Pathog*, vol. 12, no. 1, p. e1005349, Jan. 2016, doi: 10.1371/journal.ppat.1005349.

[102] R. J. Johnston *et al.*, "The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8 + T Cell Effector Function," *Cancer Cell*, vol. 26, no. 6, pp. 923–937, Dec. 2014, doi: 10.1016/j.ccell.2014.10.018.

[103] J. Tauriainen *et al.*, "Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals," *Sci Rep*, vol. 7, no. 1, p. 40354, Jan. 2017, doi: 10.1038/srep40354.

[104] J. Blazkova *et al.*, "Correlation Between TIGIT Expression on CD8+ T Cells and Higher Cytotoxic Capacity," *J Infect Dis*, vol. 224, no. 9, pp. 1599–1604, Nov. 2021, doi: 10.1093/infdis/jiab155.

[105] N. Stanietsky *et al.*, "The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity," *Proceedings of the National Academy of Sciences*, vol. 106, no. 42, pp. 17858–17863, Oct. 2009, doi: 10.1073/pnas.0903474106.

[106] K. A. Holder, K. Burt, and M. D. Grant, "TIGIT blockade enhances NK cell activity against autologous HIV-1-infected CD4 $^{+}$ T cells," *Clin Transl Immunology*, vol. 10, no. 10, Jan. 2021, doi: 10.1002/cti2.1348.

[107] D. K. Gill *et al.*, "Equivalence of ELISpot Assays Demonstrated between Major HIV Network Laboratories," *PLoS One*, vol. 5, no. 12, p. e14330, Dec. 2010, doi: 10.1371/journal.pone.0014330.

[108] A. C. Karlsson *et al.*, "Comparison of the ELISPOT and cytokine flow cytometry assays for the enumeration of antigen-specific T cells," *J Immunol Methods*, vol. 283, no. 1–2, pp. 141–153, Dec. 2003, doi: 10.1016/j.jim.2003.09.001.

[109] K. R. Demers *et al.*, "Temporal Dynamics of CD8+ T Cell Effector Responses during Primary HIV Infection," *PLoS Pathog*, vol. 12, no. 8, p. e1005805, Aug. 2016, doi: 10.1371/journal.ppat.1005805.

[110] E. R. Roberts, D. G. Carnathan, H. Li, G. M. Shaw, G. Silvestri, and M. R. Betts, "Collapse of Cytolytic Potential in SIV-Specific CD8+ T Cells Following Acute SIV Infection in Rhesus Macaques," *PLoS Pathog*, vol. 12, no. 12, p. e1006135, Dec. 2016, doi: 10.1371/journal.ppat.1006135.

[111] X. Yu *et al.*, "The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells," *Nat Immunol*, vol. 10, no. 1, pp. 48–57, Jan. 2009, doi: 10.1038/ni.1674.

[112] C. L. Ventola, "Cancer Immunotherapy, Part 3: Challenges and Future Trends.," *P T*, vol. 42, no. 8, pp. 514–521, Aug. 2017.

[113] G. M. Chew *et al.*, "TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection," *PLoS Pathog*, vol. 12, no. 1, p. e1005349, Jan. 2016, doi: 10.1371/journal.ppat.1005349.

[114] J. Tauriainen *et al.*, "Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals," *Sci Rep*, vol. 7, no. 1, p. 40354, Jan. 2017, doi: 10.1038/srep40354.

[115] X. Yin *et al.*, "Expression of the Inhibitory Receptor TIGIT Is Up-Regulated Specifically on NK Cells With CD226 Activating Receptor From

HIV-Infected Individuals," *Front Immunol*, vol. 9, Oct. 2018, doi: 10.3389/fimmu.2018.02341.

[116] H. Takata *et al.*, "Delayed differentiation of potent effector CD8 $^{+}$ T cells reducing viremia and reservoir seeding in acute HIV infection," *Sci Transl Med*, vol. 9, no. 377, Feb. 2017, doi: 10.1126/scitranslmed.aag1809.

[117] C. Riou *et al.*, "Differential Impact of Magnitude, Polyfunctional Capacity, and Specificity of HIV-Specific CD8 $^{+}$ T Cell Responses on HIV Set Point," *J Virol*, vol. 88, no. 3, pp. 1819–1824, Feb. 2014, doi: 10.1128/JVI.02968-13.

[118] X. Zhou, S. Yu, D.-M. Zhao, J. T. Harty, V. P. Badovinac, and H.-H. Xue, "Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1.," *Immunity*, vol. 33, no. 2, pp. 229–40, Aug. 2010, doi: 10.1016/j.immuni.2010.08.002.

[119] F. Gounari and K. Khazaie, "TCF-1: a maverick in T cell development and function," *Nat Immunol*, vol. 23, no. 5, pp. 671–678, May 2022, doi: 10.1038/s41590-022-01194-2.

[120] R. L. Rutishauser *et al.*, "TCF-1 regulates HIV-specific CD8+ T cell expansion capacity," *JCI Insight*, vol. 6, no. 3, Feb. 2021, doi: 10.1172/jci.insight.136648.

[121] H. Takata and L. Trautmann, "Transforming dysfunctional CD8+ T cells into natural controller–like CD8+ T cells: can TCF-1 be the magic wand?," *Journal of Clinical Investigation*, vol. 132, no. 11, Jun. 2022, doi: 10.1172/JCI160474.

[122] S. M. Henson and A. N. Akbar, "KLRG1--more than a marker for T cell senescence.," *Age (Dordr)*, vol. 31, no. 4, pp. 285–91, Dec. 2009, doi: 10.1007/s11357-009-9100-9.

[123] N. S. Joshi *et al.*, "Inflammation Directs Memory Precursor and Short-Lived Effector CD8+ T Cell Fates via the Graded Expression of T-bet Transcription Factor," *Immunity*, vol. 27, no. 2, pp. 281–295, Aug. 2007, doi: 10.1016/j.immuni.2007.07.010.

[124] S. Wang, Q. Zhang, H. Hui, K. Agrawal, M. A. Y. Karris, and T. M. Rana, "An atlas of immune cell exhaustion in HIV-infected individuals revealed by single-cell transcriptomics," *Emerg Microbes Infect*, vol. 9, no. 1, pp. 2333–2347, Jan. 2020, doi: 10.1080/22221751.2020.1826361.

[125] F. Sallusto, D. Lenig, R. Förster, M. Lipp, and A. Lanzavecchia, "Two subsets of memory T lymphocytes with distinct homing potentials and effector functions.," *Nature*, vol. 401, no. 6754, pp. 708–12, Oct. 1999, doi: 10.1038/44385.

[126] M. R. Wills *et al.*, "Human virus-specific CD8+ CTL clones revert from CD45ROhigh to CD45RAhigh in vivo: CD45RAhighCD8+ T cells comprise both naive and memory cells.," *J Immunol*, vol. 162, no. 12, pp. 7080–7, Jun. 1999.

[127] L. Yin *et al.*, "Antiretroviral therapy corrects HIV-1–induced expansion of CD8+CD45RA+CD27−CD11abright activated T cells," *Journal of Allergy and Clinical Immunology*, vol. 122, no. 1, pp. 166-172.e2, Jul. 2008, doi: 10.1016/j.jaci.2008.04.029.

[128] M. Rolot and B. G. Dewals, "Unconventional CD45RA+ memory CD8 T cells to control HIV infection during antiretroviral therapy," *Cell Mol Immunol*, vol. 17, no. 9, pp. 897–898, Sep. 2020, doi: 10.1038/s41423-020-0478-8.

[129] D. Wang *et al.*, "A comprehensive profile of TCF1+ progenitor and TCF1− terminally exhausted PD-1+CD8+ T cells in head and neck squamous cell carcinoma: implications for prognosis and immunotherapy," *Int J Oral Sci*, vol. 14, no. 1, p. 8, Dec. 2022, doi: 10.1038/s41368-022-00160-w.

[130] F. J. Beurskens *et al.*, "Exhaustion of cytotoxic effector systems may limit monoclonal antibody-based immunotherapy in cancer patients.," *J Immunol*, vol. 188, no. 7, pp. 3532–41, Apr. 2012, doi: 10.4049/jimmunol.1103693.

[131] S. Daubeuf, M. A. Lindorfer, R. P. Taylor, E. Joly, and D. Hudrisier, "The direction of plasma membrane exchange between lymphocytes and accessory cells by trogocytosis is influenced by the nature of the accessory cell.," *J Immunol*, vol. 184, no. 4, pp. 1897–908, Feb. 2010, doi: 10.4049/jimmunol.0901570.

[132] R. P. Taylor and M. A. Lindorfer, "Fcγ-receptor–mediated trogocytosis impacts mAb-based therapies: historical precedence and recent developments," *Blood*, vol. 125, no. 5, pp. 762–766, Jan. 2015, doi: 10.1182/blood-2014-10-569244.

[133] X. Chen *et al.*, "An Fc-Competent Anti-Human TIGIT Blocking Antibody Ociperlimab (BGB-A1217) Elicits Strong Immune Responses and Potent

Anti-Tumor Efficacy in Pre-Clinical Models," *Front Immunol*, vol. 13, Feb. 2022, doi: 10.3389/fimmu.2022.828319.

[134] E. Vendrame *et al.*, "TIGIT is upregulated by HIV-1 infection and marks a highly functional adaptive and mature subset of natural killer cells," *AIDS*, vol. 34, no. 6, pp. 801–813, May 2020, doi: 10.1097/QAD.0000000000002488.

[135] N. Joller *et al.*, "Cutting Edge: TIGIT Has T Cell-Intrinsic Inhibitory Functions," *The Journal of Immunology*, vol. 186, no. 3, pp. 1338–1342, Feb. 2011, doi: 10.4049/jimmunol.1003081.

[136] R. J. Johnston *et al.*, "The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8 + T Cell Effector Function," *Cancer Cell*, vol. 26, no. 6, pp. 923–937, Dec. 2014, doi: 10.1016/j.ccell.2014.10.018.

[137] S. Ben Mkaddem, M. Benhamou, and R. C. Monteiro, "Understanding Fc Receptor Involvement in Inflammatory Diseases: From Mechanisms to New Therapeutic Tools," *Front Immunol*, vol. 10, Apr. 2019, doi: 10.3389/fimmu.2019.00811.

[138] M. Paccagnella, A. Abbona, N. Denaro, M. Merlano, and O. Garrone, "Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) in Cancer," in *Handbook of Cancer and Immunology*, Cham: Springer International Publishing, 2023, pp. 1–21. doi: 10.1007/978-3-030-80962-1_11-1.

[139] E. M. B. Scholz and A. D. M. Kashuba, "The Lymph Node Reservoir: Physiology, HIV Infection, and Antiretroviral Therapy," *Clin Pharmacol Ther*, vol. 109, no. 4, pp. 918–927, Apr. 2021, doi: 10.1002/cpt.2186.

[140] D. Niebel *et al.*, "DNA methylation regulates TIGIT expression within the melanoma microenvironment, is prognostic for overall survival, and predicts progression-free survival in patients treated with anti-PD-1 immunotherapy," *Clin Epigenetics*, vol. 14, no. 1, p. 50, Dec. 2022, doi: 10.1186/s13148-022-01270-2.

[141] S. Marwitz, S. Scheufele, S. Perner, M. Reck, O. Ammerpohl, and T. Goldmann, "Epigenetic modifications of the immune-checkpoint genes CTLA4 and PDCD1 in non-small cell lung cancer results in increased expression," *Clin Epigenetics*, vol. 9, no. 1, p. 51, Dec. 2017, doi: 10.1186/s13148-017-0354-2.

[142] K. E. Pauken *et al.*, "Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade," *Science (1979)*, vol. 354, no. 6316, pp. 1160–1165, Dec. 2016, doi: 10.1126/science.aaf2807.

[143] A. L. Hung *et al.*, "TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM," *Oncoimmunology*, p. e1466769, May 2018, doi: 10.1080/2162402X.2018.1466769.

[144] J. Duraiswamy, K. M. Kaluza, G. J. Freeman, and G. Coukos, "Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors.," *Cancer Res*, vol. 73, no. 12, pp. 3591–603, Jun. 2013, doi: 10.1158/0008-5472.CAN-12-4100.

[145] F. Hatefi *et al.*, "Combined Blockade Of PD-1 and TIGIT is not Sufficient to Improve the Function Of CD8+ T-Cells in Chronic Lymphocytic Leukemia.," *Asian Pac J Cancer Prev*, vol. 23, no. 7, pp. 2225–2231, Jul. 2022, doi: 10.31557/APJCP.2022.23.7.2225.

[146] M. N. Wykes and S. R. Lewin, "Immune checkpoint blockade in infectious diseases," *Nat Rev Immunol*, vol. 18, no. 2, pp. 91–104, Feb. 2018, doi: 10.1038/nri.2017.112.

[147] T. A. Rasmussen, J. L. Anderson, F. Wightman, and S. R. Lewin, "Cancer therapies in HIV cure research," *Curr Opin HIV AIDS*, vol. 12, no. 1, pp. 96–104, Jan. 2017, doi: 10.1097/COH.0000000000000328.

[148] N. Patsoukis *et al.*, "PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation," *Nat Commun*, vol. 6, no. 1, p. 6692, Mar. 2015, doi: 10.1038/ncomms7692.

[149] M. M. Kamiński *et al.*, "T cell Activation Is Driven by an ADP-Dependent Glucokinase Linking Enhanced Glycolysis with Mitochondrial Reactive Oxygen Species Generation," *Cell Rep*, vol. 2, no. 5, pp. 1300–1315, Nov. 2012, doi: 10.1016/j.celrep.2012.10.009.

[150] D. O'Sullivan *et al.*, "Memory CD8+ T Cells Use Cell-Intrinsic Lipolysis to Support the Metabolic Programming Necessary for Development," *Immunity*, vol. 41, no. 1, pp. 75–88, Jul. 2014, doi: 10.1016/j.immuni.2014.06.005.

[151] A. J. Marko, R. A. Miller, A. Kelman, and K. A. Frauwirth, "Induction of Glucose Metabolism in Stimulated T Lymphocytes Is Regulated by Mitogen-Activated Protein Kinase Signaling," *PLoS One*, vol. 5, no. 11, p. e15425, Nov. 2010, doi: 10.1371/journal.pone.0015425.

6. Appendix

6.1: Health Research Ethics Approval

From: administrator@hrea.ca <administrator@hrea.ca>
Sent: Monday, November 6, 2023 8:47 PM
To: Grant, Michael <mgrant@mun.ca>
Cc: administrator@hrea.ca
Subject: HREB - Approval of Ethics Renewal 20180836

Researcher Portal File #: 20180836

Dear Dr. Michael Grant:

This e-mail serves as notification that your ethics renewal for study HREB # 2017.220 – The influence of cytomegalovirus infection on maturation and aging of the immune system in HIV-infected individuals – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from 23 Nov 2023 to 23 Nov 2024.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an "Event".

The ethics renewal will be reported to the Health Research Ethics Board at their meeting dated 16 Nov 2023.

Thank you,

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