Immunometabolism in chronic HIV-1 infection

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All the experiments for this study have been carried out at the Institute for HIV Research, University Hospital Essen, at the University of Duisburg-Essen or at another equivalent facility.

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Die Experimente der vorliegenden Arbeit wurden am Institut für HIV Forschung, der Universität Duisburg-Essen oder an einer anderen gleichwertigen Einrichtung durchgeführt.

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Some parts of this thesis have been published in JCI Insight article: Effect of HIV infection and antiretroviral therapy on immune cellular function (Korencak et al. 2019; PMID: 31217351). These parts are cited throughout the thesis (e.g. in figure legend).

Detailed contribution of other colleagues to this thesis might be found on page 88.

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1. INTRODUCTION

1.1. HIV

In 1981, cases of *Pneumocystis carinii pneumonia* were documented in five gay men in Los Angeles (CDC 1981). At the same time, there were reports of men in New York and California with Kaposi´s Sarcoma (Hymes et al. 1981). In 1982, the symptom complex was named AIDS (acquired immune deficiency syndrome)(CDC 1982). Later, in 1984, the cause of AIDS was identified – the human T-lymphotropic virus, type 3 (HTLV-III), today known as HIV (Marx 1984).

Since the virus has spread around the globe, it caused the death of more than 35 million people. Currently, there are more than 37 million people living with HIV and the yearly rate of mortality is 1 million (WHO 2019). The life expectancy of individuals infected with HIV increased rapidly from approximately 6-10 years to a normal life span of a healthy person due to introduction and refinement of antiretroviral therapy (ART) (May et al. 2014).

In 2015, the United Nation Program on HIV/AIDS (UNAIDS) presented a strategy to end AIDS epidemic and decrease the number of new infections, being that, by year 2020, 90% of all people living with HIV will know their HIV status, 90% of all people diagnosed with HIV will receive suitable ART, and 90% of all people on ART treatment will have complete viral suppression (UNAIDS 2015). Despite this ambitious plan, there is still a considerate percentage of people, mostly in developing countries, who have no access to treatment. Figure I shows prevalence of HIV in the world.

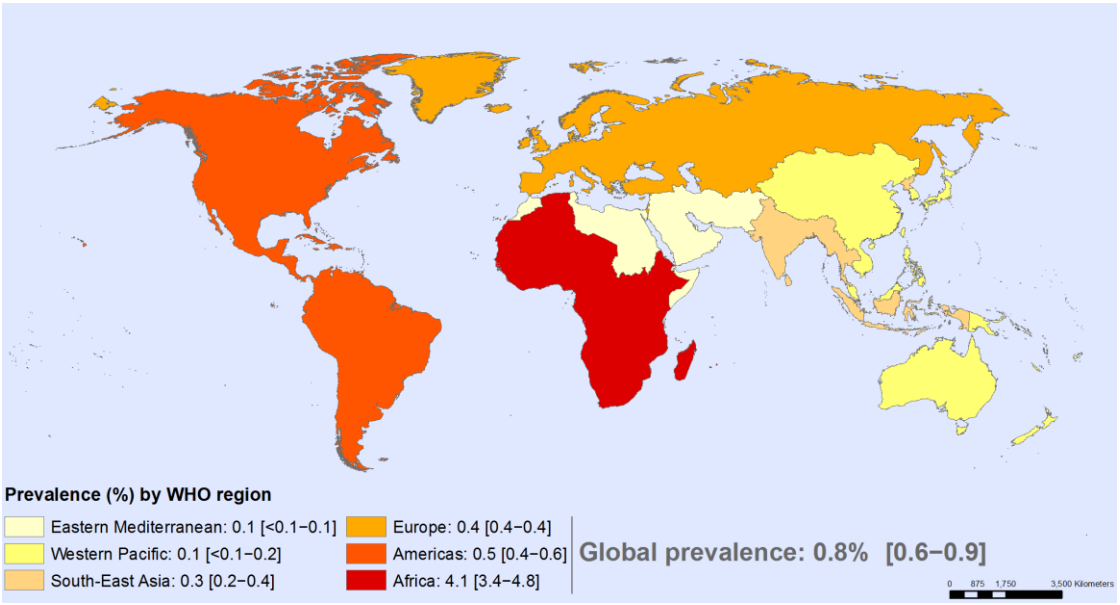

Figure I: Prevalence of HIV

Prevalence of HIV among adults aged 15 to 49 from 2017 by WHO regions (WHO 2017)

1.1.1. HIV-1 structure and replication strategy

HIV-1 belongs to the family of *Retroviridae*. All viruses from this family have very similar structure **(Figure II)**. The virus is enveloped by a lipid membrane, which is derived from the host cell (Jacobo-Molina et al. 1993). On the surface are glycoproteins (gp120). These are connected to the virus through interactions with transmembrane protein (gp41). HIV's matrix is composed of a protein p17, which covers the inner part of viral membrane. HIV-1 capsid is normally an asymmetric cone formed by capsid proteins (p24) and encapsulates two copies of viral genome, which are stabilized by ribonucleoprotein complex. It also contains three essential viral enzymes encoded by the virus: protease, reverse transcriptase, and integrase (Turner and Summers 1999; Chen 2016; Watts et al. 2009; Becerra, Bildstein, and Gach 2016).

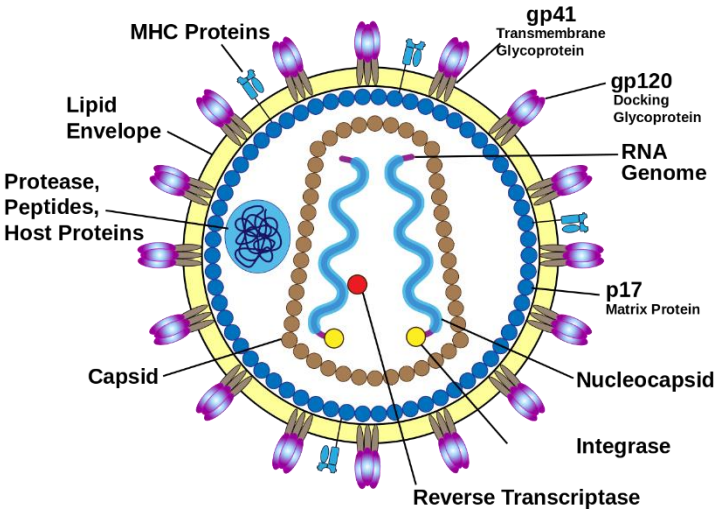

Figure II: Schematic structure of HIV-1

Schematic structure of HIV-1 with all structures, enzymes and proteins (Tomé 2013)

HIV-1 binds to the cells through cellular CD4 receptor **(Figure III)**. Binding occurs via interaction between viral gp120 and cellular CD4 proteins. However, to enter the cell and start the replication process, the virus requires additional cell-protein to promote the fusion of cellular and viral membranes, including CXCR4 and CCR5 (Clapham and Weiss 1997; Doranz et al. 1996; Moore 1997). After membrane fusion and uncoating, reverse transcription is carried out by reverse transcriptase. After successful transcription, the transcript – viral DNA – is transported into the nucleus where it is integrated into host genome by using the viral enzyme - integrase (Telesnitsky and Goff 1997; Freed, Englund, and Martin 1995). Next, the integrated DNA is transcribed into RNA, some of which undergo RNA splicing to make mature messenger RNAs (mRNAs). mRNAs are transported out of nucleus where they are translated into regulatory proteins Tat and Rev. Rev migrates into nucleus, binds to full unspliced RNAs and allows them to leave nucleus (Pollard and Malim 1998). Some of these full length RNAs are used as a new copies of the virus genome while other are translated into structural genes Gag and Env. Afterwards, Gag binds to HIV RNA, they move to the surface of the cell and assemble into a noninfectious immature virion. Virions are pushed out of the cells by a process called budding. Protease enzyme breaks up the polyprotein and allows the virus to assemble to its mature infectious form (Emerman and Malim 1998; Wiegers et al. 1998).

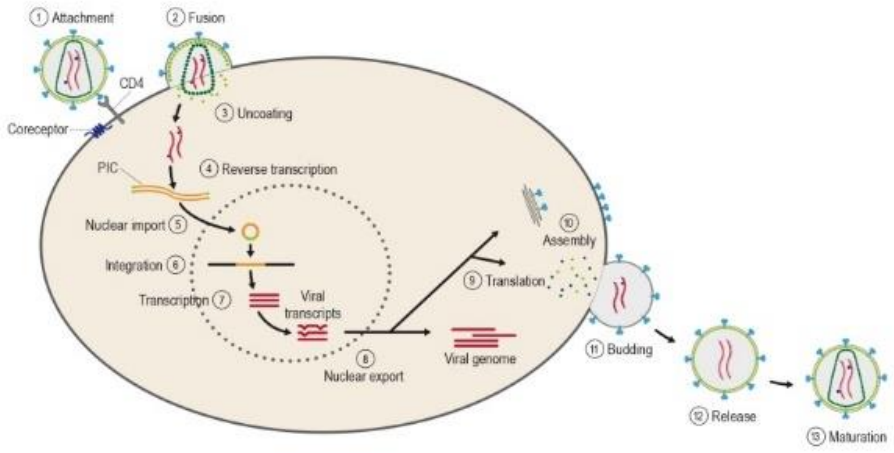

Figure III: Individual steps of HIV-1 replication

Schematic description of HIV-1 replication process with individual phases (Becerra, Bildstein, and Gach 2016)

1.1.2. Transmission of HIV-1

There are several mechanisms by which a human can be infected with HIV-1. Most commonly through unprotected sexual (anal or vaginal) intercourse with an HIV-infected, viremic individual. Second-most common, by sharing injection needles with an HIV-infected person, and thirdly via transmission occurring from HIV-infected mothers to the newborn during the labor or breastfeeding. Other ways of transmission, like via blood transfusion are extremely rare and occur only in countries where blood is not routinely screened for HIV (Bartholomew and Jones 2006). The particular mechanisms of transmission can vary in frequency among different countries. In 2014, 40% of new HIV-1 diagnoses occurred in men who have sex with men (MSM) in Western Europe but only

28% in Central Europe. For people who inject drugs (PWID) it was 5% in Central Europe and 3% in Western Europe (Becerra, Bildstein, and Gach 2016; Baggaley et al. 2013).

1.1.3. Acute HIV-1 infection

Acute HIV-1 infection refers to a transient symptomatic illness showing uncontrolled viremia and widespread destruction of immune cells following HIV acquisition (Kahn and Walker 1998). It is characterized by sequential appearance of viral markers and antibodies in the blood (Fiebig et al. 2003). Systematic progress of HIV-1 infection is shown in **Figure IV**.

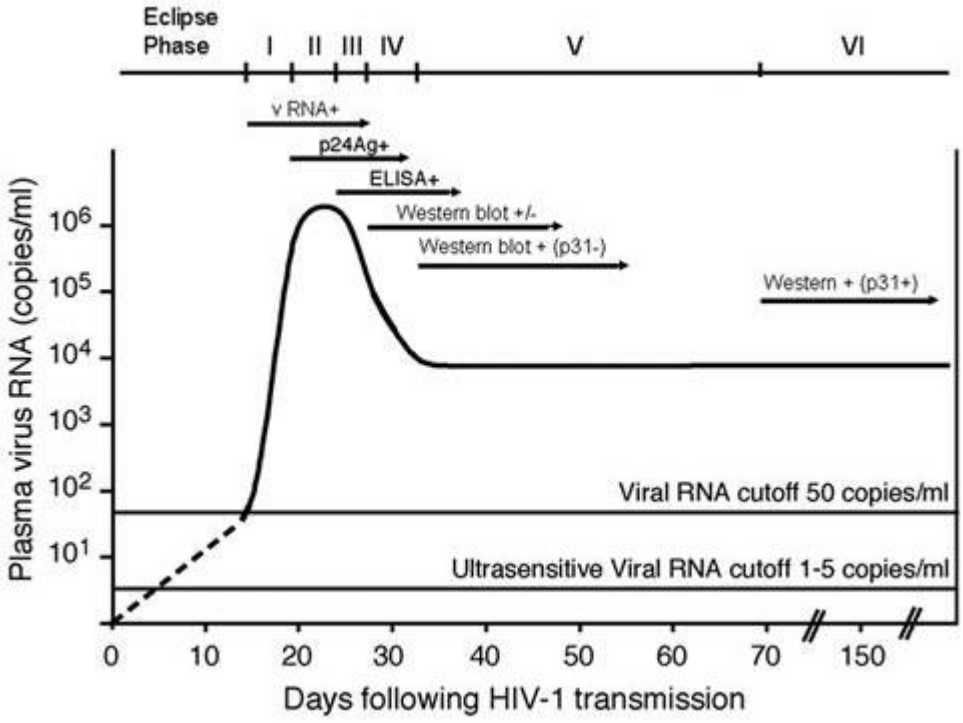

Figure IV: Fiebig stages of acute HIV-1 infection (Fiebig et al. 2003)

Few days after transmission, there is an initial eclipse phase, in which HIV-1 infection is established in local tissues at the exposure site. There is no spread of the virus into the circulation at detectable levels at this time point. After dissemination of HIV-1 into lymphoid tissue and systemic circulation, HIV-1 replication reaches peak levels (Li et al.

2009; Fiebig et al. 2003). During the ramp-up phase of acute HIV-1 infection, the infected individuals develop an intense inflammatory response characterized by high levels of cytokines and chemokines characterized as a cytokine storm (Stacey et al. 2009). Acute HIV-1 infection is often associated with an acute retroviral syndrome, which can include fever, rash, night sweats, severe fatigue, headache, diarrhea, and other non-specific clinical and laboratory abnormalities (Cooper et al. 1985). The onset of symptoms usually occurs 2 weeks after HIV-1 acquisition together with the peak viremia (Stacey et al. 2009; Lindback et al. 2000). Acute HIV-1 infection is characterized by positive nucleic acid testing and a non-reactive or indeterminate Western Blot (Ananworanich et al. 2008). The 2nd generation enzyme immunoassay (EIA) detects IgG to HIV and was until recently used as a screening standard in HIV diagnostic. However, the time between infection and reactivity (window period) is 25-35 days (Branson and Stekler 2012). The 4th generation antigen antibody combination EIA, which detects p24 antigen, HIV IgM and IgG is shortening the window period and is now replacing the 2nd generation EIA (Robb et al. 2016).

Several studies have proven that the role of HIV-1-specific cellular immune responses are crucial for the initial control of viremia. Appearance of HIV-1-specific CD8 T cells has been temporally associated with the initial decline of viremia (Koup et al. 1994; Borrow et al. 1994). These CD8 T cells have the ability to kill HIV-infected cells (Yang et al. 1997) and furthermore, it has also been demonstrated that strong early CD8 T cell responses during primary HIV-1 infection are associated with lower viral set point and can determine long-term disease outcome (Streeck et al. 2009; Streeck et al. 2014). Acute HIV-1 infection results in massive CD4 T cell depletion in all body compartments which often occurs together with clinical symptoms (Streeck and Nixon 2010). Furthermore, not only decline in CD4 T cell count but also impairment of CD4 T cell functions is a characteristic for acute HIV-1 infection. Impairment of CD4 T cell helper function subsequently results in the functional impairment of HIV-1-specific CD8 T cells (Lichterfeld et al. 2004). Because the CD4 T cells are the main target of HIV-1, their role in acute HIV-1 infection has not been the primary focus of the scientific community, but rather studies focus more on HIV-1-specific CD8 T cell responses (Streeck et al. 2014). More recently, there are studies showing that HIV-1-specific CD4 T cell responses also

play an important role in controlling viral replication in HIV-1 infection and may predict disease outcome (Harari et al. 2004; Pitcher et al. 1999; Soghoian and Streeck 2010).

1.1.4. Chronic HIV-1 infection

The face of chronic HIV-1 infection has significantly changed over last years due to ART. Before introducing ART, HIV-1-infected individuals developed AIDS and the life expectancy was approximately 10 years. Nowadays people infected with HIV-1 can live for decades as observed in healthy population (Nakagawa, May, and Phillips 2013). ART results in full control of viremia and partial restoration of the immune system, which prevents development of AIDS. However, ART does not fully restore the health of an HIV-infected individual. People who are on ART therapy might develop new health problems referred as “non-AIDS morbidity”, including cardiovascular disease, kidney disease, cancer, liver disease, or neurocognitive disease (Deeks, Lewin, and Havlir 2013; Freiberg et al. 2013). Direct toxicity of antiretroviral medicaments, together with other lifestyle factors such as smoking or alcohol use, can contribute to these complications, although each new generation of antiretroviral drugs has been associated with less toxicity (Scherzer et al. 2012; Ryom et al. 2013).

1.1.5. Antiretroviral treatment

To date, several classes of antiretroviral medication with distinct mechanisms have been discovered. Common ART regimens use a combination of three antiretroviral agents against at least two distinct molecular targets. A standard “treatment backbone” consist of two nucleoside reverse transcriptase inhibitors in combination with one drug from other class (**Figure V**) (Arts and Hazuda 2012).

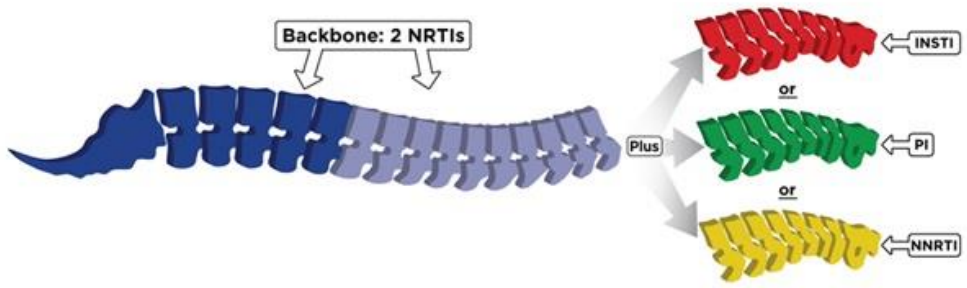

Figure V: HIV treatment backbone (HHS 2016)

There are over 30 individual or combined substances, which are approved and being used for HIV-1 treatment. According to their mode of action, they can be divided into 5 different drug classes:

1. Nucleoside reverse transcriptase inhibitors (NRTIs)
2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
3. Protease inhibitors (PIs)
4. Integrase inhibitors
5. Entry inhibitors

1.1.5.1. NRTIs

NRTIs were the first class of drugs approved by the US Food and Drug Administration (FDA) (Young 1988).The target of NRTIs is the reverse transcriptase. They act as alternative substrates and compete with physiological nucleosides. These analog nucleosides have only a minor modification in the ribose molecule. DNA synthesis is aborted after incorporation of nucleoside analogs because phosphodiester bridges are not formed to stabilize the double strand DNA. NRTIs are administrated as prodrugs, which require host cell entry and phosphorylation by cellular kinases. (Hart et al. 1992; Mitsuya et al. 1985; Nakashima et al. 1986; Furman et al. 1986). The first nuclease analogue introduced on the market in 1987 was zidovudine (AZT). The use of NRTIs is associated with many long-term side effects including lactate acidosis, myelotoxicity, polyneuropathy, or pancreatitis but also with metabolic disorders, in particular lipoatrophy (Galli et al. 2002; Mallal et al. 2002). Negative effects were also observed in mitochondria, which require nucleosides for proper function. Mitochondria's metabolism is disrupted by incorporating nucleoside analogues due to high affinity to mitochondrial polymerase gamma, which leads to mitochondrial degeneration. It is also possible that besides mitochondrial damage, other mechanisms contribute to the toxicity (Gerschenson and Brinkman 2004; Lewis 2003; Kohler and Lewis 2007).

Currently, there are nine FDA-approved NRTIs: abacavir (ABC), didanosine (ddI), lamivudine (3TC), stavudine (d4T), zalcitabine (ddC), zidovudine (AZT), tenofovir disoproxil fumarate (TDF), emtricitabine (FTC) and tenofovir alafenamide (TAF). As with all antiretroviral therapies, treatment with any of these might result in an HIV-strain with decreased drug susceptibility. HIV resistance to NRTIs can be achieved by two mechanisms - discrimination or extinction pathway. In discrimination pathway, the resistant mutations cause changes in amino acids in the primary structure of reverse transcriptase, which results in increased selective-ability of the enzyme to incorporate the correct and natural nucleosides instead of NRTIs. In the extinction pathway, the primary structure of the reverse transcriptase is changed in a way, which facilitates the removal of chain terminators NRTI-triphosphate from the 3´ end of the DNA chain after it has been incorporated (Meyer et al. 1999; Meyer et al. 2002; Marcelin et al. 2004; Yahi et al. 2005; Boyer et al. 2002).

1.1.5.2. NNRTIs

NNRTIs were first described in 1990. They inhibit the reverse transcription by directly binding to the enzyme and restraining its function. NNRTIs are small hydrophobic chemical compounds that bind directly and non-competitively to a hydrophobic pocket near to, but not overlapping, the active site of the reverse transcriptase. The binding results in a change in structural conformation of residues, which are important for an optimal ability of the enzyme to catalyze DNA synthesis (Hsiou et al. 1996; Jacobo-Molina et al. 1993; Rodgers et al. 1995; Sluis-Cremer, Temiz, and Bahar 2004; Spence et al. 1995).

To date, there are five NNRTIs available. The first generation includes nevirapine (NVP), delavirdine (DLV), and efavirenz (EFV) which were introduced to the market in 1996 (D'Aquila et al. 1996). Second generation represents etravirine (ETV), which was approved for HIV-1 treatment in 2008. The last drug from this class, rilpivirine (RLP), was approved in 2011. NNRTI resistance generally results from amino acid substitution in the NNRTI-binding pocket (Tantillo et al. 1994). Resistance appears fast due to the long half-life of NNRTI and low level of resistance barrier. Once it occurs, it almost

always generates additional cross-resistance to other NNRTIs (Cozzi-Lepri et al. 2012). Despite observed resistance, both randomized and large cohort studies showed very good efficacy of NNRTI when they are combined with nucleoside analogues (Dejesus et al. 2011; Soriano et al. 2011; Daar et al. 2011).

1.1.5.3. PIs

PIs are substrate analogs for the HIV-1 enzyme protease. Protease cuts the viral gag-pol polyprotein into functional subunits. After blocking the enzyme with PIs, polyprotein cannot be cut and non-infectious virus is produced. PIs are small chemical compounds that mimic the natural gag-pol peptide substrate for protease but they do not allow the enzyme to cut it due to a chemical modification. Thanks to this, the active site of the enzyme is occupied by the PIs which block access to the natural substrate – gag-pol polyprotein (Cameron et al. 1998; Youle 2007).

There are several PIs approved for HIV-1 treatment: amprenavir (APV), atazanavir (ATV), darunavir (DRV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir, and tipranavir (TPV). All PIs must be boosted with pharmaco-enhancers to achieve sufficient plasma concentration. Currently two drugs are used as boosters – RTV or cobistat. RTV is a potent inhibitor of subunit of cytochrome hepatic enzyme. Inhibition of this enzyme increases the plasma concentration of all PIs (Kempf et al. 1997). If mutation occurs, it affects the binding of the drug to the active site of the enzyme by changing the association and dissociation ability and the protease become available for its natural substrate (van Maarseveen and Boucher 2006).

1.1.5.4. Integrase inhibitors

Integrase inhibitors represent the most recently discovered class of HIV-1 medicaments. They inhibit HIV-1 enzyme integrase, which is responsible for integrating viral DNA into host genome. The process of integration has multiple steps. First, the integrase binds to viral DNA in the cytoplasm forming so called pre-integration complex. Afterwards, the pre-integration complex is processed by removing dinucleotide at each end of DNA and

producing new 3´ hydroxyl ends. Third step starts after the pre-integration complex reaches nucleus through nuclear pore. Integrase binds to the host chromosomal DNA and mediates irreversible binding of viral and cellular DNA. This step can be inhibited by integrase strand transfer inhibitors (INSTIs), which represent all approved drugs from integrase inhibitors class. The last step is a repair of gaps by cellular DNA repair enzymes in DNA sequence, which might happened during the integration process (Hazuda et al. 2004; Lataillade and Kozal 2006).

First approved INSTI was raltegravir (RAL), then dolutegravir (DLG) and quinolone derived INSTI elvitegravir (EVG). Last year, 2018, a new INSTI was approved for HIV-1 treatment – bictegravir (BIC) (Sato et al. 2006; Deeks 2018). The drugs are generally well tolerated, however, the long-term cytotoxicity is not known (Eron et al. 2013). Genetic mutation in the viral integrase gene may results into a drug resistant integrase, which can catalyze integration of viral DNA. There is no evidence of cross-resistance within the INSTIs (Wirden et al. 2009).

1.1.5.5. Entry inhibitors

Entry inhibitors interfere with the receptor-mediated entry of the virus into the host cell. The entry process has more phases and according to these, we can subdivide entry inhibitors into two classes: co-receptor antagonists and fusion inhibitors. To successfully enter the host cell, HIV-1 needs a so-called co-receptor as well as the CD4 receptor. Two of the most important co-receptors are CCR5 and CXCR4 (Alkhatib et al. 1996; Doranz et al. 1996). Currently, there is one CCR5 antagonist approved for HIV-1 treatment: maraviroc (MVC). It binds allosterically to CCR5, induces conformational changes, which result in inhibition of binding to viral gp120 (Dittmar et al. 1997; Chan and Kim 1998). A second class of entry inhibitors are fusion inhibitors. An FDA-approved fusion inhibitor is enfuvirtide. This drug is a peptide chain that mimics the HR2 structure of gp141 which binds to HR1 region and facilities the fusion of viral envelope and cell membrane. Binding of the drug into HR1 region prevents binding of HR2 and blocks the fusion (Kilby et al. 2002).

1.2. Immune system

Immune system consists of many different cells and molecules, which defend the body against bacteria, viruses, parasites, and other immunogens. The immune system is spread throughout the whole body and involves many cells, tissues and organs. It can recognize foreign or damaged cells and clear them through numerous mechanisms (Murphy 2012).

1.2.1. Innate immune system

The innate immune system is evolutionarily the oldest defense mechanism present in all multicellular organisms. The first defense against pathogens are physical barriers like skin or gut mucosa and chemical barriers such as antimicrobial proteins secreted on the mucosal surfaces. If a pathogen overcomes these barriers, mechanisms of the innate immune system will try to eliminate it. One part of innate immune system is the complement system, which can directly recognize and destroy foreign organisms utilizing phagocytic white blood cells like macrophages and neutrophils. These can ingest and kill pathogens by secreting toxic enzymes. Innate immune responses occur respectively very fast, right after exposure to foreign antigen, in contrast to adaptive immunity, where immune responses can develop after several days (Beutler 2004; Murphy 2012). The innate immune system has variety of cells to use in a fight against foreign antigen. These cells are neutrophils, eosinophils, natural killer (NK) cells and a group of antigen presenting cells (APCs) – macrophages and dendritic cells. These cells contribute to the clearance of antigen through the presentation of pattern recognition receptors to the pathogen-associated molecular patterns (PAMPs). PAMPs are microbial products, usually essential proteins presented on the surface of the pathogen. These are recognized by the pattern recognition receptors on the innate immune cells surface. One large class of pattern-recognition receptors are toll-like receptors (TLRs). There are 10 different TLRs in human and they are responsible for recognizing molecules mostly on microbial surface. Schematic representation of human TLRs is shown on **Figure VI**.

After recognition, inflammatory cytokines, chemokines, or co-stimulatory molecules can be expressed (Tang et al. 2012).

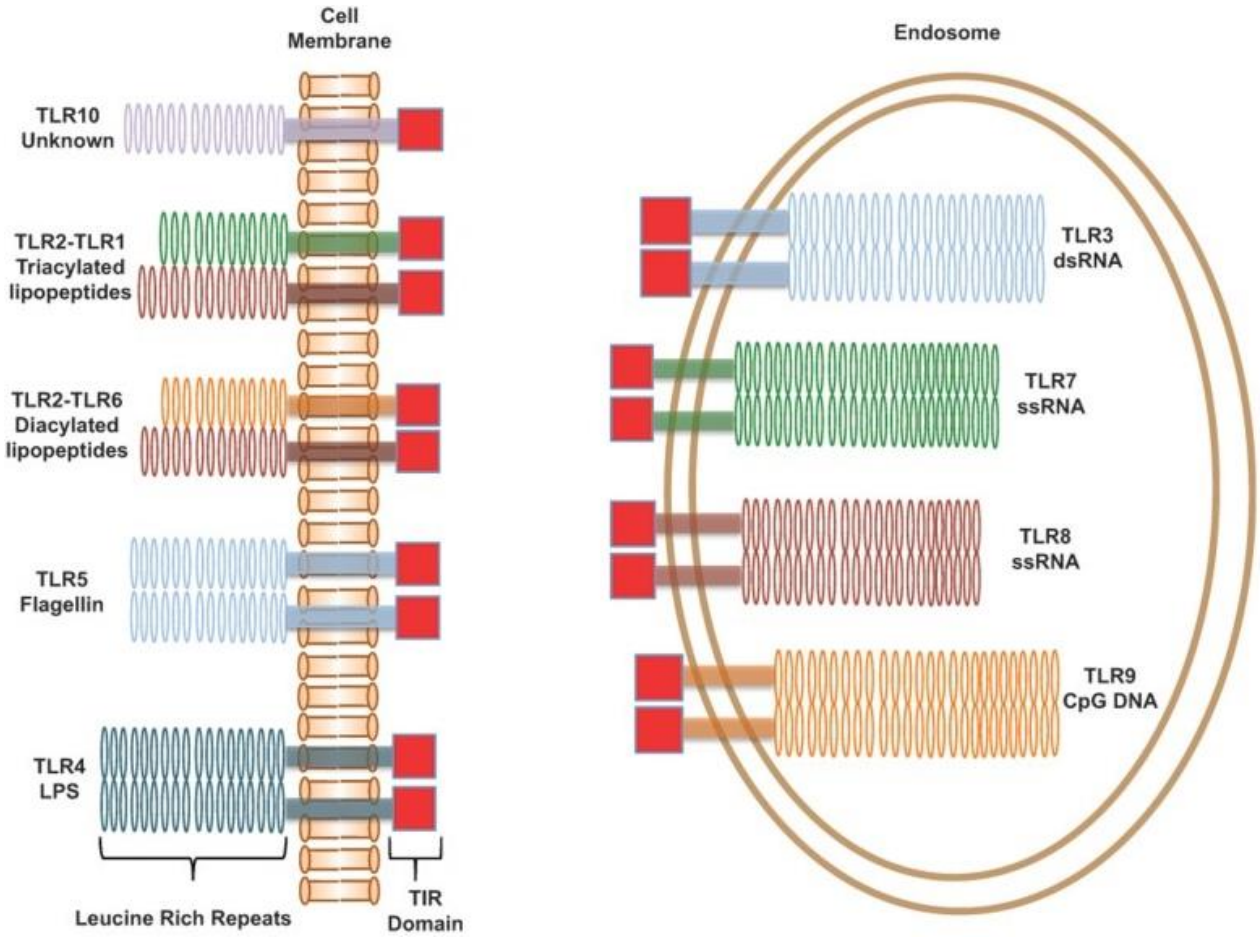

Figure VI: Schematic representation of human toll-like receptors, their localization, structure and ligands (Godfroy et al. 2012)

NK cells

NK cells develop in the bone marrow from the same progenitor as T lymphocytes and B lymphocytes. They are larger than T and B cells, have distinctive cytoplasmic granules containing cytotoxic proteins, and are functionally identified by their ability to kill certain tumor cell lines *in vitro* without the need of specific immunization (Lanier 2008; Murphy

2012). NK cells kill cells by releasing cytotoxic granules containing cytotoxic granzymes and the pore-forming protein – perforin. They are released onto the surface of a target cell, which the NK cell recognized and bound via cell surface receptors and their contents penetrate to the cell membrane and induce programmed cell death. However, unlike T cells, killing by NK cells is triggered by germline-encoded receptors that recognize molecules on the surface of infected or malignantly transformed cells (Argentati et al. 2000).

NK cells can be activated in response to interferons or cytokines. Activated NK cells serve to contain virus infections while the adaptive immune response is generating antigen specific cytotoxic T cells and neutralizing antibodies that can clear the infection. If NK cells are defend to body against viral infections and other pathogens, they must have a mechanism to distinguish infected cells from uninfected healthy cells. This is achieved by a combination of direct recognition of changes in cell surface glycoprotein composition, which is induced by metabolic stress such as malignant transformation or viral and bacterial infection, together with a recognition of altered self, which involves changes in the expression of major histocompatibility complex (MHC) molecules. NK cells are able to sense changes in the expression of MHC class I molecules by integrating the signals from two types of surface receptors, which together control the NK cell´s cytotoxic activity and cytokine production (Borrego et al. 2002; Boyington and Sun 2002). Activating receptors trigger the NK cell to kill its target. Several classes of activating receptors are expressed by NK cells, including immunoglobulin-like and C-type lectin families. Their stimulation causes release of cytokines such as INF-γ and release of cytotoxic granules (Brown et al. 2001). Second set of receptors, called inhibitory receptors, act to prevent NK cells from killing a normal host cell (Trowsdale 2001).

1.2.2. Adaptive immune system

The adaptive immune system is more capable of eliminating pathogens compared to innate immune system because of the specific recognition function of lymphocytes. The adaptive immune system consists of T and B lymphocytes, which can recognize and

respond to individual antigens by means of antigen receptors on the surface of lymphocytes. All lymphocytes in the human body create collectively a massive collection of different antigen receptors, which gives the adaptive immune system an opportunity to respond to an almost endless variation of foreign antigen. After overcoming an infection, activated lymphocytes and antibodies produced by B cells might persist to prevent immediate re-infection. They also provide long-lasting immunity which will later result in much faster immune response, when encountering the same pathogen (Murphy 2012).

T lymphocytes

Progenitor T cells are produced in the bone marrow after which they are transported into the thymus where they undergo final differentiation with fully developed TCR receptors along with either CD4 or CD8 receptor. In the first steps of differentiation, double positive CD4+ CD8+ cells are produced (Carpenter and Bosselut 2010). Double positive T cells with TCR receptor, which do not interact with self-MHC complexes undergo apoptosis. This process is called negative selection. Negative selection also occurs if the antigen binding avidity is too strong. This mechanism prevents mature T cells reacting to self-antigen. Positive selection and survival occurs only if antigen binding is moderate avidity. Along with positive and negative selection, double positive thymocytes differentiate into single positive CD4+ or CD8+ cells and exit the thymus as recent thymic emigrants into the blood and lymphoid tissues. (Germain 2002).

CD4 T cells

CD4 T cells are an important part of adaptive immune system. They help to coordinate, suppress or regulate immune responses and therefore they are also called T helper cells. CD4 T cells express different cytokines and according to the cytokine expression we can divide them into different subsets: Th1, Th2, Th9, Th17, Th22, Treg (regulatory) and Tfh (follicular helper) (Raphael et al. 2015; Zhu and Paul 2010). Different cytokine expression profile of each subset is shown on **Figure VII.**

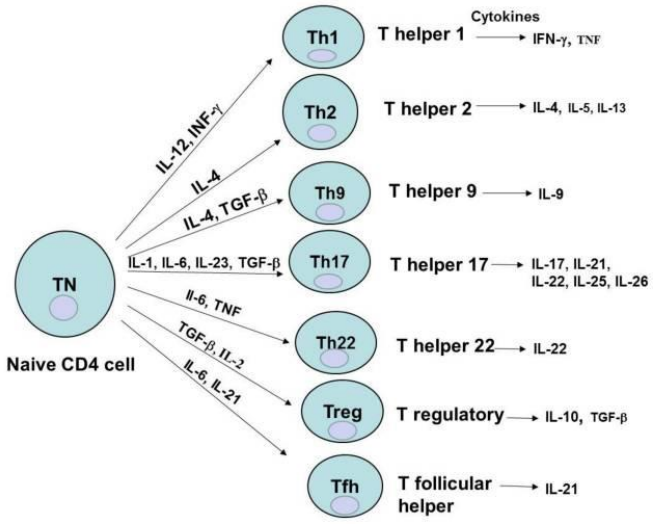

Figure VII: CD4 T cell subsets (Golubovskaya and Wu 2016)

After APCs presents an antigen to naïve T cells, T cells become activated, proliferate, and differentiate into effector cells. T cells recognize foreign antigens only when they are displayed on body´s own cells. APCs display on their surface peptide fragments of pathogens proteins. These foreign peptides are delivered to the cell surface by specialized host-cell glycoproteins – the MHC molecules. There are two classes of MHC molecules, both different in their structure and expression pattern in the tissues. MHC class I molecules present peptides from pathogens, commonly viruses, to CD8 cytotoxic T cells, which are specialized to kill any cell they specifically recognize. Because viruses can infect any nucleated cell, almost all of these cells express MHC class I molecules, although the level of expression varies from one cell type to another. In contrast, the main function of CD4 T cells that recognize MHC class II molecules is to activate effector cells of the immune system. Thus, MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages. These effector cells then migrate to the infection site and can help destroy the pathogen. While the effector cells are relatively short-lived, memory cells live much longer and are part of the immune memory. They are located in secondary lymphoid organs (central memory T cells, T_{CM}) or in the recently infected tissue (effector memory T cells, T_{EM}). When the organism encounters the same pathogen again, the memory T cells proliferate and immune

response occurs within a relatively short time (Golubovskaya and Wu 2016; Rosenblum, Way, and Abbas 2016).

CD8 T cells

CD8 T cells are also called cytotoxic CD8 T cells due to their role in immune defense against intracellular pathogens like viruses or bacteria, and for tumor surveillance. Just like CD4 T cells, they are generated in thymus and together with TCR receptor they express CD8 receptor which consists form α and β chain (Terry et al. 1990; Golubovskaya and Wu 2016). They can be divided into subsets according to their cytokine expression profile. These include Tc1, Tc2 and Tc17. Tc1 CD8 T cells are very similar to Th1 CD4 T cells. They also produce TNFα, IFNγ and IL-2 (Tajima et al. 2011). The differentiation from naïve to cytotoxic CD8 T cells requires co-stimulation from APCs and peptide presentation via MHC Class I. After CD8 T cell recognizes its antigen and becomes activated, it has 3 possible mechanisms for how to kill the infected or malignant cell. First is the secretion of cytokines, in particular TNFα and IFNγ cytokines, which have strong anti-tumor and anti-microbial effects. The second way the CD8 T cells deal with infected cells is the production and release of cytotoxic granules. These granules, also found in NK cells, contains two families of proteins – perforin and granzymes. Perforin forms a pore in the membrane of target cells, similar to the membrane attack complex of complement. This pore allows granzymes to enter the infected or malignant cell. Granzymes are serine proteases which cleave the proteins inside the cell, shutting down the production of viral proteins and ultimately resulting in apoptosis of the cell. The cytotoxic granules are released only in the direction of the target cell, aligned along the immune synapse, to avoid non-specific bystander damage to healthy surrounding tissue. CD8 T cells are able to release their granules, kill an infected cell, move to a new target and kill again. This is often referred as serial killing (Boyman et al. 2012; Janeway CA Jr 2001). Finally, the last mechanism of how CD8 T cells destroy infected cells is through the Fas/FasL interactions. Activated CD8 T cells express FasL on their surface and this binds to the Fas receptor on the surface of target cells. Their binding initiates a caspase cascade, which results in apoptosis of target cells. Because both Fas and FasL are expressed on CD8 T cells, it is also a mechanism how CD8 T cells can kill each other, called fratricide. It happens during the contraction

phase at the end immune response to eliminate immune effector cells (Janeway CA Jr 2001).

B lymphocytes

B lymphocytes also called B cells differentiate in the bone marrow, but the early B cell development and commitment to B cell lineage occurs in the foetal liver prenatally. B cells represent part of the adaptive humoral immune system and are responsible for mediating the production of antigen-specific immunoglobulin (Ig), called antibodies. Immunoglobulins consist of two identical heavy (IgH) and light (IgL) chains which are joined by disulphide bond (LeBien and Tedder 2008). Mature B lymphocytes exist as pools of intra-epithelial B1-type and follicular B2-type cells in the peripheral, secondary lymphoid organs of the immune system (Parkin and Cohen 2001). In the first phase hematopoietic progenitors and precursors are induced by chemokines, cell contact and cytokines. By at least 10 transcription factors-controlled gene expression program commits them to the second , B-lymphoid restricted phase, in which they enter stepwise V (D) J rearrangements at the IgH and IgL loci. First, the emerging repertoires of IgH chains are probed for fitness to assemble in a later full Ig molecule by pairing with surrogate L chain and form pre-B cell receptors. The fittest pre-B cell receptors allow the widest proliferative expansion of the corresponding pre-B cells. After this first checkpoint for fitness, precursor B cells enter IgL chain gene rearrangements. The resulting repertoires of IgM on immature B cells are selected at a second checkpoint for auto-reactivity at a cellular site that still needs to be defined. Cells expressing IgM with high avidity for autoantigens are deleted, unless they manage to change their specificity of their Ig away from auto-reactivity by secondary IgL chain gene rearrangements, a process called “editing.” Cells expressing IgM with low avidity may be positively selected by autoantigens to enter the B1 compartments, whereas 90% of all newly generated immature B cells never make it to the periphery and cells with no avidity to autoantigens are ignored and can transit to the periphery, probably mostly to the B2 pools, as long as they express IgM on their surface (Melchers 2014).

In mature B cells occurs a mechanism referred as immunoglobulin class switching. Class switching takes place after activation of B cells via its receptor (BCR) to generate different classes of antibodies all with the same variable region, but the constant region of the heavy chain is changed. Naïve mature B cells produce both IgM and IgD, which are the first two heavy chain segments in the immunoglobulin locus. After activation, B cells start to proliferate and if they encounter specific signaling molecules via their CD40 and cytokine receptors, they undergo antibody class switching to produce IgG, IgA or IgE antibodies (Stavnezer, Guikema, and Schrader 2008).

1.2.3. HIV-1 and immune system

Acute HIV-1 infection is associated with typical symptoms as sore throat, fever, swollen glands or rash. These symptoms are not specific to HIV-1 but rather an effect of immune activation which is induced as a response to HIV-1, but also occurs in other viral infections (Deeks and Walker 2004). There is a massive release of different cytokines including IFNγ, TNFα, IL-6, IL-10, IL-15 (Stacey et al. 2009). The frequency of activated T cells increases during acute HIV-1 infection where up to 50% of CD8 T cells are activated (Papagno et al. 2004). ART can partially reverse these pro-inflammatory pathways, but this effect is incomplete and the chronic immune activation persists indefinitely (Deeks and Walker 2004). CD4 T cells also play an important role in HIV-1 infection. Firstly, they are the major target of the virus and they are being massively depleted after HIV-1 infection (Cohen et al. 2010; Fiebig et al. 2003). Studies have shown an imbalance in the distribution of CD4 T cell subsets, increased markers for activation and exhaustion and decreased effector functions in HIV-1 infection (Khaitan and Unutmaz 2011; Giorgi and Detels 1989). Additionally to CD4 T cell´s helper function, these cells have also ability to kill infected cells. Cytotoxic CD4 T cells have an important role in controlling the viral infections, including HIV-1, as well as cytotoxic CD8 T cells (Johnson et al. 2015).

ART treated individuals with undetectable viral load show higher levels of immune activation than healthy controls and this is associated with increased morbidity and mortality (Paiardini and Muller-Trutwin 2013). The chronic immune activation is based on

clinical observations and blood analyses from chronically HIV-1-infected individuals. These analyses describe an escalation in expression of activation markers (CD38, HLA-DR) and proliferation marker (Ki-67) on both CD4 and CD8 T cells. Increased number of CD38+ HLA-DR+ CD8 T cells was associated with CD4 T cell deletion and development of AIDS (Giorgi and Detels 1989). Additionally, CD38 expression on HIV-1-specific CD8 T cells correlates with HIV viral load (Doisne et al. 2004). Most of the cytokines involved in chronic immune activation during HIV-1 infection can be produced by different cell types. It is known that T cells are the major source of IL-10, while TGF-β is mostly produced by Treg cells (Konkel et al. 2017). Plasmacytoid dendritic cells (pDC) secret type I IFN. Other cells of the immune system like B cells and NK cells contribute to this stage as well by secreting chemokines, which are able to modify cellular distribution (Paiardini and Muller-Trutwin 2013).

Without ART treatment, the majority of HIV-1 infected individuals progress to AIDS. However, there is a small percentage of infected people who demonstrate control of the virus, namely, the long term non-progressors and elite controllers (Yan et al. 2013). These individuals can maintain low or undetectable viral loads without any ART. Previous studies have shown that HIV-specific CD8 T cells from elite controllers have enhanced cytotoxic function compared to progressors (Saez-Cirion et al. 2007; O'Connell et al. 2009). In the majority of elite controllers is the spontaneous control of HIV-1 infection associated with normal CD4 T cell count. Just in a small proportion of these individuals was observed CD4 T cell decline despite undetectable levels of viremia (Kamya et al. 2011). HIV-specific T cell responses are thought to play a key role in viral load decline during primary infection and determining the subsequent viral load set point. The initial appearance of HIV-specific CD8 T cells is closely associated with the drop in plasma viremia that occurs during acute infection (Eller et al. 2016). But not only HIV-specific CD8 T cells impact the outcome of HIV. It has been shown that HIV-specific CD4 T cells are significantly associated with maintenance of low viremia during chronic HIV infection (Ranasinghe et al. 2012).

During the course of HIV-1 infection, approximately 50% of HIV-infected individuals develop neutralizing antibodies (nAbs) capable of inhibiting more than 50 viral isolates (Hraber et al. 2014). nAbs are specific types of antibodies that have the ability to act on

specific sites on microorganisms such as viruses that aid in establishing an infection. In viral infections, antibodies interrupt transmission primarily in two ways: by blocking virus entry into the uninfected target cells by way of disengaging virus–receptor interaction or via antibody-dependent cell-mediated cytotoxicity (ADCC). For complex viruses such as HIV-1 and influenza, which display very high variations in their genotypes, antibodies capable of efficiently tackling the breadth of these genetic variations are essential. These antibodies are referred to as broadly neutralizing antibodies (bnAbs) (Rusert et al. 2016). As already mentioned above, during the course of natural HIV-1 infection, infected individuals typically develop type-specific cross-neutralizing antibodies, but only about 1% of them are found to develop broad and very potent bnAbs (Simek et al. 2009). Some of these potent bnAbs have shown promising results in animal models, as well as in humans, and have shown modest and transient suppression of viremia, thus opening a very interesting and important avenue of exploring how bnAbs can be successfully used as therapeutic agents to complement ART in comprehensive treatment of HIV-infected patients (Kumar et al. 2018; Halper-Stromberg and Nussenzweig 2016).

1.3. Immunometabolism

Cell signals regulate the metabolic activity to control the growth, proliferation and function of the cells (MacIver, Michalek, and Rathmell 2013). In immune cells, specific alterations in metabolic pathways are associated with immune effector functions, in particular, production of different sets of cytokines (O'Neill, Kishton, and Rathmell 2016; Mesquita and Rodrigues 2018) . Individual immune cell subsets have different functions in immunity, therefore they use several different metabolic pathways to generate appropriate levels of energy to support cell survival and to produce metabolic intermediates, which are important for cellular growth and proliferation (Wang, Luan, and Medzhitov 2019). Despite the fact that these metabolic pathways are quite different in terms of end product, they are closely linked due to shared fuel inputs and sometimes products from one pathway serve as fuel for another one (O'Neill, Kishton, and Rathmell 2016).

1.3.1. Glycolysis

Glycolysis begins with the uptake of glucose from extracellular environment. Afterwards, glucose is processed in cellular cytosol in a complex 10-step reaction cascade with mostly pyruvate as the end product (**Figure VIII**). Compared to other metabolic pathways, glycolytic metabolism is quite inefficient in terms of generating adenosine triphosphate (ATP).

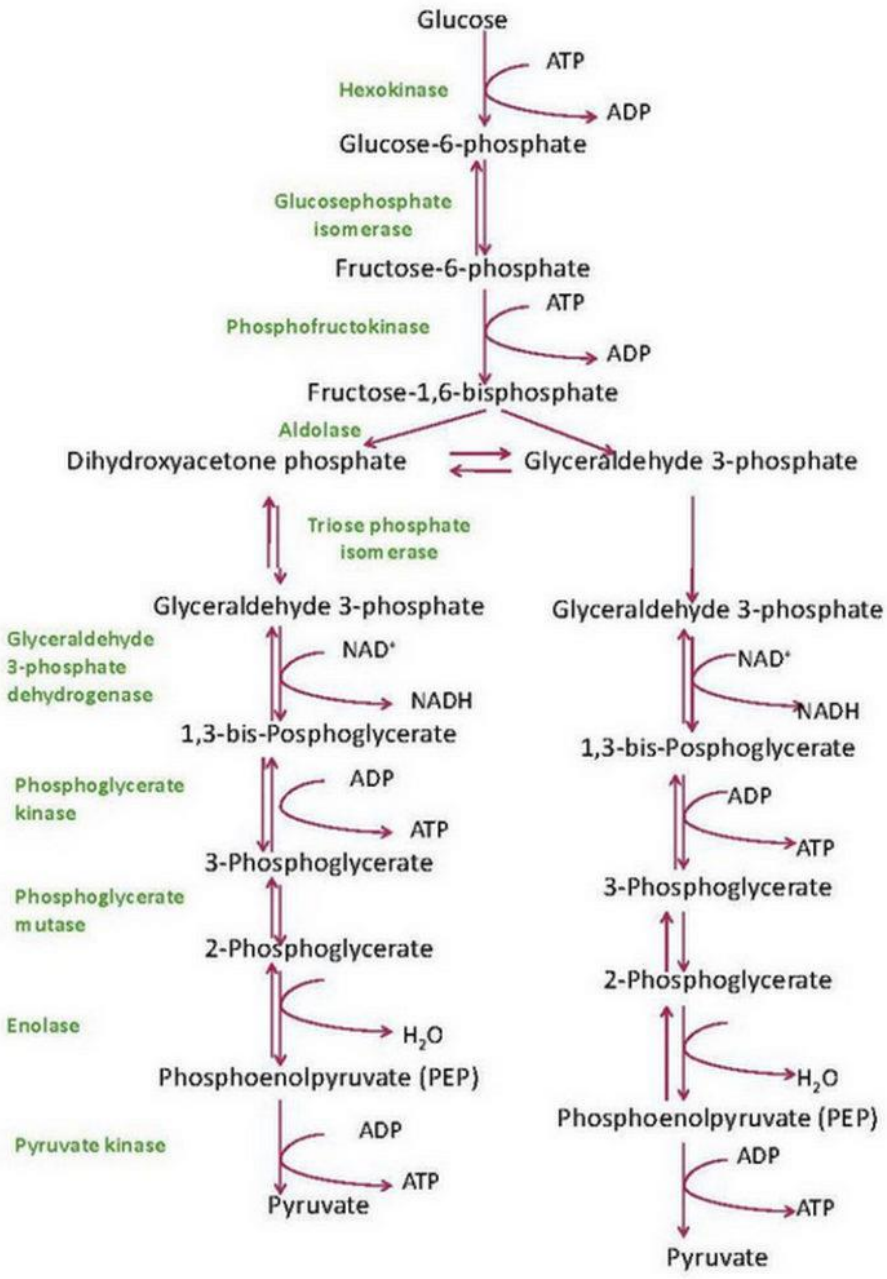

Figure VIII: Glycolysis (Rajesh 2016)

Apart from other benefits for the cells, like diversion of intermediate products to support anabolic growth, glycolytic metabolism is also reducing NAD^+ to NADH. To maintain the glycolytic flux, cells very often reduce pyruvate to lactate to recycle NADH and keep NAD^+ levels high. Apart from this, glycolysis has a key role in providing biosynthetic

intermediates for ribose synthesis, which is subsequently used to build nucleotides, amino acids, and fatty acids. Glucose is considered as the dominant and essential metabolic pathway of rapidly proliferating cells (O'Neill, Kishton, and Rathmell 2016).

Glycolysis is involved in many immune processes. Studies have indicated that both activated macrophages and activated T cells have a huge glucose demand (Newsholme et al. 1986; Palmer et al. 2015). Glycolysis provides only two molecules of ATP from one molecule of glucose, whereas oxidative phosphorylation generates 36 molecules of ATP from one glucose molecule. However, the glycolytic pathway can be activated via induction of enzymes which are involved in it. Comparing this to oxidative phosphorylation, which requires complete mitochondrial biogenesis is boosting of glycolysis much faster process. Activating signals like growth factors increase glucose uptake and glycolysis, which provides ATPs, supports the citric acid (TCA) cycle, and provides intermediates to pentose phosphate pathway (PPP). Additionally, the activating signals promote glycosylation reactions and synthesis of key biomass products such as serine, glycine, alanine and acetyl-Co A for lipid synthesis (Renner et al. 2015; O'Neill, Kishton, and Rathmell 2016). Enhanced glycolysis has been observed in lipopolysaccharide (LPS)-stimulated macrophages and DCs (Rodriguez-Prados et al. 2010; Krawczyk et al. 2010), in activated NK cells (Donnelly et al. 2014), as well as in activated effector T cells (Michalek et al. 2011) and activated B cells (Doughty et al. 2006). All effector T cell subsets increase glycolysis after activation and this increase is most significant in Th17 (Shi et al. 2011), Th1, Th2 (Michalek et al. 2011) and activated CD8 T cells (Gubser et al. 2013). Increased glycolysis can be therefore considered as a hallmark for metabolic change in most immune cells undergoing activation, for example as a response to stimulation of PRRs, cytokine receptors, or antigen receptors. Increased glycolysis gives the cells sufficient amount of ATP and intermediates to perform their effector functions, in case of T cells it is production of effector cytokines (Shi et al. 2011; O'Neill, Kishton, and Rathmell 2016).

When looking at molecular signature associated to glycolysis during immune cell activation, it has been shown that LPS induces activation of hypoxia-inducible factor 1α (HIF-1α). HIF-1α is a transcription factor which is crucial for induction of many genes involved in glycolysis (Tannahill et al. 2013; Shi et al. 2011). Another major player in

metabolism is the gene for mechanistic target of rapamycin (mTOR). mTOR is the key metabolic regulator of immunity. It consists of two subunits mTORC1 and mTORC2. They can sense amino acids and growth factors and subsequently promote mRNA translation and lipid synthesis to support cell growth. Apart from this, mTOR is also involved in signaling cascades which involve T cells and monocytes differentiation (O'Neill and Hardie 2013; Pollizzi et al. 2015; Laplante and Sabatini 2013).

1.3.2. TCA cycle

TCA cycle also known as Krebs cycle **(Figure IX)** occurs inside the mitochondrial matrix and is one of the major metabolic pathways in most of quiescent or non-activated and non-proliferative cells. TCA cycle and oxidative phosphorylation are highly efficient in

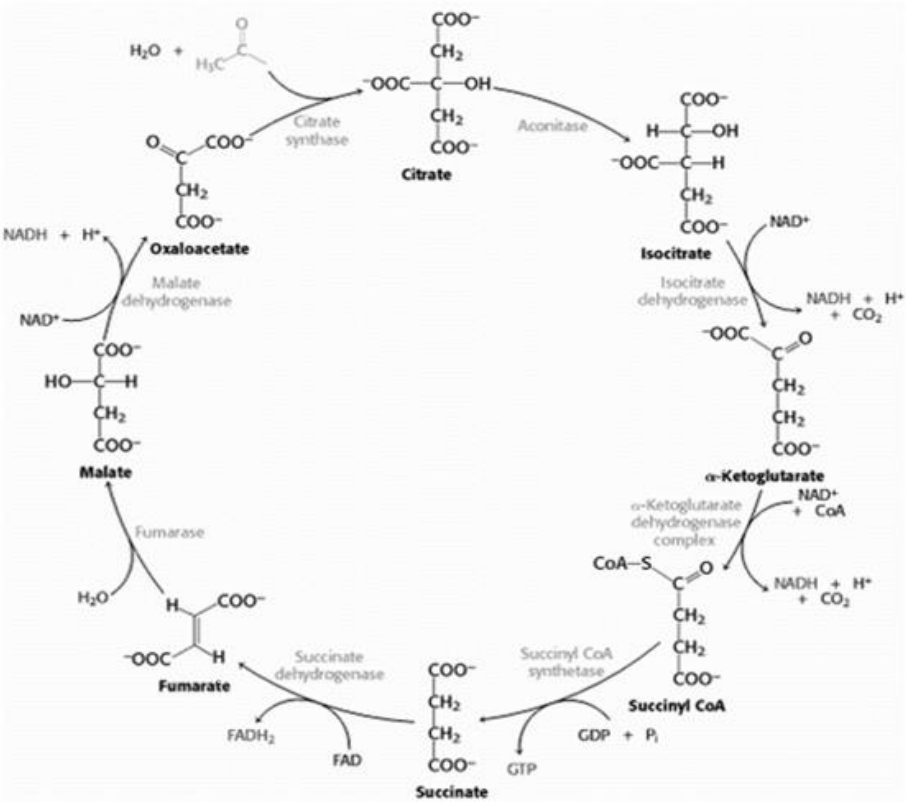

Figure IX: TCA cycle (Chhabra 2019)

generating ATP for cells, which require energy and longevity. In TCA cycle, glycolysis-generated pyruvate and fatty acids are converted into acetyl coenzyme A (acetyl-CoA), which joins the TCA cycle aldol condensation with oxaloacetate to form citrate **(Figure VIII)**. Glutamine is also an important fuel for TCA cycle because it directly converts into α-ketoglutarate. The two most important products of TCA cycle are NADH and $FADH_2$, which support oxidative phosphorylation by transferring electrons into electron transport chain (ETC) **(Figure X)** (O'Neill, Kishton, and Rathmell 2016).

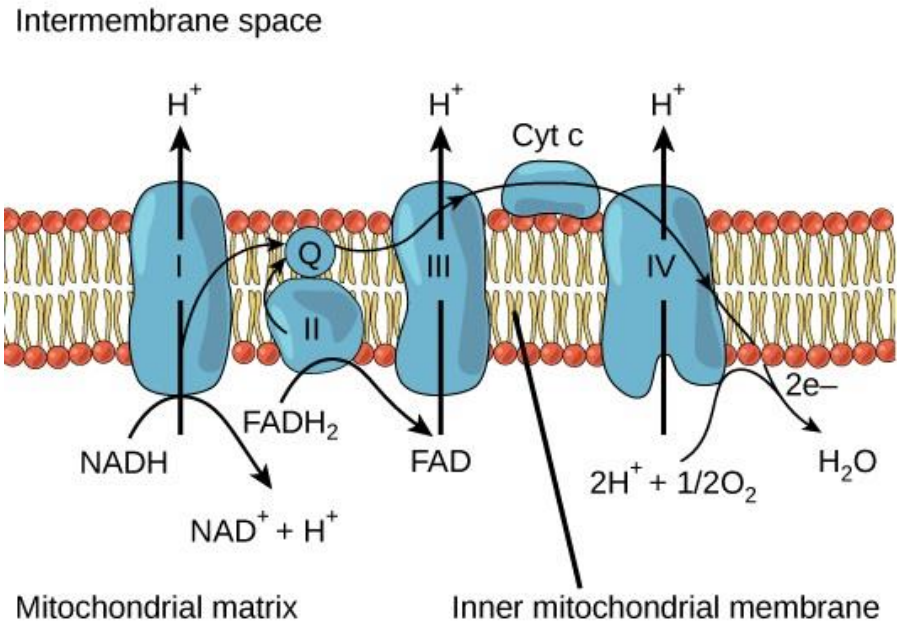

Figure X: Electron transport chain (Lumen 2015)

Both TCA cycle and oxidative phosphorylation are fully functional in almost all T cell subsets (Michalek et al. 2011). However, there is a shift towards glycolysis and away from TCA cycle in effector T cells. Moreover, TCA cycle is the dominant pathway in memory CD8 T cells (O'Sullivan et al. 2014).

1.4. Aim of the thesis

The overall goal of this PhD thesis is to assess the impact of ART-treated and untreated chronic HIV-1 infection on cellular metabolism and functions of immune cells, including NK cells, B cells, CD4 and CD8 T cells.

Chronic viral infections negatively impact the immune system in many ways, ranging from chronic immune activation and exhaustion, to reduction in effector-functions until cell death. Increased metabolism has been associated with effector and activated cells, however, knowledge about metabolic activity during chronic infections is relatively sparse. Therefore, in the first part of the project, we decided to compare the metabolic profile of immune cells through analysis of glycolysis and oxidative phosphorylation, which were isolated from chronically HIV-1-infected treatment-naïve individuals to those from HIV negative individuals. After analyzing metabolism, we investigated the possibility of a correlation between measured metabolic parameters and previously described chronic immune activation and exhaustion.

The second part of the project was focused on immune cell metabolism from individuals on ART treatment with fully suppressed viral load and restored CD4 T cell count.

As a follow-up, we investigated the impact of individual drug regimens on respiratory activity andCD4 T cell cytokine secretion.

Lastly, to elucidate the data we obtained from this study, we examined possible mechanisms by measuring mitochondrial ROS, membrane potential, and mitochondrial mass.

2. MATERIAL AND METHODS

2.1. Materials and devices

15/50ml falcon tubes	TPP, Switzerland
96-well microplates	Starlab, Germany
24-well microplates	Starlab, Germany
Antimycin A	Agilent, USA
Automatic pipettor	Thermo Fischer Scientific, Germany
BD FACS Celesta	BD Bioscience, Germany
Biocoll (ficoll)	BioChrome, Germany
BSA	Sigma-Aldrich, Germany
Carbone dioxide incubator	BINDER, Germany
Cell culture flasks T75	Greiner Bio One, Germany
Cell strainer (0.44µm)	TPP Techno Plastic Products, Germany
Centrifuge (Eppendorf 5804R)	Eppendorf, Germany
Cryotubes	Oehmen, Germany
Cryo-freezing container	Sigma-Aldrich, Germany
DMSO	Sigma-Aldrich, Germany
DynaMag2	Thermo Fischer Scientific, Germany
EDTA	Sigma-Aldrich, Germany
EVE cell counting slides	NanoEn Tek, USA
FACS tubes	BD Bioscience, Germany
FBS	BioChrome, Germany

FCCP	Agilent, USA
HEPES	Sigma-Aldrich, Germany
Human T cell activator	Thermo Fischer Scientific, Germany
L-Glutamine	Sigma-Aldrich, Germany
Liquid nitrogen tank	Techlab, Germany
MACS LS columns	Miltenyi, Germany
MACS separator magnet	Miltenyi, Germany
Microtubes 1,5/2ml	Eppendorf, Germany
Oligomycin	Agilent, USA
PBS	Life Technologies, UK
pH meter	Oehmen, Germany
Pipettes (5,10,25ml)	Greiner Bio One, Germany
Pipette filter tips	Starlab, Germany
Poly-D-Lysine	Sigma-Aldrich, Germany
RPMI 1640 medium	Life Technologies, UK
Rotenone	Agilent, USA
Seahorse XFe 96 Analyzer	Agilent, USA
Seahorse cartridge	Agilent, USA
Seahorse microplate	Agilent, USA
Sodium pyruvate	Sigma-Aldrich, Germany
Trypan Blue 0.4%	NanoEn Tek, USA
Vortex test tube vortex	NeoLab Migge, Germany
Water bath GFL	Oehmen, Germany

2.2. Kits

Annexin V Viability kit	Thermo Fischer Scientific, Germany
CD4, CD8, NK, B –cells Isolation kit	Miltenyi, Germany
Mitochondrial stress test	Agilent, USA
DNA mini isolation kit	Qiagen, Germany
Mitochondrial stress test	Agilent, USA
MitoSox Red superoxide ind.	Thermo Fischer Scientific, Germany

2.3. Oligos

primer	accession	5´sequence	3´sequence
phosphoglycerate kinase 1	NM_000291.3	AGA GCC CAC AGT TCC ATG GT	GCA AAG TAG TTC AGC TCC TTC TTC
glucose transporter 1	NM_006516.2	GGG CAT GTG CTT CCA GTA TG	GCG ATC TCA TCG AAG GTC CG
glyceraldehyde-3-phosphate dehydrogenase	NM_002046.3	GCA GGG GGG AGC CAA AAG GG	GGT GCA GGT GGC ATT GCT GATG

2.4. Methods

For the purpose of this work, HIV-1 will from now on be referred to as HIV.

Study populations

Group of HIV-positive treatment-naïve (n=11) and HIV-positive treated (n=12) individuals was recruited from HIV and STD clinic (HPSTD) at the University Hospital in Essen. Blood from healthy uninfected controls was provided by the Department of Transfusion Medicine.

Ethics committee approval

None of the funding sources were involved in the design or carry-out of the study. The study was approved by IRB at the University Duisburg-Essen. Informed consent was obtained from all participants involved in the study.

Isolation of Peripheral Blood Mononuclear Cells (PBMC)

EDTA blood samples were obtained from both HIV-positive treatment-naïve and treated individuals. Buffy coats were obtained from healthy controls. PBMC were isolated by standard density-gradient protocol and cryopreserved as previously described (Streeck et al. 2007). Briefly, blood samples were gently pipetted on a ficoll layer and centrifuged. The middle phase containing PBMC was then transferred into new falcon tube and the cells were washed twice with R10 medium (RPMI-1640 supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicilline, 100 µg/ml streptomycine and 10 mM HEPES). Cells were then cryopreserved or directly used for further experiments.

Cell Subset Separation and Stimulation Conditions

Cryopreserved PBMC were thawed in R10 media and allowed to rest overnight at 37°C, 5% CO_2. Different cell types were subsequently enriched by magnetic separation using Multisort Isolation Kits (Miltenyi Biotec), achieving more than 90% purity. Cells were then stimulated using anti-CD3/CD28 beads (Gibco, Life Technologies) at a cell-to-bead ratio of 1:1 in the presence of IL-2 (30 IU/ml) (eBioscience) for 3 days. Cells were cultured in R10 media in humidified atmosphere (5% CO_2) at 37°C.To assess the impact of antiretroviral medications CD4 T cells were incubated in presence of different ART regimens – emtricitabine (FTC), zidovudine (AZT), tenofovir (TDF), lamivudine (3TC), rilpivirine (RLP), raltegravir (RAL), dolutegravir (DLG), elvitegravir (EVG), darunavir ethanolate (DRV), ritonavir (RTV) (Selleckchem). Concentration of the antiretroviral medications for each analysis are described in Table 03.

Metabolic Assay

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were determined using a Seahorse XFe Extracellular Flux Analyzer according to manufacturer´s protocol (Pelletier et al. 2014). Briefly, cells were plated on poly-D-lysine coated 96-well polystyrene Seahorse plate at density of 200,000 cells per well. Cells were equilibrated in non-buffered DMEM medium supplemented with 2 mM glutamine, 2 mM sodium pyruvate and 10 mM glucose for 45 min at 37 °C with 0% CO_2 atmosphere prior the experiment. Metabolic assays were performed using the following concentrations of subsequently injected compounds: oligomycin 1 µM, mitochondrial uncoupler p-trifluormethoxy carbonyl cyanice phenol hydrazine (FCCP) 1 µM, Antimycine A and rotenone (both 0.5 µM). At each time interval, the OCR and ECAR values were measured as indicators mitochondrial respiration and glycolysis respectively.

Oroboros metabolic assay

Oxygen consumption rate (OCR) was assessed by using Oroboros instrument according to manufacturer SUIT protocol. Briefly, control cells and DLG-treated cells were pipetted in individual instrument chambers. Afterwards, different compounds were injected subsequently to reach the final concentration – pyruvate (5mM), malate (2mM), ADP (2,5mM), cytochrome C (10uM), glutamine (10mM), succinate (10mM), UCCCP (5uM), rotenone (0,5uM) and Antimycine A (2,5uM). Data were analyzed using Oroboros software.

PD1 blocking

Cryopreserved PBMC from HIV positive treatment-naïve individuals were thawed and allowed to rest overnight at 37°C, 5% CO_2. On the next day, CD8 T cells were isolated using CD8 Isolation Kit (Miltenyi). Cells were then stimulated using anti-CD3/CD28 beads (Gibco, Life Technologies) at a cell-to-bead ratio of 1:1 in the presence of IL-2 (30 U/ml) (eBioscience) and anti-PD1-leaf (clone: EH12.2H7) for 3 days. Afterwards, the metabolic assay was performed as described above.

Phenotypic assessment by flow cytometry

Cryopreserved PBMC were thawed and rested overnight as described above. One million PBMC from each individual was passed through 40 µm cell strainer (BD Falcon) to obtain single cell suspensions. Cells were washed with phosphate buffered saline (PBS) and stained with viability dye Zombie Aqua (Biolegend). Cells were subsequently washed with staining buffer (PBS supplemented with 2% FCS) and stained with fluorescently conjugated antibodies: anti-CD3-AL488 (clone UCHT1; Biolegend), anti-CD4-AF700 (clone RPA-T4; Biolegend), anti-CD8-APC-Cy7 (clone RPA-T8; Biolegend), anti-CD38-BV605 (clone HIT2; Biolegend), anti-HLA-DR-APC (clone L243; Biolegend), anti-PD-1-PE (clone EH12.2h7; Biolegend). Data was collected at FACS Aria III (BD) and analyzed with FlowJo Software version 10 (TreeStar).

Assessment of ex-vivo proliferation by flow cytometry

Cryopreserved PBMC from HIV positive treated individuals were thawed and rested overnight as described above. Afterwards approximately 3 million PBMC from each individual were washed with phosphate buffered saline (PBS) and stained with the cell division tracker carboxyfluorescein succinimidyl ester (CFSE, Biolegend). Therefore, cells were resuspended in 1 ml PBS and stained with 1.25 µM CSFE at 37 °C, 5% CO_2 for 20 min. Subsequently the staining was quenched by adding 5 times the original staining volume of R10 media. Cells from each individual were centrifuged, resuspended in R10 Media and transferred in 24-well polystyrene plate (Starlab) at concentration of 1 million/ml. Cells were stimulated with 5 µg/ml SEB or left unstimulated as a control at 37 °C, 5% CO_2 for 5 days. On the 5th day cells were washed with PBS and stained with viability dye Zombie Aqua (Biolegend) for 30 min. After washing with staining buffer (PBS supplemented with 2% FCS) cells were stained extracellularly for 20 min with fluorescently conjugated antibodies: anti-CD3-APC-Cy7 (clone UCHT1; Biolegend), anti-CD4-BV421 (clone RPA-T4; Biolegend), anti-CD8-AF647 (clone C8/144B; Biolegend). Data was collected at FACS Celesta (BD) and analyzed with the proliferation tool of FlowJo Software version 10.0.7 (TreeStar).

Assessment of T cell polyfunctionality

Cryopreserved PBMC isolated from healthy donors were thawed and rested overnight as described above. Cells were transferred in 24-well polystyrene plate (Starlab) at concentration of 1 million/ml and stimulated with 200 ng/ml SEB in presence of different ART regiment at 37 °C, 5% CO_2 for 3 days. On day 3, cells were re-stimulated with 5µg/ml SEB, and incubated in the presence of co-stimulatory molecule CD28/CD49d (BD Bioscience) together with anti-CD107a-PE-Cy5 (clone H4A3; BD Bioscience) for 6 hours. After 1 hour Golgi Stop and Golgi Plug (BD Bioscience) were added and cells were incubated for additional 5 hours. Cells were then washed with PBS and stained with viability dye Zombie Aqua (Biolegend). After washing with staining buffer (PBS supplemented with 2% FCS) CytoFix/CytoPerm Solution (BD Bioscience) was added and cells were incubated for 20 min at 4 °C. Cells were then washed with 1x Perm/Wash Buffer (BD Bioscience) and stained intracellularly with fluorescently conjugated antibodies: anti-CD3-PacificBlue (clone UCHT1; Biolegend), anti-CD4-BV421 (clone RPA-P4; Biolegend), anti-CD8-APC-Cy7 (clone RPA-T8; Biolegend),anti-MIP1β-PE (clone D21-1351; BD Bioscience), anti-TNFα-AF700 (clone Mab11; Biolegend), anti-IFNγ-PECy7 (cloneB27; Biolegend), anti-IL-2-FITC (clone MQ1-17H12; Biolegend). Data was collected and analyzed as above.

Protein isolation and HIF-1α immunoblot

CD 4 T cells were activated in presence of NRTI (FTC), NNRTI (RLP), INSTI (DLG) and PI (DRV) for three days. Hypoxic incubation was performed in a Ruskinn Invivo2 300 workstation under 1 % O_2 for four hours. Cells were lysed under normoxic or hypoxic conditions with a common immunoblot lysis buffer (150 mM NaCl, 20 mM TRIS pH7.5, 1 % NP40, 5 mM EDTA) containing 10 % freshly added proteinase inhibitor (Roche) for 20 min on wet ice. Lysates were centrifuged at 3600 rpm, 4°C for 5 min in a microcentrifuge and supernatants containing cellular proteins were collected and stored at −80°C. Protein concentration was measured with the Bio-Rad DC protein assay. 50 µg of whole cell lysate has been subjected to 7.5 % SDS-PAGE and transferred onto a nitrocellulose membrane (0.2 µM pore size; Schleicher & Schuell Microscience, Dassel, Germany). HIF-1α protein has been detected by a monoclonal anti- HIF-1α antibody (BD

Transduction; Cat. No. 610958), β-Actin (Sigma Aldrich, Cat. No. A2103-200UL) served as loading control.

RNA isolation and gene expression analysis by qPCR

CD4 T cells have been treated as indicated for immunoblot. Total RNA was isolated by the acidic guanidinium thiocyanate-phenol-chloroform extraction method(Chomczynski and Sacchi 1987). One microgram of total RNA was reverse-transcribed into cDNA, and quantification of HIF target genes was performed by real-time PCR (SYBRGreen PCR reaction with Blue S Green qPCR mix (Biozym), Bio-Rad CFX 96 Touch^{TM} qPCR system). The primer sequences used for qualitative and quantitative PCR are listed in section 2.3. Oligos. Data has been analyzed with $\Delta\Delta C_t$ method and are indicated as fold change over DMSO treated control.

Assessment of cell viability

Viability of cells was repeatedly assessed by using trypan blue staining. Briefly, 10 µl of cell suspension was mixed with 10 µl of 0.4 % trypan blue and loaded onto a cell counting slide. Afterwards, the number of life cells was determined using an automatic cell counter EVE (NanoEn Tech, USA). An exception was made for the non-nucleoside reverse transcription inhibitor (NNRTI) efavirenz (EFV), which showed cytotoxicity at low plasma concentrations *in vitro* and was therefore excluded from these studies. For the determination whether the living cells undergo early apoptosis cells were stained repeatedly with Annexin V. Cells were washed with staining buffer (PBS supplemented with 2% FCS) and stained extracellularly for 20 min with fluorescently conjugated antibodies: anti-CD3-APC-Cy7 (clone UCHT1; Biolegend), anti-CD4-BV421 (clone RPA-T4; Biolegend). Afterwards cells were washed with Annexin V Binding Buffer (Biolegend) and stained with anti-AnnexinV-FITC (Biolegend) antibody for 15 min. Subsequently 400 µl of Annexin V Binding Buffer were added to the cells. Data was collected at FACS Celesta (BD) and analyzed with FlowJo Software version 10.0.7 (TreeStar).

Measuring mitochondrial reactive oxygen species (mtROS)

To detect mtROS, MitoSox Red mitochondrial superoxide indicator (Thermo Fisher) was used according to the manufacturer´s protocol. Shortly, cells were washed with warm

Hank´s balanced salt solution with calcium and magnesium (HBBS/Ca/Mg) and resuspended in 1 ml of 5 µM MitoSox reagent (supplied with the kit). Cells were incubated at 37 °C, 5% CO_2 for 10 min. Afterwards, cells were washed 3 times with warm HBBS/Ca/Mg. Data was collected and analyzed as above.

Mitochondrial DNA content

Total cellular DNA was isolated using DNA mini isolation kit (Qiagen). Real-time PCR was performed using the thermo-cycler qTower 2.0 (Analytic Jena) innuMIX qPCR MasterMix SyGreen (Analytic Jena) and mitochondrial DNA content assessed as previously described(Venegas and Halberg 2012).

Assessment of biological, antiviral activity in vitro

Cryopreserved PBMC isolated from HIV positive donors were thawed, rested overnight and activated with 1µg/ml of PHA for 3 days. They were then seeded in a 6-well polystyrene plate at density of $1.5x10^6$ cells/ml and infected with 67ng/ml of HIV-1 US-1 virus. 5-fold plasma concentration of different ART regimens (DLG, RAL, EVG as well as TDF, TFV) was added into the suspension and cells were incubated at 37°C, 5% CO2 for another 3 and 4 days. Cells were harvested, washed with PBS and stained with viability dye Zombie Aqua (Biolegend) for 30 min at RT. Cells were subsequently washed with staining buffer (PBS supplemented with 2% FCS) and stained with fluorescently conjugated antibody anti-CD8-AF647 (clone RPA-T8; Biolegend) for 20min at 4°C. Performing the intercellular staining cells were washed again with staining buffer and incubated with CytoFix/CytoPerm Solution (BD Bioscience) for another 20 min at 4 °C. After washing with 1x Perm/Wash Buffer (BD Bioscience), cells were intracellularly stained with fluorescently conjugated antibodies: anti-CD3-APC-Cy7 (clone UCHT1; Biolegend), anti-CD4-BV421 (clone RPA-P4; Biolegend) and anti-p24-PE (clone KC57; Beckman coulter) for 30min at 20°C. Data was collected at BD FACS Celesta and analyzed with FlowJo (Version 10.1) as described above.

FCS concentration test

Cryopreserved PBMC isolated from HIV positive donors were thawed, rested overnight and on the next day CD4 T cells were isolated using CD4 isolation beads (Miltenyi).

Cells were then stimulated with anti-CD3/CD28 beads (Gibco, Life Technologies) at a cell-to-bead ratio of 1:1 in the presence of IL-2 (30 U/ml) (eBioscience) and DLG (1-fold and 5-fold plasma concentration) for 3 days. Cells were cultured in R10 media supplemented with 10%, 30% or 50% FCS in humidified atmosphere (5% CO_2) at 37°C. Afterwards, the metabolic assay was performed as described above.

Statistical Analysis

Prism (GraphPad Software, San Diego, CA) was used for statistical analysis. Data are presented as mean ± SD for technical replicates, or mean ± SEM for biological replicates. Differences between treatment categories was estimated with ANOVA. Statistical significance is indicated in all figures by the following annotations: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3. RESULTS

3.1. Individual immune cell subsets have very different metabolic activity

Before investigating whether HIV infection, treated or untreated, has any impact on cellular metabolism, we assessed metabolic profile of immune cells from healthy individuals. We used an extracellular flux analyzer to study cellular metabolic pathways. Glycolysis was determined based on extracellular acidification rate (ECAR) and oxidative phosphorylation, also called cellular respiration, was assessed according to oxygen consumption rate (OCR). Representative plots **(Figure 01)** show how metabolic activity differs among CD4 and CD8 T cells, NK cells and B cells. Each cell subset has quite distinct demand on oxygen and glucose.

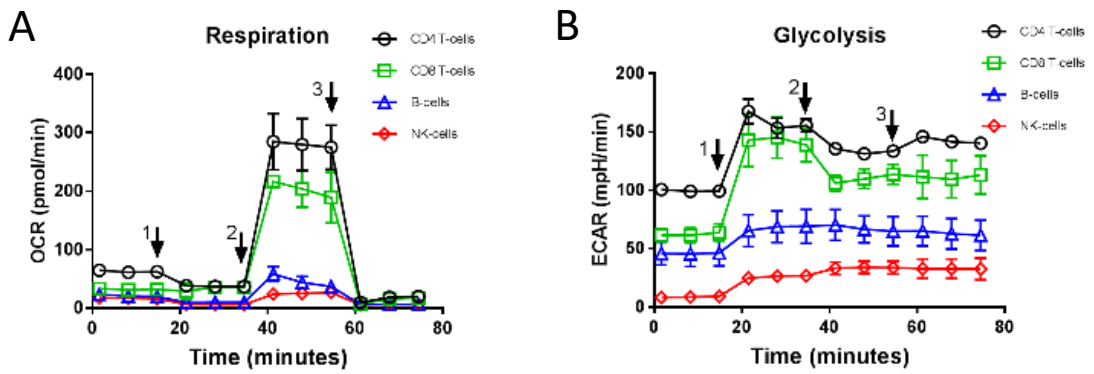

Figure 01: Representative plots of OCR and ECAR.

(A) Representative plot of oxidative consumption rate (OCR) showing subsequent injections of test components (1) oligomycin, (2) FCCP and (3) rotenone with Antimycine A. (B) Representative plot of extracellular acidification rate (ECAR) with the same injection strategy as previous test. Published (Korencak et al. 2019).

As presented on **Figure 02**, CD4 T cells have significantly highest metabolism on both levels – glycolysis and oxidative phosphorylation. CD8 T cells display lower metabolic activity, however, if required, they are able to increase glycolysis to levels comparable to CD4 T cells **(Figure 02-E)**. Metabolism of B cells and NK cells was overall low,

indicating that individual cell subsets have different metabolic requirements to maintain their functions.

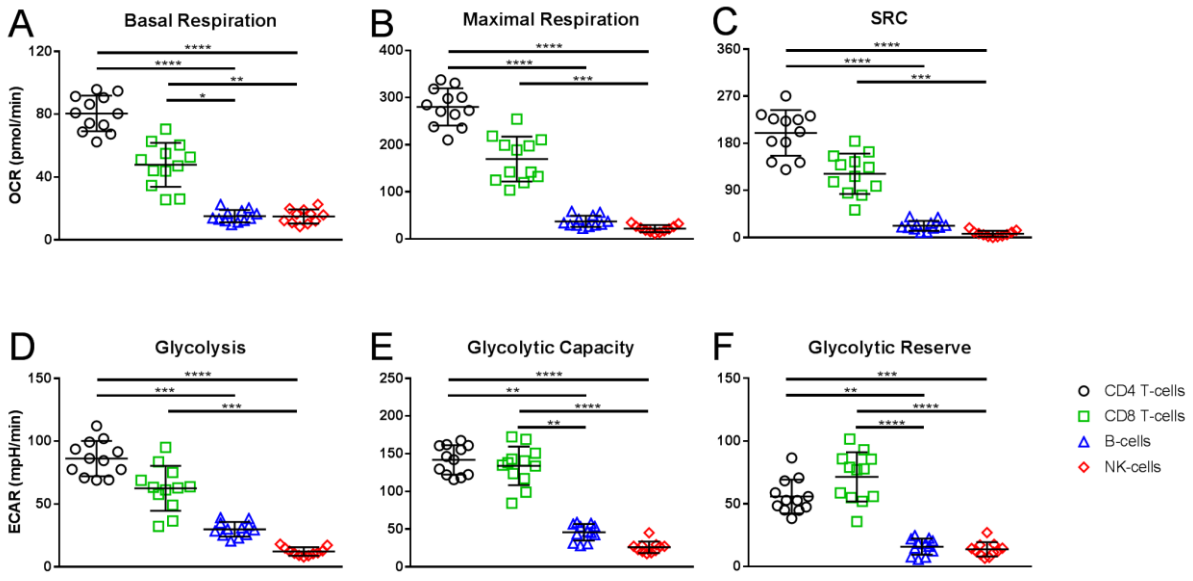

Figure 02: Changes in metabolic activity of CD4 and CD8 T cells, NK cells and B cells.

(A,B,C) Different demand of oxygen of each cell type in basal and maximal respiration and from these resulting different SRC. Significantly highest respiration of CD4 T cells, followed by CD8 T cells compared to B cells and NK cells. (D,E,F) CD4 T cells are showing the highest ECAR, but their maximal glycolytic capacity is equal to CD8 T cells. B cells and NK cells have significantly lower ongoing glycolysis. Data are representative of n=12 HIV^- individuals and shown as mean ± SD. * indicates $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ by One-Way ANOVA. Published (Korencak et al. 2019).

3.2. Cellular respiration of chronically HIV-infected treatment-naïve individuals is significantly disrupted and it is linked to immune activation and exhaustion

When having a picture how metabolism is shaped among immune cells in healthy individuals, we decided to assess, whether HIV untreated infection might have an impact on any of the metabolic pathways. Therefore, we analyzed and compared glycolysis and oxidative phosphorylation from HIV-positive treatment-naïve individuals (n=12) with HIV-

negative healthy controls (n=12). We observed that both basal and maximal respiration were significantly decreased in T cells and NK cells from HIV-infected treatment-naïve individuals, but with no impact on B cells **(Figure 03-A)**.

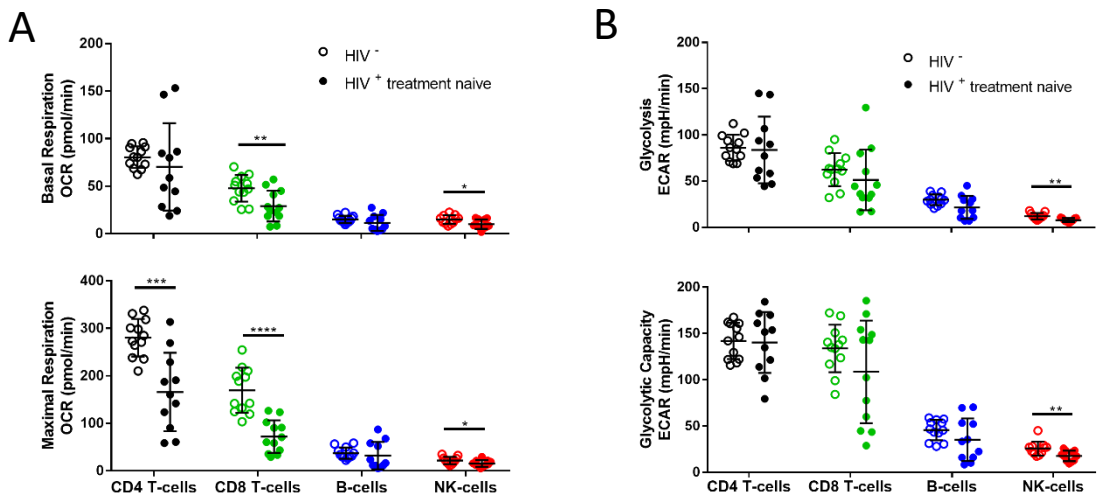

Figure 03: Respiration and glycolysis in chronically HIV-infected treatment-naïve individuals.

(A) Comparison of basal and maximal mitochondrial respiration between HIV treatment-naïve individuals and healthy controls demonstrating reduced basal and maximal respiration of CD4 and CD8 T cells and NK cells HIV^{+} treatment-naïve individuals. (B) No differences in glycolysis and glycolytic capacity were observed among CD4 and CD8 T cells and B cells. Plots show individual values with the mean ± SD. Statistical significance was assessed by Kruskal-Wallis test with Dunn´s multiple comparison test and. RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

Interestingly, only NK cells showed reduction in glycolysis, while other cell types were able to maintain ECAR levels compared to healthy controls **(Figure 03-B)**. This indicates that cells during HIV infection might predominantly rely on glycolysis. Indeed, when we looked at OCR/ECAR ratio **(Figure 04)**, ratio which shows the relative contribution of

oxidative phosphorylation and glycolysis to energy production, we observed higher OCR/ECAR ratio in HIV negative individuals, indicating that the energy in these cells is mainly produced in oxidative phosphorylation whereas the lower OCR/ECAR ratio in HIV-infected treatment-naïve individuals indicates a shift towards glycolysis.

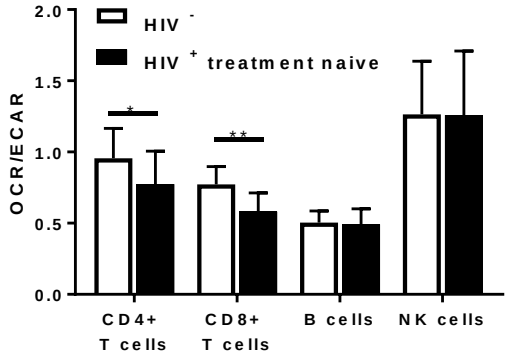

Figure 04: OCR/ECAR ratios of HIV negative and chronically HIV-infected treatment-naïve individuals.

OCR/ECAR ratio comparison between HIV negative and chronically HIV-infected treatment-naïve individuals in CD4 and CD8 T cells, B cells and NK cells. Bar chart shows the mean value ± SD. Statistical significance was assessed by Kruskal-Wallis test with Dunn´s multiple comparison test and. RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

Chronic immune activation is a well-known phenomenon during HIV infection. We investigated whether there is an association between the metabolic profile and immune activation, measured as expression of HLA-DR and CD38. We found a strong correlation between expression of activation markers and metabolic parameters in CD8 T cells **(Figure 05-A)**. Increase in chronic cellular activation resulted into decreased metabolism. Secondly, we also analyzed whether there is a link between expression of programmed-death-1 (PD-1), a marker for T cell exhaustion, and metabolism. We observed that CD8 T expressing PD-1 were metabolically less active than cells with lower expression of this exhaustion marker **(Figure 05-B)**.

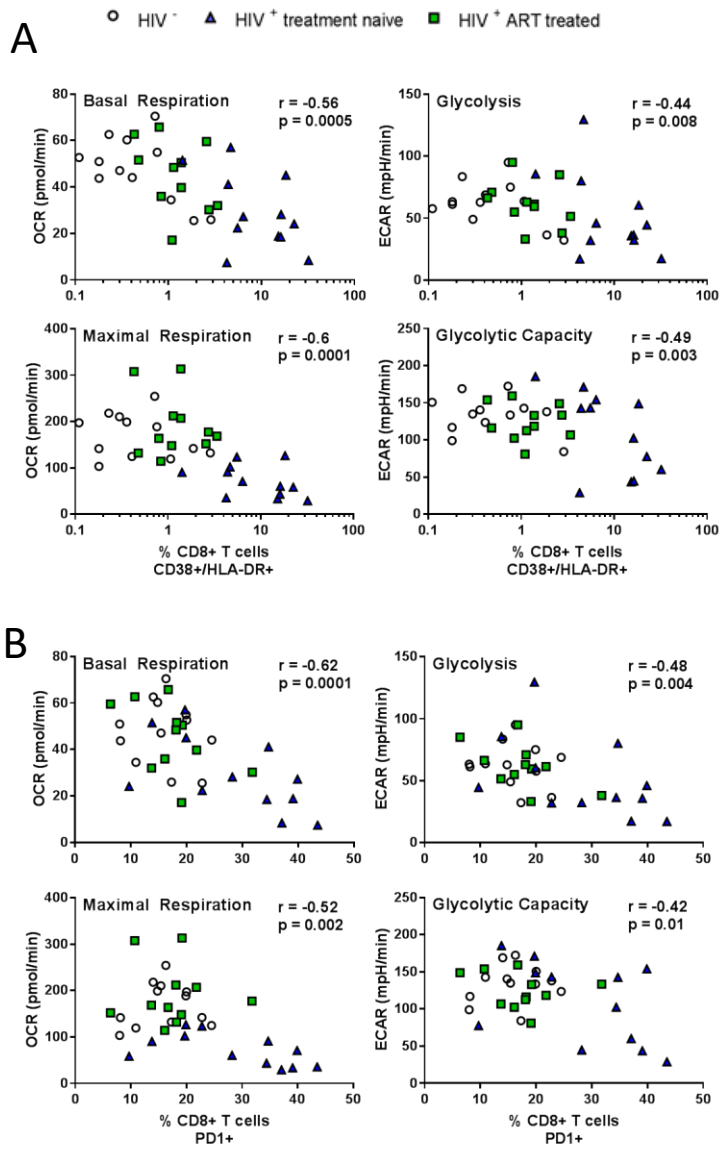

Figure 05: Correlation between bioenergetics profiles and immune activation and exhaustion in CD8 T cells.

Negative correlation between activation (characterized as $CD38^{+}$,$HLA\text{-}DR^{+}$) resp. between exhaustion (characterized as $PD\text{-}1^{+}$) and metabolism in CD8 T cells. Statistical significance was assessed by Pearson correlation test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

Interestingly, we did not find these associations in CD4 T cells, neither for immune activation **(Figure 06-A)**, neither for exhaustion **(Figure 06-B)**.

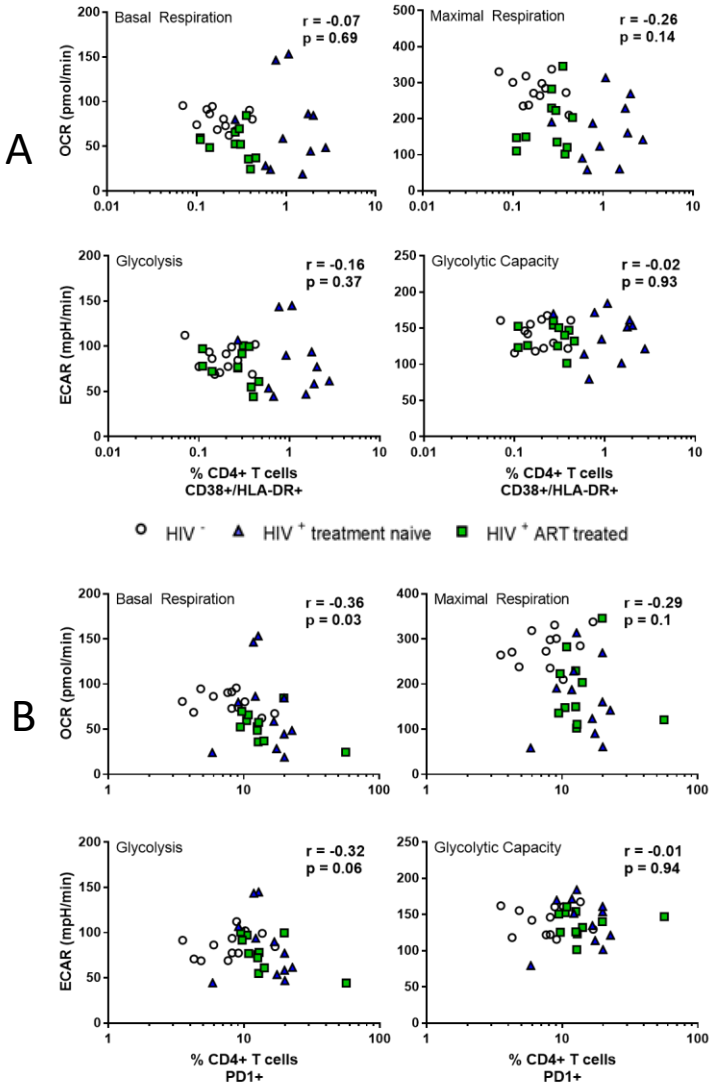

Figure 06: Correlation between bioenergetics profile of CD4 T cells and metabolism.

Correlation between activation (characterized as $CD38^{+}$,$HLA\text{-}DR^{+}$) and metabolism without any significances. Correlation between exhaustion (characterized as $PD\text{-}1^{+}$) and metabolic parameters. Statistical significance was assessed by Pearson correlation test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

It has been shown that usage of PD-1 blocking antibody might enhance effector function of T cells. Therefore we decided to use the same approach to restore the metabolic profile of the cells. We cultured CD8 T cells isolated from chronically HIV-infected treatment-naïve individuals (n=5) in presence of PD-1 blocking antibody and measured oxidative phosphorylation and glycolysis. We did not see a significant increase on basal respiration and glycolysis level, but the difference became significant when looking at maximal respiration and glycolytic capacity **(Figure 07)**.

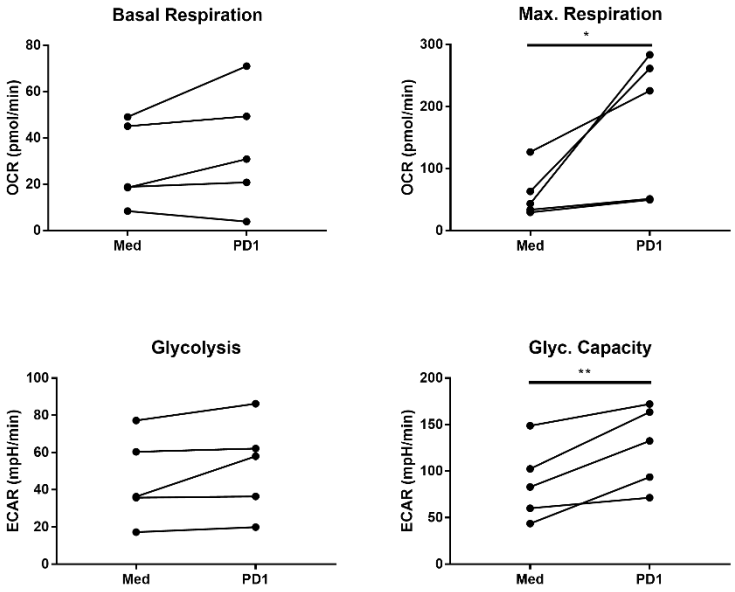

Figure 07: Effect of PD-1 blocking antibody on metabolic activity in CD8 T cells.

CD8 T cells isolated from chronically HIV-infected individuals (n=5) cultured in the presence of PD-1 blocking antibody have significantly higher maximal respiration and glycolytic capacity compared to cells cultured without it. Statistical significance was assessed by Student t test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

Taken together, these data show that T cells exhaustion and chronic immune activation is directly link to the metabolic dysfunction observed in chronic HIV Infection.

3.3. ART has a negative impact on metabolism and proliferation of CD4 T cells

Next, we wondered whether it is possible to restore the impaired metabolism by initiating ART, as previous studies shown reduction in immune activation and exhaustion after ART (Khaitan and Unutmaz 2011; Paiardini and Muller-Trutwin 2013). We compared both glycolysis and oxidative phosphorylation between HIV-negative (n=12) and HIV-positive treatment-experienced individuals (n=12). We found that ART is able to restore metabolic profile of CD8 T cells, B cells and NK cells **(Figure 08)**.

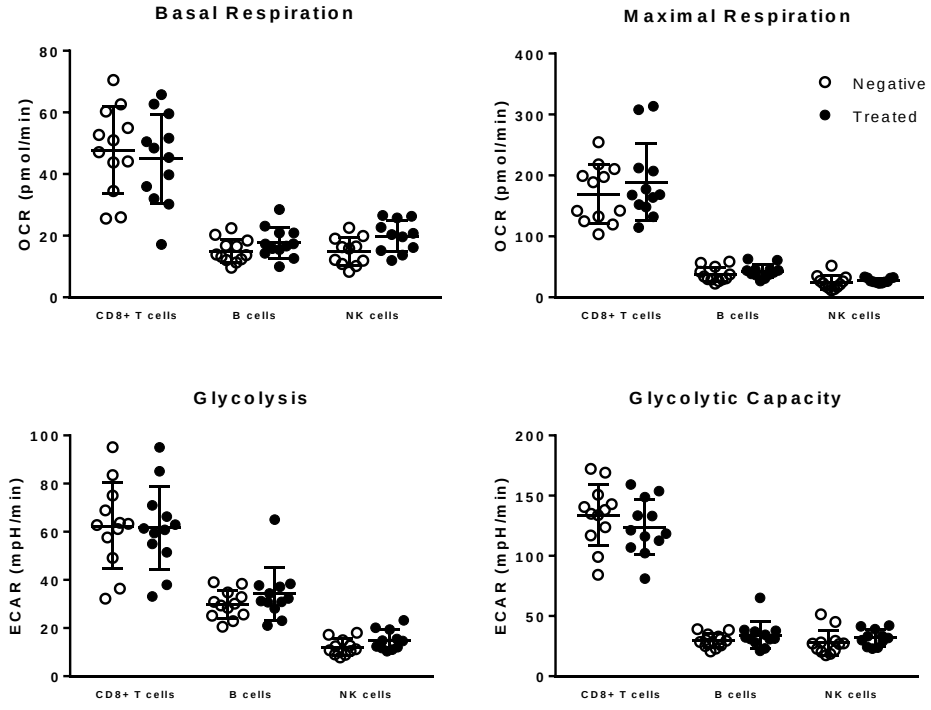

Figure 08: Effect of ART on metabolic phenotype

ART can revert metabolic phenotype. (A) Restored respiratory capacity of ART treated individuals in both basal and maximal respiration. (B) No differences in glycolysis and glycolytic capacity between ART treated and HIV negative individuals. Data are representative of n=12 HIV^- and HIV^+ ART treated individuals and shown as mean ± SD. * indicates $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ by One-Way ANOVA. Published (Korencak et al. 2019).

However, we noticed that basal and maximal respiration of CD4 T cells remains significantly lower even after ART **(Figure 09-A)**, without any impact on glycolysis **(Figure 09-B)**.

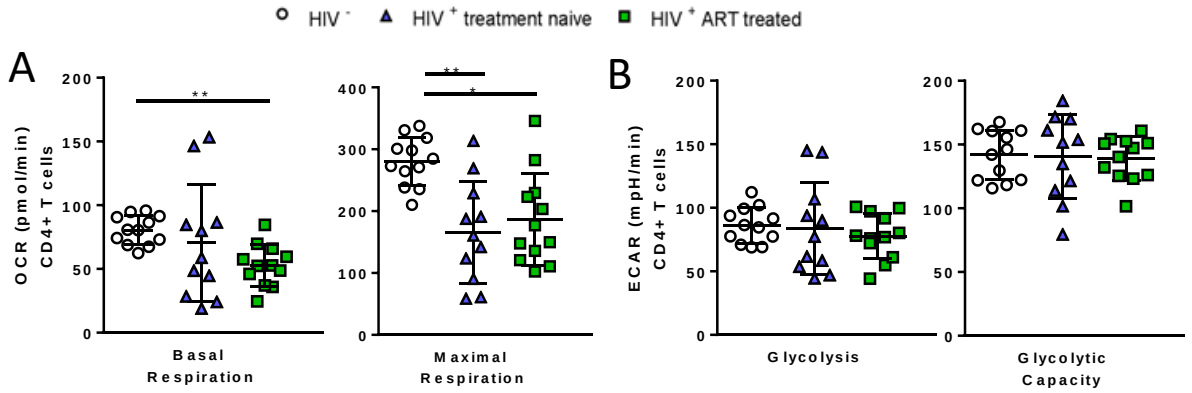

Figure 09: Changes in respiration in CD4 T cells from HIV-infected ART-treated individuals

CD4 T cells from HIV^{+} treatment-naïve (n=11) and ART-treated individuals (n=12) display reduced basal and maximal respiration compared to HIV-negative (n=12). (A) Comparison of basal and maximal mitochondrial respiration between HIV treatment-naïve and treated individuals and healthy controls demonstrating reduced basal and maximal respiration of CD4 T cells in ART treated individuals (B) No differences in glycolysis and glycolytic capacity of CD4 T cells in the three groups. Statistical significance was assessed by Kruskal-Wallis test with Dunn´s multiple comparison test and. RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

Moreover, we did not see only an impairment in the respiration of CD4 T cells from HIV-positive ART-treated individuals, but also a shift towards glycolysis as indicated by the OCR/ECAR ratio **(Figure 10)**.

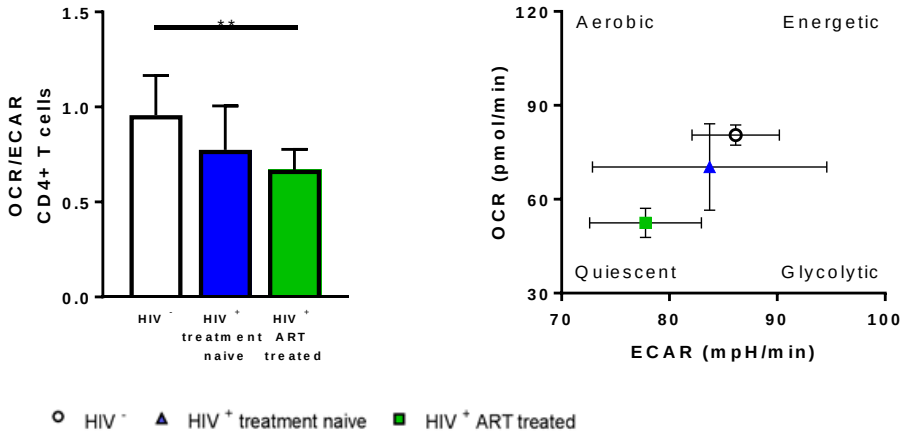

Figure 10: OCR/ECAR ratios of HIV negative, chronically HIV-infected treatment-naïve and HIV-infected ART-treated individuals

Schematic graphs of energetic profiles between ART-treated, chronic untreated and healthy individuals as shown in differences in OCR/ECAR ratio highlighting differences in the bioenergetics profiles between the three groups. Bar chart shows the mean value ± SD. Statistical significance was assessed by Kruskal-Wallis test with Dunn´s multiple comparison test and. RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

To understand the cause of the metabolic impairment in CD4 T cells we stratified all individuals by treatment regimens **(Table 01)**. Interestingly, DLG as a part of the triple therapy showed lower basal and maximal respiration compared to other non-INSTI ART regimens **(Figure 11)**.

List of Subjects

Subject	Gender	Age	Viral load (copies/ml)	Treatment	Storage Time (Days)
HIV⁺ treatment-naïve individuals					
35201242	m	55	295500	N/A	365
36203392	m	39	23010	N/A	15
35150397	m	41	7222	N/A	406
36166981	m	43	18360	N/A	76
36103741	m	34	8622	N/A	137
36224244	m	39	93400	N/A	16
B8535	m	N/A	54200	N/A	3670
B8952	m	N/A	36800	N/A	3729
36242355	m	38	34880	N/A	8
B512	m	N/A	156000	N/A	N/A
36276785	m	49	169	N/A	36
n=11	100% m	42 ± 7	66196 ± 88701	N/A	845.8 ± 1510.8
HIV⁺ ART treated individuals					
35186251	m	46	<40	DLG, ABC+3TC	395
35166402	m	67	<40	3TC, ETV, RAL, RTV, DRV	401
35137603	m	40	<40	DRV, RTV, RAL	403
35206003	m	27	<40	TDF+FTC+RLP	368
35150752	m	37	<40	TDF+FTC, RAL	370
36027663	m	27	<40	TDF+FTC, RAL	189
35189653	m	38	<40	TDF+FTC, RAL	399
36184623	m	45	<40	TDF+FTC+RLP	30
36095698	m	46	<40	TDF+FTC, DLG	146
35261700	m	29	<40	TDF+FTC, RAL	261
36128388	m	51	<40	AZT+3TC+ABC, TPV,RTV	159
31699824	m	52	<40	ABC+3TC+DLG	141
n=12	100% m	42 ± 12	<40	N/A	271.8 ± 133.2

Table 01: List of participants in *in-vitro* study

Characteristics of individuals. Plus-minus values are means ±SD. All participants in this study were men with no difference in age between these two groups. All participants receiving ART treatment had undetectable levels of viral RNA. Published (Korencak et al. 2019).

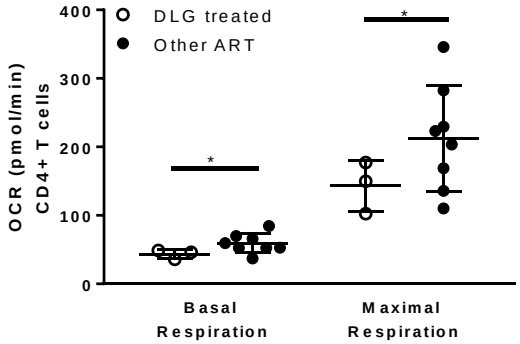

Figure 11: Respiration of DLG-treated CD4 T cells

Decreased oxidative phosphorylation of ART-treated HIV^{+} individuals receiving DLG-containing regimen (open circles) compared to PI, NNRTI (black circles) containing regimens. Published (Korencak et al. 2019).

Next, we investigated whether the negative impact of INSTI-containing regimens on cellular respiration can have also an impact on proliferation of the cells. For this purpose we conducted an *ex vivo* study involving 54 participants, who had been on the same treatment for at least 6 months and show full viral suppression **(Table 02)**.

A

Individuals Ex-Vivo Proliferation

Demographics		Overall	INSTI	NNRTI	PI	P-value
Age						0.1274
	Mean ± std	47.7 ± 13.0	47.8 ± 12.3	42.0 ± 12.3	52.3 ± 13.7	
Gender						0.1000
	Male	50	21	14	15	
	Female	4	3	0	1	
CD4 count‡						
	Median	573,5	596	494	539	0.1470
Days on treatment						
	Mean ± std	819 ± 790	365 ± 173	617 ± 360	1570 ± 1055	<0.0001
Storage Time (Days)						
	Mean ± std	554.5 ± 253.6	377.7 ± 117.9	594.1 ± 236.8	785.2 ± 221.7	<0.0001

B

INSTI		n
DLG	ABC+3TC	12
EVG	FTC+TAF	12
	total:	24
NNRTI		
RLP	FTC+TAF	13
RLP	FTC+TDF	1
	total:	14
PI		
RTV+DRV	FTC+TDF	11
RTV+ATV	FTC+TDF	3
LPV	FTC+TDF	1
RTV+ATV	FTC+TAF	1
	total:	16

Table 02: List of participants in *ex-vivo* study

(A) Characteristics of individuals used in ex vivo proliferation assay. Plus-minus values are means ±SD. (B) Individual ART regimens. All participants in this study had two NRTI as a back bone in combination with a third drug from INSTI, NNRTI or PI class listen in first column.50% of INSTI users were receiving DLG and 50% EVG. All of NNRTI users received RLP and majority of PI users received RTV in combination with DRV. Published (Korencak et al. 2019).

Individuals treated with INSTI-based ART regimen had significantly lower proliferation index compared to those, who were on PI-based or NNRTI-based regimens, but also compared to healthy controls **(Figure 12)**.

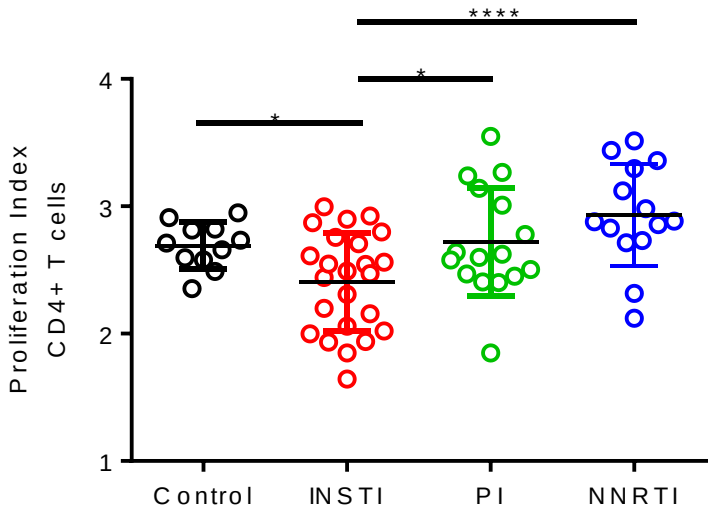

Figure 12: *Ex-vivo* proliferation of CD4 T cells

Significantly decreased proliferation of CD4 T cells of ART-treated HIV^{+} individuals receiving INSTI-containing regimen (red circles) compared to PI (blue circles) and NNRTI (green circles) containing regimens. Statistical significance was assessed by Kruskal-Wallis test with Dunn´s multiple comparison test and. RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

3.4. Two drugs, DLG and EVG from INSTI class negatively affect metabolism of CD4 T cells

To define the impact of commonly used anti-HIV drugs from each class on cellular metabolism, we treated CD4 T cells with antiretroviral medication at previously described plasma concentrations **(Table 03)** (Gish et al. 2002; Bergshoeff et al. 2004; Avihingsanon 2015; Yilmaz et al. 2009; Yilmaz 2009; Elliot et al. 2016; Bruno et al. 2001; Dickinson et al. 2015; Boffito et al. 2011). Before that, we confirmed the biological activity of DLG, EVG, RAL and TDF in *in vitro* experiment **(Figure 13)**.

Antiretroviral	Abbreviation	Plasma Concentration (ng/ml)		Chosen Concentration (ng/ml)	Source
		Median	Range		
Emtricitabine	FTC	717	21-1072	1000	Gish et al., 2002
Zidovudine	AZT	1150	710-1850	1500	Bergshoeff et al., 2004
Tenofovir DF	TDF	149	120-193	200	Avihingsanon et al., 2015
Tenofovir	TFV	NA*	NA*	200	
Lamivudine	3TC	2077	1264-2893	1500	Bruno et al., 2001
Rilpivirine	RLP	139	128-168	200	Dickinson et al., 2015
Raltegravir	RAL	448	37-5180	1000	Yilmaz et al., 2009
Dolutegravir	DLG	3908	3571-4245	4000	Elliot et al., 2016
Elvitegravir	EVG	1675	1557-1884	2000	Elliot et al., 2016
Darunavir ethanolate	DRV	3930	1800-12900	5000	Yilmaz et al., 2009
Ritonavir	RTV	746	646-1045	1000	Boffito et al., 2011

*variable plasma concentrations according to the prodrug

Table 03: Plasma concentrations of all antiretroviral medications

List of tested ART drugs with previously determined plasma concentration and concentration chosen in this study. Published (Korencak et al. 2019).

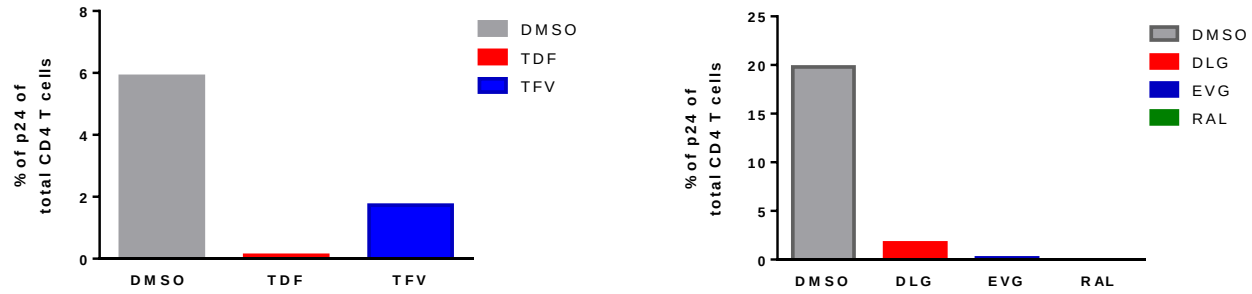

Figure 13: Biological antiviral activity of TDF, DLG, EVG and RAL

In vitro confirmation of biological antiviral activity of INSTI as well as TDV and TFV. (A) Comparison of HIV inhibitory activity of integrase inhibitors DLG, EVG and RAL showing that all 3 drugs were in their active form and able to inhibit HIV replication as measured by percentage of p24+ CD4 T cells. (B) Prodrug TDF (Tenofovir Disoproxil Fumarate) and its already cleaved form Tenofovir (TFV) showed comparable inhibition of HIV replication. Bar charts represent measurement of n=1 individual. Published (Korencak et al. 2019).

As shown on **Figure 14-A**, most of the tested antiretrovirals did not have any impact on respiration, however DLG and EVG (both INSTI) significantly decreased both basal and maximal respiration. Interestingly, we did not observe any alterations in glucose metabolism **(Figure 14-B)**.

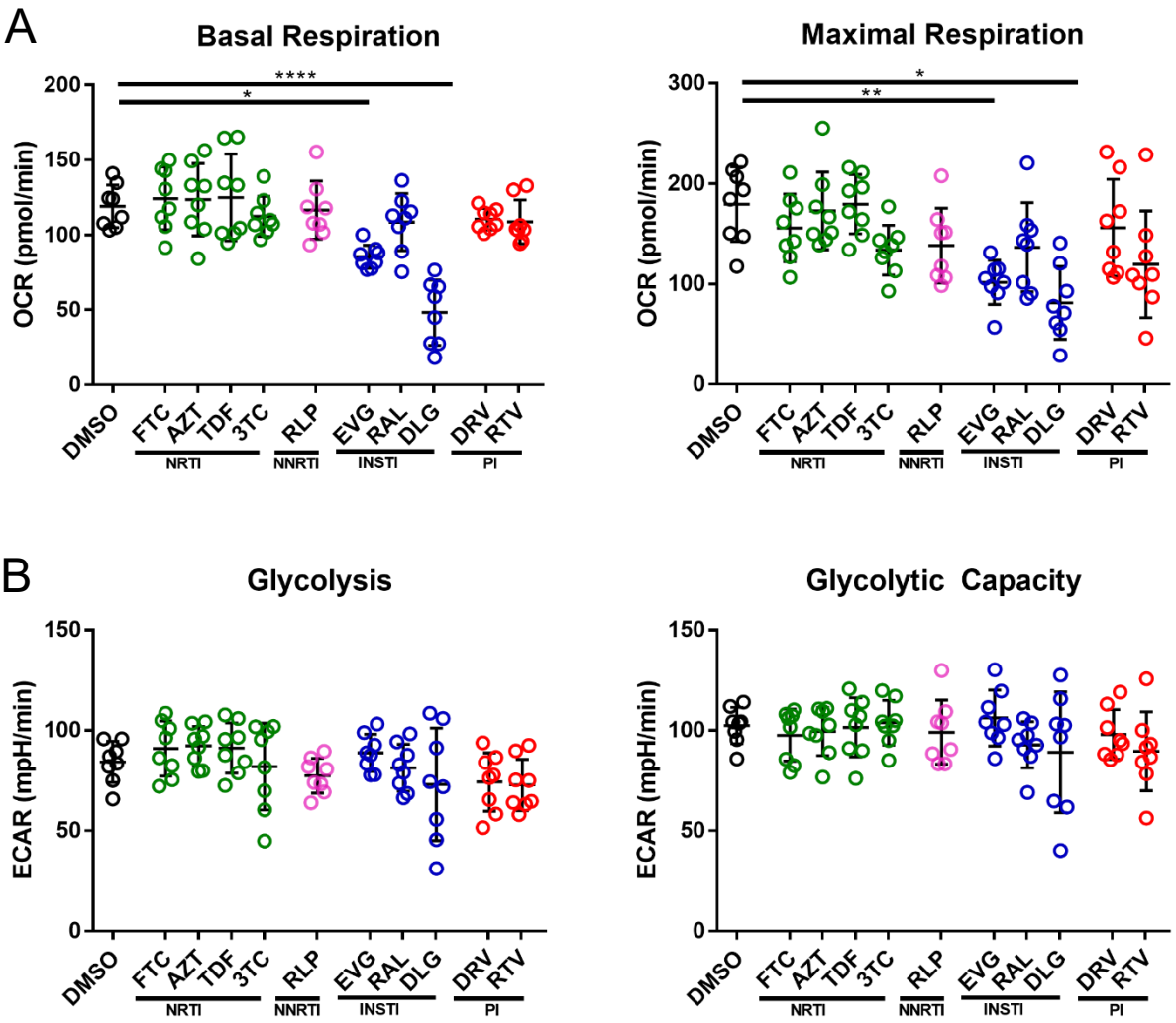

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Figure 14: Respiration of CD4 T cells treated with individual antiretroviral drugs

Assessment of impact of ARTs on CD4 T cell metabolism and functionality (n=8). (A) Basal and maximal respiration of CD4 T cells exposed to different ARTs for 3 days. Significant reduction in OCR was observed for CD4 T cells treated with DLG and EVG. (B) All ART show no impact on

glycolysis and glycolytic capacity. Plots show individual values with the mean ± SD. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

We next investigated whether we can detect any impact on glucose metabolism by western blot analyze of HIF1α, a transcription factor which induces transcription of genes involved in glucose metabolism. No changed in HIF1α were observed between cells treated with INSTI and other HIV drug classes **(Figure 15-A)**. Additionally, we performed RT-PCR to see whether there are differences in expression of genes for glucose transporter Glut1 or glycolytic enzyme PGK1. Similar as in previous experiment, no significant changes were observed **(Figure 15-B)** indicating that both INSTI – DLG and EVG – specifically interfere with mitochondria. Due to decreased respiration capacity, we hypothesized, that this interference is happening in electron transport chain located in inner mitochondrial membrane.

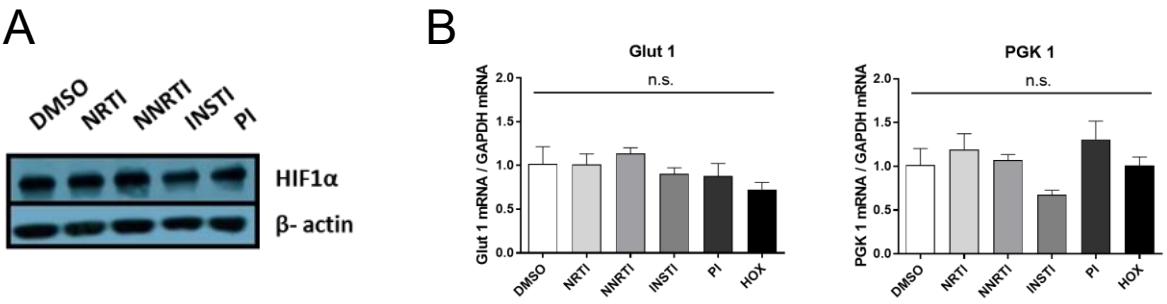

Figure 15: Western blot and RT-PCR of genes involved in metabolism.

HIF1α protein expression (A) and Glut1 and PGK1 mRNA levels (B) of cells treated with DMSO, NRTI (represented by FTC), NNRTI (represented by RLP), ISNTI (represented by DLG) and PI (represented by DRV) showing no differences. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test with no significant results. Published (Korencak et al. 2019).

We were curious, whether is the negative effect of DLG and EVG dose-depended. To find out, we treated CD4 T cells with the same drugs as previously, but at concentration 5-times higher than plasma concentration. The negative effect of both INSTI, EVG and DLG, was more profound than expected, causing complete metabolic shutdown in case of EVG, and bringing metabolism to minimum in case of DLG **(Figure 16)**. To ensure, that the decreased metabolism is not a result of a potential cytotoxicity of the drugs, we performed two independent viability assays. Both assays, the one based on Trypan Blue staining and also the one based on Annexin V staining, showed no cytotoxic effect **(Figure 17)**. Additionally, we decided to titrate down DLG to determine concentration, which has no impact on respiration. It was approximately 16-fold lower than plasma concentration **(Figure 18)**.

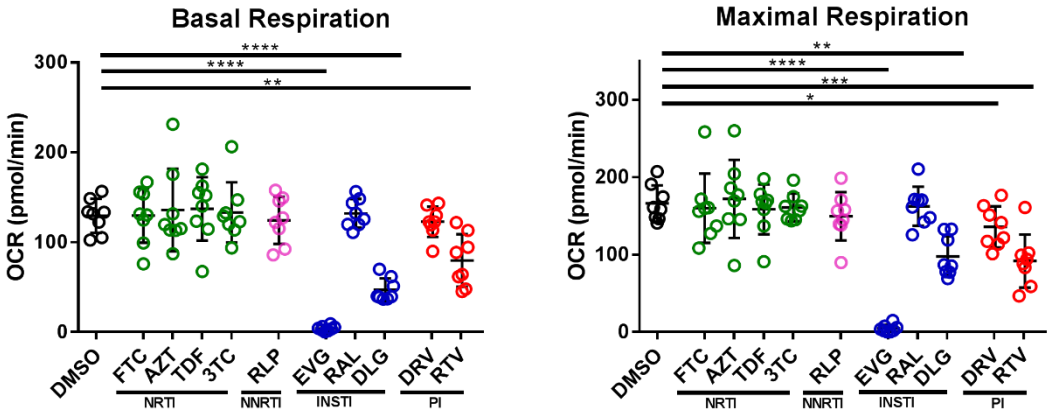

Figure 16: Effect of 5-fold plasma concentration ART on cellular respiration

Assessment of changes in respiration in the presence of 5-fold higher plasma concentration of ART. Significant decrease in basal (A) and maximal respiration (B) of CD4 T cells treated with DLG and EVG indicating dose-dependent reduction in metabolism. Plots show individual values with the mean ± SD. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

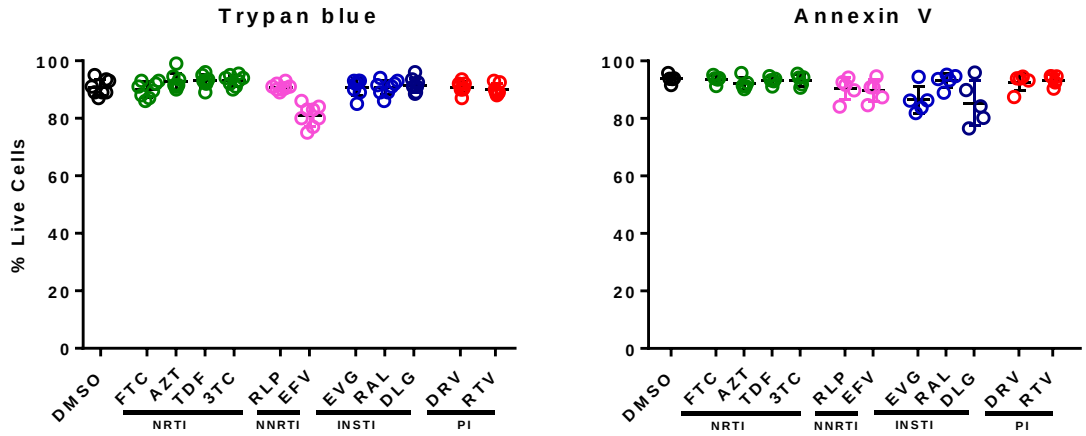

Figure 17: Viability test of CD4 T cells after ART treatment.

Assessment of cell viability in the presence of different ART over a time period of 3 days. Viability assed by Trypan blue and by Annexin V. Plots show individual values with the mean ± SD. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

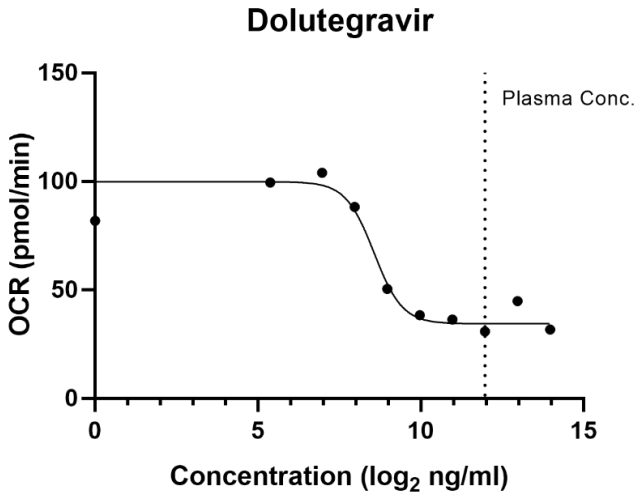

Figure 18: Titration curve of DLG

Titration curve of dolutegravir. The highest concentration of 16000 ng/ml (4-fold plasma concentration) was double diluted down to 125 ng/ml (1/32-fold plasma concentration). Additional concentration of 41.5ng was added into experiment as well as DMSO control. All concentrations are shown as log2. Published (Korencak et al. 2019).

Next, we used another approach to confirm interference of INSTI in mitochondrial electron transport chain. By adding different substrates into reaction and following uncoupling of mitochondrial membrane we could observe changes in oxygen consumption. For this purpose we used Oroboros instrument. As shown on **Figure 19**, cells treated with DLG had only mild increase in oxygen consumption after ADP injection compared to control cells (left arrows). The same pattern was observed after uncoupling mitochondrial membrane (right arrows).

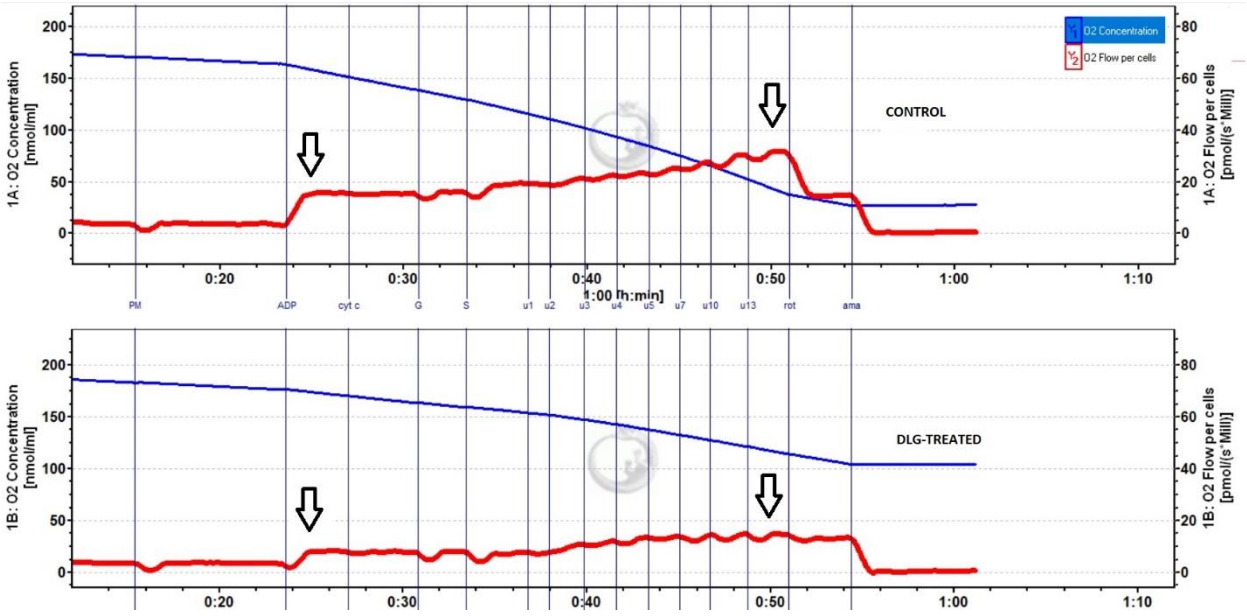

Figure 19: Oxygen consumption of CD4 T cells measured by Oroboros

Graphs showing O2 flow per 1 million cells (red line) and O2 concentration in reaction chamber over the course of experiment (blue line). Cellular respiration was decreased (indicated by

arrows) in cells, which were treated with DLG (lower graph) compared to control cells (upper graph).

3.5. DLG and EVG shift multifunctional immune response of CD4 T cells into monofunctional response

Studies have shown that multifunctional CD4 T cells immune responses might provide better help to CD8 T cells in terms of clearing an infection (Ferre et al. 2010; Emu et al. 2005; Teigler et al. 2017). The multifunctional profile of CD4 T cells pre-treated with antiretroviral substances was assessed by using a 5-marker functional panel after super antigen staphylococcus enterotoxin B (SEB) stimulation. Representative gating strategy is shown on **Figure 20**.

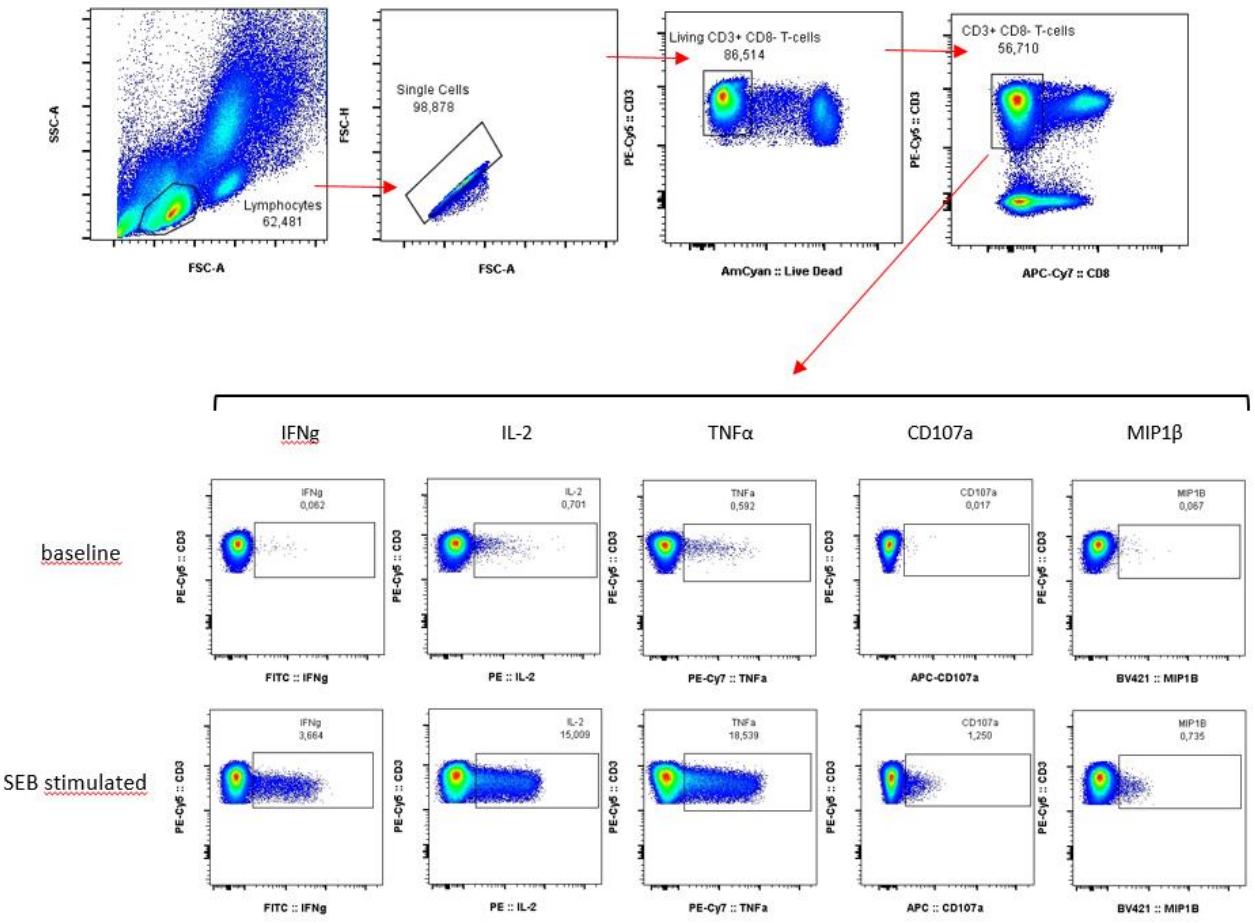

Figure 20: Representative gating strategy for ICS

Published (Korencak et al. 2019).

We observed that percentage of cytokine secreting CD4 T cells was significantly decreased when the cells were pre-treated with DLG and EVG, but not other drugs. The strongest effect was in case of IL-2 and MIP-1β **(Figure 21)**. Moreover, these two drugs also changed previously multifunctional immune response into a monofunctional response **(Figure 22)**.

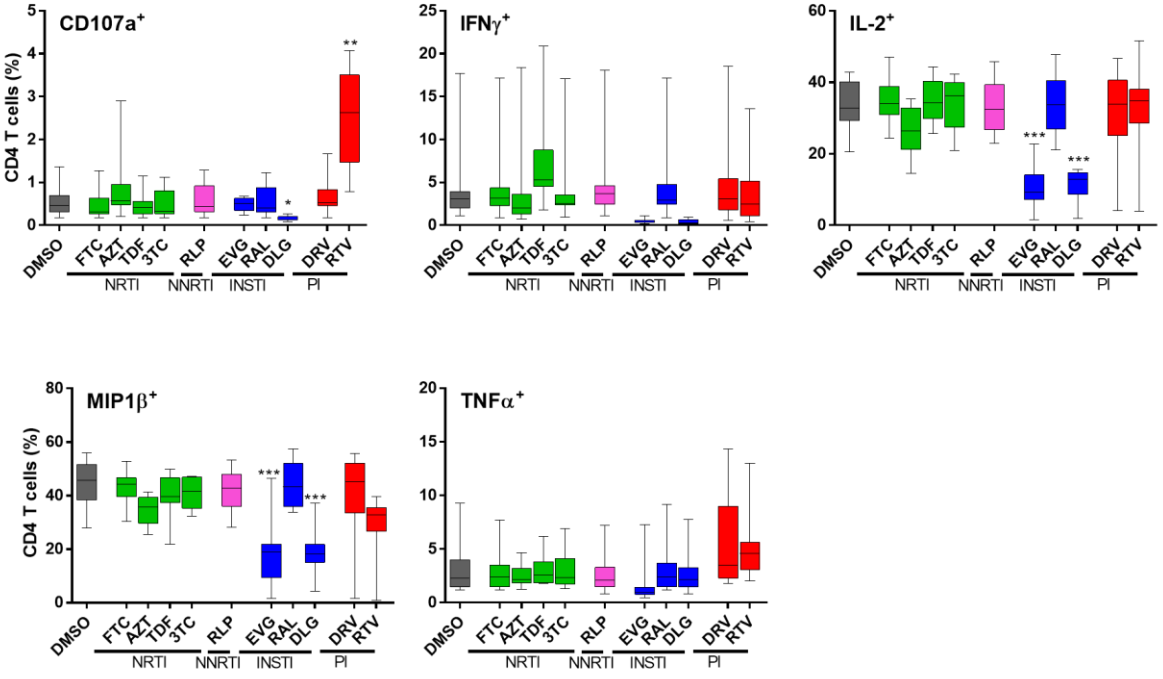

Figure 21: Multifunctional profile of CD4 T cells after ART-treatment

Assessment of CD4 T cell polyfunctionality after stimulation with SEB in the presence of different ART. Overall reduced functionality was observed in the presence of DLG and EVG for CD107a, IFNγ, IL-2, and MIP1β. Box plots show the median value within 5-95 percentile. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

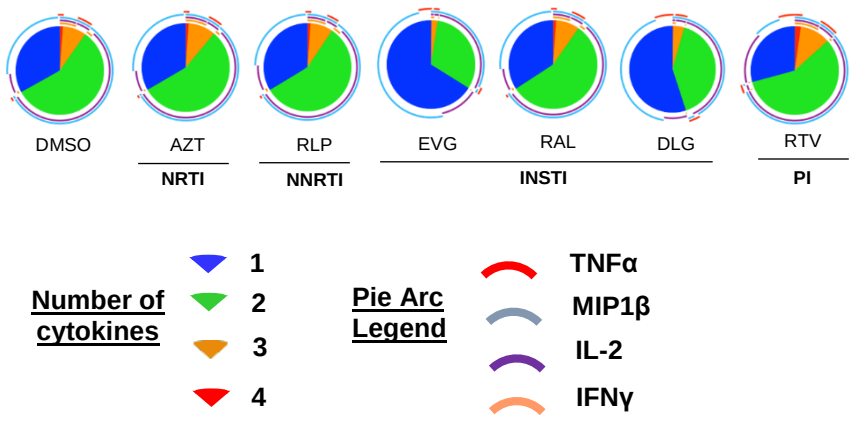

Figure 22: SPICE analyze of immune responses of CD4 T cells

SPICE analyze showing a shift in the polyfunctionality profile of CD4 T cells stimulated in the presence of DLG and EVG towards a TNFα dominating monofunctional response. Published (Korencak et al. 2019).

3.6. INSTI show mitochondrial toxicity

EVG is a modified quinolone antibiotic (Sato et al. 2006) of which some have shown mitochondrial toxicity (Suto et al. 1992). Taking in consideration previous findings, in particular, significantly impaired oxidative phosphorylation, we hypothesized that INSTI might interfere with mitochondria. It has been demonstrated that NRTIs can block polymerase-γ in mitochondria leading to a decrease in mitochondrial mass, measured as mitochondrial DNA (mtDNA) copy numbers (Lewis and Dalakas 1995). Therefore we decided to perform the same analyze of mtDNA copy numbers in CD4 T cells treated with individual antiretroviral substances for 3 days. Surprisingly, while we expected lower mitochondrial mass in cells which were treated with DLG or EVG and showed very low respiratory capacity, we observe an opposite effect – a significant increase in mtDNA copy number **(Figure 23-A)**. We hypothesized, that the cells are cumulating defective mitochondria, which are not able to provide oxygen for cellular processes, but instead, generating reactive oxygen species (ROS), which cause cell damage. To verify this hypothesis we performed an experiment in which we measured mitochondrial ROS

(mtROS) in CD4 T cells treated with ART substances. As expected, we observed a massive increase in mtROS production in cells treated with DLG and EVG, but also with RTV **(Figure 23-B)**. To our surprise, mitochondrial membrane potential was not affected **(Figure 24)**.

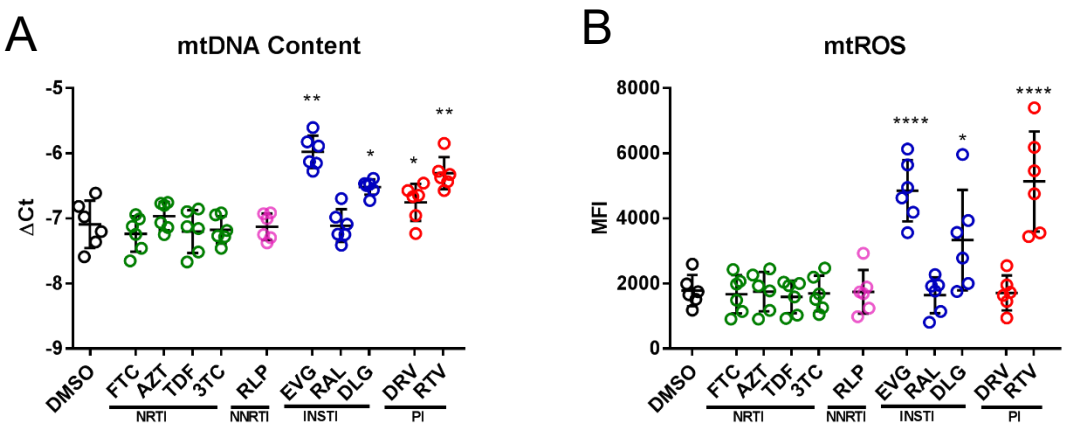

Figure 23: Mitochondrial toxicity of INSTI in CD4 T cells

Shift in mtROS production and mitochondrial content (measured as amount of mtDNA) after ART exposure for three days (n=6). (A) CD4 T cells exposed to DLG, EVG and RTV showed increased production of mtROS. (B) Increased mtDNA content of CD4 T cells upon incubation with DLG, EVG and RTV for 3 days. Plots show individual values with the mean ± SD. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). Published (Korencak et al. 2019).

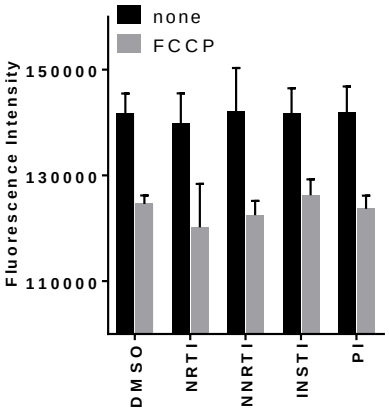

Figure 24: Mitochondrial membrane potential of CD4 T cells

Membrane potential without and with mitochondrial uncoupler FCCP. No differences between cells treated with INSTI class and other drug classes. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test with no significant results. Published (Korencak et al. 2019).

3.7. Increased serum concentration in culture media does not reverse the negative effect of INSTI on cellular metabolism and functionality

It is known that both INSTI – DLG and EVG are highly protein bound in human plasma. The interacting partner is serum albumin. Standard culture media contains 10% fetal cow serum (FCS). To prove that the negative effect of INSTI on metabolism is not due to the inability of the drug to bind serum albumin because of its low concentration in culture media, we set up an experiment in which we tested three different FCS concentrations in culture media – 10%, 30% and 50% and tested both 1-fold and 5-fold plasma concentration of DLG. As expected, we observed an increase in respiration in cells cultured in media with 30% or 50% FCS **(Figure 25-A)**.

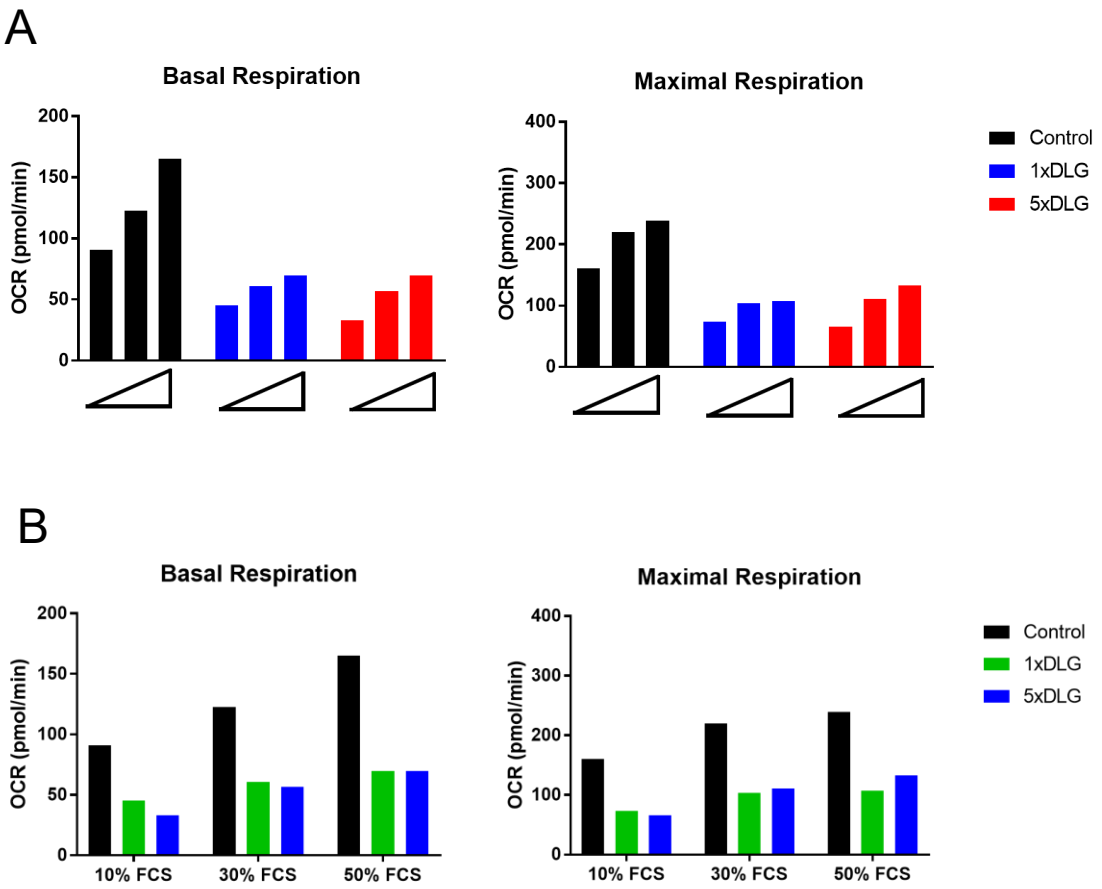

Figure 25: Impact of increased FCS concentration on respiration

(A) Increasing FCS concentration (triangles) 10%, 30% and 50% was corresponding to increased basal and maximal respiration in CD4 T cells, which were treated with 1-fold plasma concentration of DLG or 5-fold. (B) Basal and maximal respiration of cells treated with DLG remains decreased compared to control cells even in high concentration FCS in culture media. No statistical analyze was perform due to n=1.

However, this increase was not only observed in cells treated with DLG, but also in control cells. This is not surprising as FCS provides growth and proliferation factors to the cells. On **Figure 25-B**, we can see that the negative effect of DLG on metabolism persists even with very high serum concentration in culture media and cellular respiration of cells treated with DLG remains low also in high FCS concentration in media.

4. DISCUSSION

Antiretroviral therapy has changed the HIV infection outcome from a progressive, very often fatal condition, to a manageable chronic disease, which persists in the body (Deeks, Lewin, and Havlir 2013). People are no longer dying on AIDS, if they have access to appropriate treatment, but have a life-span comparable to the heathy population (May et al. 2014). Despite many great benefits, like restoration of immune cellular function, reduction in T cell activation or complete viral suppression, ART might induce many side-effects, referred as non-AIDS morbidity (Farahani et al. 2017). Therefore, a significant part of current research focuses on how to improve life quality of HIV-infected individuals by using novel approaches. Just recently, scientists started to pay more attention to a new field of research – immunometabolism. Many events in the cell, like immune activation or exhaustion, are tightly bound to the cellular metabolism (O'Neill, Kishton, and Rathmell 2016). Thus, immunometabolism might offer new strategies in effort to fight HIV. In our study, we describe that metabolism varies among different cell types with metabolically most active CD4 T cells. We found that untreated HIV infection rapidly decreases both cellular respiration and glycolysis, and this is linked to chronic immune activation. However, impaired metabolism might be reverted by ART. Interestingly, in case of CD4 T cells, the metabolism stays low even after ART initiation. We were able to identify two drugs from the INSTI class – DLG and EVG, which were responsible for perpetual metabolic shutdown of CD4 T cells. Moreover, these drugs also affected immune cellular responses in terms of decreased cytokine secretion. We assume that both drugs interfere in mitochondria, which was proven by increased mitochondrial ROS and mitochondrial mass.

A study analyzing metabolic differences between CD4 and CD8 T cells has shown, that CD4 T cells are metabolically more active (Renner et al. 2015). Our findings are consistent with this study. We found that CD4 T cells have the highest rate of glycolysis and cellular respiration. Moreover, we also analyzed the metabolic profile of B cells and NK cells. These cell subsets, in particular NK cells, have significantly lower metabolism compared to T cells. This demonstrates that cells have different metabolic requirements depending on their function in the immune system. It has been described, that HIV infection might have an impact on cellular metabolism. Datta et al. found that HIV

disrupts mitochondrial metabolism in macrophages (Datta et al. 2016). Another study showed that T cells increase mitochondrial mass and expression of genes involved in glycolysis during chronic HIV infection (Aounallah et al. 2016). We analyzed metabolic profile of HIV-infected treatment-naïve individuals and compared it to the healthy controls. We found that CD4 and CD8 T cells, B cells and NK cells isolated from these individuals have significantly impaired both basal and maximal respiration. Furthermore, we discovered a link between chronic immune activation in CD8 T cells and oxidative phosphorylation or glycolysis respectively.

Although many previous studies have shown that restoration of immune cellular function of T cells is possible after ART initiation (Rajasuriar, Wright, and Lewin 2015; Streeck et al. 2006), we observed contrary to this. We noticed that metabolism of CD4 T cells remain significantly decreased even in individuals undergoing ART treatment. Today, ART include a combination of antiretroviral agents resulting in decreased morbidity and mortality associated with HIV and AIDS. ART dramatically suppresses viral replication and reduces the HIV RNA below detectable levels, with following reconstitution of the immune system (Arts and Hazuda 2012). INSTI represent the newest class of anti-HIV drugs and regimens including INSTI have become the recommended first-line treatment strategy (Alejos et al. 2019). Integrase is an essential enzyme in the HIV replication process, and because of the lack of its homologue in human cells, it represents an ideal target (Hajimahdi and Zarghi 2016). Here we describe previously unknown impact of INSTI on metabolism and immune cellular function of CD4 T cells. We observed that while CD8 T cells, B cells and NK cells were able to restore their metabolic profiles after ART treatment, the metabolism of CD4 T cells remained significantly decreased, especially in individuals receiving INSTI-containing regimens. Impaired oxidative phosphorylation was detected when we exposed the cells to two drugs from the INSTI class – DLG and EVG, suggesting mitochondrial toxicity. Mitochondrial dysfunction during HIV treatment had been described more than two decades ago (Lewis and Dalakas 1995). It has been shown, that the mitochondrial pathogenesis is due to NRTI-induced inhibition of DNA polymerase-gamma, an essential enzyme in mitochondrial replication (Lewis 2003). Another possible mechanism involves the inhibition of endogenous nucleotide kinase followed by generation of ROS resulting in mutations in

both, nuclear and mitochondrial DNA (Gerschenson and Brinkman 2004). We showed that the cells treated with INSTI displayed changes in ROS production as well as mitochondrial mass, indicating that the mechanism of mitochondrial toxicity might be similar as seen in NRTI.

EVG was derived from quinolone antibiotics and therefore is its chemical structure very similar to them (Sato et al. 2006). A study has described that these antibiotics induce mitochondrial dysfunction by interfering with the electron transport chain in mitochondria and producing high levels of ROS (Kalghatgi et al. 2013). The effect of DLG and EVG we observed was comparable with this study. We noticed significantly higher levels of mitochondrial ROS in INSTI-treated cells compared to those, which were treated with other drug regimens. Moreover, we saw an increase in mitochondrial DNA. These findings indicate an interference of INSTI with mitochondria.

Apart from the pathologic changes in metabolism, which we were able to link to mitochondrial toxicity, we also observed changes in immune responses in cells treated with EVG and DLG. After TCR-triggered stimulation using SEB, we saw a massive decrease in cytokine production, in particular MIP1-β and IL-2. The cells lost their ability to secrete several cytokines at once, providing polyfunctional immune response, and switched to monofunctional response dominated by secretion of TNF-α. Interestingly, TNF-α production has been previously linked to increased oxidative stress and increased ROS in mice (Habtetsion et al. 2018).

Although many previous studies and clinical trial showed safety and efficacy of INSTI compared to individuals receiving PI- or NNRTI-based regiments (Jacobson and Ogbuagu 2018; Bruzzese et al. 2018), the data from our study are in contrast to this. There is still more evidence indicating the possible toxicity of INSTI. A study on a large cohort of pregnant women revealed that the prevalence of neural-tube defects in newborns was higher when the mother was on DLG-containing regiment compared to other ART (Zash et al. 2019). Another study documents a case of acute myocarditis after a switch to DLG (Eyer-Silva, Rosa da Silva, and da Cunha Pinto 2019), and neuropsychiatric adverse events have been also associated with INSTI usage (Hoffmann and Llibre 2019).

In conclusion, we have shown that untreated HIV infection is associated with changes in metabolism of immune cells. It disrupts cellular respiration, which is in correlation with overall immune activation in case of CD8 T cells. We were able to prove that while other cell types were able to restore their metabolic profiles after ART initiation, the metabolism of CD4 T cells remains decreased. We identified two drugs –DLG and EVG, both INSTI, which were responsible for the metabolic shut down. Moreover, these drugs not only impacted metabolism, but also caused a degradation of multifunctional immune response of CD4 T cells to a monofunctional TNF-α dominated stress response. We identified an interference of INSTI with mitochondria, however there is still a gap in knowledge explaining the mechanism of INSTI-induced toxicity.

5. SUMMARY

Metabolism plays a key role in a cell's ability to maintain their viability and fulfil their effector functions. It has been shown that cells in chronically HIV-infected individuals become exhausted and undergo a progressive loss of hierarchical functions, but the changes in their cellular metabolism remain unclear. Discovering and finding potential changes in the metabolism might offer a new potential targets in cure of the infection.

To understand changes in immune metabolism in subjects with chronic HIV infection, we firstly assessed differences in metabolism of different cell subsets. We described that individual immune cell subsets have quite distinct metabolic requirements. We showed that the metabolism of T cells, in particular CD4 T cells is significantly higher compared to B cells or NK cells.

When we compared the metabolic parameters between chronically HIV-infected treatment-naïve and HIV negative individuals, we observed that the HIV infection negatively affects metabolism, mainly cellular respiration. This was in negative correlation with immune activation and exhaustion.

The progression of HIV infection and immune dysfunction can be slowed down or even reverted with appropriate ART. We analyzed whether is metabolism of immune cells restored after ART initiation. CD8 T cells, NK cells and B cells were able to perform both glycolysis and oxidative phosphorylation compared to healthy subjects, however CD4 T cells maintained low respiration. To see which class or better which drug is responsible for the metabolic shut down, we performed set of *in vitro* experiments when we treated CD4 T cells with individual drug regimens. We saw a very strong impact of two drugs from INSTI class – DLG and EVG. They both not only significantly reduced cellular respiration but also weaken the ability of the cells to secrete cytokines.

Due to the fact that EVG was developed from quinolone antibiotics which were shown to cause mitochondrial toxicity, we decided to see whether there are some physiological changes in mitochondria of our treated cells. We found, that the cells which were treated with either EVG or DLG significantly increased the mitochondrial mass and at the same time also mtROS. In this case, more mitochondria does not mean better respiration of

the cells. Increased mtROS indicates that the cell might have more mitochondria, but these are not functional.

Taken together, our data demonstrate a substantial disruption in the metabolic activity of lymphocytes during chronic HIV infection that is restored through antiretroviral therapy. However, two INSTI, DLG and EVG, diminish the metabolic activity in CD4 T cells, leading to a switch in functionality and impairment of overall function.

6. ZUSAMMENFASSUNG

Der Stoffwechsel spielt eine Schlüsselrolle für die Fähigkeit einer Zelle, ihre Lebensfähigkeit zu erhalten und ihre Effektorfunktionen zu erfüllen. Es hat sich gezeigt, dass Zellen bei chronisch HIV-infizierten Personen erschöpft sind und fortschreitend wichtige Funktionen verlieren. Allerdings bleiben die Veränderungen in ihrem Zellstoffwechsel während einer HIV-Infektion bisher unklar. Das Entdecken und Auffinden möglicher Veränderungen im Stoffwechsel könnte neue potenzielle Ziele für die Heilung der Infektion darstellen.

Um die Veränderungen des Immunstoffwechsels bei Patienten mit chronischer HIV-Infektion zu verstehen, haben wir zunächst die Unterschiede im Stoffwechsel verschiedener Zelltypen untersucht. Wir haben beschrieben, dass einzelne Untergruppen von Immunzellen ganz unterschiedliche Stoffwechselanforderungen zu haben scheinen. Wir haben gezeigt, dass der Metabolismus von T-Zellen, insbesondere der CD4-T-Zellen, im Vergleich zu B-Zellen oder NK-Zellen signifikant erhöht ist.

Beim Vergleich der Stoffwechselparameter zwischen behandelten chronisch HIV-infizierten, therapienaiven und HIV-negativen Personen stellten wir fest, dass die HIV-Infektion den Stoffwechsel, hauptsächlich die Zellatmung, negativ beeinflusst. Dies korrelierte negativ mit der Immunaktivierung und Erschöpfung.

Das Fortschreiten der HIV-Infektion und der Immunschwäche kann durch eine entsprechende ART-Therapie verlangsamt oder sogar rückgängig gemacht werden. Wir analysierten, ob der Metabolismus von Immunzellen nach ART-Initiation ebenfalls wiederhergestellt werden kann. CD8-T-Zellen, NK-Zellen und B-Zellen waren im Vergleich zu gesunden Probanden in der Lage, sowohl Glykolyse als auch oxidative Phosphorylierung durchzuführen. CD4-T-Zellen dagegen behielten eine niedrige Zellatmung bei. Um herauszufinden, welche Klasse oder sogar welches Medikament für den Stoffwechselstillstand verantwortlich ist, haben wir eine Reihe von *In-vitro*-Experimenten durchgeführt, bei denen wir CD4-T-Zellen mit einzelnen Medikamenten behandelt haben. Wir konnten einen sehr starken Einfluss von zwei Medikamenten aus der INSTI-Klasse identifizieren - DLG und EVG. Sie reduzierten nicht nur signifikant die

Zellatmung, sondern schwächten auch die Fähigkeit der Zellen, Zytokine zu exprimieren.

Die Struktur und Entwicklung von EVG entstammt aus Chinolon-Antibiotika, von denen bereits gezeigt werden konnte, dass sie mitochondriale Toxizität verursachen. Aufgrund dieser Tatsache haben wir uns dazu entschieden, mögliche physiologische Veränderungen in den Mitochondrien nach Behandlung der Zellen mit einzelnen Medikamenten zu untersuchen. Wir fanden heraus, dass in Zellen, die entweder mit EVG oder DLG behandelt wurden, die mitochondriale Masse und gleichzeitig auch mtROS signifikant erhöht waren. In diesem Fall bedeuteten mehr Mitochondrien allerdings keine bessere Zellatmung und eine erhöhte Konzentration von mtROS deutete darauf hin, dass die Zelle möglicherweise eine erhöhte Anzahl an nicht funktionieren Mitochondrien besitzt.

Zusammengenommen zeigen unsere Daten eine erhebliche Störung der Stoffwechselaktivität von Lymphozyten während einer chronischen HIV-Infektion, die durch eine antiretrovirale Therapie behoben werden kann. Zwei INSTI-Medikamente, DLG und EVG, verringern jedoch die Stoffwechselaktivität in CD4-T-Zellen, was zu einer Verschiebung der Funktionalität und einer Beeinträchtigung der Gesamtfunktion führt.

7. LIST OF PUBLICATIONS

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Contribution

Experiments conducted only by the author (Marek Korencak):

Figures: 01-04

Figures: 07-11

Figure: 14

Figure: 16

Figures: 18-20

Figures: 23-25

Experiments conducted with support of others:

Figures: 05, 06 (Bruce Schultz, MTA, Institute for HIV Research)

Figure: 17 (Enrico Richter, PhD student, Institute for HIV Research)

Figures: 21, 22 (Enrico Richter, PhD student, Institute for HIV Research)

Experiments conducted by others:

Figures: 12, 13 (Enrico Richter, PhD student, Institute for HIV Research)

Figure: 15 (Buena de los Reyes, MTA; Sandra Winning, PhD, Institute for Physiology)

Patrick Juszczak (Institute for HIV Research) was involved in data collection, in particular information about the treatment of study participants.

Some experiments from other co-authors from the JCI Insight article: Effect of HIV infection and antiretroviral therapy on immune cellular function (Korencak et al. 2019; PMID: 31217351) were not published in the final version of the publication.

Abbreviations

AF	alexa fluor
AIDS	acquired immune deficiency syndrome
APC	allophycocyanin
BV	brilliant violet
CD	cluster of differentiation
CO2	carbon dioxide
Cy7	cyanin dye 7
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ECAR	extracellular acidification rate
FACS	fluorescence-activated cell sorting
FCS	fetal cow serum
FITC	fluorescein isothiocyanate
HCV	hepatitis C virus
HIV	human immunodeficiency virus
INF	interferon
INI	integrase inhibitor
INSTI	integrase strand transfer inhibitor
LCMV	lymphocytic choriomeningitidis
NK	natural killer
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor

OCR	oxygen consumption rate
PAMP	pathogen-associated molecular patterns
PD-1	programmed cell death protein 1
PI	protease inhibitor
PMBC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PHA	phytohaemaglutinin
RNA	ribonucleic acid
ROS	reactive oxygen species
RPMI	Roswelt park memorial insitute medium
TCR	T cell receptor

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Erklärungen

Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. 2, g) der Promotionsordnung der Fakultät für Biologie zur Erlangung der Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema „Immunometabolism in chronic HIV infection“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Marek Korencak befürworte und die Betreuung auch im Falle eines Weggangs, wenn nicht wichtige Gründe dem entgegenstehen, weiterführen werde.

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