



# Inflammatory Cytokines in Persistent Low-Level Viremia in Human Immunodeficiency Virus-1 Patients on Combination Antiretroviral Therapy in Western Kenya

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## Abstract

HIV-1 low level viremia is a consequence of virologic failure and has been shown to be caused by dysregulated cytokine responses in HIV-1 cART patients. Very little has been done to elucidate the level of IL-17, IFN- $\gamma$ , IL-10 and TGF- $\beta$  in HIV-1 patient on cART in Kenya. Compared to genotypic and phenotypic tests, cytokine assays are cheaper and so employing the use of IL-17, IFN- $\gamma$ , IL-10 and TGF- $\beta$  may offer one of the solutions to prediction of low-level viremia in HIV-1 cART patients, as these cytokines may be key in development of therapies to minimize low level viremia in HIV-1 patients on cART. This review summarizes the latest understanding of pro-inflammatory (IL-17, IFN- $\gamma$ ) and anti-inflammatory (IL-10, TGF- $\beta$ ) cytokine activity in peripheral blood of HIV-1 patients, as potential predictors of low-level viremia, and virologic failure in HIV-1 patients on cART, and gives proposals on mechanisms to develop therapies based on these cytokines, to be used in minimizing low level viremia occurrence.

**Keywords:** HIV-1; Persistent Low-level viremia; Combination antiretroviral therapy; Interleukin-17; Interleukin-10; Interferon gamma gamma; Transforming growth factor beta

## Introduction

Combination antiretroviral therapy (cART) is meant to suppress and maintain viral load (VL) in human immunodeficiency virus-1 (HIV-1) patients at lowest detectable levels using the conventional viral load testing platforms. After periods of suppression, patients on cART have often reverted from viral suppression to viremia [1]. Viremia in HIV-1 patients on cART may lead to development of HIV-1 drug resistant mutants and has been associated with virological failure, and this has a consequence of morbidity and mortality in the patients. Cytokines dysregulation could exacerbate persistent low-level viremia development and subsequent virologic failure in HIV-1 patients on HAART. To gain insight into the immunological predictors' persistent low-level viremia in HIV-1 patients on cART, the levels of IL-17, IFN- $\gamma$ , IL-10 and TGF- $\beta$  in HIV-1 patients on first line with low level viremia and cART adherent may be explored to relate the level of the cytokines to HIV-1 persistent

low-level viremia, virologic failure, and disease progression. Combination antiretroviral therapy (cART), previously called highly active antiretroviral therapy (HAART) suppresses human immunodeficiency virus-1 viral replication to undetectable levels, delays development of mutant strains, and improves immunological status of human immunodeficiency virus-1 (HIV-1) patients [2,3]. An important global sustainable goal geared towards ending the human immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDs) epidemic by 2030 is achievable through strategies which include attaining viral load suppression in over 95% human immunodeficiency virus-1 patients on combination antiretroviral therapy [4]. In efficiently controlling human immunodeficiency virus-1 progression, combination antiretroviral therapy has transformed the pandemic into a manageable chronic disease, however eradication remains an objective due to virus persistence and rebound [5]. Viral load is reported to drop when patients are introduced to and successfully respond to combination antiretroviral therapy [6].

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Studies have shown that, despite viral suppression, low level viremia still occurs in human immunodeficiency virus-1 patients on combination antiretroviral therapy [7,8]. Persistent low level viremia has been associated with viral genotype resistance, adherence difficulties, acquired immunodeficiency syndrome events, and virologic failure [9,10].

## Cytokines

Cytokines are proteins secreted by cells of the body to act on other cells, or on the cells that produced them through regulation and influencing of immune response [11]. An imbalance in T helper 1 (Th1) and T helper 2 (Th2) cytokines in human immunodeficiency virus-1 patients on combination antiretroviral therapy could provide some of the answers revolving around virologic rebound and low level viremia (Ma et al., 2019a). There is need for insight into human immunodeficiency virus-1 - immune system interaction in the wake of persistent low-level viremia, with the objective of maintaining viral suppression and minimizing low level viremia in human immunodeficiency virus-1 patients on combination antiretroviral therapy, thereby contributing to the advancing field of immunology. Characterization of cytokines that constitute human immunodeficiency virus-1 reservoir maintenance is important in pathogenesis of human immunodeficiency virus-1, as cytokines could be used in monitoring of disease prognosis during therapy, to maintain the viral load at lowest detectable levels, which would minimize virologic failure. To add to this, cytokines immunotherapy could be a promising therapeutic option in human immunodeficiency virus-1 patients on combination antiretroviral therapy (cART). The cytokines, while working in cell signalling, mediate cell-to-cell interaction in higher organisms, through forming complex networks which are altered in infections such as human immunodeficiency virus-1 [12]. Being modifiers and effectors of innate and adaptive immune system inflammation, cytokines are responsible for intercellular communication and immune regulation [13]. These proteins are produced by T lymphocytes, which are the major effectors in cellular immunity, and work by mediating inflammation and regulating other immune cells [14]. The multifaceted interaction of cytokines in viral infections has been shown to be involved in disease pathogenesis [15]. Cytokines, are biomarkers of inflammation and may be used in monitoring disease progress and also for therapy. The various immune biomarkers found in human immunodeficiency virus-1 infection have been associated with disease progression, and during the infection a number of cytokines including interferon gamma are produced.

Cytokines are categorized into pro-inflammatory and anti-inflammatory, and their release leads to the release and production of other cytokines, bringing out the “cytokine storm” phenomenon. It has been shown that, in human

immunodeficiency virus-1 infection, various immune biomarkers have been associated with disease progression. T cell activation and biomarkers of inflammation are thought to predict human immunodeficiency virus-1 disease progression, as high levels have been found in peak viremia [16]. While pro-inflammatory cytokines are against infection and injury, anti-inflammatory cytokines limit the injurious effects of pro-inflammatory cytokines [17]. There have been suggestions that the immunologic profiles and cytokine expression in human immunodeficiency virus-1 patients is more proinflammatory than immunoregulatory [18]. In acute human immunodeficiency virus-1 infection, it has been shown that, cytokine storm that ensues, is followed by the production of immunoregulatory cytokines. Additionally, Human immunodeficiency virus-1 (HIV-1) infection has been shown to lead to upregulation of various cytokines, and the persistence has been demonstrated even in combination antiretroviral therapy inhibition of viral replication. In human immunodeficiency virus-1 infection, there is a general tendency of increased cytokine levels due to progression of immunodeficiency, and when combination antiretroviral therapy has not been initiated and the trend is reversed on combination antiretroviral therapy initiation [19]. The cytokine suppresses the regulator of viral transcription, and exhibit non-cytolytic antiviral activity [20]. It has been suggested that, in early stages of human immunodeficiency virus-1 infection, Th1 response dominates, while during chronic infection, Th2 response is predominant, leading to increased production of interleukin 10, among other immunosuppressive cytokines [21]. Human immunodeficiency virus-1 (HIV-1) disease progression leads to enhanced secretion of pro-inflammatory cytokines, as several studies have revealed a correlation between the cytokines, with human immunodeficiency virus-1 viral load [22]. As earlier noted, cytokines which increase over the course of chronic human immunodeficiency virus-1 infection include the anti-inflammatory proteins such as interleukin 10 and transforming growth factor -Beta. It has been suggested that, various cytokines release is a characteristic of increased viral replication [23]. Similarly, both proinflammatory and immunosuppressive cytokines have been shown to inhibit viral replication by favouring human immunodeficiency virus-1 latency [24], as the increased production of pro inflammatory cytokines is an important facet of human immunodeficiency virus-1 induced chronic immune activation.

## Interleukin-17

Interleukin-17 is a cytokine produced by cells of immunity, notably Th17 cells, gamma delta T cells, natural killer T cells (NKT cells), Cell differentiation antigen 8 T cells, neutrophils, natural killer cells (NK cells), mast cells, and microglia. As interleukin-17 is a proinflammatory cytokine which mediates immunopathology and inflammation, this pro-inflammatory

cytokine as produced by Th17 cells recruits neutrophils and monocytes to the site of infection, and its activation leads to downstream of chemokines and cytokines such as MCP-1, IL-21, IL-8, IL-6 and IL-1 [25]. In a study done by elevated interleukin-17 was associated with human immunodeficiency virus-1 transmission [26]. Interleukin-17 has been shown to play a critical host immune response protective role in infections such as viral, bacterial, and fungi. In maintaining tissue integrity, interleukin-17 plays a pivotal role particularly at epithelial barrier sites, via generating protective immune responses to microbes. Additionally, the pro-inflammatory function exerted by interleukin-17 plays a critical role in inflammatory conditions, occasioned by stress proteins, microbial metabolites, and pathogen Associated Molecular Patterns (PAMPs) [27]. Alongside a myriad of inflammatory cytokines, interleukin 17 has been found to be a foremost proinflammatory cytokine which plays a salient role in generation of a protective immune response [28]. Interleukin 17 has further been thought to characterize human immunodeficiency virus-1 disease severity. Interleukin 17 and concerted actions of other proinflammatory and immunoregulatory cytokines, by which the final immunological response and severity of the viral infection is depended. This may partially explain the pathogenic and protective functions of interleukin 17, in disparate settings of inflammation.

While interleukin 17 (IL-17) has been shown to play a pivotal role against viruses through regulation of the immune responses, it is worthwhile noting that some studies have found reduced levels of interleukin 17 in human immunodeficiency virus-1 patients on combination antiretroviral therapy [29]. None the less, contradicting findings have found higher levels of interleukin 17 in human immunodeficiency virus-1 patients with viral loads less than 50 copies/ml [30], signifying there could still be levels of inflammation even in patients responding well to combination antiretroviral therapy. Other known functions of interleukin 17 include the promotion of neutrophil migration to a site of inflammation and playing a crucial protective role during intestinal inflammation. Additionally, Interleukin -17 also plays a significant role in promoting cytotoxic T – cell activity and enhancing Th1 immune response, modulating antiviral B-cell activities, inducing protective inflammatory responses. Interleukin 17 has been known to stimulate fibroblasts into the secretion of other cytokines such as Prostaglandin E2 (PGE2), granulocyte-colony stimulating factor (G-CSF), IL-6, and IL-8 [31]. Some studies have postulated that Interleukin -17 is produced in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients, with undetectable viral load. Interleukin 17 has also been indicated as a therapeutic target and biomarker in sepsis.

In limiting viral infection-induced pathology, interleukin 17 mediates protective immune responses, inhibits detrimental

inflammations, and contributes to maintenance of tissue integrity. Interleukin-17 has protective effects against viruses, bacteria, and parasitic infections, and high levels could predict a greater risk of sepsis progression. Studies have shown an increasing human immunodeficiency virus-1 replication association with interleukin 17 inflammatory activities. The potential to recruit phagocytes at high notch is what makes interleukin 17 response to be potentially dangerous in the midst of the many benefits. Interleukin 17 has been thought to play an important function in repair, pathology, and barrier surface protection. In response to invading virus, inducing excessive neutrophil migration and activation, antagonizing development of T regulatory cells, inducing Th2 immune responses, and promoting fibrosis development, are some of the mechanisms used by interleukin 17 in contributing to tissue damage during viral infections. Although reported mostly as associated with immunopathology, interleukin 17 plays a vital role in host defense. The successful operation of interleukin 17 in immunity arises partly from the synergistic activities with other factors, the role as a counterpart of interferon gamma, and the self-sustaining feedback loop.

It has been suggested that, in human immunodeficiency virus-1 infection, high levels of interleukin 17 correlate with high viral load [32] as higher interleukin 17 cytokine levels have been found in uncontrolled human immunodeficiency virus-1 infected patients. Through induction of innate-like acute immune defenses, such as chemokines, interleukin 17 signals mostly in non-hematopoietic cells. Additionally, interleukin 17 acts through increasing serum concentrations for GM-CSF and G-CSF and via acute phase proteins, to hasten the development of other required subsets of immune cells such as macrophages and neutrophils, over eosinophils and basophils. Generally, interleukin 17 is thought to have the main function of protection of tissues against the invasion of microbes, through the swift recruitment of phagocytes to the site of infection. This further kicks off antimicrobial factor induction, as well as maintaining recruited cells, and enhancing access to tissues. When chronically directed to inappropriate targets, interleukin 17 through the pro-inflammatory effect contribute to pathogenic inflammation. Thus, interleukin 17 centrally orchestrates immunity in humoral and cellular immunity in line with IL-4 and IL-13, and interferon gamma respectively. During functioning, interleukin 17 can recruit a large number of polymorph nuclear cells, and has the capability of maintaining these population of cells at the sites of infection. In the course of various infections, interleukin 17 is produced by lymphoid cells which include Tc17, gamma delta T cells, and Th17 cells, in the course of varied infections.

### **Interferon Gamma (IFN- $\gamma$ )**

Interferon Gamma(IFN- $\gamma$ ), as produced by leukocytes, was discovered in 1965 by Fredrick Wheelock, as the only member of

the type II interferon family as a soluble macromolecule antiviral factor, with strong pleiotropic immunomodulatory effects on innate and adaptive [33]. This pro-inflammatory cytokine has been shown to be produced mostly by T cells and natural killer cells, and works by increasing neutrophil and monocyte function, macrophage activation, has antiviral activities, and works on MHC-1 and II expression on cells. High levels of interferon gamma were associated with human immunodeficiency virus-1 disease progression. It has been well stipulated that interferon gamma, whose original function is natural antiviral activity may be effective in viral infections and disseminated multi-organ invasion. Studies have reported increased interferon gamma in successful combination antiretroviral therapy. Interferon gamma works by mediating the innate and adaptive immune responses. Interferon gamma is among the cytokines that characterize human immunodeficiency virus-1 disease severity. Interferon gamma is also known for influencing transcriptional regulation of a number of genes, as some studies have reported extreme susceptibility to infectious diseases when there is a disruption of interferon gamma gene or its receptor. During a cytokine storm, lysis of immune cells or T cell activation triggers interferon gamma release, which leads to immune cells activation, and subsequent pro-inflammatory cytokine release [34]. Interferons are important in control of various virus infections. As a signature cytokine in activated T cells, interferon gamma is the most potent macrophage activator, in addition to the modulation of both adaptive and innate immune networks. Interferon gamma has been reported as a key driver of cellular immunity, and orchestrates milliard protective functions in the process of heightening of immune responses in infections. Interferon gamma induces antigen specific regulatory B cells and T cells, which act in a counter-regulatory fashion in an immune reaction, leading to prevention and control of excess immune responses such as the occurrence in a cytokine storm that may be fatal. Patients with chronic human immunodeficiency virus-1 infection display CD4+ T cells, with upregulated interferon stimulated genes. Interferon gamma is among the cytokines thought to predict human immunodeficiency virus-1 disease progression. The functioning of interferon gamma is through enhancing of increasing leukocyte infiltration, affecting cellular apoptosis and proliferation. Persistent interferon signalling has been found in pathogenic primates. The expression of many interferon regulated genes is a characteristic of interferon response to human immunodeficiency virus-1 infection, and leads to an anti-viral state is establishment in both bystander and infected cells [35]. Various roles are played by interferon gamma in human immunodeficiency virus-1 pathogenesis (Roff et al., 2014). As stipulated earlier, while cells involved in the secretion of interferon gamma include activated CD4 T cells and Cell differentiation antigen 8 T cells, natural killer cells (NK cells), gamma delta T cells, natural killer T cells(

NKT cells) , B cells, monocytes, dendritic cells, and macrophages. The cytokine has been found to be produced from the leukocytes upon stimulation with a mitogenic plant lectin, phytohemagglutinin, and known to activate innate cell-mediated immunity, and stimulate adaptive antigen-specific immunity [36]. Some studies have postulated that interferon gamma is produced in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients, with undetectable viral load. Interferon gamma in human immunodeficiency virus-1 infection is detected early, at the acute phase, and remains continually produced throughout the course of infection. Some of the advantages of interferon gamma as a therapeutic agent are: Its effectiveness in viral infections has strongly been predicted, as a virus-specific antiviral therapeutics agent, it can be used in epidemics, and in new viral infections. The fact that interferon gamma is able to enhance natural killer cell and cytotoxic T cell activity against human immunodeficiency virus-1 infected cells signifies the relevance of this cytokine in the control of human immunodeficiency virus-1 replication.

Interferon gamma (IFN- $\gamma$ ) can through antigen presentation cells, amplify antigen presentation by cognate T-cell interaction, induce antiviral responses, and increase Reactive Nitrogen Intermediates (RNIs) and Reactive Oxygen Species (ROS). For viral infections, interferon gamma is used as a therapeutic agent, as it interferes with viral multiplication, eliciting potent anti-viral activity. The strong antiviral activities induced by interferons makes them a primary actor in pathogen defense. As a regulator of host immune response to a number of microbes, Interferon gamma has been shown to be involved in human immunodeficiency virus-1 pathogenesis. Additionally, interferon gamma has been shown to confer antiviral state through modulation of T cell and B cell differentiation and maturation, and can also activate local immune cells such as dendritic cells. It has been shown that, interruption of antiretroviral therapy predicts virologic rebound in human immunodeficiency virus-1 patients through the expression of type I interferon-associated genes. Interferon gamma activities revolve around immune modulation, proinflammation, and immune activation. During active infection, high levels of interferon gamma are produced, and acts on the regulation of antigen presentation by APCs, and the induction of class switching in B cells. Interferon gamma proinflammatory activities involve enhancing host immune responses through activation of phagocytic cells leading to oxidative burst stimulation and release of degradative enzymes. A lack of, or deficiency in interferon gamma secretion may lead to death as a result of susceptibility to infectious diseases. In early viral infections, pattern recognition receptors (PRR) engagement is triggered after recognition of pathogen associated molecular patterns (PAMPs), or danger associated molecular patterns (DAMPs). Molecular pattern recognition initiates antiviral state in antigen presenting cells and

the production of interferon gamma, which triggers innate immune response [37]. In addition to the induction of the maturation of macrophages toward a proinflammatory phenotype and antigen presentation, activation of macrophages and neutrophils diapedesis to the site of infection is promoted by interferon gamma. A study done by revealed elevated levels of serum IFN $\gamma$  in human immunodeficiency virus-1 patients prior to treatment with combination antiretroviral therapy, and decreasing levels with the initiation of combination antiretroviral therapy, while other studies have however demonstrated higher levels of interferon gamma even after combination antiretroviral therapy initiation, signifying that IFN $\gamma$  may either prevent or augment the pathogenesis of human immunodeficiency virus-1.

The proinflammatory antiviral response and immune regulation has made interferon gamma a potential biomarker that is used in immune competence and antiviral response evaluation in human immunodeficiency virus-1 patients. It has been shown that, in playing the key role in innate and adaptive immune responses, interferon gamma (IFN- $\gamma$ ) promotes differentiation of naive CD4+ cells into effector Th1 T cells leading to the antiviral activities, and immunity against intracellular infections. Interferon gamma (IFN- $\gamma$ ) also facilitates leucocyte migration, and the growth and maturation of other cells types such as glial cells, mesenchymal cells, and dendritic cells. In interfering with viral life cycle, interferon gamma inhibits virus entry, both at the extracellular and intracellular stages, inhibits viral replication, and disrupts gene expression, preventing translation. Interferon gamma has been shown to steadily increase throughout the acute stage of human immunodeficiency virus-1 infection. There is a postulation that interferon gamma levels decrease during chronic human immunodeficiency virus-1 infection to levels similar to healthy individuals. From findings of recent studies, human immunodeficiency virus-1 infection has been associated with the induction of interferon gamma response. Elevated levels of interferon gamma in human immunodeficiency virus-1 patients on combination antiretroviral therapy have been suggested to either control or enhance human immunodeficiency virus-1 disease, based on the human immunodeficiency virus-1 infection clinical stage. Several studies have found high levels of interferon gamma as sustained in human immunodeficiency virus-1 patients despite combination antiretroviral therapy, and with the high levels, there is a lower rate of comorbidities.

Interferon gamma (IFN- $\gamma$ ) plays a critical role in human immunodeficiency virus-1 pathogenesis through enhancing host resistance to infection, as evidenced by study findings in which higher levels of interferon gamma were found in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients [38] and the levels remain high in human immunodeficiency virus-1 patients on combination antiretroviral therapy. On the contrary, in their study observed reduced

interferon gamma levels in both combination antiretroviral therapy naïve as well as combination antiretroviral therapy experienced human immunodeficiency virus-1 patients, with a positive correlation with CD4 count, and a negative correlation with human immunodeficiency virus-1 viral load. Additional studies have reported, increase in interferon gamma in successful combination antiretroviral therapy [39], while levels are reduced before therapy and 12 months into therapy, [40] signifying that the cytokine response may be un affected in combination antiretroviral therapy, probably due to residual virus in immune sanctuaries, which necessitates persistent immune activation. There is an increasing suggestion that interferon gamma protects the host against viral infections, by inhibiting viral entry at intracellular and extracellular levels [41]. Furthermore, low viral load has been shown to correlate positively with interferon gamma human immunodeficiency virus-1 specific CD4+ T cells. Interferon gamma steadily increases in acute stage of human immunodeficiency virus-1 infection, and levels decline in chronic disease, to levels similar to those in healthy individuals. It has been postulated that Immunologic profiles, and cytokine expression is proinflammatory than immunoregulatory in human immunodeficiency virus-1 patients. Also, infection with human immunodeficiency virus-1 results in antigen presentation modification in dendritic cells and macrophages, leading to anergic state in T helper cells specific to human immunodeficiency virus-1.

Being a proinflammatory cytokine and in regulator of cytotoxic T cell response , interferon gamma, through direct activation of phagocytic cells triggers oxidative burst, and the release of degradative enzymes as synergy with other cytokines ensues [42]. Interferon gamma has been known to prevent viral replication and promotion of innate and adaptive immunity and so the proinflammatory antiviral response and immune regulation characteristics upgrades this cytokine as a biomarker for consideration in evaluating antiviral response and immune competence in human immunodeficiency virus-1 infection. Interferon gamma induces the secretion of proinflammatory cytokines by fibroblasts, epithelial cells, and endothelial cells and since it is produced in cytokine storm in acute stage of human immunodeficiency virus-1 infection, this cytokine is thought to affect the development of cytotoxic T lymphocyte functions in controlling human immunodeficiency virus-1 viral load. As human immunodeficiency virus-1 efficiently replicates in tissues and triggers the upregulation of a variety of cytokines, such as IFN-gamma, higher levels of interferon gamma have been found in combination antiretroviral therapy non-responders, and in combination antiretroviral therapy patients, a transient viremia of more than 1000copies/ml has been associated with high levels of this cytokine [43]. It may be plausible to note that, since some studies reported high levels of IFN  $\gamma$  in combination antiretroviral



therapy patients, as well as a number of patients in the same study recording low levels, the probable suggestion to this observation could be that, this may be attributed to genetic characteristics of human immunodeficiency virus-1 and individual differences in immune responses against human immunodeficiency virus-1, and no other confounding clinical events.

### **Interleukin-10 (IL-10)**

Interleukin-10, an anti-inflammatory cytokine is a cytokine synthesis inhibitory factor (CSIF), which works by attenuating T cells and Th17 cell response through regulatory CD4+ T cells. Pro inflammatory cytokines have been shown to impact the antigen sensitivity of Cell differentiation antigen 8+ T cells, while the interleukin 10, which is produced during chronic infection was associated with reduced Cell differentiation antigen 8+ T cell antigen sensitivity during the establishment of chronic infection. Additionally, this cytokine is thought to regulate immune homeostasis in health and disease. It is produced by T cells, B cells, and macrophages, among other immune cells, and inhibits mononuclear cell function and cytokine production [44,45]. This study found high levels of interleukin 10 in human immunodeficiency virus-1 patients with persistent low level viremia compared to interleukin 10 level in human immunodeficiency virus-1 suppressed patients, which agrees with the findings by Gorenec and team in which the levels of interleukin 10 were high in human immunodeficiency virus-1 patients on antiretroviral therapy, during the stage of chronic human immunodeficiency virus-1 infection. Threshold for CD8+ T cells activation is increased directly by interleukin 10. Additionally, the signalling of interleukin 10 inhibits Th1 cytokines production, and it stimulates Th2 cytokine production. Interleukin 10 was associated with seroconversion in human immunodeficiency virus-1 patients. Immunoregulatory cytokine interleukin 10 facilitates the early events of viral persistence. In the anti-inflammatory environment, high levels of interleukin 10 constrains the proliferative capacity of Th1 cells, and suppresses pro inflammatory responses which are meant to clear infection. Some studies have found that interleukin 10 is produced in human immune deficiency virus-1 combination antiretroviral therapy naïve patients, with undetectable viral load. It has been reported that, CD8+ T cells antigen sensitivity is decreased in chronic infection, and is directly mediated by interleukin 10. Interleukin modulated Co-inhibitory receptors expression, Immune metabolism, Tfl frequencies, gene signatures and proteins associated to cell survival, and maintenance of memory T cells, and this is associated with human immunodeficiency virus-1 reservoir persistence. Additionally, there are however, latently infected long lived memory T cells which persist, and therefore the residual virus prevents the eradication of the infected cells [46]. It is now postulated that, reservoir establishment and

persistence in human immunodeficiency virus-1 patients on combination antiretroviral therapy is contributed to by interleukin 10. Interleukin 10 characterizes human immunodeficiency virus-1 disease severity. Encoded by interleukin 10 gene in humans, interleukin -10 has been shown to be a key immunoregulatory cytokine. In inhibiting immune responses, interleukin 10 works by upregulating membrane suppressor molecules such as PD-L1, and PD-1 expression, leading to immune response activation suppression, through shifting inflammatory to anti-inflammatory immunity.

Interleukin 10 has also been shown to impair the capacity of TCR signal transduction of CD8+ T cells. For a strong and functional and proliferative response, T cell activation should ensue in organized process which involves three signals comprised of recognition of antigen, through T cell receptor of professional antigen presentation cell (APC) presented respective cognate antigen, co-stimulation receptor where the T cells bind to their respective ligand, and the termination signal, which comes later as immune response effector phase, followed by elimination of the pathogen. The Mgat5, which is a glycosyltransferase which enhances glycan branching on glycoproteins surface is induced by interleukin 10. Being an immunosuppressive cytokine, interleukin 10 signals through signal transducer and activator of transcription (STAT3) in regulation of T follicular helper germinal centre formation, and cell differentiation. Interleukin 10 directly restricts CD8+ T cells activation function through a regulatory loop in chronic viral infection such as human immunodeficiency virus-1 infection by modifying cell surface glycosylation. As seen earlier, In T cell exhaustion interleukin 10 is also involved, and this is quite different for chronic activation, as T cell persistent exposure to constant antigen leads to T-cell exhaustion. The capacity of CD8+ T cells to develop effector functions in low level antigen also called functional avidity or antigen sensitivity is dependent on efficient pathogen control. This happens particularly through inhibiting of the activation and maturation of innate immune cells such as macrophages, natural killer cells, and dendritic cells, and on the other hand, expanding the t regulatory cells, resulting to disease persistence. Impairment of CD8+ T cells function has been shown to be maintained by residual human immunodeficiency virus-1 replication, which enables the virus to persist even in combination antiretroviral therapy [47]. It has been postulated that suboptimal immune control of infections such as human immunodeficiency virus-1 ensues in T-cell exhaustion, interleukin 10 induction plays an important role in viral persistence establishment.

While interleukin 10 is produced by T regs as part of response by the body to chronic infection established by upregulation of pro-inflammatory cytokines, this critical component of in the immunosuppression network, is required to dampen the activities of proinflammatory cytokines after pathogen encounter.



Additionally, In human immunodeficiency virus-1 infection, high levels of interleukin 10 coincides with chronic disease progression, thus interleukin 10 induction features more in chronic infections. It has been reported by some studies that, there is a correlation between human immunodeficiency virus-1 reservoirs in human immunodeficiency virus-1 patients on combination antiretroviral therapy with interleukin 10 levels, and both transforming growth factor beta and interleukin 10 contribute to the immunosuppressive function of T regulatory cells. In cases where combination antiretroviral therapy is interrupted, viral rebound occurs. Clonal expansion takes place in human immunodeficiency virus-1 infected cells, and the clonally expanded cells increase with time. It has been postulated that, more than 50% of the clonally expanded cells are maintained through latent reservoirs. Viral rebound is contributed to by the human immunodeficiency virus-1 infected clonally expanding cells. While combined antiretroviral therapy (cART) reduces human immunodeficiency virus-1 transmission, maintains a good state of health, and suppresses human immunodeficiency virus-1 multiplication, it has been suggested that persistent virus leads to T cell exhaustion which is albeit established early in infection, and is a huge barrier to immune control of human immunodeficiency virus-1, greatly hindering the elimination of the virus. As such, there is a very fragile balance between progressive T cell exhaustion, and T cell mediated virus control. It is plausible to note that, starting combination antiretroviral therapy as soon as possible after diagnosis can reduce viral load to undetectable levels, and in the process restore immunological function.

Ideally, studies have reported that, when combination antiretroviral therapy is initiated, a greater number of human immunodeficiency virus-1 patients have a reduction in human immunodeficiency virus-1 viral load. Human immunodeficiency virus-1 affects the CD4 receptors on immune cells leading to improper functioning of the immune system, however, it has been well articulated that so far, combination antiretroviral therapy only reduces viral replication, but does not cure human immunodeficiency virus-1. In absence of effective therapy, inflammation characteristic of human immunodeficiency virus-1 infection ensues, and it is associated with immune cells changes, failure of immune reconstitution under combination antiretroviral therapy, a decrease in antiviral response, and a damage to organs. Interleukin 10 has been shown to promote the differentiation of CD4+ T cells into a Th2 phenotype, and increased values have been found in human immunodeficiency virus-1 infection. The formation of galectin 3-mediated membrane lattice was promoted by increased CD8+ T cell N-glycan branching, and restricted key glycoprotein and so increasing T cell activation required antigenic threshold. The serum levels of interleukin 10, which is a prototypical anti-inflammatory cytokine plays the

immunosuppressive role, and its serum levels are associated with high viral load, and human immunodeficiency virus-1 progression, and levels are decreased in effective combination antiretroviral therapy. Combination antiretroviral therapy decreased the level of interleukin 10. As reported earlier, Low level viremia of 500-999 copies /ml was associated with virologic failure [48]. Some studies have suggested that viral suppression was found in human immunodeficiency virus-1 combination antiretroviral therapy suppressed patients for a couple of years, and the improper immune activation has been associated with development of low-level viremia.

In the course of controlling and elimination of foreign substances, activated T cells may inflict irreparable damage to target cells. Though it is produced by regulatory CD4+T cells, interleukin-10 is produced by other cells that include macrophages, dendritic cells, natural killer cells, monocytes, and neutrophils. Induced toll-like receptor (TLR) stimulated B cell activation suppression occurs when interleukin 10 and transforming growth factor beta work in synergy. Anti-inflammatory cytokines are immunoregulatory inhibitors of excess inflammatory response resulting from pro-inflammatory cytokines. During its functioning, interleukin-10 suppresses innate and adaptive immunity, and is produced by a variety of cells white blood cells. Interleukin-10 is elicited as an immunosuppressive cytokine in innate immune responses to viral infections and other pathogens and protects the tissues against the effects of inflammatory responses due to infection. Being a multifunctional cytokine in viral infections, including human immunodeficiency virus-1 infection, interleukin 10 plays a role in T cell impairment of function in persistent viral infections and its blockade leads to enhanced viral control. Interleukin 10 limits high inflammation, as the cytokine balances proinflammation induced by pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). Cellular immune responses and antigen presentation are inhibited by interleukin 10 in human immunodeficiency virus-1 infection by reducing IL-12 and IL-2 production leading to an immunoregulatory state induction in macrophages and dendritic cells. Production of proinflammatory cytokines and chemokines is restricted by interleukin 10, thereby preventing Th1 differentiation. On innate immune cells inflammatory activities, interleukin-10 represses phagocytosis, antigen expression, and release of immune mediators. Interleukin 10 as a regulatory cytokine balances the immune response through blocking exaggerated T cell response, by binding to the inhibitory receptors. High levels of interleukin 10 are found in severe sepsis patients, and often is linked to mortality. Interleukin-10 high levels have been found in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients, and the levels found to reduce when the patient is on combination antiretroviral therapy [49] signifying there is



increased inflammation with human immunodeficiency virus-1 progression. Interleukin-10 has also, seen to promote the action and survival of Foxp3 regulatory T cells [50].

Together with transforming growth factor beta, Interleukin -10 as an inhibitory cytokine, reinforces regulatory T cell suppressive function, maintaining a state of immune homeostasis. The positive correlation in interleukin 10 and human immunodeficiency virus-1 viremia and has been found to be a useful marker of human immunodeficiency virus-1 disease progression. Some studies found high levels of interleukin 10 in plasma at combination antiretroviral therapy initiation, compared to levels six months later. While combination antiretroviral therapy naïve human immunodeficiency virus-1 patients exhibit increased levels of interleukin 10, and its production in human immunodeficiency virus-1 infection is associated with human immunodeficiency virus-1 progression to AIDS , it has been postulated that combination antiretroviral therapy introduction reduces the inflammatory response to human immunodeficiency virus-1 and this lowers the levels of interleukin 10 in human immunodeficiency virus-1 patients. Interleukin-10 both limits antigen presentation and modulates the local cytokine micro-environment, preventing robust T cell responses. It has been suggested that, CD4+CD25+FoxP3+ T regs which produce interleukin 10 maintain a balance between immunosuppression and overactive responses and can be infected by human immunodeficiency virus-1 virus, and that, in human immunodeficiency virus-1 infection T regs regulate the immune system through T cell suppression of inflammation and spread of virus [51,52]. It is postulated that all subsets of T cells can produce interleukin -10, and at the peak of an inflammatory response, the main source of interleukin 10 is antiviral CD4+ T cells and CD8+ T cells. Additionally, interleukin-10 limits the release of reactive oxygen intermediates from cells of immunity, induction of nitric oxide synthase, and the production of nitric oxide.

### Interleukin-10 Effects on Antiviral T Cells

Studies have shown that blocking of interleukin 10 enhances Th1 memory development and function, promotes Th1 priming, and increases germinal center Th1 cells. While CD8+ T cells kill infected cells by recognizing virus presented on MHC I molecules via antigen presenting cells, Th1 cells allow the CD8+ T cells differentiation into effector cytotoxic lymphocytes, and this activity is modulated by interleukin-10, which act as a regulatory effector. The suppression of immune activation on the other hand promotes latent viral reservoir formation and limits viral clearance. Interleukin-10 produced in antiviral immunity by APCs and NK cells is a counterbalance to proinflammatory state which protects tissue damage. Additionally, the evasion of the immune response, and host regulated immunosuppression can impair viral

clearance, moreover, in T cell exhaustion, effector functions of CD4+ T cells and CD8+ T cells are lost. As earlier suggested, interleukin 10 targets many cells, and it's produced by different types of cells, leading to a wide anti-inflammatory activity. In the control of viral infections, natural killer cells and natural killer T cells are pivotal arm of innate immunity through the production of interleukin-10, additionally, Macrophage activity has been seen as the main target of the interleukin-10 inhibitory effects. Interleukin-10 promotes cytokine production, NK cell proliferation, and cytotoxicity.

### Interleukin 10 and Antiviral Cellular Responses

Studies have suggested that cytotoxic CD8+ T lymphocytes (CTL) are used to eliminate viruses, and other intracellular pathogens. The suppressive effect of interleukin 10 targets specific genes such as lipopolysaccharides, and inhibition of transcription. Similarly, recognition of danger associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) drives the antiviral state in antigen-presenting cells (APC) which initiate innate immune response, and interleukin-10 is induced in macrophages, dendritic cells, natural killer cells, monocytes, neutrophils and T cells. Interleukin-10 inhibits T cell responses via antigen presenting cells (APCs) and can also limit T cell responses by acting through induction of no responsiveness or anergic state directly on CD4 T cells. It is worthwhile noting that, viruses trigger pattern recognition receptors (PRR) engagement during early phase of infections after danger-associated molecular patterns (DAMPs) or pathogen associated molecular patterns (PAMPs) recognition, followed by a milliard of cytokines including interleukin 10.

### Interleukin-10 and Virus Clearance

Interleukin- 10 has been shown to act as a brake on inflammation, however, effects on antiviral immune response depends on site of infection, the virus, and immune response timing. As seen earlier, levels of interleukin-10 have been found to correlate viral loads in human immunodeficiency virus-1 patients. Some studies have postulated that, high interleukin-10 levels may be protective in early human immunodeficiency virus-1 responses, and in acute infection, the cytokine may become detrimental as virus persistence is promoted. Also the signalling of interleukin 10 also inhibits Th1 cytokines production, and stimulates Th2 cytokines production, thus, the anti-inflammatory response caused by interleukin 10 impacts negatively on effector T cell, favouring persistence of human immunodeficiency virus-1 infection. In an immune response at peak level, interleukin 10 could enhance CD8 T cells activity, and limit antigen presenting cell inflammation, and it is at this phase that interleukin 10 is produced by CD4+ CD25+ Treg cells.

## Interleukin 10 in Persistent Viral Infections

Human immunodeficiency virus-1(HIV- 1), being a persistent viral infection has a high rate of morbidity and mortality and also lacks efficient therapy, not to mention a functional cure. Interleukin-10 has been shown to be induced in human immunodeficiency virus-1 infection, and is an immunosuppressive cytokine that dampens proinflammatory responses, after virus encounter. Since in the course of chronic human immunodeficiency virus-1 infection antiviral cytokines are not produced in plenty by CD4+ T cells and CD8+ T cells, leading to persistent infections, T cell gene expression changes such as elevated interleukin 10 production, transforming growth factor beta and inhibitory receptor induction occur. As mentioned earlier, innate and adaptive immune cells that produce interleukin 10 include dendritic cells, B cells, and macrophages, which, after they secrete the cytokine, the produced interleukin 10 binds interleukin 10 receptor on the immune cells to trigger T cell anergic state and to reduce antigen presentation. It is worthwhile noting that, interleukin 10 can alter antiviral T cell function, through its effects on antigen presenting cells, as the proliferation of antiviral Th1 cells and cytokine production is limited by interleukin 10. While the control of inflammatory responses from viral infections requires interleukin 10 regulatory mechanisms, reservoir establishment and human immunodeficiency virus-1 persistence is aggravated by the secretion of interleukin-10.

## Transforming growth factor-beta (TGF-β),

Transforming growth factor -beta (TGF-β) is an anti-inflammatory cytokine produced by T cells and B cells, and works by inhibiting haematopoiesis, T cell and B cell proliferation, as well as promotion of wound healing. Transforming growth factor-beta (TGF-β), a pleiotropic cytokine with potent immune regulatory properties in the immune system is produced by T regulatory cells, whose numbers are reported to be increased in lymphoid tissues and mucosa of human immunodeficiency virus-1 combination antiretroviral therapy naïve patients, and are associated with disease progression [53]. In normal development and homeostasis transforming growth factor beta plays an important role [54]. Chronic inflammatory response in human immunodeficiency virus-1 patients on combination antiretroviral therapy is partially counteracted by anti-inflammatory processes, which ironically exacerbates immunosuppression and also causes development of non-communicable non-AIDS related disorders. It has been reported that, transforming growth factor beta downregulates the release of perforins and granzymes, synthesis of interferon gamma and Fas ligand expression, and collectively this contributes to CD8+ T cells cytotoxicity. TGF beta downstream signalling and dysregulation leads too many diseases. Transforming growth

factor-beta (TGF-β) level has been found to be elevated in HIV-1 non adherent patients and not in combination antiretroviral therapy adherent, and healthy controls [55]. Suggestions available so far include the blockage of transforming growth factor beta, which would serve as human immunodeficiency virus-1 functional cure, when applied to viral latency reduction [56]. During a normal inflammatory response, transforming growth factor beta signalling plays a key role.

Higher levels of transforming growth factor beta have been found in human immunodeficiency virus-1 patients in acute, sub-acute and chronic infection when compared to the negative controls [57]. Transforming growth factor beta has been shown to inhibit proliferation of resting memory CD4+ T cells by inhibition of cell cycle and limitation of induction of apoptosis. The suppressive functions of T regulatory cells is through production of transforming growth factor beta, which inhibits T-helper (Th)1 and Th2 cell differentiation and proliferation achieved through inhibition of production of the transcription factors, GATA-3 and T bet. Transforming growth factor beta is encoded by 33 genes in mammalian cells, as a secreted, heterodimeric, and homodimer proteins which controls the differentiation of cells [58]. While transforming growth factor-beta, secreted by natural killer cells has been shown to have a negative regulatory role in HIV infection, increased transforming growth factor beta has been reported in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients as reported, in innate immune system, Transforming growth factor beta inhibits natural killer (NK) cell interferon gamma production and CD16 activation induced antibody-dependent cellular cytotoxicity (ADCC) induced.

## Transforming Growth Factor-Beta in Human Immunodeficiency Virus-1 Infection

Transforming growth factor -Beta (TGF-β) is an anti-inflammatory immune regulatory inhibitor of excess inflammatory response resulting from pro-inflammatory cytokines activity. In limiting immune activation, TGF beta reduces the availability of activated CD4+ T cells, thus supporting human immunodeficiency virus-1 replication and spread V. This cytokine has been shown to remain persistently elevated in human immunodeficiency virus-1 combination antiretroviral therapy and combination antiretroviral therapy naïve patients, to counteract immune destruction by cytotoxic CD8+ T cells and the cytopathic effects of human immunodeficiency virus-1, contributing to CD4+ T cells depletion, resulting in immunosuppression and subsequent development of AIDS. Transforming growth factor beta deregulation has been shown to lead to anomalies and disease in the development process. Transforming growth factor-beta (TGF-β) levels have been shown

to be high in human immunodeficiency virus-1 patients before commencement of combination antiretroviral therapy, and remain high 12 months into treatment. Transforming growth factor beta has been listed among major cytokines that cause of immunosuppression in human immunodeficiency virus-1 infection, by targeting both innate and adaptive immune systems and profibrotic activity as well as suppressing the effects on CD4+T cells, in addition to regulation of the proliferative and effector functions of CD8+ T cells. Transforming growth factor beta characterizes human immunodeficiency virus-1 disease severity. Antiretroviral therapy (ART) should be given to all HIV-1 patients as soon as viremia is detected [59]. Similar findings in a different study found decreased transforming growth factor beta in patients with non-progressive human immunodeficiency virus-1 infection, and increased levels were observed in patients with progressive human immunodeficiency virus-1 infection.

The signalling of transforming growth factor beta has been shown to promote human immunodeficiency virus-1 infection in both resting memory and activated CD4+ T cells. Other studies have implicated Transforming growth factor beta in regulation of humoral immune responses through suppression of proliferation, survival, and differentiation of B cells into antibody secreting B cells and the mechanisms are thought to be responsible for the suppressive effects of transforming growth factor beta in IL-2, IL-4, and interferon gamma cytokine production. The blockade of transforming growth factor beta has been suggested to promote establishment of latency reservoir early in human immunodeficiency virus-1 infection. To note, high levels of transforming growth factor beta are produced in human immunodeficiency virus-1 infection in combination antiretroviral therapy naïve and combination antiretroviral therapy experienced patients and leads to immunosuppression contributing to progression of acquired immunodeficiency syndrome in combination antiretroviral therapy naïve patients. The majority of patients can start with the now 2-drug regime, or 3-drug regime which include integrase strand transfer inhibitor. Currently, a long term acting (4 weeks or 8 weeks), based on availability and regulatory body approval may be adopted. For neutrophils some studies have reported that migration as well as degranulation is inhibited by transforming growth factor beta while others have reported the cytokine as having potent chemotactic and activating factors for neutrophils. Transforming growth factor beta has also been shown to inhibit activation and maturation of dendritic cells as well as inducing dendritic cell apoptosis, impeding expression of costimulatory molecules (CD40,CD80, CD86),antigen presenting capacity, HLA class II molecules, production of tumour necrosis factor-alpha, Interferon alpha, IL-12, and migration. Additionally, TGF beta may have the potential of directly enhancing virus replication by blocking adaptive human immunodeficiency virus-1 control responses, which include virus

specific CD8+ T cells responses as well as humoral immunity (Dickinson et al., 2020). Early in inflammation, transforming growth factor beta upregulates T regulatory cell production and promotes Th17 differentiation and later in inflammation, this cytokine may inhibit proliferation of T regulatory cells thereby inhibiting immune responses, and thus Th17/CD4+CD25+FoxP3+ T regs balance is important in maintenance of a normal immune function (Theron et al., 2017a). Human immunodeficiency virus-1 patients continue to experience low-grade, persistent systemic inflammation, even on attaining viral suppression after combination antiretroviral therapy introduction. Human immunodeficiency virus-1 patients with nonprogressive infection have been reported to have lower TGF beta levels as compared to the progressors. Transforming growth factor beta also increases CD69 expression and decreases CD25 expression on CD4+ T cells, showing it can modulate differentiated CD4+ T cells. The anti-inflammatory response that ensues exacerbates immunosuppression and also predisposes the patient to non-AIDS-related, non-communicable disorders. As reported, plasma transforming growth factor beta is elevated in human immunodeficiency virus-1 patients, as opposed to seronegative individuals, which correlates with T cell levels, high, and disease progression (Dickinson et al., 2020). Studies have shown that transforming growth factor beta which is an anti-inflammatory cytokine remains elevated in both virally suppressed and unsuppressed patients, which may be confounded by residual virus in the suppressed patients, and T regs are protected from apoptosis and promotion of induced Tregs differentiation during thymic development by transforming growth factor beta. Some studies have attributed secondary immunosuppression to having been caused by over production of transforming growth factor beta (TGF- $\beta$ ), as a cause of immunosuppression in human immunodeficiency virus-1 infection.

### **Transforming growth factor beta one adaptive immune system**

Transforming growth factor-beta (TGF- $\beta$ ) has been shown to inhibit Th1 and Th2 cell proliferation and differentiation through inhibition transcription factors, T bet, and GATA-3 production. BLIMP-1, which is a transcriptional repressor is upregulated by transforming growth factor beta 1, thereby promoting human immunodeficiency virus-1 latency and reservoir formation [60]. Transforming growth factor-beta (TGF- $\beta$ ) also negatively affects the proinflammatory functions of macrophages such as inhibition of MyD88-dependent Toll-like receptor signalling, expression of inducible nitric oxide synthase, and matrix metalloproteinase, and has been shown to be induced by human immunodeficiency virus-1 trans activator of transcription (Tat), which could be behind the immunosuppressive effects of Tat. Some study has also reported

this cytokine to be systemically induced early in human immunodeficiency virus-1 infection, and been demonstrated to remain upregulated throughout infection. Throughout its working mechanism, transforming growth factor beta suppresses the production of cytokines such as interleukin (IL)-2, and interferon (IFN)- $\gamma$ , and  $\beta 2$  subunit of the IL-12 receptor (IL-12R) on CD4+ T cells loss, leading to IL-12 unresponsiveness. Additionally, transforming growth factor beta also inhibits immune responses through CD4+, CD25+, Foxp3+ Tregs regulation, and so suppressing T cell functions.

### **Effects of TGF- $\beta$ on cells of the innate immune system**

Transforming growth factor beta inhibits NK cell interferon gamma production and also CD16 activation induced ADCC. Macrophages proinflammatory activities such as inhibition of matrix metalloproteinase-12 and expression of inducible nitric oxide synthase are negatively affected by transforming growth factor beta. Similarly, transforming growth factor beta downregulates MyD88-dependent Toll-like receptor signalling pathway. Rapid secretion of numerous cytokines following human immunodeficiency virus-1 infection is a characteristic of the innate immune system, and the cytokines play a crucial role in control of the virus, as well as disease pathogenesis. Additionally, TGF beta maintains a resting state in immune cells through blocking of cell activation and proliferation [61]. After viremic spread, TGF beta, which is a pleiotropic cytokine is induced rapidly, and remains upregulated throughout infection. Establishment of human immunodeficiency virus-1 latency reservoirs may be exacerbated by transforming growth factor beta upregulation through the increasing of resting memory CD4+ T cells, and also homing lymphoid organ of central memory CD4+ T cells that are infected.

TGF beta plays an important role during human immunodeficiency virus-1 infection, in regulation of CD8+ T cells, and as reported, blocking the activities of TGF beta may decrease immune activation, and limit pathogen clearance. It has been reported that, human immunodeficiency virus-1 is promoted by transforming growth factor beta in resting and activated memory T cells, and that even after many years of combination antiretroviral therapy, high levels of transforming growth factor beta have been reported. Transforming growth factor beta has been shown to upregulate the expression and frequency of CCR5 human immunodeficiency virus-1 coreceptor and so in CCR5-tropic virus, augmenting viral infection of activated and resting CD4+ T cells. The pleiotropic effects of interferon gamma is on proliferation, activation, and differentiation of many immune cells, and upon human immunodeficiency virus-1 infection, reactivation has been shown to increase in the presence of transforming growth factor beta. While human immunodeficiency virus-1 latency in memory CD4+ T cells is supported by

transforming growth factor beta Sydney [62], reactivation of human immunodeficiency virus-1 reservoirs and immune responses has been suggested to occur through blockade of transforming growth factor beta, and enhances anti-human immunodeficiency virus-1 immune responses. As viremia escalates, human immunodeficiency virus-1 infection leads to a cytokine storm, with an elevated myriad of cytokines and chemokines as a result of acute human immunodeficiency virus-1 viral replication. Human immunodeficiency virus-1 persists latently as reservoirs in CD4+ T cells during combination antiretroviral therapy (cART).

### **Increased Blood and Tissue Levels of TGF- $\beta$ in HIV Infection**

Viral burden and human immunodeficiency virus-1 latency has been shown to be increased by transforming growth factor beta, as high levels have been observed in human immunodeficiency virus-1 combination antiretroviral therapy naïve patients, and the high levels have been found in lymphoid tissues, cerebrospinal fluid and in the blood of human immunodeficiency virus-1 patients. Direct cytopathic effects of human immunodeficiency virus-1 and destruction by cytotoxic CD8+ T cells contribute majorly to depletion of CD4+ T cells leading to progressive immunosuppression which culminates in AIDs development. Transforming growth factor-beta (TGF- $\beta$ ), alongside interleukin 10 in human immunodeficiency virus-1 infection, have been postulated to play negative roles, working against the pro-inflammatory cytokine activities. Increased levels of transforming growth factor beta have been associated with proliferation of defective T cells and B cells, and in human immunodeficiency virus-1 infection, additionally, the production of transforming growth factor beta has also been shown to be contributed by human immunodeficiency virus-1 proteins.

### **Effects of TGF- $\beta 1$ on host, promoting HIV-1 reservoir load**

Human immunodeficiency virus-1 receptor CCR5 are increased leading to a high number of integrated HIV DNA, and the resultant is suppression of miR-9-5p by transforming growth factor beta 1 leading to upregulation of transcriptional repressor BLIMP-1, causing latency and increased viral load. Transforming growth factor beta-1 also inhibits immune responses indirectly via regulation of CD4+, CD25+, Foxp3+ Tregs which play the role of suppression of T cell functions activities which are regulated indirectly by transforming growth factor beta in the process of inhibiting immunoresponses.

### **Interleukin 17/Interleukin 10 ratio in human immunodeficiency virus-1 infection**

Human immunodeficiency virus-1 (HIV-1) control by the host innate immune system is essential, nonetheless, the excessive antiviral activity should be carefully regulated to prevent high inflammation and accompanying tissue damage at any phase of the infection. Interleukin-17 and interleukin-10 cytokines have been found to be related to TH 17, and T regs respectively, as they are expressly produced by the cells [63]. Interleukin-17 from Th17 cells stimulates chemotaxis of neutrophils, dampens cytotoxic T cell activity, and induces a Th2 skewed immune response, while T regulatory cells inhibit excessive immune responses and promotion of early recruitment of virus-specific CD8+ T cells. Interleukin-10, from CD4+CD25+FoxP3+ T regs and interleukin 17 from Th17 T cells balance is a state of equilibrium that allows quick protective immune responses against infectious agents while curtailing the potential for causing harm to the host. A balance of Th17/Treg ratio is pivotal in fine tuning the inflammatory response to viral infections and if this balance is properly maintained, the pathologic effects of interleukin 17 may be held in check, and beneficial functions will outweigh the pathogenic effects. Studies have shown some imbalance in interleukin 17/ interleukin 10 in human immunodeficiency virus-1 patients and this imbalance has been suggested to be the main promoter of human immunodeficiency virus-1 replication. Some studies have suggested that lower levels of interleukin 17 are associated with high interleukin 10 release. As earlier suggested, it has been shown that, Th17 and CD4+CD25+FoxP3+ T regs in inflammatory outcomes and development have opposite roles. Other studies have postulated that in human immunodeficiency virus-1 infection, interleukin 17 plays both a pathogenic and protective roles. It is plausible that balancing of mucosal Th17/ CD4+CD25+FoxP3+ T regs ratio at combination antiretroviral therapy initiation leads to a good virologic response to combination antiretroviral therapy and maintenance of higher CD4+ T cell counts [64]. Additionally, it has been found that, early in inflammation, Th17 provides a link between adaptive and innate immunity and maintains mucosal barrier integrity while CD4+CD25+FoxP3+ T regs inhibits activities of T lymphocytes to reduce excess autoimmune symptoms, thus reducing body resistance to pathogens thereby preventing inflammation [65]. To note, Th17, being very permissive to human immunodeficiency virus-1 infection may promote the intracellular replication of virus, and thus, presence of these cells correlates with human immunodeficiency virus-1 pathology. Thus, the induction of expression of inflammatory factors such as CXC chemokines, and G-CSF is done by interleukin 17, in addition to participation in immune responses against viruses. Conclusively, it is worth noting that the excess activity of interleukin 17 when not counteracted by interleukin 10, can lead to a proinflammatory effect, leading to autoimmunity and tissue damage.

## Viral load in combination antiretroviral therapy

Highly active antiretroviral therapy (HAART) currently referred to as combination antiretroviral therapy (cART) reduces morbidity and mortality in the majority of human immunodeficiency virus-1 infected individuals and is used as a life time therapy because as at this period, treatment does not clear the human immunodeficiency virus-1 reservoir. Combined antiretroviral therapy improves the quality of life for human immunodeficiency virus-1 patients, as more strides are being made towards improving the clinical management and outcomes for at risk populations. First line combination antiretroviral therapy involves a combination of three to four ARVs, including integrase inhibitors (INIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Protease inhibitor (PIs), Nonnucleoside reverse transcriptase inhibitors (NNRTIs), and fusion inhibitor (FIs) [66]. ART should be given to all human immunodeficiency virus-1 patients as soon as viremia is detected. It has been postulated that, systemic inflammation is decreased by combination antiretroviral therapy, but rarely achieved to levels are comparable to human immunodeficiency virus-1 non infected individuals. Viral load and CD4+ T cell count are currently being utilized in prediction of human immunodeficiency virus-1 disease outcome [67], but the levels may not give early prediction of drug resistance in human immunodeficiency virus-1 patients. It has been postulated that, detectable viral load after close to 6 months of combination antiretroviral therapy could be related to the progression of human immunodeficiency virus-1 infection and it has been reported that there is a shift from Th1 to Th2 cytokine profile which has been associated with human immunodeficiency virus-1 disease progression [68].

The majority of patients can start with the now 2-drug regime, or 3-drug regime which include integrase strand transfer inhibitor. Currently, a long term acting (4 weeks or 8 weeks), based on availability and regulatory body approval. In cases where combination antiretroviral therapy is interrupted, viral rebound occurs. Clonal expansion takes place in human immunodeficiency virus-1 infected cells, and the clonally expanded cells increase with time. It has been postulated that, more than 50% of the clonally expanded cells are maintained through latent reservoirs. Viral rebound is contributed to by the human immunodeficiency virus-1 infected clonally expanding cells. In human immunodeficiency virus-1 patients on combination antiretroviral therapy, the viral load decrease is accompanied with an increase in CD4+ T cells and CD8+ T cells [69]. Combination antiretroviral therapy has been known to reduce human immunodeficiency virus-1 viral load to undetectable levels, and halts human immunodeficiency virus-1 replication. Clonal expansion of in human immunodeficiency virus-1 infected cells is driven by among others, homeostatic proliferation, antigen driven



proliferation, and human immunodeficiency virus-1 site-dependent integration proliferation. The persistence of human immunodeficiency virus-1 human immunodeficiency virus-1 in latent reservoir is a great barrier to cure. Therapy safety and effectiveness monitoring before and during administration is key. Higher levels of low level viremia was associated with virologic failure. Studies have shown that, episodes of high human immunodeficiency virus-1 viral load are preceded by persistent viremia and a possibility of resistance development.

Laboratory tests which may include genotyping, human immunodeficiency virus-1 RNA level, and CD4 cell count are recommended, at specified points for co-infections and for the general population infected by human immunodeficiency virus-1 before and during therapy [70]. Presumably, on combination antiretroviral therapy initiation, viral suppression should be attained within 24 weeks, and in clinical management, within 6 months of combination antiretroviral therapy initiation, additionally, in the event of first line combination antiretroviral therapy failure, second line is initiated, and is comprised of NRTIs and ritonavir-boosted protease inhibitor. Virologic failure is noted to be rare, though switching therapy may be done for convenience, among other reasons. To note, virologic suppression has been shown to be achieved in more than 90% of first line combination antiretroviral therapy patients since at least one drug targets a step of human immunodeficiency virus-1 replication, thus, most immune biomarkers are reported to be normalized in human immunodeficiency virus-1 combination antiretroviral therapy suppressed patients, and move towards human immunodeficiency virus-1 negative levels. In human immunodeficiency virus-1 patients on combination antiretroviral therapy, immune dysregulation is shown to be minimized due to human immunodeficiency virus-1 suppression and immune recovery, is exhibited by an increase in CD4+T cell count, following successful combination antiretroviral therapy, additionally, cytokines are also normalized, improving the stability of the system, as the goal of combination antiretroviral therapy is to reduce human immunodeficiency virus-1 related mobility and mortality through inhibiting of human immunodeficiency virus-1 replication. It has been suggested that combination antiretroviral therapy failure in human immunodeficiency virus-1 patients can be identified in three ways; immunologically, virologically, or clinically [71].

### **Virologic Failure in Combination Antiretroviral Therapy**

Some studies have defined virologic failure by a persistently detectable viral load of over 1000copies/ml within a three months interval after starting combination antiretroviral therapy [72]. Dolutegravir (DTG)-based therapies has been recommended as

the preferred first-line antiretroviral therapy option [73]. Persistent low level viremia has been associated with virologic failure, AIDS, genotype resistance, and adherence difficulties. While some studies have established Resistance -associated mutations to be found in the gag and Tat genes of human immunodeficiency virus-1 [74], other studies have delinked viral resistance or less drug concentration to persistent low level viremia [75]. There is a recommendation to lower human immunodeficiency virus-1 virologic failure threshold from 1000copies/ml to 50 copies/ml. For a regimen switch, tolerability, drug resistance history, treatment history and drug adherence should be considered. Reasons for virologic failure include: patient adherence -related factors such as high pill burden and missed clinic appointments, and regimen related factors such as reduced efficacy and sub-optimal pharmacokinetics. Combination antiretroviral therapy initiation in human immunodeficiency virus-1 patients reduces T cell activation, as there is immune reconstitution [76].

Once the CD4+ T cell count hits below 200 cells/ml, there is a recommendation against routine monitoring in patients who are clinically well. Viral suppression remains below the UNAIDS target of 90% achievable by 2020 in human immunodeficiency virus-1 patients on combination antiretroviral therapy. (“Incidences and Factors Associated with Viral Suppression or Rebound among HIV Patients on Co [77]. The Kenya 2018 combination antiretroviral therapy guidelines define persistent low level viremia as having detectable viral load, of less than 1000copies/ml on two or more consecutive tests done after previous human immunodeficiency virus-1 suppression. The goals of antiretroviral therapy have been known to be prevent onward transmission of human immunodeficiency virus-1 infections, Prolong the life expectancy and improve quality of life, reduce human immunodeficiency virus-1 non-infectious and infectious morbidities, provide durable and maximum suppression of viral load, and reduce the adverse effects of treatment. Persistent immune activation is characteristic of viral replication, increased pro-inflammatory cytokines, and loss of the gut mucosa’s integrity, and can predict the occurrence of depletion of CD4+ T cells. Notably, the viral load has been shown to be maintained by combination antiretroviral therapy to levels below detection limit for the majority of treated patients. High viral rebound rate (41% of the study population) was found in human immunodeficiency virus-1 patients on combination antiretroviral therapy. Well-tolerated, and sustainable treatment, alongside good and enhanced adherence is all necessary in order to achieve suppression of human immunodeficiency virus-1 replication in human immunodeficiency virus-1 on combination antiretroviral therapy.

### **HIV viral load testing**



Effective combination antiretroviral therapy leads to viral load reduction to below 50 copies/mL in human immunodeficiency virus-1 patients, however events of persistent low level viremia, with varying virological consequences are still eminent. Restoration of pathogen specific immune function improves with an increase in CD4+ T cell count, and this is the result of prolonged human immunodeficiency virus-1 suppression, which in addition, reduces human immunodeficiency virus-1 related mortality and morbidity. A study reported some of the factors associated with viral suppression to include widow status, good adherence, and world health organization stage I, while the factors associated with viral rebound included WHO stage II, 36 months duration on ART, and poor adherence to antiretroviral therapy. Risk factors for low level viremia include higher baseline viral load measurements, non-adherence to medication, low CD4 cell count at base line, and Non-nucleotide reverse transcriptase inhibitors (NNRTI) use, among others, therefore intensifying and modifying combination antiretroviral therapy has the potential, and leads to a decrease in virologic failure. It has been suggested that, when human immunodeficiency virus-1 is poorly controlled, this leads to the risk of emergence of drug resistance, transmission of human immunodeficiency virus-1 infection, and death [78]. Clonal expansion and viral reservoir size have been postulated as the possible causes of persistent low level viremia. Additionally, it has been observed that patients with low level viremia have high chances of virologic failure. It has been shown that, patients who adhere to treatment resume a near to normal life style, although adherence has been reported as key challenge and focus in human immunodeficiency virus-1 patients on antiretroviral therapy. While some studies have reported good progress in attaining of undetectable viral load for more than 2 years, which points to the achievement of the united nation's 2030 objective of human immunodeficiency virus-1 control, there seems to be more incidences of viral rebound in human immunodeficiency virus-1 patients on combination antiretroviral therapy.

While persistent low level viremia has been associated with virologic failure, alteration of immune status, and emergence of drug resistance, there has been no reported association between occurrence of blips and immunologic and virologic failure. Some of the known causes of unsuppressed human immunodeficiency virus-1 during combination antiretroviral therapy most often are taking inappropriate combination antiretroviral therapy, infection with drug-resistant strains, and combination antiretroviral therapy non-compliance. Viral suppression was listed as part of the UNAIDS 2014 sustainable development goals. While studies have found and reported that persistent low level viremia can lead to viral shedding, and human immunodeficiency virus-1 reservoir expansion, combination antiretroviral therapy is meant to maintain undetectable viral load, avoid emergence of drug

resistance, and decrease human immunodeficiency virus-1 transmission, thereby keeping low level viremia at bay, [79]. Without a serious and extremely compelling reason, antiretroviral therapy should not be stopped, instead, in cases of reported drug toxicity, attempts to switch regime should be exploited. Recent reports from the world health organization (WHO) guidelines has placed treatment failure at confirmed viral load of greater than 1000 copies/ml, while the US has placed virologic failure at 200copies/ml viral load. Based on this, and considering the current records, world-wide, 35% of human immunodeficiency virus-1 patients had achieved viral suppression, as reported by UNAIDS, 2019 report. The viral load should be sufficiently suppressed, should attempts to switch a regime due to toxicity ensue, to avoid the development of drug resistance to the new drug. Available diagnostics enable detection and identification of virologic suppression with effective treatment, to be below undetectable levels, based on many standard assays sensitivity. Thus, human immunodeficiency virus-1 treatment success is defined by viral load below detection level, when done by conventional testing algorithms. To achieve the UNs 90-90-90 target by 2020 as forecasted, would be important towards AIDS pandemic elimination by 2030. ("Incidences and Factors Associated with Viral Suppression or Rebound among HIV Patients on Combination Antiretroviral Therapy from Three Counties in Kenya," 202. Recent studies have further reported that combination antiretroviral therapy does not eradicate residual viremia, as has been evidenced by many patients after years of treatment, since viral persistence is still reported in many instances.

Virologic suppression has been defined as a confirmed viral load below detection level, while virologic failure is defined as the inability to achieve or maintain a viral load of less than 200copies /ml by or after one year of starting combination antiretroviral therapy. The objective of providing antiretroviral therapy should always be to provide a virologic suppression achievable regime. Since low-level viremia has been shown to be a predictor of virologic failure in human immunodeficiency virus-1 patients on combination antiretroviral therapy, it has been postulated that human immunodeficiency virus-1 being one of the ten causes of mortality in adults, is uncontrolled in low level viremia, and exacerbates the pandemic on rebound. Currently, WHO does not have guidelines on change of clinical care for human immunodeficiency virus-1 viral load of less than 1000copies/ml, and this allows occurrence of low level viremia in human immunodeficiency virus-1 patients on combination antiretroviral therapy. Achieving these targets depends squarely on trend monitoring of viral suppression and viral rebounds, and by understanding the factors revolving around viral rebound, in order to effect interventions. Viral load testing has been shown to be key in human immunodeficiency virus-1 monitoring. Statistics



have shown that, approximately at least one out of five patients undergoing combination antiretroviral therapy have experienced episodes of detectable viremia in form of blips, while 4-10% of patients on combination antiretroviral therapy have shown persistent low level viremia. Low level viremia has been found as a grey zone between undetectable viral load, and virologic failure. Virologic failure has been defined as a confirmed viral load of more than 50 copies /ml on viral load measurements taken 2-3 months apart consecutively, while under intensive and optimal adherence counselling.

Human immunodeficiency virus-1 viral load of less than 100copies/ml in patients on combination antiretroviral therapy has been seemingly thought of as insufficient, however it is the persistence of low level viremia in this occurrence that has harmful effects. Some studies found, at next viral load testing, patients with viral load of less than 200copies/ml had high odds of viral non-suppression, and low level viremia at the next viral load. Other studies have indicated that patients with persistent low level viremia without regimen change will progress to virologic failure. Previous studies and guidelines had indicated treatment failure to be a viral load of 1000 copies/ml, but robust evidence now suggests, a viral load of more than 50 copies/ml can result to virologic failure. The European AIDS Clinical Society defines virologic failure as a viral load greater than 50copies/ml, and recommends a change of therapy, which is more stringent than WHO, that requires a change in therapy when the viral load is greater than 1000copies/ml. In Kenya, at the initiation of ART, viral load is recommended, and for effective monitoring, this should be followed by a six-month interval viral load testing. It is now revealed that human immunodeficiency virus-1 transmission can occur even with a viral load of 200copies/ml of blood. In a study conducted by, the findings indicated a low level viremia in 16% of the study population, and 11.4% patients had virologic non-suppression. Similarly, from the adjusted risk, it was found that patients at increased risk of virologic failure were those with low level viremia. Additionally, in Africa however, WHO guidelines has set the virologic failure threshold at a viral load of more than 1000copies/ml, and a combination antiretroviral therapy switch to be done at this point [80]. To note, monitoring of viral load recommendation is at six months, and 12 months upon combination antiretroviral therapy initiation, and thereafter, monitoring should be done annually. Studies have shown, viral replication even at these low levels, when it is sustained, is capable of leading to virologic failure, as well as accumulation of drug resistance mutations. Based on UNAIDS report 2019, the achievement by Kenya of the 90-90-90 so far is 89% for the first “90”, 77% for the second “90”, and no data for the third “90”. (UNAIDS 2019).

A lot of inflammation has been observed in low level viremia and various agencies have defined low level viremia differently; for

instance, the European Acquired Immune Deficiency Syndrome (AIDS) Clinical Society (EACS) defines low level viremia as a viral load between 20 to 50 copies/ml, the Department of Health and Human Services guidelines (the USA, 2016), defines low level viremia as viral load between 50 to 200copies, while WHO guidelines have defined low level viremia as viral load between 50 to 999copies/ml. Some of the factors associated with persistent low level viremia have been shown to include genotypic resistance, vaccinations, concomitant infections, intermediate viral loads (200-399) copies/ml, High viral loads (400-999) copies/ml, baseline CD4 count, and combination antiretroviral therapy adherence difficulties [81]. Some of the reasons attributable to high viral loads have been reported as drug absorption difficulties or drug-drug interactions altered pharmacokinetics leading to inadequate antiretroviral therapy drug levels, transmitted and acquired prescribed antiretroviral therapy resistance, and most commonly, patient adherence inadequacy. Patients who have virologic non-suppression need a repeat viral load testing, as well as adherence counselling, while patients with more than two tests consecutively indicating virologic non-suppression need a review for combination antiretroviral therapy review. The global human immunodeficiency virus-1 guidelines recommend preferably viral load as the combination antiretroviral therapy monitoring strategy, thus, the human immunodeficiency virus-1 programmes that base on WHO guidelines, have considered patients with viral loads of less than 1000copies/ml to be virologically suppressed. In first line failing antiretroviral therapy, it is recommended that there should be enhanced adherence counselling, followed by a repeat viral load measurement in 2-3 months.

While viral load testing is an important tool used in monitoring combination antiretroviral therapy response in human immunodeficiency virus-1 patients, undetectable human immunodeficiency virus-1 viral loads as done by routine assays has often been considered as a marker for successful combination antiretroviral therapy. As seen earlier, some studies have found virologic non-suppression in human immunodeficiency virus-1 patients. Factors associated with virologic rebound have been found to include nutrition, adherence problems, and rural residency [82]. A great concern to physicians is the puzzle of patients presenting with low level viremia, despite self-reported adherence, as some observational studies found an association between very low level viremia with subsequent virologic rebound. For human immunodeficiency virus-1 patients, virologic suppression is the hallmark of successful ART. In resource limited settings, WHO recommends measurement of viral load as a preferred strategy for combination antiretroviral therapy response in human immunodeficiency virus-1 patients. It has however been postulated that undetectable viral load varies by laboratory assay used, and varied technical properties. The



UNAIDS “90-90-90” proposed target by the year 2020 alludes that 90% viral suppression should have been achieved by patients on ART, 90% status knowledge by all infected, and a further 90% human immunodeficiency virus-1 diagnosed patients should have been put on ART [83]. Should the result of the viral load test come as less than 50 copies/ml, the recommendation is that, the patients may be introduced to DTG+ the same two NRTs the patient was on. If on the other hand the outcome of the viral load result is more than 50copies /ml, then it is recommended, a switch to a second line regime that includes two NRTs + DTG, considering regime algorithms. Proper human immunodeficiency virus-1 suppression reduces virus transmission, development of resistant mutations, and improves clinical outcomes, and switching of combination antiretroviral therapy in human immunodeficiency virus-1 patients to 100 copies/ml has led to more patients with low level viremia attaining viral suppression.

As viral load testing is being embraced as the means to verify virologic suppression, drug resistance testing is not routinely done in the majority of human immunodeficiency virus-1 care centers possibly due to the high cost, and technical requirement to undertake the testing [84]. As reported earlier, human immunodeficiency virus-1 virologic failure has been defined by a viral load of more than 1000copies/ml as defined by WHO guidelines. In resource endowed settings, virologic failure is however defined as two consecutive viral loads of less than 200copies/ml, and this should trigger a switch of combination antiretroviral therapy. Drug resistant mutations and adherence inconsistencies have been suggested as the major barriers to sustained virologic suppression in human immunodeficiency virus-1 patients on combination antiretroviral therapy [85]. Practically, low level viremia study is hindered by problems in carrying out human immunodeficiency virus-1 genotyping, occasioned by less plasma human immunodeficiency virus-1 - RNA required for successful genome amplifications. Drug resistance mutations were reported in 15% of the sampled patients, who had experienced bounds of virologic failure defined by a viral load of more than 1000copies/ml. As reported earlier, the efficacy in antiretroviral is done through monitoring of the viral load and CD4 cell count, and key to note, the endpoint for ART efficacy is reported as a sustained virologic suppression of less than 50 copies /ml, and CD4+ T cell count monitoring can be stopped on achieving a count of 400 cells/ml.

Since HIV-1 drug resistance testing helps in choosing the right combination antiretroviral therapy, predicting virologic failure, and preserving the regime in use, some studies have estimated a 0.4%-38.7% low level viremia experience in human immunodeficiency virus-1 patients on combination antiretroviral therapy. Using different virologic failure threshold across agencies poses different definitions to low level viremia. Design mechanisms to address adherence barriers such as food security,

non-disclosure, alcohol use and depression, as well as the misconceptions around antiretroviral therapy. In order to monitor treatment efficacy, the world health organization (WHO), in 2013 recommended the use of viral load testing. It has however been noted that, very few concerned entities have implemented this at large scale. A number of studies have observed an association between low level viremia and drug resistance mutations in human immunodeficiency virus-1 patients on combination antiretroviral therapy and suggested viral load testing as the best monitoring tool to ascertain virologic suppression, though this may come in handy too late when routine viral load testing is done at three to six month follow-up time. The most desired outcome in human immunodeficiency virus-1 patient’s management is viral suppression, which in the current era, can effectively be achieved by antiretroviral therapy. 37% of patients on ART were found to have virologic failure. It is also hypothesized that subsequent virologic failure and impairment of combination antiretroviral therapy options are the result of low level viremia. Effective combination antiretroviral therapy is paramount to eradication of human immunodeficiency virus-1, and WHO has listed combination antiretroviral therapy as one of the 90-90-90 targets, where the third 90 was to ensure 90% viral suppression in human immunodeficiency virus-1 patients on combination antiretroviral therapy by the year 2020.

A study found that, on initial routine viral load test, virologic failure was found in patients whose virologic suppression was low. Dire consequences may however be experienced by some patients on ART who may revert to viral rebound. Studies have suggested that transmitted human immunodeficiency virus-1 drug resistance among other clinical characteristics, is associated with the time to viral suppression and virologic failure [86] additionally, two viral loads in sequence, of greater than or equal to 1000copies/ml was considered by WHO 2016 as virologic failure [87-89]. As seen before, recent studies have postulated that, in patients on first line combination antiretroviral therapy, transmitted drug resistance may lead to virological failure, as there has been reported immune activation in human immunodeficiency virus-1 patients even with combination antiretroviral therapy. It is suggested that, for human immunodeficiency virus-1 patients on combination antiretroviral therapy with viral loads more than 200 copies/ml, there is a requirement for frequent viral load testing to help in planning for regime switch [90]. From the results of a CDC survey that was done in 2015, it was reported that only two countries among seven sub-Saharan countries tested the viral load of more than 85% of human immunodeficiency virus-1 patients on ART, and four countries tested less than 25% human immunodeficiency virus-1 patients on ART. Sustained antiretroviral therapy which has increased the viral suppression success and a reduction in the



HIV-AIDs related deaths has been the focus on the fight against human immunodeficiency virus-1.

It has been reported that by 2018, 53% of human immunodeficiency virus-1 patients on combination antiretroviral therapy had suppressed viral load while 47% had detectable viral loads at varied levels, and that drug regimen has been considered as an independent factor associated with virologic failure [91]. Human immunodeficiency virus-1 RNA levels estimation is the standard way of determining human immunodeficiency virus-1 replication [92]. Routine viral load testing was introduced in Kenya in 2013. The use of human immunodeficiency virus-1 VL monitoring for identification of combination antiretroviral therapy failure has been recommended by WHO guidelines on treatment of human immunodeficiency virus-1 [93]. As earlier reported, after periods of combination antiretroviral therapy, viral load has still been detected in human immunodeficiency virus-1 patients. Combination antiretroviral therapy successes include good clinical outcomes, however when first line combination antiretroviral therapy fails, the benefits of combination antiretroviral therapy reduce, and virologic failure may result. Virologic failure is when there is a human immunodeficiency virus-1 plasma VL of  $\geq 1000$  copies/ml after previously attaining a human immunodeficiency virus-1 plasma VL of  $\leq 1000$  copies/ml [94,95]. It has been found that, while routine viral load testing has been recommended by WHO for human immunodeficiency virus-1 patients on ART, access to the resource-intensive and expensive laboratory test remains suboptimal. Current management guidelines for virologic failure is by adherence intervention after first detected virologic failure, and a viral load test repeat three months thereafter; a second line combination antiretroviral therapy is recommended if the second VL confirms virologic failure [96,97]. In many cases, intermittent levels of low-level viremia followed by a return to suppression without a change in therapy-“blips” are experienced prior to virologic failure [98,99]. Among the patients on first line antiretroviral therapy, viral suppression has been reported, based on epidemiological studies, and as such, if streamlined care is utilized, there is the hope of human immunodeficiency virus-1 eradication. It has been reported that human immunodeficiency virus-1 Patients on combination antiretroviral therapy and with viral blips are at risk of virologic failure [100,101]. While drug resistant viruses are found in human immunodeficiency virus-1 patients with prolonged viral load decline [102], persistent viremia has been associated with high risk of virologic failure, and development of drug resistant mutants.

Viral load measurement has been used for decades in high resource settings and this is majorly what is being used to determine response to combination antiretroviral therapy [103]. The dangers associated with viral rebound include treatment failure, the potential for human immunodeficiency virus-1

transmission, antiretroviral therapy resistance, and an increased vulnerability to other illnesses. Most patients were found to have viral loads greater than 1000copies /ml during three visits, of which, some mutations were recorded, and out of the mutations, a number resulted into virologic failure [104]. In Kenya viral load testing is done at six months and twelve months after combination antiretroviral therapy initiation, then annually thereafter, for patients with undetectable VL. For patients with viral loads  $\geq 1000$ copies/ml, a follow-up is done as per guideline algorithms, in which the patient receives a human immunodeficiency virus-1 viral load repeat test three months later. A second viral load within this time frame surmounts to treatment failure [105]. Viremia likely occurs in patients who took long to be initiated on combination antiretroviral therapy, and also the presence of virus in reservoirs in immunological niches such as lymph nodes. The presence of residual virus leads to persistent immune activation, and this could lead to viremia, and further culminate to virologic failure that comes with morbidity and mortality [106].

The increased risk of human immunodeficiency virus-1 mortality and morbidity also hampers the achievement of UNAIDS agenda 95-95-95 targets by 2030. As earlier reported, WHO 2016 guidelines have placed a human immunodeficiency virus-1 viral load of  $\geq 1000$ copies/ml threshold, that a repeat viral load test within 6 weeks has to be done, together with enhanced counselling, and human immunodeficiency virus-1 viral loads of greater than 1000 copies/ml require a switch to second line combination antiretroviral therapy [107]. Based on research studies so far, viral suppression as a result of viral rebound has not received much attention in Kenya, though reports available indicate upscaled viral load uptake. Viral load compared to immunologic and clinical indicators in human immunodeficiency virus-1 patients on combination antiretroviral therapy helps in identifying non-adherent patients and early detection of treatment failure. Virologic failure as defined by WHO is a viral load threshold of 1000copies/ml, a point at which the risk of emergence of drug resistance and subsequent virologic failure has been shown to occur, and is reporter to be a function of persistent low-level viremia of between 50-999copies/ml. More reports involving virologic rebound after periods of suppression have been recorded. Further and more recent studies have postulated that higher levels of viral load and persistent low level viremia in human immunodeficiency virus-1 patients on combination antiretroviral therapy increased the risk of virologic failure. Patients on combination antiretroviral therapy have been shown to by some studies to reach HIV RNA of less than 50 copies /ml blood within 3-6 months after initiation of combination antiretroviral therapy. Additionally, intermittent low-level viremias have been reported in up to 50% of human immunodeficiency virus-1 patients on combination antiretroviral therapy. More factors associated with virological failure include



the patient being at WHO stage 3 and 4 at combination antiretroviral therapy initiation [108].

Recent studies have found that there were frequent bouts of low-level viremia in human immunodeficiency virus-1 patients on combination antiretroviral therapy, and that the persistent viremia below 1000 copies/ml also increases risk of virologic failure [109]. A number of factors have been reported as to be associated with human immunodeficiency virus-1 viral suppression, some of which include the right combination of drug regimen, fair and good adherence to antiretroviral therapy, WHO stage 1 diagnosis, and increased treatment duration. It has been suggested that, human immunodeficiency virus-1 RNA detection during long term combination antiretroviral therapy indicates drug resistance and emerging virological failure, which is characterized by repeated human immunodeficiency virus-1 RNA values of greater than 50-1000copies/ml [110]. To note, high income countries define virological failure based on viral load thresholds of 50-200 copies per ml, while for low- income countries, as reported before, WHO guidelines define virological failure as viral loads of 1000 copies per ml. Increased potential for human immunodeficiency virus-1 drug resistance, and human immunodeficiency virus-1 transmission can occur as a result of progression of low-level viremia to treatment failure. The quality of life in human immunodeficiency virus-1 patients on combination antiretroviral therapy is improved to near normal levels as combination antiretroviral therapy effectively suppresses human immunodeficiency virus-1 replication. Unfortunately, for some patients, after achieving viral suppression, the patients are not able to maintain the suppression, rather they experience viral rebound, which apart from increasing the risk of potential for transmission, there is also the risk of treatment failure.

During combination antiretroviral therapy, detectable viral load of 50-990copie per ml define low level viremia, and the low level viremia still occurs in some percentage even after standardized combination antiretroviral therapy. In their study, reported 26.6% of the sampled patients, had experienced low level viremia, and had increased risk of virologic non-suppression and virologic failure. Clinical interventions are initiated in high income countries upon detection of viral loads that are greater than 50 copies/ml, which may not be the case for resource limited settings. Low level viremia is a risk factor for human immunodeficiency virus-1 transmission and may impact clinical and immunological outcomes of patients. Transitioning to DTG reduced the risk of virologic non-suppression and the subsequent virologic failure. So far, from a global perspective, only half of the human immunodeficiency virus-1 patients initiated on antiretroviral therapy have experienced viral suppression. There was low prevalence of high viral loads and virologic failure in patients who joined adherence clubs. There is need to establish and expand adherence clubs, and streamline the models to match

the objectives, as well as strengthen adherence counselling at the various care centers, upon the results of cytokines. Failure of combination antiretroviral therapy in human immunodeficiency virus-1 patients in low and middle-income countries has been defined by WHO as viral load of greater than 1000copies /ml. As opposed to blips which are a single human immunodeficiency virus-1 viral load of more than 50 copies per ml, followed by virologic suppression, low level viremia is defined by two or more episodes of human immunodeficiency virus-1 viral load of higher than 50 copies per ml, and so far, has reported prevalence of between 5% to 30% [111-130]. There is no specific interventions in treatment and monitoring of human immunodeficiency virus-1 patients even with repeated low level viremia based on the current WHO guidelines. 10.1% of the 2795 human immunodeficiency virus-1 patients on combination antiretroviral therapy experienced low level viremia and subsequent virologic failure. To attain viral suppression, it has been suggested that the patient management could involve good adherence to counselling strategy and considering nevirapine-based regimens. Studies have suggested that during combination antiretroviral therapy, detectable viral load of 50-990copie per ml define low level viremia. Some studies suggest genotyping, for low level viremia in human immunodeficiency virus-1 patients on combination antiretroviral therapy, to minimize virologic failure. Low level viremia while the patient was combination antiretroviral therapy predicts the risk of virologic failure, and this suggests frequent viral load monitoring with intensive adherence support. Because of the increased risk of virologic failure, patients with low level viremia may require intensified monitoring. Persistent low level viremia has been found in human immunodeficiency virus-1 patients on combination antiretroviral therapy. Risk factors for low level viremia include higher baseline viral loads, low CD4 + T cell counts prior to combination antiretroviral therapy, non- nucleoside reverse transcriptase use, and non-adherence to medication, among other factors. More than half of patients with persistent viremia exhibit virologic failure. According to WHO guidelines 2016, Virologic failure is persistently detectable VL  $\geq$  1000copies/ml in two consecutive VL measurements within 3 months interval and with adherence, after at least six months on combination antiretroviral therapy. This study aimed at investigating the levels of Th17, interferon gamma, CD4+CD25+FoxP3+ T regs and transforming growth factor beta and viral load in human immunodeficiency virus-1 infected patients on combination antiretroviral therapy with and without viremia, attending AMPATH clinic at Moi Teaching and Referral Hospital -Eldoret, Kenya.

## Conclusion

By analyzing the level of cytokines, the study obtained evidence for varying levels of the target cytokines, suggesting that the

interleukin 17, interferon gamma, interleukin 10, and TGF beta play a significant role in human immunodeficiency virus-1infection progression. Low level viremia is linked to cytokine release, and the levels of cytokines predict human immunodeficiency virus-1replication and progression. There was low level viremia in human immunodeficiency virus-1patients on combination antiretroviral therapy, evidenced by the cytokine levels in sampled patients over the study period. The viral load in low level viremia patients leads to virologic failure. The higher levels of cytokines in the patients with low level viremia signify viral replication, and subsequent viral failure. This study suggests that a state of long-term immune activation is maintained by human immunodeficiency virus-1infection. The chronic activation results to a general loss of immune function in T cells that would lead to immune system degradation and subsequent development of AIDS. There was an imbalance in the proinflammatory and immunoregulatory cytokines analyzed, a sign that the immune system was trying to clear an ensued infection, and on the other hand trying to balance the effects of pro-inflammation by release of the immunoregulatory cytokines, in a bid to strike some balance. This study demonstrates a strong correlation between human immunodeficiency virus-1rebound and the levels of both pro-inflammatory and anti-inflammatory cytokines, as HIV1-1 proliferation seems to affect cytokine production. Human immunodeficiency virus-1progression may be controlled by a balance between pro-inflammatory and anti-inflammatory cytokines. The Pro- and anti-inflammatory cytokines can be used to monitor human immunodeficiency virus-1disease prognosis during therapy, particularly in low regular viral load monitoring resource limited settings, to look out for persistent low-level viremia in the wake of looming virological failure. Therefore, addressing pro-inflammatory and anti-inflammatory cytokines as significant predictors of persistent low-level viremia is highly recommended in this study setting.

## Recommendation

Monitoring benchmarks for programmes should be revised for low level viremia, to track human immunodeficiency virus-1progress control and to strengthen clinical outcomes. Systems should also be designed, for combination antiretroviral therapy patients to benefit from Supportive services which may include close monitoring and enhancing patient-physician relationship as important features to successfully achieving virologic control, and identifying biomarkers for remission. Other Immune markers investigation should be considered, to determine utility in monitoring disease progression, thus, an extensive replica follow-up of this research is needed to validate the usefulness of interleukin 17, interferon gamma, interleukin 10, and TGF  $\beta$  as predictors of virological failure in persistent low level viremia in human immunodeficiency virus-1patients on combination

antiretroviral therapy. Additionally, these findings warrant further investigation to incorporate more cytokines (Both pro-inflammatory and anti-inflammatory), as the conclusions need to be verified in large, well-designed studies.

## Conflict of Interest

None

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None

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