SAMHD1 AND HIV1 INFECTION: A NEW APPROACH

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ABSTRACT
Sterile alpha motif and histidine aspartate domain containing protein 1 (SAMHD1) is a new hope for the treatment of AIDS. Millions of people are suffering from AIDS since there is no definite treatment available for this life threatening disease at present. Moreover several hurdles are associated with the treatment procedure. Several strategies have been developed to counteract this syndrome, out of which HAART is the best one. This antiretroviral therapy represses the viral growth rate hence prolongs the patient’s life. However it cannot fully cure the infection, thus it is necessary to develop such strategy which can fully cure this life threatening infection. Some components have been identified in the different researches as the potential anti HIV agents, among which we observed SAMHD1 has an effective antiretroviral activity. So, in this review, we have tried to characterize the SAMHD1 and have also discussed about the approaches that can be used for the treatment with SAMHD1. Research development of treatment procedure with SAMHD1 can open up a new era which can save million of life suffering from AIDS.

KEYWORDS: SAMHD1, AIDS, HAART, HIV.

INTRODUCTION:
Human immunodeficiency virus (HIV) was identified as a human infectious pathogen in 1958. It is a retrovirus and it generally infects CD4+ T cells [Barre-Sinoussi et al., 1983, Laguette et al., 2012]. Eventually the infection leads to chronic activation of immune system, functional impairment and consequent loss of CD4+ cells [Hazenberg et al., 2003, Fu et al., 2016]. At present, near about 30 million people are being infected with the virus and consequently 2 million people becomes infected per year worldwide [Hertoghs et al., 2015]. AIDS is a state of pathological alteration in humans where weakness in immune response [Che et al., 2010] allows life threatening opportunistic infections and cancers to occur. Activation of CD4+ T cells can be impelled by non-replicating HIV1 and it can also cause excessive CD4+ T cells depletion by cell lysis and apoptosis [Doitsh et al., 2013, Holm et al., 2005]. Although these activated CD4+ T cells are extremely permissive to HIV-1 infection but the resting/quiescent CD4+T cells are turbulent to HIV-1 infection [Zack et al., 1990, Eisele et al., 2012, Gao et al., 1993, Wu, 2012, Baldauf et al., 2012].

Sterile alpha motif and histidine-aspartic domain containing protein 1 (SAMHD1) is a cellular enzyme that inhibits the ability of retroviruses specifically HIV-1 to infect myeloid cells, non cycling cell types which includes monocytes, dendritic cells, macrophages and resting CD4+ T cells [Goldstone et al., 2011, Powell et al., 2011, Wang et al., 2014, Berger et al., 2011, Dragan et al., 2013, Puigdomenech et al., 2013, St. Gelais et al., 2012, Descours et al., 2012]. SAMHD1 was discovered in the year 2000 as a component of human innate immune system and named dendritic cell derived IFN-gamma induced protein (DCIP) [Li et al., 2000]. In most human organs specifically in tumorous to HIV-1 restriction activity of SAMHD1. The C terminus located outside the catalytic core domain confers stability and after identification of the C terminus independently by two groups, the structural basis for dGTP stimulated dNTPase activity was revealed [Ja et al., 2013, Zhu et al., 2013]. Several proteins called restriction factors are identified in the cells which arrest the replication cycles of SAMHD1 such as TRIM 5 alpha, APOBEC3G, Tethrin [Sheehy et al., 2002, Sternlau et al., 2004, Neil et al., 2008]. Tripartite motif (TRIM) 5 alpha: It interferes with the uncoating step by binding to the viral capsid (CA) and then disorders the lattice [Laguette et al., 2012]. But human TRIM 5 alpha harbors amino acid substitution which abrogates its restriction potential [Yap et al., 2005]. Apolipoprotein B mRNA-editing enzyme catalytic polypeptide link 3G (APOBEC3G / A3G): It is a type of cytidine deaminase which disrupts the early steps of viral life cycle [Stopak et al., 2003, Mariani et al., 2003]. Vif binds with A3G and degrades it polyubiquitination and proteasome in infected cells thus prevents the entry of it into nascent viral particles [Yu et al., 2003]. Also HIV counteract to A3G in Vif independent manner [Dang et al.,...
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2008]. Bone marrow stromal cell antigen 2 (BST-2) / Tethrin: It tethers nascent viral particles into the plasma membrane thus prevents their release and reinfec tion of new target cells [Neil et al., 2008]. Another viral auxiliary protein Vpu interacts with BST-2 and sequesters it away from its site of action and in the absence of Vpu, other viral proteins inhibits it [Kirchhoff, 2009]. SAMHD1 is of great importance in contrast to previously recognized HIV-1 restriction factors due to the fact that SAMHD1 does not meet a counterattack developed by HIV-1 [Cribier et al., 2013]. The enzyme reverse transcriptase of HIV1 with low Km binds to dNTPs with high affinity [Diamond et al., 2004] which conforms lower levels of reverse transcription in non-cycling cells [Ayinde et al., 2012, Lahouassa et al., 2012, Kim et al., 2012, Amie et al., 2013] whereas reverse transcriptase of HIV2 has lower affinity to dNTPs [Fujita et al., 2012]. It is a deoxynucleoside triphosphate phosphohydrolase which cleaves deoxynucleoside triphosphates (dNTP) into deoxynucleosides (dN) and inorganic triphosphates (iP). [Powell et al., 2011, Goldstone et al., 2011, Kim et al., 2012, White et al., 2013, Hrecka et al., 2011, Amie et al., 2013, Beloglozava et al., 2013] as well as lowers the concentration of dNTPs below the level required for reverse transcription of HIV1 [Lahouassa et al., 2012, Franzolin et al., 2013, Schaller et al., 2012]. Inhibition of reverse transcription prevents the synthesis of full length double stranded DNA and also disorders later stages of the viral life cycle which includes nuclear translocation and integration of proviral DNA [Baldauf et al., 2012, Goujon et al., 2013]. Restriction function of SAMHD1 has been recognized in non-cycling cells such as monocytes [Berger et al., 2011], macrophages [Dragin et al., 2013], dendritic cells [Puigdomenech et al., 2013, St. Gelais et al., 2012] and resting CD4+ T cells [Descours et al., 2012].

MECHANISM OF HIV-1RESTRICTION:

Limiting reverse transcription by dNTPase:

SAMHD1 is usually accepted as a host restriction factor for HIV1 infection which controls dNTP pools in immune cells. It cleaves deoxynucleoside triphosphates at the alpha-phosphate position which generates inorganic triphosphates and deoxyribonucleosides , thus depleting dNTP pool which is required by cellular DNA polymerase [Baldauf et al., 2012, Goldstone et al., 2011]. So the early steps of reverse transcription are blocked due to low levels of dNTPs in resting cells. HIV1 can initiate reverse transcription but cannot complete the full length HIV1 cDNA synthesis, SAMHD1 can inhibit the synthesis of full length double stranded DNA, impede later stages of viral life cycle which includes nuclear translocation, integration of proviral DNA and gap repair during HIV1 integration [Zack et al., 1990, Gao et al., 1993, Diamond et al., 2004, Lahouassa et al., 2012]. The overall mechanism is discussed in figure1.

Structure of SAMHD1 helps in restriction:

Tetramerization of SAMHD1 is required for its biological activity and efficient restriction of HIV-1 infection and the intracellular localization of SAMHD1 protein has no role in inhibition of infection [Brandariz-Nuñez et al., 2012, Hofmann et al., 2012]. Earlier it was identified that N terminal truncated protein of SAMHD1 is dimeric [Goldstone et al., 2011] but by using a combination of biochemical and virologic approaches they identified the functional organization of SAMHD1 and its tetrameric state in monocytes cells which strongly restricts HIV-1 infection in contrast to its dimeric form [Yan et al., 2013]. Also the oligomerization of SAMHD1 is not efficient in restriction activity [White et al., 2013, Brandariz-Nuñez et al., 2012]. Chemical cross-linking studies showed that the tetrameric form is regulated by its C terminus which is essential for its full activity to deplete dNTP pool as well as to inhibit HIV1 infection. Also C terminus of SAMHD1 protein has a docking site for Vpx protein which degrades SAMHD1 by proteasomal degradation and the C terminus mostly divergent in SAMHD1 proteins from different primates and vertebrate species [Laguet et al., 2012, Ahn et al., 2012]. Variable C terminal domain and conserved SAM domain is present in vertebrate SAMHD1 proteins which indicates its importance in cellular function. Deletion of N terminal region and SAM domain of SAMHD1 mislocalizes it into cytoplasm which confers no change in its restriction activity. The dNTPase activity of SAMHD1 is induced by deoxyguanosine triphosphate (dGTP) binding at a predicted allosteric sites of HD domain [Goldstone et al., 2011, Powell et al., 2011]. Also HD domain is required for the activity of SAMHD1 in restriction of infection in non-dividing cells [Laguet et al., 2011]. The other domains of SAMHD1 are not well established.

Nuclease activity of SAMHD1:

Besides the dNTPase activity of SAMHD1, metal dependent 3'-5' exonuclease activity also may contribute to HIV1 infection restriction by binding and degrading retroviral genomic RNA or transcribed viral mRNA, and also cDNA products from reverse transcription [Beloglozava et al., 2013, Goncalves et al., 2012, Tüngler et al., 2013]. It was found that SAMHD1 is a nucleic acid binding protein preferably with RNA over DNA. The fluorescence cross-correlation spectroscopy reveals that SAMHD1 specifically binds with single stranded RNA and DNA and the nucleic acid binding and SAMHD1 complex formation are correlated with each other. The

**FIGURE 1:** General mechanism of action of SAMHD1. dNTPs are cleaved into deoxynucleotides (dN) and inorganic phosphates (P_i). Due to lack of dNTPs, reverse transcription (RT) is inhibited.

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interaction between nucleic acid and SAMHD1 complex formation requires HD domain and C terminal region of SAMHD1 but not the SAM domain. This phenomenon established by the mutations linked with Aicardi-Goutières syndrome (AGS), showed both impaired nucleic acid binding and SAMHD1 complex formation. These results suggest the role of SAMHD1 in nucleic acid metabolism, linked with cell proliferation and cell cycle regulation. SAMHD1 specifically breaks 3’ overhangs of double stranded DNA/RNA substrates and RNA in blunt ended DNA/RNA duplexes.

REGULATION
Phosphorylation mediated regulation:
SAMHD1 expression can be regulated by phosphorylation which is a post-translational modification [Herold et al., 2017]. SAMHD1 is simultaneously expressed in both cycling and non-cycling cells but it can inhibit HIV-1 infection only in non-cycling cells [Baldauf et al., 2012, Descours et al., 2012]. In contrast to non-cycling cells, the SAMHD1 is phosphorylated in cycling cells at the position S92 of threonine (T922), which is mediated by cyclin dependent kinase CDK1/2 [Cribier et al., 2013, White et al., 2013, Ballana et al., 2014, Tang et al., 2015, Ruiz et al., 2015]. Human phospho-proteome studies indicates phosphorylation at serine residues 33 and 93 [Bian et al. 2014, Zhou et al., 2013, and another study showed that the gross SAMHD1 phosphorylation is conferred by N-terminal phosphorylation [Badia et al., 2017].

Cell cycle dependent regulation
An enzyme, ribonucleotide reductase (RNR) is a key enzyme in de novo synthesis of dNTP in contrary to SAMHD1. It converts ribonucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs) which after subsequent phosphorylation converts into deoxyribonucleotides (dNTPs). SAMHD1 is downregulated while RNR is upregulated during the S-phase of the cell cycle mediating dNTP pool expansion for nuclear DNA synthesis [Aye et al., 2015]. SAMHD1 is highly expressed in G0 and G1 [Ballana et al., 2014].

Promoter methylation:
Lower expression of SAMHD1 may be associated with malignant diseases on mRNA and protein level [de Silva et al., 2013, de Silva et al., 2014]. Several studies suggested that lower expression is associated with promoter methylation and elevation or reduction of dNTP levels bear mutagenic potentials [Bester et al., 2011, Kunz et al., 1988, Chabosseau et al., 2011, Meuth et al., 1989, Wilkinson et al., 1989]. Later studies on lung cancer, revealed that mRNA level of SAMHD1 was much higher than the protein level, further suggested that post-transcriptional and or post-translational modification may occur. There are also several other mechanisms by which SAMHD1 can be regulated. SAMHD1 can also be regulated by inhibiting the formation of SAMHD1 heterotetramer by the binding of single stranded DNA and RNA species of 60 nucleotides to SAMHD1 dimers [Seamon et al., 2015]. Recently it has been reported that inhibition of SAMHD1 tetramerization and dNTPase activity is mediated by crosslinking cysteine residue 522 to either C341 or C350 in an oxidative intracellular environment [Mauney et al., 2017] and is further suggested by the fact that malignant tumours are usually exposed to higher levels of intracellular oxidative stress [Costa et al., 2014]. Histone acetylation may be also involved in regulation of SAMHD1 expression [Wang et al., 2014]. The H206 and D207 residues of HD domain play a crucial role in dNTPase activity of SAMHD1, whereas mutations to either H206 and D207 abrogates ssDNA binding as well as inhibits tetramer formation, which may suggest that SAMHD1 may regulate its dNTPase activity through NA binding [Ji et al., 2013, White et al., 2013, Beloglazova et al., 2013, Seamon et al., 2015]. Some studies show that SAMHD1 can be induced by IFN following IFN stimulatory DNA treatment [Rice et al., 2009] and also by a combination of IL-12 and IL-18 [Pauls et al., 2013].

APPLICATIONS:
The application of SAMHD1 as the antiretroviral medicine is a challenge for scientists. Though lots of treatment strategies are present to counteract the AIDS but it is true that no one strategy can cure the lethal syndrome fully. So research is going on the inhibition of AIDS. In these circumstances, we strongly believe that SAMHD1 has the potential to act as an antiretroviral agent which can inhibit HIV to some extent. To apply this agent, three approaches are hypothesized by us.

Approach 1:
Nucleoside reverse transcriptase inhibitors (NRTIs), which are agents lacking 3’ OH moiety of ribose sugar ring that precisely retards HIV1 infection and is a key ingredient of highly active antiretroviral therapy (HAART) which maintains low viral load [Erb et al., 2000, Bangsberg et al., 2001]. It is administered as a nucleoside derivative which facilitates crossing of cellular membrane. After entering into the cell, host nucleoside and nucleotide kinases phosphorylates NRTIs and converts them into their triphosphate form. NRTI-TPs are used by viral RTs as a substrate over dNTPs. DNA chain elongation is inhibited after the incorporation of NRTI-TPs into proviral DNA due to the inhibition of formation of the phosphoester bond with an incoming dNTP [De Clercq et al., 2009]. But NRTIs redundantly interacts with several host molecules such as DNA polymerase gamma, mitochondrial DNA polymerase etc leading to off-target effects [Feng et al., 2001, Feng et al., 2004, Lee et al., 2003]. It was identified that SAMHD1 influences the activity of NRTIs against HIV1 infection [Amie et al., 2013]. So, the combination of SAMHD1 and NRTI can efficiently fight against HIV1 infection. It is demonstrated in figure 2.

FIGURE 2: APPROACH 1: NRTI mediated treatment for AIDS. SAMHD1 influences the formation of NRTI triphosphate (NRTI-TP) which can mimic the role of dNTPs and inhibits reverse transcription of HIV.
Approach 2:
p53, a tumor suppressor gene, plays a key role in restriction of HIV1 infection. p53 can be induced by interferons Type 1 in human immune cells after HIV1 infection [Genini et al., 2001, Imbeault et al., 2009, Yoon et al., 2015, Takaoka et al., 2003] and the restriction employed by p53 can be done by various mechanisms such as – inhibition of LTR promoter, suppression of Tat by phosphorylation etc [Duan et al., 1994, Gualberto et al., 1995, Bargonetti et al., 1997, Li et al., 1995]. Recently, scientists suggested that p53 affects HIV1 reverse transcription [Bakhanashvili, 2001, Bakhanashvili et al., 2004]. Low level laser therapy (LLLT) can induce the expression of p53 at certain level required to inhibit the viral infection [Lugongolo et al., 2017] Also the increased expression of p53 further induces the expression of its downstream gene p21, p21, a cyclin dependent kinase inhibitor, has an antiretroviral function by inhibiting CDK2 dependent phosphorylation of SAMHD1 [Ballana et al., 2014, Leng et al., 2014, Allouch et al., 2014]. Both the p53 and p21 combinely restricts HIV1 early stage replication [Shi et al., 2018]. It is demonstrated in figure 3.

FIGURE 3: APPROACH 2: Low level laser therapy (LLLT) mediated treatment for AIDS. LLLT induced the expression of p53 which lead to the over expression of p21. These events lead to the down regulation of CDK2 that can reduce the phosphorylation of SAMHD1 (inactivation).

Approach 3:
IFNs are chemokines which activates innate immunity by enhancing the expression of Inter Stimulated Genes (ISGs). There are several types of ISGs that helps in antiretroviral restriction and one of them is Interferon Induced Transmembrane (IFITM) [Schoggins et al., 2011, Bailey et al., 2014, Narayana et al., 2015]. IFITMs are localized into the cell surface and it inhibits the entry of HIV1 into the host cell and it is also reported that it inhibits the activity of Vpx proteins which can efficiently degrade SAMHD1. Thus it can be hypothesized that IFITM treated cells are required to use SAMHD1 as the remedy for HIV2 infection [Roesch et al., 2018]. It is demonstrated in figure 4. But extensive study is required in this field.

FIGURE 4: APPROACH 3: Interferon Induced Transmembrane (IFITM) for AIDS (especially for HIV2 treatment). VPX degrades SAMHD1 efficiently. VPX protein activity is inhibited by IFITM. So there is no reduction in the antiretroviral activity of SAMHD1.

CONCLUSION
Day by day, the rate of AIDS occurrence is increasing. So it has become very important to develop a new treatment strategy for AIDS. So, keeping the fact in mind, in this review we have focused on SAMHD1 as a viral restriction factor. SAMHD1 may open a new possible way to treat the HIV1 infection. SAMHD1 is a dNTPase, thus it blocks reverse transcription of HIV1 as dNTPs are key molecule in this mechanism. Here we have discussed some approaches for the application of SAMHD1 on restriction of HIV1 infection. This review work may prove to give encouragement and updated valuable information for characterizing SAMHD1 and use it as a remedy for AIDS.
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**Abbreviation:**

HIV: Human immunodeficiency virus; SAMHD1: Sterile alpha motif and histidine-aspartic domain containing protein 1; dNTPase: deoxynucleoside triphosphohydrolase; TRIM: Tripartite motif; APOBEC3G / A3G: Apolipoprotein B mRNA-editing enzyme catalytic polypeptide link 3G; dN: deoxynucleosides; iPPTP: inorganic triphosphates; AGS: Aicardi-Goutieres syndrome; LLT: Low level laser therapy.