Indexed, Abstracted and Cited: ISRA Journal Impact Factor, International Impact Factor Services (IIFS), Directory of Research Journals Indexing (DRJI), International Institute of Organized Research and Scientific Indexing Services, Cosmos Science Foundation (South-East Asia), International Innovative Journal Impact Factor, Einstein Institute for Scientific Information {EISI}, Directory of Open Access Scholarly Resources, Science Indexing Library (UAE), Swedish Scientific Publication (Sweden), citefactor.org journals indexing, Directory Indexing of International Research Journals

World Journal of Biology and Medical Sciences

Published by Society for Advancement of Science®

ISSN 2349-0063 (Online/Electronic)

Volume 3, Issue- 4, 37-47, October to December, 2016

Journal Impact Factor: 4.197

WJBMS 03/04/50/2016

www.sasjournals.com

A Double Blind Peer Reviewed Journal / Refereed Journal wjbmedsc@gmail.com/wjbms.lko@gmail.com

REVIEW ARTICLE

Received: 09/07/2016 Revised: 08/11/2016 Accepted: 15/11/2016

Biotechnology Advances for HIV/ AIDS treatment

Rahul U. Hude and Swati C. Jagdale

Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, S. No. 124, Kothrud, Pune- 411038. Savitribai Phule Pune University, Maharashtra, India

ABSTRACT

The spreading of the Human Immunodeficiency Virus (HIV) is relentless and pandemic thus the increasing deaths from Acquired Immune-Deficiency Syndrome (AIDS) it need the urgency for an effective treatment against AIDS. Current HIV drugs pose many challenges. Incidence and prevalence of AIDS is down sharply since the introduction of the Highly Active Anti-Retroviral Therapy (HAART). Researchers worldwide under took divert research efforts and are trying to develop more effective drug dosage to cure AIDS/HIV. In spite of successful involvement and treatment protocols, an HIV vaccine would be the ultimate strategy to control and prevention. There have been careful efforts for vaccine development. Many scientific approaches have gained popularity and in late 1980s they introduce broadly reactive neutralizing antibodies (BnAbs). Scientists have hypothesized that induction of broadly reactive neutralizing antibodies (BnAbs) by stimulation of right sequences of somatic hyper mutations could be capable of effective neutralization and viral elimination. Genome editing (GE) based HIV therapy is modification of infection-related genes to produce HIV resistant cells followed by reinfusion of the modified cells into patients. Gene therapy offers the promising approach to prevent progressive HIV infection by sustained interference with viral replication in the absence of chronic chemotherapy. Lots of research is going on and clinical trials are carried out from researcher to demonstrate biotechnology based strategies for the effective treatment on

HIV replication. Present chapter deals with various recent techniques and antiretroviral drug therapy based on biotechnology and their future scope to treat HIV/AIDS.
Key words: Human Immunodeficiency Virus (HIV), Acquired Immune-Deficiency Syndrome (AIDS), Biotechnology, Vaccines and Gene Therapy.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the main causative agent of Acquired Immune-Deficiency Syndrome (AIDS). It considered as one of the major public health problem and social stability. It is a global pandemic and is leading infectious disease resulting in significant morbidity and mortality. Globally each year about 5 million peoples affected by this virus. Over 3 million, including 500,000 children die because of AIDS The scale infection of HIV/AIDS epidemic has exceeded all expectations since its first recognition [**Sharma et al., 2012**]. According to UNAIDS and WHO report on HIV/AIDS it was estimated that about 1.6 millions died and 2.3 millions have acquired new infection and approximately 35.3 million people currently living with HIV-1. According to UNAIDS reports a 52% reduction in new HIV infections among children and a combined 33% reduction among adults and children since 2001 [**UNAIDS, 2013**]. AIDS still remains one of the most serious and large humanitarian crises nevertheless and despite prevention policies and anti-retroviral therapies.

HIV is concentrated in specific anatomic sites such as lymphoid organs (spleen, lymph nodes and GALT), central nervous system, testicles and female genital tract. HIV, like other common infectious pathogens, gains access to the host through mucosal membranes in both horizontal (sexual intercourse) and vertical transmission (child delivery and breast-feeding) and after initial replication, latently establishes itself in different viral sanctuaries [**Sharma et al., 2012**, **Agrawal et al., 2013**]. HIV targets the cells responsible for establishing and maintaining immunity. If a level of protective immunity is established, it can be eliminated when the virus attacks immunologically competent B- and T-cells, thereby destroying the cells that provide for long-term immunity. Although work continues to expand our knowledge of the pathogenesis of HIV infection, the prospects for developing an effective prophylactic vaccine still remains elusive in spite of recent successful trial in macaques [**Titti** et al., 2007 and **Hansen et al., 2013**]. As a matter of fact, we do not know which antigen(s), which immunization regimen and which arm of the immune system play a key role in protective immunity.

Current HIV drugs pose many challenges. The development of antiviral drugs can be hindered by several factors such as low efficacy of antiviral agents, less solubility of the compound(s); some drugs have short half life, poor bioavailability when administered in the form of conventional dosage form and systemic toxic side-effects [**Sharma et al., 2012**, **Agrawal et al., 2013**]. Currently available anti HIV drugs can be classified into seven categories and more than twenty five individual drugs are available in the market as conventional dosage forms. The antiretroviral therapy used for HIV/AIDS treatment is associated with dose-dependent cellular toxicity and sub-optimal pharmacokinetic properties. The major clinical toxicities associated with anti-HIV drugs are peripheral neuropathy, pancreatitis and lypidodystrophy necessitating dose reduction or discontinuance of the treatment. An effective antiretroviral therapy, on a long term basis is required to maintain viral suppression and reduction of disease progression. Thus, there is still a need to improve several properties of anti-HIV agents, mainly focusing in enhancing their elimination half-life and maintaining the plasma concentration of the drug within the

therapeutic range to avoid dose limiting toxicities [**Agrawal et al., 2013**, **Patil and Shirote** 2011- **Zhang et al., 2014**].

Biotechnology based HIV/AIDS treatment

Presently various category of anti-HIV agents are used to treat HIV. They have various toxic side effects and treatment cost is so expensive. Researchers worldwide under took divert research efforts and are trying to explore more effective drug dosage to cure AIDS/HIV. Even the best HIV drugs will be too costly for the massive number of peoples who need them. So to stop the rapidly spreading HIV infection worldwide, the best way to stop an infectious disease which will be safe and cost effective is through a vaccine. Vaccine works by imitating a key feature of a disease causing agent. Scientist cannot use either killed or live HIV in a vaccine. Because if killed virus revive and become infectious; similarly live HIV can mutate into lethal form and cause the infectious HIV disease. Thus researchers are trying to develop new vaccine model possible by biotechnology. Development of HIV/AIDS vaccine started in late 1980s. Till upto date more than 100 prophylactic vaccine candidates were tested worldwide for clinical phase 1 trials. Some candidates moved to 2a phase, while few of them advanced to phase 2b and phase 3 efficacy studies. Among all only one candidate shown modest efficacy so far clinically tested for efficacy. The reminders have failed to demonstrate efficacy and in one case there was a trend towards possible enhanced risk of development of HIV infection, acquired through risk behavior, in those vaccinated [**Your world, Biotechnology and you, Biotechnology and AIDS, (2000)**, **Rebecca** et al., 2016 and **Jose, E. (2013)**].

Although some results have been obtained; therapeutic vaccine approaches almost failed to provide adequate level of efficacy. Since the introduction of the Highly Active Anti-Retroviral Therapy (HAART) that completely changed the prognosis for HIV infected patients. The prospects for keeping down the HIV infection and preventing the development of AIDS have improved substantially. The incidence and prevalence of AIDS is down sharply since the introduction of the HAART therapy. This dramatic improvement has not been without hardships and complications (costs of therapy, adverse effects on heart, liver, kidney and compliance etc). Moreover, HIV strains have emerged that are resisting anti-viral therapy, thus making the complete eradication of the virus from the body almost impossible and threatening to derail the effectiveness of HAART. In fact, the virus resides in latent reservoirs within memory CD4+ T-cells and cells that significantly contribute to the generation of elusive replication competent viral genotypes such as the monocyte-macrophages. These cells are typically concentrated in specific anatomic sites (liver, kidney, lungs, lymphoid tissue, testes, gut and the central nervous system). The eradication of the virus from such reservoirs is censorious to the effective long term treatment of HIV/AIDS patients. Therefore, there is a great need to explore and develop new alternative or synergistic and cost effective approaches to i) fight the virus at the entry port ii) eradicate virus from the reservoirs iii) avoid the need of lifetime treatments and iv) provide save delivery to target population (neonates, pregnant women and adults) [**Lafeuillade and Stevenson (2011)** and **Johnston and Barre-Sinoussi, 2012**].

A number of viral vectors and alternative delivery systems are being researched for advanced HIV vaccines. These include vectors such as DNA vectors, mRNA vectors, adenoassociated virus (AAV), Semliki forest virus (SFV),Venezuelan equine encephalitis virus (VEE), vesicular stomatitis virus (VSV), Sindbis virus (SIN), herpes simplex virus (HSV), nonreplicating adenovirus (Ad), Measles virus (MV), modified vaccinia virus Ankara (MVA),

canarypox (ALVAC), and nanoformulations. Table 1 shows examples of viral vectors and alternative HIV vaccine delivery systems [**Muni et al., 2015 and Marjorie Robert-Guroff (2007)**].

Table 1. Examples of viral vectors and alternative HIV vaccine delivery systems [Muni et al., 2015 and Marjorie Robert-Guroff (2007)].

Examples of vectors	Examples of vaccine
Nonreplicating adenovirus vectors (Ad)	Mixture of 4 rAd5 vectors that express HIV-1 subtype B Gag-Pol fusion protein and envelope (Env) from subtypes A, B, and C
Adeno-associated virus (AAV)	Adeno-associated virus based HIV-1 subtype C vaccine (tgAAC09)
Venezuelan equine encephalitis virus (VEE) Sindbis virus (SIN)	Recombinant trimeric HIVΔV2gp140Env protein
Herpes virus (HSV)	Recombinant herpes simplex virus (HSV) envelope and Nef antigens of simian immunodeficiency virus
Measles virus (MV)	Recombinant measles virus vaccines expressing HIV-1 clade B envelope Glycoprotein
Modified vaccinia virus Ankara (MVA)	Modified vaccinia virus Ankara-vectored HIV-1 clade A vaccine
Vesicular stomatitis virus (VSV)	Recombinant vesicular stomatitis virus-(rVSV-) based vectors expressing HIV-1 env 89.6P gp160
Canarypox (ALVAC)	HIV-1 canarypox vaccine (vCP1452)
Semliki forest virus (SFV)	Self-amplifying rSFV2gen RNA encoding HIV-1C antigens
DNA vectors	HIV-1 env/rev DNA vaccine
mRNA vectors	MS2 VLP-mediated RNA vaccine
Nanoformulations	Fullerenol: nanoformulation of virus sized nanoparticles with dual-function nanoadjuvants to simulate immune responses to the HIV DNA vaccine

After being developed and then largely abandoned in the twentieth century, many antibody preparations have been tested for safety and efficacy in preclinical model and are now in clinical trials. Interest in using antibodies to treat infectious diseases is being fuelled in part by the emergence of drug-resistant microorganisms, the relative inefficacy of antimicrobial drugs and of therapeutic vaccine approaches in infected patients and the fact that antibody-based therapies are the only means to provide immediate immunity against biological weapons [**Trkola** et al., 2005, **Ferrantelli** et al., 2007 and **Shingai et al., 2013**].

Broadly Reactive Neutralizing Antibodies (BnAbs)

In HIV infection protective immune response is an ultimate challenge because of the virus characteristics. HIV virus rapidly mutates leads to many changes in their structure therefore should elicit a number of antibodies which are capable of neutralizing many genetically

different strain. Most of the infected HIV patients produce monoclonal type antibodies response capable of to give some level of protection against HIV virus. Similarly virus develops resistance to these antibodies and thrives in the host superseding the humoral and immune response. However there are some notable exception are observed to this nonconventional BnAbs in a very small percentage of HIV infected peoples. This production of BnAbs was measured by standardized neutralization assays. Such known BnAbs are developed during first three year of natural infection. Researcher are trying to focus and have opinion that vaccine regimens should focus on inducing useful BnAbs for the neutralization of virus strain ultimately providing high level of protection. Currently, as potential candidates for inducing BnAbs production; many neutralizing antibody targets are being researched. These include receptor binding sites on gp 41 molecules such as conserved helices and membraneproximal external region (MPER) of gp 41, binding sites on gp 120 for CD4 receptors and CCR5 or CXCR4 coreceptors; variable regions like V1, V2, and V3 on gp 120. Indeed, passive administration of potent neutralizing antibodies protected macaques from infection or delays HIV-1 rebound after cessation of antiretroviral therapy. However, the treatment of HIV-infected individual with antibodies conferred at best only transient reduction of viral load likely due to their pharmacokinetic and limited ability to kill infected cells in the densely packed lymphoid and non lymphoid tissues. Though this is very promising, bnAbs also have shortcomings. BnAbs are very rarely produced and the mechanisms for inducing them through feasible vaccination regimes are not yet fully understood. BnAbs undergo extensive somatic hypermutation thereby developing extreme specificity for viral strains as well as increasing the breadth and potency of HIV viral neutralizations. However, it is difficult to induce BnAbs production because of several levels of somatic hyper mutations needed for the process which takes months to years, by which time the virus develops newer and resistant mutations [**Melissa** et al., 2009, **Devin** et al., 2013, **Yuxing** et al., 2009, **Hua** et al., 2013, **Hugo** et al., 2010, **Peter and John**, 2012, **Ann** et al., 2009 and **Wayne**, 2016].

Cell derived liposomes

M. Machluf et. al (2011) first time developed and demonstrated the proof of concept of cell derived liposomes expressing CCR 5 as a new targeted drug delivery system that can be naturally target infected HIV cell and possibility of treating HIV by viral reservoir depravation without using any antiretrovirals. They prepared Cell-derived-liposomes from the cytoplasmatic membranes of cells expressing CCR5, the human receptor for gp 120, that is found on the surface of virions and HIV-infected cells. The specific targeting and cytotoxicity of the cell-derived liposomes towards gp 120 expressing cells were studied. The CCR 5-conjugated cell derived liposomes can specifically bind and fuse with their target cells and subsequently deliver their content into the infected cells leading to their specific destruction. Such a research opens the door for future studies [**Tomer et al., 2011**].

Many recent technological advances in the field of immunoglobulin research and given the need for new antimicrobial therapies; single chain antibodies (scFV) generated considerable interest regarding their therapeutic applications against HIV infection. With respect to the whole Ig molecule, scFvs offer numerous advantages such as high solubility, small size, refolding capability and good tissue penetration in vivo. Also, genetic engineering technologies have provides the way to harness and reformat individual recombinant Abs thereby tailoring their utility for therapeutic applications. Nevertheless, scFV approach, although able to block virus life cycle, could not sufficient however to attack the provirus in

the infected cells. This is the major hurdle to cure HIV [**Philippe** et al., 2013 and **Alexander et al., 2013**].

Genome editing based HIV therapy

Genome editing (GE)-based HIV therapy is achieved by modification of infection-related genes to produce HIV resistant cells followed by reinfusion of the modified cells into patients. The ultimate goal is to achieve a functional or actual cure for HIV infection. Despite multiple potential targets for GE-based HIV therapies, CCR5 is the most feasible owing to the naturally existing CCR5 d32 genotype which confers resistance to HIV. A recent clinical trial of infusion of modified autologous CD4+ T cells proved safety and efficacy within the limits of the studies. However, long-term evaluation of the safety and efficacy is required before GE-based HIV therapy is ready for clinical implementation [**Yusa et al., 2012**].

Table 2. New GE technologies [Wan-Gang, 2015].

Nuclease	Origin	Delivery tool	Advantages	Disadvantages
ZFN	Eukaryotes	AV, AAV, LV	Compatible with various viral vectors; each ZF module (30 amino acids) recognizes three sequence-specific nucleotides; widely used in various cell types and multiple organisms including humans	Design of ZF arrays for sequence recognition is required; off-target effects
TALEN	Xanthomonas	AV	Each TALE repeat (33–35 amino acids) recognizes one nucleotide; widely used in various cell types and multiple organisms including humans	Design of TALE arrays for sequence recognition is required; the binding site starts with a T base; larger size than ZFN; off-target effects
CRISPR/ Cas	Bacteria	Plasmids	RNA-guided DNA endonuclease; targets multiple sites simultaneously to produce large gene fragment deletions; much simpler design (a short RNA for the gene of interest); potential application in various hosts including humans	The target sequence needs to be preceded by PAM; off-target effects

AV, Adenovirus; AAV, adeno-associated virus; CRISPR/Cas, clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9; LV, lentivirus; PAM, protospacer adjacent motifs; TALE, transcription activator-like effector; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

Advances in GE-based therapies

The technology that underpins GE is crucial for successful therapy, and will make future therapeutic advancements possible. Transcription activator-like effector nucleases (TALENs), TALEs are proteins secreted by numerous species of Xanthomonas to modulate gene expression in host plants and to facilitate bacterial colonization and survival. Interestingly enough, these proteins bind to DNA in a specific way and can be modified for targeting a given genome sequence. TALEs can be in turn converted into DNA scissors by connecting them to a site specific endonuclease (TALENs) that should have the ability to selectively cleave the HIV provirus; zinc-finger nucleases (ZFNs), and the clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) systems have all been used in GE-based therapies. New GE technologies and their details are shown in Table 2. The ZFN system has been the most widely used in GE studies targeting HIV infection. Although there are only a few reports of GE-based HIV therapies using the TALEN and CRISPR/Cas 9 systems, these two GE tools may have great potential. The major strategy for GE-based HIV therapies is to produce engineered immune cells that are resistant to HIV infection or replication [**Mark et al., 2013 – Feng et al., 2011**]. Table 3 summarizes some recent GE based HIV therapies [**Wan-Gang, 2015]**.

Table 3. Summary of recent GE based HIV therapies [Wan-Gang, 2015].

Target gene	Editing tool	Target Cell
CCR5	ZFN	Autologous CD4+ T cells (clinical trial)
CCR5	ZFN	Autologous CD4+ T cells (clinical trial)
CCR5	ZFN	Autologous CD4+ T cells (clinical trial)
CCR5	ZFN	Autologous hematopoietic stem cells (clinical trial)
CCR5	ZFN	Induced pluripotent stem cells, embryonic stem cells
CCR5	ZFN	Hematopoietic stem, progenitor cells
CCR5	ZFN	Hematopoietic stem, progenitor cells
CCR5	ZFN	TZM-bl cells
CCR5	ZFN/TALEN	293 T cells
CCR5	CRISPR/Cas9	293 T cells
CCR5	CRISPR/Cas9	iPSCs
CCR5 (RF)	ZFN	Jurkat cells, JLTRG-R5 cells
CXCR4	ZFN	T cells
CXCR4	ZFN	T cells
Provirus (LTR)	ZFN	Infected T cells
Provirus (Pol)	ZFN	TZM-bl cells, HeLa-derived JC53-BL cells
Provirus (LTR)	CRISPR/Cas9	Infected T cells
Provirus (LTR)	CRISPR/Cas9	Microglial, promonocytic, and T cells
LEDGF/p75	TALEN	293 T cells, Jurkat cells
TSPO	CRISPR/Cas9	293 T cells

Abbreviations: CCR5, C-C chemokine receptor type 5; CCR5 (RF), restriction factors inserted into the CCR5 locus; CXCR4, C-X-C chemokine receptor type 4; CD4, cluster of differentiation 4; CRISPR/Cas, clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9; LEDGF, lens epithelium-derived growth factor; LTR,

long terminal repeat; TALEN, transcription activator-like effector nuclease; TSPO, mitochondrial translocator protein; ZFN, zinc-finger nuclease.

Human gene therapy is based on the introduction of new genes into the cells of an individual with the intention to achieve a curative effect. Gene therapy also is an alternative treatment for a wide range of infectious diseases that cannot be treated by standard clinical procedures. In the past years, cleavage-based genome methods have been developed based on the generation of nucleases for genome engineering by linking cleavage domain of the FokI restriction enzyme to a designed zinc finger protein. The therapeutic potential of such approaches have been noted and clinical trials have been designed using human cells precisely modified by designed nucleases [**Manjunath et al., 2013**, **Wan-Gang, 2015**].
Several different anti–HIV-1 gene therapy approaches have been tested in hematopoietic cells over the past 15 years. These approaches can be classified into two categories (I) Protein-based agents (including intrabodies, intrakines, dominant-negative proteins, fusion inhibitors and zinc-finger nucleases). (II) RNA-based agents (including ribozymes, antisense, aptamers and RNA interference (RNAi)). Protein-based inhibitory agents: proteins can be directed to inhibit viral or cellular targets. RNA-based inhibitory agents: Ribozymes are antisense RNAs that can inhibit HIV replication by enzymatically cleaving targeted mRNAs. Lots of research is going on and clinical trials are carried out from researcher to demonstrate protein and RNA based strategies for the effective treatment on HIV replication [**John et al., 2007**].

ACKNOWLEDGEMENTS

Author's are sincerely thankful to Dr. B. S. Kuchekar, Principal and management of MAEER's Maharashtra Institute of Pharmacy, Pune, India for all support and facility.

REFERENCES

http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_unaids_global_report_2012_with_annexes_en.pdf (Accessed on May 2016).
http://www.who.int/hiv/data/global_treatment_report_presentation_2013.pdf Accessed on May 2016).
http://www.unaids.org/en/media/unaids/contentassets/documents/factsheet/2014/20140716Fact Sheet_en.pdf Accessed on May 2016).

Sharma P., Chawla A., Arora S. and Pawar P., (2012). Novel drug delivery approaches on antiviral and antiretroviral agents. *Journal of Advanced Pharmaceutical Technology & Research*. 3(3): 147-159.

UNAIDS report on the global AIDS epidemic. (2013).

Rohini Agrawal, N., Joseph, A. and Mukerji, A. (2013). Polymeric Prodrugs: Recent Achievements and General Strategies. *Journal of Antivirals and Antiretrovirals*, S15: 1-12.

Titti, F., Cafaro, A., Ferrantelli, F., Tripiciano, A., Moretti S., et al., (2007). Problems and emerging approaches in HIV/AIDS vaccine development. *Expert Opin Emerg Drugs* 12: 23-48.

Hansen, S., Piatak, M., Ventura, A., Hughes, C., Gilbride, R., et al., (2013). Immune clearance of highly pathogenic SIV infection. *Nature* 502: 100-104.

Patil, S. and Shirote, P., (2011). Prodrug approach: an effective solution to overcome side-effects. *International Journal of Medical and Pharmaceutical Sciences.* 1(7): 1-13.

Zhang, Y., Gao, Y., Wen, X. and Ma, H. (2014). Current prodrug strategies for improving oral absorption of nucleoside analogues. *Asian Journal of Pharmaceutical Sciences*. 9: 65-74.

Your world, Biotechnology and you, Biotechnology and AIDS, (2000). Published by The biotechnology institute Pennsylvania, 9(2): 1-16.

Rebecca, L., Tie Qun, Z. and Ivana, K. (2016). Review of efficacy trials of HIV-1/AIDS vaccines and regulatory lessons learned. A review from a regulatory perspective. *Biologicals*. 44: 73-89.

Jose, E. (2013). A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine*. 31: 3502– 3518.

Lafeuillade, A. and Stevenson, M. (2011). The search for a cure for persistent hiv reservoirs. *AIDS Reviews*. 13: 63-66.

Johnston, R. and Barre-Sinoussi, F. (2012). Controversies in HIV cure research. *Journal of the International AIDS Society* 15: 16.

Muni, R., Venkataraghavan, R., Anshul, S., Nancy, S. and Sandeep, A., (2015). HIV vaccine: recent advances, current roadblocks, and future directions. *Journal of Immunology Research* 2015:1-9.

Marjorie Robert-Guroff (2007). Replicating and non-replicating viral vectors for vaccine development. *Current Opinion in Biotechnology*. 18(6): 546–556.

Trkola, A., Kuster, H., Rusert, P., Joos, B., Fischer, M., et al. (2005). Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nature Medicine*. 11: 615-622.

Ferrantelli, F., Buckley, K., Rasmussen, R., Chalmers, A., Wang, T., et al., (2007). Time dependence of protective post-exposure prophylaxis with human monoclonal antibodies against pathogenic SHIV challenge in newborn macaques. *Virology*. 358: 69-78.

Shingai, M., Nishimura, Y., Klein, F., Mouquet, H., Donau, O., et al., (2013). Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* 503: 277-280.

Melissa, D. S., Wasima, R., et al., (2009). Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *Journal of Virology*. 83(14): 7337–7348.

Devin, S., Uri, L., et al., (2013). The effects of somatic hypermutation on neutralization and binding in the pgt 121 family of broadly neutralizing hiv antibodies. *PLOS Pathogens*. 9(11): 1-20.

Yuxing, Li., Krisha, S., et al., (2009). Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals. *Journal of Virology.* 83(2): 1045–1059.

Hua Xin, L., Rebecca, L., et al., (2013). Coevolution of a broadly neutralizing HIV1 antibody and founder virus. *Nature*. 496(7446): 1-11.

Hugo, M., Johannes, F. S., et al., (2010). Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. *Nature*. 467(7315): 591–595.

Peter, D. K. and John, R. M. (2012). Human antibodies that neutralize HIV-1: identification, structures, and b cell ontogenies. *Immunity*. 37(3): 412–425.

Ann, J. H., Pascal, P., et al., (2009). Effective, low titer, antibody protection against low-dose repeated mucosal shiv challenge in macaques. *Nature Medicine*. 15(8): 951–954.

Wayne, C. K., (2016). A shot at AIDS. *Current Opinion in Biotechnology*. 42:147–151.

Tomer, B., Naama, T. Et al., (2011). Cell derived liposomes expressing CCR5 as a new targeted drug-delivery system for HIV infected cells. Journal of Controlled Release. 151: 139–148.

Philippe, P. M., Robin, J. V. and Nardos, G. T., (2013). *In Vivo* Applications of Single Chain Fv (Variable Domain) (scFv) Fragments. *Antibodies*. 2:193-208.

Alexander, F., Masoud, A., et al., (2013). Gene therapy using a secreted single chain variable fragment targeting CCR5 to inhibit HIV infection. *Journal of Antiviral and Antiretroviral*. 5(4): 85-91.

Mark, J. O., Colby, G. S., et al., (2013). TALEN-based gene correction for epidermolysis bullosa. *Molecular Therapy*. 21(6): 1151–1159.

Ting, Li and Bing, Y., (2013). TAL effect or nuclease (TALEN) engineering. *Methods in Molecular Biology*. 978: 63-72.

Victoria, M. B., Ying, W., et al., (2012). *In vivo* genome editing using high efficiency TALENs. *Nature*. 491(7422): 114–118.

Berdien, B., Mock, U., et al., (2014). TALEN-mediated editing of endogenous T-cell receptors facilitates efficient reprogramming of T lymphocytes by lentiviral gene transfer. *Gene Therapy*. 21: 539–548.

Qiurong, D., Youn-Kyoung, L., et al., (2013). A TALEN genome editing system to generate human stem cell based disease models. Cell Stem Cell. 12(2): 238–251.

Florian, H., Grainne, K., et al., (2013). E-TALEN: a web tool to design TALENs for genome engineering. *Nucleic Acids Research*. 41(20): 1-7.

Tomonori, K., Arslan, A., Et al., (2013). An efficient strategy for TALEN-mediated genome engineering in Drosophila. *Nucleic Acids Research*. 41(17): 1-9.

Xavier, M. A., Sharma, R., et al., (2013). Robust ZFN-mediated genome editing in adult hemophilic mice. *Blood*. 122 (19): 3283-3287.

Gupta, A., Christensen, R.G., et al., (2012). An optimized two-finger archive for ZFN-mediated gene Targeting. *Nature Methods*. 9(6): 588–590.

Yusa, K., Rashid, S. T., et al., (2012). A ZFN/piggyBac Step Closer to Autologous Liver Cell Therapy. Hepatology. 55(6): 2033-2035.

Manjunath, N., Guohua Yi., et al., (2013). Newer gene editing technologies toward hiv gene therapy. *Viruses*. 5: 2748-2766.

Thomas, G., Charles, A. G., et al., (2013). ZFN, TALEN and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*. 31(7): 397–405.

Maarten, H., Ignazio, M., et al., (2013). Differential integrity of TALE nuclease genes following adenoviral and lentiviral vector gene transfer into human cells. *Nucleic Acids Research*. 41(5): 2-14.

Tessa, G. M., Jose, M. C., et al., (2014). CHOPCHOP: a CRISPR/Cas9 and TALEN web tool for genome editing. *Nucleic Acids Research*. 42: W401–W407.

Yanjiao, S., Yuting, G., et al., (2014). CRISPR/Cas-mediated genome editing in the rat via direct injection of one-cell embryos. Nature Protocols. 9(10): 2493-2512.

Luke, A. G., Max, A. H., et al., (2014). Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell*. 159(3): 647–661.

Brian Owens, (2014). Zinc-finger nucleases make the cut in HIV. *Nature Reviews Drug Discovery*. 13:321-322.

Jens, B., Ulla, B., (2010). *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. *Annual Review of Phytopathology*. 48:419–436.

Adam, J. B., Sebastian, S., et al., (2010). TAL effectors: finding plant genes for disease and defense. *Current Opinion in Plant Biology*. 13:394–401.

Michelle, C., Tomas, C., et al., (2010). Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*. 186: 757–761.

Jeffrey, C. M., Siyuan, T., et al., (2011). TALE nuclease architecture for efficient genome editing. *Nature Biotechnology*. 29(2): 143-150.

Feng, Z., Le, C., et al., (2011). Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nature Biotechnology. 29(2): 149-154.

Wan-Gang, Gu, (2015). Genome editing-based HIV therapies. *Trends in Biotechnology*. Article in press: 1-8.

John, J. R., Carl, H J., et al., (2007). Genetic therapies against HIV. *Nature Biotechnology*. 25 (12): 1444-1454.

Corresponding author: Dr. Swati C. Jagdale, Professor and Head, Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, Pune, (MS), 411038, India

Email: jadgaleswati@rediffmail.com swati.jagdale@mippune.edu.in

Contact number: +91-9881478118