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GENOMIC RECOMBINATION BETWEEN HIV-1 AND VZV VACCINE STRAIN WITH VIROPLASMID LIKE ACTIVITY IN HIV - A THOUGHT

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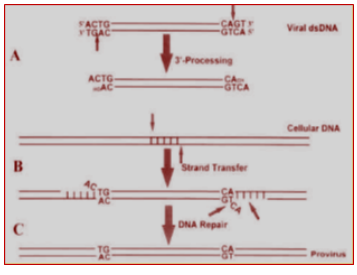
ABSTRACT The integration of Human Immunodeficiency Virus-1 (HIV-1) DNA into the host DNA is a critical step in the HIV life cycle. Hence, structural, pharmacological and biochemical study of retroviral DNA integration has been the cornerstone of intensive research in the past three decades. In this context, the hypothesis purpose is to explore the possibility of recombination between HIV-1 and Varicella-zoaster vaccine (VZV) viruses and the impact of genetic recombination on viral integration and other stages of HIV life cycle. I make use of the principles of HIV integrase (IN) enzyme action on the HIV-1 DNA ends during 3' processing operation (Fig. 1). During replication of HIV-1 in the cytoplasm, IN produces cleavage of the dinucleotide "GT" at each DNA 3'end and generates dinucleotide "CA" 5' overhang at both ends. Varicella vaccine DNA when subject to restriction enzyme digestion that produces double stranded DNA with "GT" dinucleotide 5' overhangs at both the ends. The digested DNA and ligase enzyme is transfected to HIV negative peripheral blood mononuclear cells (PBMC) and coculture with HIV-1 positive PBMC. Because of complementarity phenomenon, annealing occurs between DNA of HIV 3'hydroxyl ends with DNA of VZV 5' phosphate ends. The hypothetical recombinant HIV particles may provide a significant insight for the development of novel classes of genetic mechanisms such as cells and gene therapy, anti-HIV drugs or vaccines that work synergistically or at least augments with the existing pharmacotherapy.

KEYWORDS : Recombination, HIV, Varicella, Vaccine, Viroplasmid.

INTRODUCTION:

HIV possess high rate of genetic variation, one of the major obstacles for its successful eradication. It occurs as a result of mutations due to error-prone reverse transcription, high levels of viral turnover, retroviral recombination and selection pressure from the immune system. In HIV, recombination occurs much more frequently than mutation, and is a major cause of viral diversi fication.[21,22,24,28,40,43,52,69,72,73]



The key step in the HIV lifecycle is the formation of the provirus, from cDNA produced during reverse transcription process. Integration is promoted by the viral IN enzyme, which catalyzes two distinct reactions. The first reaction is 3' processing, catalyzes an endonucl eolytic cleavage at the 3′ site of the conserved CA, which generally releases a terminal GT dinucleotide from the U5 and U3 ends of HIV-1 DNA in the cell cytoplasm (Fig. 1). The resulting viral DNA is then transferred into the nucleus and IN uses 3'-OH groups formed during 3' processing DNA in a staggered fashion and covalent joining of the HIV 3'-hydroxyl groups with the host DNA 5' phosphate ends and establishes new phosphodiester bond between them with the help of host co-factors.[6,7,14,17,19,29,32,34,36,37,44,45,47,51,55,56,61-63,65,70,74]

HIV-1 IN generally cleaves two bases symmetrically at both the ends of U5 and U3 linear double-stranded DNA regions (Fig. 1), While the sequences of HIV-2 IN may not always act symmetrically and cleaves two and three bases, respectively, from the U3 and U5 ends of the linear double-stranded DNA prior to integration.[37,49]

In HIV infection two different forms of latency exits preintegration and postintegration latency and thus we need to target the multiple steps of viral life cycle in order to eradicate HIV in addition to HAART treatment.[10,12,13,27,30,38,46,51,61,69]

Varicella-zoster virus (VZV) causes varicella (chickenpox), generally a mild rash disease of childhood but rarely causes serious disease in adults and immunosuppressed persons. The live, attenuated VZV Oka and Oka/ Merck strain vaccine is safe and effective for preventing childhood infection. VZV vaccine possesses multiple antigenic epitopes offer as recombinant vaccines against other infectious diseases.[48,67,71,72]

MATERIALS AND METHODS

1. HIV positive and negative blood sample collection:

After obtaining informed consent, blood samples will be collected from normal donors and infected individuals by venepuncture tubes containing EDTA anticoagulant. About 10 ml of blood will be collected for screening and coculture. A total of 10 HIV-1 positive samples will be collected for the study and all the HIV positive samples will be retested by a rapid test method. All HIV-1 positive samples will be also screened for HIV-2, HBV and HCV. Only negative samples for these infections will be included in the study.

Similarly, 10 HIV donor blood (negative) samples will be collected in EDTA anticoagulant tubes and all the HIV negative samples will be tested by a rapid test method. About 10 ml of blood will be collected for screening and coculture. All HIV negative samples also will be screened for HBV and HCV. Only negative samples for these infections will be included in the study.

Additionally, after obtaining informed consent from the parents of 5 children, blood samples of recently chickenpox vaccinated children will be collected and tested for IgM and IgG antibodies. The seropositive blood will be used for confirmation of recombination between HIV-1 and VZV genomes.

2. Processing of HIV-1 positive blood sample:

PBMC will be separated as per standard protocol. PBMC will be stained to know viability of the cells with trypan blue staining (viable PBMC are clear whereas nonviable PBMC are blue) and cells will be counted with hemacytometer.

3. Processing of HIV negative blood sample:

(1) PBMC will be separated as above for positive blood samples. (2) Stimulation PBMC: donor PBMC will be stimulated as per the standard protocol.

4. Variped (Varivax) and Varilrix vaccine DNA extraction:

Variped is the live attenuated Oka/Merck strain of varicella-zoster virus and Varilrix is the live attenuated Oka strain. The vaccines DNA will be extracted as per DNA extraction kit protocol.

TABLE 1: HUMAN HERPESVIRUS 3 STRAIN VARILRIX, COMPLETE GENOME, GENBANK: DQ008354.1, 5' OVERHANG: GT (FROM NCBI)

Pick all	Enzyme	Specificity	Total cuts	Compatible cuts	% activity in			
					1.1	2.1	3.1	CS

☐	BceAI	ACGGC(N)$_{12}$ NN	275	18	100	100	100	100
☐	FauI	CCCGCNNNN NN	302	27	100	50	10	100
☐	Hpy188I II	TCNNGA	395	12	100	100	10	100

TABLE 2: SEQUENCE DIGESTED WITH: BceAI AND FauI WITH STICKY ENDS (BOTH ENDS WITH 5' GT NUCLEOTIDE OVERHANGS): VARILRIX VACCINE (FROM NCBI)

	BceAI	FauI
1	28 * 16916/16918	30 * 17055/17057
2	142 * 67139/67141	119 * 67353/67355
3	200 * 91831/91833	173 * 91794/91796
4	235 * 107091/107093	216 * 107120/107122

TABLE 3: SEQUENCE DIGESTED WITH: FauI AND Hpy188III WITH STICKY ENDS (BOTH ENDS WITH 5' GT NUCLEOTIDE OVERHANGS): VARILRIX VACCINE (FROM NCBI)

	FauI	Hpy188III
1	300 * 124728/124730	395 * 124650/124652

5. VZV DNA restriction enzyme digestion:

The extracted VZV DNA will be subjected to restriction sequential digestion with the combination of two enzymes such as (1) BceAI and FauI, (2) BceAI and Hpy188III and (3) FauI and Hpy188III. This digestion method yields double stranded VZV DNA with both ends GT dinucleotide 5' overhangs. The restriction enzyme digestion will be performed as per manufacture's instruction.

TABLE 4: HUMAN HERPESVIRUS 3 STRAIN VARIPED (VARIVAX), COMPLETE GENOME GENBANK: DQ008355.1, 5' OVERHANG: GT (FROM NCBI)

Pick all	Enzyme	Specificity	Total cuts	Compatible cuts	% activity in			
					1.1	2.1	3.1	CS
☐	BceAI	ACGGC (N)$_{12}$NN	275	18	100	100	100	100
☐	FauI	CCCGCN NNNNN	301	27	100	50	10	100
☐	Hpy188I II	TCNNGA	394	12	100	100	10	100

TABLE 5: SEQUENCE DIGESTED WITH: BceAI AND FauI WITH STICKY ENDS (BOTH ENDS WITH 5' GT NUCLEOTIDE OVERHANGS): VARIPED (VARIVAX) VACCINE (FROM NCBI)

	BceAI	FauI
1	28 * 16916/16918	30 * 17055/17057
2	142 * 67136/67138	120 * 67350/67352
3	200 * 91828/91830	174 * 91791/91793
4	235 * 107088/107090	217 * 107117/107119

TABLE 6: SEQUENCE DIGESTED WITH: FauI AND Hpy188III WITH STICKY ENDS (BOTH ENDS WITH 5' GT NUCLEOTIDE OVERHANGS): VARIPED (VARIVAX) VACCINE (FROM NCBI)

	FauI	Hpy188III
1	299 * 124722/124724	394 * 124644/124646

Human herpesvirus 3 strain Variped (Varivax) and Varilrix complete genome obtained from The National Center for Biotechnology Information (NCBI), Bethesda, MD USA, National Library of Medicine, online resources. The full length VZV DNA was subjected to restriction enzymes digestion by blast from NCBI online resources. Out of many enzymes digestion, only 3 restriction enzymes generated 5' GT cohesive ends shown in the table 1, of which combination of any two enzymes produced only at 5 sites double stranded continuous stretch VZV DNA ends have 5' GT cohesive at both the ends shown in the tables 2 and 3. Similarly, Human herpesvirus 3 strain Variped (Varivax) complete genome processed as for Varilrix and DNA digestive fragments shown in the tables 4, 5 and 6.

6. VZV DNA transfection to HIV negative PBMC:

The restriction enzyme digested VZV DNA will be transfected to HIV negative PBMC by standard transfection protocol.

7. Ligase enzyme transfection to negative PBMC:

Ligase will be transfected to HIV negative PBMC as protein transfection protocol.

NOTE:

Trypan blue stain will be performed after donor PBMC separation, stimulation of PBMC, VZV DNA transfection, ligase enzyme transfection and positive PBMC separation.

8. HIV-1 Qualitative PBMC MicrococultureProtocol[2]:

Normal donor PBMC will be separated from the whole blood by density gradient method and then resuspend the cells in IL-2-growth medium. This is followed by transfection of VZV DNA and Ligase to donor PBMC. In two wells of a 24-well tissue culture plate 1 x 10^6 PHA stimulated VZV and ligase transfected negative PBMC will be added with 1x10^6 patient PBMC and final volume will be adjusted to 2 ml with growth medium. 2 ml of sterile water will be put in the corner wells to maintain humidity. Negative PBMC separately coculture to verify the absence of HIV infectivity. Lastly, only HIV coculture will be performed with positive HIV-1 sample to know the replication of HIV as positive control and incubated at 37°C with 5% CO_2. On days 4, 11 and 18 carefully remove 1 ml of supernatant from the well without disturbing the cells and fed with an equal volume of fresh growth medium only. On days 7, 14 and 21, 1.0 ml of medium will be removed without disturbing cells and replaced with 1 ml fresh growth medium containing 5 x 10^5 PHA-stimulated negative cells and culture will be maintained for 21 days.

9. HIV-1 Qualitative PBMC Macrococulture Protocol[3]:

About 3-5 million patient PBMC will be taken in a labeled sterile cell culture flask and about 3-5 million PHA-stimulated, VZV DNA and ligase enzyme transfected negative PBMC will be added. Donor PBMC separately coculture to verify the absence of HIV infectivity. Lastly, only HIV positive sample coculture will be performed to know the replication of HIV as positive control. On days 4, 11, 18 and 25 carefully removed half of supernatant from the flask without disturbing the cells and fed with an equal volume of fresh growth medium only. On days 7, 14 and 21 half of supernatant carefully removed from the flask without disturbing the cells and will be fed with an equal volume of fresh growth medium containing 3-5 million PHA-stimulated PBMC and culture will be maintained for 28 days.

10. RECOMBINATION CONFIRMATION:

A. **Micrococulture:** On days 7, 14 and 21 about 1 ml of cultured cells will be subjected to one or more of the following tests such as HIV DNA PCR, Agglutination test with VZV seropositive sample and/or genome sequencing for confirmation of genomic recombination between HIV-1 and varicella-zoster virus. On days 4, 7, 11, 14, 18, 21 and 25 supernatant will be subjected to HIV RNA PCR and HIV-Tridot plus antigen qualitative assay for positive growth confirmation.

B. **Macrococulture:** On days 7, 14, 21 1nd 28 about 5 ml of cultured cells will be subjected to one or more of the following tests such as HIV DNA PCR, Agglutination test with VZV seropositive sample and/or genome sequencing for confirmation of genomic recombination. On days 4, 7, 11, 14, 18, 21, 25 and 28 supernatant will be subjected to HIV RNA PCR and HIV-Tridot plus antigen qualitative assay for positive growth confirmation.

RESULTS:

The hypothetical anticipation is based on the principles of IN enzyme action on the HIV-1 DNA ends during 3' processing operation (Fig. 1) and varicella restriction enzyme digested DNA ligation. When, donor DNA transfected PBMC coculture with HIV-1 positive PBMC for 4 weeks, because of complementarity phenomenon, annealing may occur between DNA of HIV 3'hydroxyl ends with DNA of VZV 5' phosphate ends.

To the best of my knowledge, the present hypothetical speculation has no comparison since no other studies performed similar kind of research and I assume IN enzymatic action may be inhibited by cohesive ends principles with VZV DNA complementary strand. In this phenomenon, the law of genetics governs the joining of phosphodiester bond between the 3'hydroxyl of HIV-1 DNA and 5'phosphate of VZV DNA. Complementary double stranded DNA formation occurs between the HIV-1 DNA and VZV DNA owing to the

fact that 5' overhangs produced by HIV-1 IN during 3' processing and VZV DNA 5' overhangs produced by restriction enzyme digestion.

If HIV-1 genome is able to combine with the digested varicella-zoster virus genome, consequently, a novel HIV-1 recombinant species may be formed. As a result, the formation of provirus may be inhibited with human genome. Various studies were conducted by many authors on IN enzyme activity inhibition strategies which are cited here for mentioning purpose only, not for comparison. The present hypothesis is compared with others' work with respect to HIV DNA end modification process. Haobo Zhou et al reported that the most important feature of the viral att sequence for integration is the sequence 59-CAXX-39. Replacing any one of the two bases substantially impairs 39-end processing and strand transfer. Alteration of both the U3 and U5 conserved CA to TG results in severe reduction in integration. A highly conserved CA dinucleotide adjacent to the 3' processing site of HIV-1 is important for both the 3' processing and strand transfer reactions. Alteration of nucleotide sequences are poor substrates for HIV-1 IN.[47,65] Aviad Levin et al and Joseph Rosenbluh et al claims that HIV integration can be blocked by peptides. Frederic D. Bushman et al have studied modifications of short region on both DNA strands at the ends of the viral DNA, block IN protein function. John Capodici et al studied inhibition of HIV-1 infection by small interfering RNA-mediated RNA Interference. Robert L Lafemina et al reported importance of substrate specificity and IN-mediated processing of an LTR substrate could be inhibited by competition with LTR and non-LTR oligonucleotides.

In the present postulation, recombination between HIV-1 and VZV compared with other studies. Many studies reported genetic recombination between HIV 1 and 2 and intra species recombination. Kazushi Motomura et al. studied genetic recombination between HIV-1 and HIV-2. Mattias Mild et al. reported intrapatient recombination between HIV Type 1 R5 and X4 Envelopes. David N Levy et al reported retroviral recombination during reverse transcription and other stages of viral life cycle.

If the digested varicella genome anneals to HIV cohesive strand at one or both ends may generate integration deficient episomal HIV species. Recombinant HIV/VZV DNA could be a future potential candidate vaccine which may act on various stages of HIV life cycle. Recombination is being used experimentally by virologists to create new vaccines for example vaccinia viruses can carry vaccinia virus DNA recombined with DNA from other sources (exogenous DNA).[64] Denise C. Hsua et al reported recombinant HIV-1 Env glycoprotein subunit vaccines, polyvalent mosaic antigens expressed on viral vectors. Donatella RM Negri et al demonstrated that non-integrating lentiviral vectors induce a strong and sustained immune response in vivo after a single injection. Wayne L. Gray reported recombinant varicella-zoster virus vaccines for expression of heterologous antigens. Yang Ou et al reported recombinant simian varicella viruses produce immune responses to simian immunodeficiency virus antigens in immunized vervet monkeys. Klaus Wanisch et al showed that Integration-Deficient Lentiviral Vectors (IDLVs) are efficient delivery vectors generate high levels of nonreplicating episomal molecules.

The HIV replication cycle offers multiple potential targets for genetic manipulation. Christopher W Peterson et al reported combinatorial anti-HIV gene therapy, CCR5 disruption, anti-HIV entry therapies, inhibition of viral uncoating and integration, disruption of HIV provirus. In the last few years rapid development has been witnessed for modification of gene expression by gene modification technologies. Berlin patient is the classical example of gene modification approach.[20,35,50]

DISCUSSION:

One of the major difficulties in treating HIV infection and generating an effective vaccine is due to high rate of genetic variation in the viral population.[52,69] Highly active antiretroviral therapy (HAART) significantly improves life span of people living with HIV/AIDS. However, persistence of HIV in reservoirs as pre and postintegration latency required lifelong medication with many complications including toxicity and emergence of multidrug resistant mutants. Cells and gene therapies offer the promising new methods of treatment for HIV infection in the absence of chronic antiviral pharmacotherapy.[35] The present exploration concept could be one of the treatment modality as cells, gene therapy or vaccine development.

The resultant recombinant HIV-1 and VZV, a novel recombinant virus may act as follows. (1). the recombinant HIV particles may act as extrachromosomal DNA as episomes, produce Integration Deficient Species (IDP), and thus prevent the formation of provirus. (2). Recombinant molecule may proliferate with or without production of antigens of one or both HIV and VZV genomes which may induce effective immune response. (3). When recombinant virus cocultivate with wild HIV, it may overgrow the wild virus by interference phenomenon and may significantly decrease the wild virus. (4). Recombinant HIV particles may be used In vivo to study viral behavior with wild virus.

Eva Poveda et al reported exosomes as new players in HIV pathogenesis, exosomes can carry different molecules in their lumens including proteins, RNA and other biological materials. It may provide novel strategies act at different aspects of HIV life cycle.

CONCLUSION:

The hypothetical HIV and VZV cocktail virus may provide a significant insight for the development of novel classes of anti-HIV drugs, vaccine or genetic mechanisms that work synergistically or at least augments with the existing pharmacotherapy. However, several problems of various virological and immunological responses have to be addressed and resolved before considering the effectiveness of this hypothesis. HAART alone is not enough to achieve functional cure but it needs enhanced immune system which can be achieved by vaccination, cells, gene therapy or other effective modes. While a solution will not be achieved by tomorrow, but the battle against HIV-1 development of new therapeutic strategies are well-underway.

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TRANSPARENCY DECLARATION

The author has declared that there are no conflicts of interest in connection with this article.

REFERENCES

1. ACTG lab Manual Preparation of PHA-Stimulated Uninfected Donor PBMC. Version 1.0, 01 October 2004.
2. ACTG Lab Manual HIV Qualitative PBMC Micrococulture Assay. Revised Version 2.0, June 2004.
3. ACTG lab Manual HIV Qualitative PBMC Macroculture Assay. Revised Version 2.0, May 2004.
4. ACTG Laboratory Technologist Committee, Version 1.0, ACTG Lab Man, PACTG PBMC Processing, Cryopreservation, and Thawing Method, 29 May 2003.
5. Adam MacNeil, Jean-Louis Sankale, Seema Thakore Meloni, Abdoulaye Dieng Sarr, Souleymane Mboup, and Phyllis Kanki. Genomic Sites of Human Immunodeficiency Virus Type 2 (HIV-2) Integration: Similarities to HIV-1 In Vitro and Possible Differences In Vivo. Journal of virology, Aug. 2006; 80 (15): 7316-7321.
6. Alan Engelman and Peter Cherepanov. The Lentiviral Integrase Binding Protein LEDGF/p75 and HIV-1 Replication. PLoS Pathogens, www.plospathogens.org March 2008; 4 (3) e1000046: 1-9. https://doi.org/10.1371/journal.ppat.1000046.
7. Alan Engelman and Peter Cherepanov. Retroviral Integrase Structure and DNA Recombination Mechanism. Microbiol Spectr. 2014; 2 (6): 1–22.
8. Alejo Erice, Kim J. Sannerud, Valeria L. Leske, Dorothee Aeppli and Henry H. Balfour, JR. Sensitive microculture method for isolation of Human Immunodeficiency Virus Type 1 from blood leukocytes. Journal of clinical microbiology, Feb. 1992; 30 (2): 444-448.
9. Alexandra Duverger, Jennifer Jones, Jori May, Frederic Bibollet-Ruche, Frederic A. Wagner, Randall Q. Cron et al. Determinants of the establishment of Human Immunodeficiency Virus Type 1 Latency. Journal of virology, April 2009; 83 (7): 3078-3093.
10. Andrea Cara, Anna Cereseto, Franco Lori, and Marvin S. Reitz, Jr. HIV-1 Protein expression from synthetic circles of DNA mimicking the extrachromosomal forms of viral DNA. The journal of biological chemistry, March 8, 1996; 271 (10): 5393–5397.
11. Andrea Cara, Francesco Guarnaccia, Marvin Reitz JR, Robert C. Gallo and Franco Lori. Self limiting, cell type dependent replication of an integrase-defective human immunodeciciency virus type 1 in human primary macrophages but not T lymphocytes. Virology 1995; 208: 242-248.
12. Andreas Meyerhans, Tanja Breinig, Jean-Pierre Vartanian, and Simon Wain-Hobson. Forms and Function of Intracellular HIV DNA. Theoretical Biology and Biophysics Group; 2004. 14–21.
13. Audrey Brussel and Pierre Sonigo. Evidence for gene expression by unintegrated Human Immunodeficiency Virus Type 1 DNA Species. Journal of virology; Oct. 2004: 11263–11271.
14. Aviad Levin, Zvi Hayouka, Markus Helfer, Ruth Brack-Werner, Assaf Friedler, Abraham Loyter. Peptides Derived from HIV-1 integrase that bind Rev stimulate viral genome integration. PLoS ONE (www.plosone.org). January 2009; 4 (1): e4155. https://doi.org/10.1371/journal.pone.0004155.
15. Aviad Levin, Ayelet Armon-Omer, Joseph Rosenbluh, et al. Inhibition of HIV-1 integrase nuclear import and replication by a peptide bearing integrase putative nuclear localization signal. BioMed Central Ltd. Retrovirology 2009; 6 (112): 1-16.
16. Cathy X. Wang and Paula M. Cannon. Clinical applications of genome editing to HIV Cure. Mary Ann Liebert, Inc. AIDS patient care and STDs 2016; 30 (12): 539-544.

17. Christophe Marchand, Allison A Johnson, Elena Semenova and Yves Pommier. Mechanisms and inhibition of HIV integration. Drug Discov Today Dis Mech, July 2006; 3 (2): 253–260.
18. Christopher W Peterson, Patrick Younan, Keith R Jerome and Hans-Peter Kiem. combinatorial anti-HIV gene Therapy: Using a multi-pronged approach to reach beyond HAART. Gene Ther. July 2013; 20 (7): 695–702.
19. Ciuffi A. The benefits of integration. Clin Microbiol Infect, 2016; 22: 324–332.
20. Daniel Stone, Hans-Peter Kiem and Keith R. Jerome. Targeted gene disruption to cure HIV. Curr Opin, HIV AIDS, May 2013; 8 (3): 217–223.
21. David N. Levy, Grace M. Aldrovandi, Olaf Kutsch and George M. Shaw. Dynamics of HIV-1 recombination in its natural target cells. PNAS , February 2005 ; 102 (5): 4204–4209.
22. David W. Goodrich and Peter H. Duesberg. Retroviral recombination during reverse transcription. Proc. Nati. Acad. Sci. March 1990; 87: 2052-2056.
23. Denise C. Hsu and Robert J. O'Connell. Progress in HIV vaccine development. Human Vaccines & Immunotherapeutics, 2017; 13 (5): 1018-1030.
24. Donald S. Burke. Recombination in HIV: An important viral evolutionary strategy. Emerging infectious diseases, 1997; 3 (3): 253-259.
25. Donatella RM Negri, Zuleika Michelini, Silvia Baroncelli, et al. Successful immunization with a single injection of non-integrating lentiviral vector. The American Society of Gene Therapy; sept. 2007; 15 (9): 1716-1723.
26. Eva Poveda and Michael L Freeman. Exosomes as new players in HIV pathogenesis – New data from IAS 2017. AIDS Rev 2017; 19: 173-175.
27. Faysal Bin Hamid, Jinsun Kim and Cha Gyun Shin. Distribution and fate of HIV 1 unintegrated DNA species: a comprehensive update. Biomed Central, AIDS Res Ther, 2017; 14 (9): 1-8.
28. Fred C. Krebs, Tricia H. Hogan, Shane Quiterio, Suzanne Gartner, and Brian Wigdah. Lentiviral LTR-directed Expression, Sequence variation, and disease pathogenesis. The Pennsylvania State University College of Medicine, Department of Microbiology and Immunology, 500 University Drive, P.O. Box 850, Hershey, PA 17033.
29. Frederic D. Bushman and Robert Craigie. Integration of human immunodeficiency virus DNA: Adduct interference analysis of required DNA sites. Proc. Natl. Acad. Sci. April 1992; 89: 3458-3462.
30. Gaël Petitjean, Yassine Al Tabaa, Edouard Tuaillon, et al. Unintegrated HIV-1 provides an inducible and functional reservoir in untreated and highly active antiretroviral therapy-treated patients. Retrovirology, 2007; 4 (60): 1-12.
31. Hammer S, Crumpacker C, D'aquila R, et al. Use of virologic assays for detection of Human Immunodeficiency Virus in clinical trials: Recommendations of the AIDS Clinical Trials Group Virology Committee. Journal of Clinical Microbiology, October 1993; 31(10): 2557-2564.
32. Haobo Zhou, Jonah Rainey G, Swee-Kee Wong and John M. Coffin. Substrate sequence selection by retroviral integrase. Journal of virology, Feb. 2001; 75 (3): 1359–1370.
33. Hollinger F B, James W. Bremer, Lawrence E. Myers, Jonathan W M Gold, Lisa Mcquay, and The NIH/NIAID/DAIDS/ACTG virology laboratories. Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. J. Clin. Microbiol. 1992; 30 (7): 1787-1794.
34. Hongmin Chen and Alan Engelman. Characterization of a Replication-Defective Human Immunodeficiency Virus Type 1 att Site mutant that is blocked after the 3' processing step of retroviral integration. Journal of virology, September 2000; 74 (17): 8188–8193.
35. James A. Hoxie and Carl H. June. Novel Cell and Gene Therapies for HIV. Cold Spring Harb Perspect Med 2012;2:a007179. doi: 10.1101/cshperspect.a007179.
36. James Dolan, Aiping Chen, Irene T. Weber, Robert W. Harrison and Jonathan Leis. Defining the DNA substrate binding sites on HIV-1 integrase. J Mol Biol. January 2009; 385 (2): 568–579.
37. Jeannette M. Whitcomb and Stephen H. Hughes. The sequence of Human Immunodeficiency Virus Type 2 circle junction suggests that integration protein cleaves the ends of linear DNA asymmetrically. Journal of virology, July 1991; 65 (7): 3906-3910.
38. Jeremy Kelly, Margaret H. Beddall, Dongyang Yu, Subashini R. Iyer, Jon W. Marsh and Yuntao Wu. Human macrophages support persistent transcription from unintegrated HIV-1 DNA. Virology, March 2008; 372 (2): 300–312.
39. John Capodici, Katalin Kariko and Drew Weissman. Inhibition of HIV-1 infection by small interfering RNA-mediated RNA interference. J Immunol 2002; 169: 5196-5201.
40. Jonathan M.O. Rawson and Louis M. Mansky. Retroviral vectors for analysis of viral mutagenesis and recombination. Viruses, 2014; 6: 3612-3642.
41. Joseph M. Watts, Kristen K. Dang, Robert J. Gorelick, et al. Architecture and secondary structure of an entire HIV-1 RNA genome. Nature, August 6 2009; 460 (7256): 711–716.
42. Joseph Rosenbluh, Zvi Hayouka, Shoshana Loya, et al. Interaction between HIV-1 Rev and Integrase Proteins. A basis for the development of anti-HIV peptides. The Journal of Biological Chemistry, May 25, 2007; 282 (21): 15743–15753.
43. Kazushi Motomura, Jianbo Chen and Wei-Shau Hu. Genetic recombination between Human Immunodeficiency Virus. Type 1 (HIV-1) and HIV-2, Two distinct human lentiviruses. Journal of virology. Feb. 2008; 82 (4): 1923–1933.
44. Klaus Wanisch and Rafael J Yáñez-Muñoz. Integration-deficient lentiviral vectors: A slow coming of age. The American Society of Gene & Cell Therapy, Aug. 2009; 17 (8): 1316–1332.
45. Kristine E. Yoder and Frederic D. Bushman. Repair of gaps in retroviral DNA integration intermediates. Journal of virology, Dec. 2000; 74 (23): 11191–11200.
46. Laurence Colin and Carine Van Lint. Molecular control of HIV-1 postintegration latency: implications for the development of new therapeutic strategies. Retrovirology 2009; 6: 1742-4690.
47. Leavitt A D, Rose R B and Varmus H E. Both substrate and target oligonucleotide sequences affect in vitro integration mediated by human immunodeficiency virus type 1 integrase protein produced in Saccharomyces cerevisiae. J. Virol. 1992; 66 (4): 2359-2368.
48. Maria G Vizoso Pinto, Klaus-Ingmar Pfrepper, Tobias Janke, et al. A systematic approach for the identification of novel, serologically reactive recombinant Varicella-Zoster Virus (VZV) antigens. Virology Journal 2010; 165 (7): 1-9.
49. Mark S. T. Hansen and Frederic D. Bushman. Human Immunodeficiency Virus Type 2 preintegration complexes: Activities in vitro and response to inhibitors. Journal of virology, Apr. 1997; 71 (4): 3351-3356.
50. Martine Aubert, Byoung Y. Ryu, Lindsey Banks, David J. Rawlings, Andrew M. Scharenberg and Keith R. Jerome. Successful targeting and disruption of an integrated reporter lentivirus using the engineered homing endonuclease Y2 I-AniI. PLoS ONE. February 2011; 6 (2): e16825, 1-12. doi: 10.1371/journal.pone.0016825.
51. Maryann Wiskerchen and Mark A. Muesing. Human Immunodeficiency Virus Type 1 integrase: Effects of mutations on viral ability to integrate, direct viral gene expression from unintegrated viral DNA templates and sustain viral propagation in primary cells. Journal of virology, Jan. 1995; 69 (1): 376–386.
52. Mattias Mild, Joakim Esbjo¨rnsson, Eva Maria Fenyo¨ and Patrik Medstrand. Frequent intrapatient recombination between Human Immunodeficiency Virus Type 1 R5 and X4 envelopes: Implications for coreceptor switch. Journal of virology, April 2007; 81 (7): 3369–3376.
53. Nan Yan, Peter Cherepanov, Janet E. Daigle, Alan Engelman and Judy Lieberman. The SET complex acts as a barrier to autointegration of HIV-1. PLoS Pathogens, March, 2009; 5 (3): e1000327, 1-13. https://doi.org/10.1371/journal.ppat.1000327.
54. Nan Yan, Elizabeth O'Daya, Lee Adam Wheelera, Alan Engelmanc and Judy Lieberman. HIV DNA is heavily uracilated, which protects it from autointegration. PNAS, May 31, 2011; 108 (22): 9244–9249.
55. Olivier Delelis, Kevin Carayon, Ali Saïb, Eric Deprez and Jean- François Mouscadet. Integrase and integration: biochemical activities of HIV-1 integrase. Retrovirology 2008; 5 (114): 1742-4690.
56. Oyebisi Jegede, John Babu, Roberto Di Santo, Damian J. McColl, Jan Weber and Miguel E Quinones-Mateu. HIV type 1 integrase inhibitors; From basic research to clinical implications. AIDS Rev 2008; 10: 172-189.
57. Paul Malcolm Keller, Kathleen Lonergan, Beverly J. Neff, David A. Morton and Ronald W. Ellis. Purification of individual varicella-zoster virus (VZV) glycoproteins gpI, gpII, and gpIII and their use in ELISA for detection of VZV glycoprotein-specific antibodies. Journal of Virological Methods, September 1986; 14 (2):177-188.
58. Protocol for HIV-1 Isolation by PBMC Co-culture. Duke Central University, August 2012.
59. Rafal Kaminski, Ramona Bella, Chaoran Yin, et al. Excision of HIV-1 DNA by gene editing: A proof-of-concept in vivo study. Gene Ther. August 2016; 23 (89): 690-695.
60. R.E.D. Laboratories, Pontbeek 61, B-1731, Zellik Belgium. Instruction manual. Procedure for the preparation of peripheral blood mononuclear cell (PBMC) pellets from whole blood.
61. Richard D Sloan and Mark A Wainberg. The role of unintegrated DNA in HIV infection. Retrovirology, 2011; 8 (52): 1-15.
62. Robert Craigie. The molecular biology of HIV integrase. Future Virol, July 2012; 7 (7): 679–686.
63. Robert Craigie. HIV Integrase, A brief overview from chemistry to therapeutics. The journal of biological chemistry 2001; 276 (26): 23213–23216.
64. Robert Fleischmann W. Jr. Genetic change in viruses. Chapter 43, viral genetics. Medical Microbiology, 4th edition, 1996, The University of Texas, Medical branch at Galveston, https://www.ncbi.nlm.nih.gov/books/NBK8439/
65. Robert L. Lafemina, Pia L. Callahan and Michael G. Cordingley. Substrate specificity of recombinant Human Immunodeficiency Virus integrase protein. Journal of virology, Oct. 1991; 65 (10): 5624-5630.
66. Sharon P. Steinberg and Anne A. Gershon. Measurement of antibodies to varicella-zoster virus by using a latex agglutination test. Journal of Clinical Microbiology, July 1991; 29 (7): 1527-1529.
67. Sueli L. Tillieux, Wendy S. Halsey, Elizabeth S. Thomas, John J. Voycik, Ganesh M. Sathe and Ventzislav Vassilev. Complete DNA sequences of two Oka strain varicella-zoster virus genomes. Journal of virology, November, 2008: 82 (22): 11023–11044.
68. Sylvain Thierry, Soundasse Munir, Eloïse Thierry, et al. Integrase inhibitor reversal dynamics indicate unintegrated HIV-1 DNA initiate de novo integration. Retrovirology, 2015; 12 (24): 1-12.
69. Timothy E. Schlub, Redmond P. Smyth, Andrew J. Grimm, Johnson Mak and Miles P. Davenport. Accurately measuring recombination between closely related HIV-1 genomes. PLoS Computational Biology, April 2010; 6 (4): e1000766. https://doi.org/10.1371/journal.pcbi.1000766.
70. Viola Ellison and Patrick O. Brown. A stable complex between integrase and viral DNA ends mediates human immunodeficiency virus integration in vitro. Proc. Natl. Acad. Sci. July 1994; (91): 7316-7320.
71. Vladimir N. Loparev, Elena N. Rubtcova, Vanda Bostik, et al. Identification of five major and two minor genotypes of varicella-zoster virus strains: A practical two-amplicon approach used to genotype clinical isolates in Australia and New Zealand. Journal of virology, December 2007; 81(23): 12758-12765.
72. Wayne L. Gray. Recombinant varicella-zoster virus vaccines as platforms for expression of foreign antigens. Advances in Virology, Vol 2013, article ID 219439 1-8. http://dx.doi.org/10.1155/2013/219439.
73. Yang Ou, Vicki Traina-Dorgeb, Kara A. Davisa and Wayne L. Graya. Recombinant simian varicella viruses induce immune responses to simian immunodeficiency virus (SIV) antigens in immunized vervet monkeys. Virology. August, 2007; 364 (2): 291–300.
74. Yasutsugu Suzuki, MunLokChew and YouichiSuzuki. Role of host-encoded proteins in restriction of retroviral integration. Front Microbiol. June 2012; 3: Article 227. doi: 10.3389/fmicb.2012.00227.