Prevention and molecular treatment of AIDS with lentivirus: A historical perspective

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*"Thirty years after the first described AIDS case, fifteen years after the advent of Highly Active Antiretroviral Therapy, miracles continue"**

Introduction

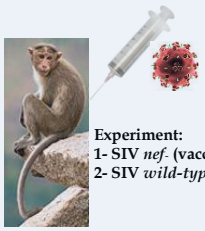
Currently, viral vectors are the most efficient vehicle on the transfer of genes to their infectivity in a high proportion of cells and with a very good capability of carrying transgenes in its modified genome genetically. In addition, note the potential to produce adverse effects in the application to humans, their immunogenicity and the risk to produce homologous recombination or toxicity.

The most widely used viral vectors are developed from adenovirus, retrovirus, vaccinia virus, AAV, poxviruses and lentivirus among others. Because of the long history of the characterization of viral particles and its genome, infectivity to the target cell, transgenic capacity and accessability to establish cellular lines helpers for the production of recombinant virus stock to infect target cells [1]. Lentivirus is the type of retrovirus responsible for the acquired immunodeficiency syndrome (AIDS) and has been seen it has very interesting properties for the establishment of these vector systems.

In recent years, these HIV-based vectors have been studied and tested for the efficacy of gene transfer, furthermore to its biological safety to exclude the possible reconstitution of a competent pathogenic replication in infected patients.

The 90's

ATTENUATED LENTIVIRUS IN GENE *NEF* WITH A COMPETENT REPLICATION



Experiment:
1- SIV *nef-* (vaccine)
2- SIV *wild-type*

Extremely low viral load

Control:
1- SIV *wild-type*

Died

The researchers believed that the *nef* gene was responsible for pathogenicity:

- SIV in rhesus monkeys affects similar to HIV in humans [2]
- If there was no *nef* at the beginning of the infection, it was believed that the antiviral immune response could be efficiently set up [3]

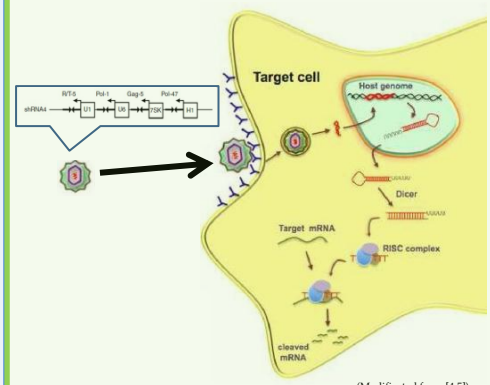
Problems:

- Cell-to-cell pathway to transmission
- *nef* wasn't the responsible for pathogenicity
  - There were other factors
  - *nef* regulated the degree of replication

NON-VIABLE = TOO MUCH RISK [2]

First years of XXI century

GENE THERAPY BASED IN RNA INTERFERENCE

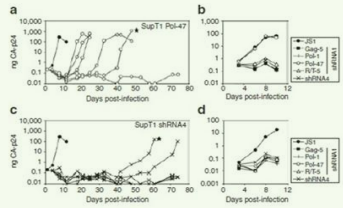


(Modificated from [4,5])

This strategy didn't get a total blockade:

- RNAi didn't muted completely the expressions of genes in a natural way
- The genetic barrier imposed was too high for the virus to overcome the inhibition of the four expressed shRNA

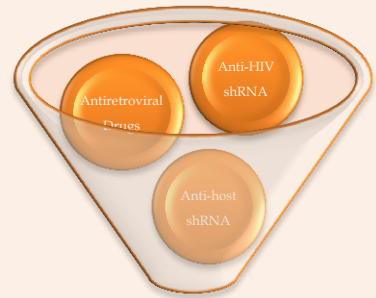
Handicap: the specificity of RNAi for the HIV-1 didn't cover the other multiples genetics subtypes of HIV [4,6].



Prevention of escape with four short hairpin RNAs (Extracted from [5]).
a and *b* are positive controls. In *c*, a initial virus replication was potently inhibited. After 40 days slow virus spread was scored in two of six cultures from every experiment but, in *d*, those virus could replicate exclusively in the control cell line, indicating that those virus emerging in *c* were not resistant.

Present

COMBINED THERAPY

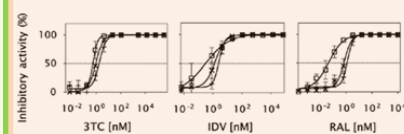


SYNERGY EFFECTS

Prevents possible breaks of the virus with targets develop with lack of redundancy [5-9]

Strategy to block the appearance of mutants and improve the antiviral activity:

- ✓ Improves the deletion if it acts at different levels of virus replication
- ✓ Inhibitory effect: Anti-HIV > anti-host
- ✓ shRNAs + Drugs = Increases susceptibility from drugs [10,11]



Additive antiviral activity of shRNAs and antiretroviral drugs (Extracted from [10]).
The possibility to have an additive effect with drugs has been evaluated through drugs dose-response curves in cellular lines expressing different shRNAs.
PM1: cellular line, **JS1**: empty viral vector, **Pol1**: single anti-HIV shRNA, **3TC**: Lamiduvina, **IDV**: Indinavir and **RAL**: Raltegravir

Conclusions and future prospects

- It is important to emphasize that, in order to fight the infection caused by HIV, it is necessary to combine different therapeutic strategies.
- The HIV is the virus which has taught more to the scientific community about the abilities and weaknesses of our own immune system in comparison to any other pathogen discovered. According to the WHO, there are approximately 34 million infected people in the world, but the high cost of antiretroviral drugs makes impossible the distribution to these developing countries. For that reason, the best option would be the development of replication competent and secure viral vector that allows *in vivo* the release of anti-HIV genes that confer resistance or selective death to the infected cells [12,13,14].
- The best alternative in order to cure the infection is through interference RNA and, specifically, through short-hairpin RNA but, before its use, appropriate vectors that are stable and will allow a lasting expression of these RNA will be needed. Because of this, lentivirus has been chosen for its effectiveness and biological safety in gene transfection, but still need to improve different aspects before it becomes clinically viable shRNA-based therapy [1].
- The HIV-1 uses a error-prone replication machinery that allows a quick adaptation to new conditions, being the cause of making drug-based therapies fail because they allowed the emergence of resistant viral mutants [15].
- RNAi seems to provide a promising strategy to fight chronic infections like AIDS and, as previously thought, using combined therapies from antiviral drugs and RNAi is giving the expected results [10,16].
- The future prospects that are expected from these strategies are very wide and if clinical phases are passed successfully, use RNAi to treat other worldwide diseases such as tuberculosis or malaria will be imminent.

*(Extracted from [17])/ **[1]** Walther W, et al. Viral vectors for gene transfer: a review of their use in the treatment of human diseases. *Drugs*. 2000; 60(2): 249-71/ **[2]** Baba TW, et al. Pathogenicity of Live, attenuated SIV after mucosal infection of neonatal macaques. *Science*. 1995; 267(5205): 1820-5/ **[3]** Chakrabarti L, et. al. Limited viral spread and rapid immune response in lymph nodes of macaques inoculated with attenuated simian immunodeficiency virus. *Virology*. 1995; 213(2): 535-48/ **[4]** Manjunath N, et al. Lentiviral delivery of short hairpain RNAs. *Adv Drug Deliv Rev*. 2009; 61(9): 732-45/ **[5]** ter Brake O, et al. Lentiviral vector design for multiple shRNA expression and durable HIV-1 inhibition. *Mol Ther*. 2008; 16(3): 557-64/ **[6]** DiGiusto DL, et. al. RNA-based gene therapy for HIV with lentiviral vector-modified CD34+ cells in patients undergoing transplantation for AIDS-related lymphoma. *Sci Transl Med*. 2010; 2(36): 36-43/ **[7]** Boden D, et al. Human Immunodeficiency virus type 1 escape from RNA interference. *J Virol*. 2003; 77(21): 11531-5/ **[8]** ter Brake O, et al. Silencing of HIV-1 with RNA interference: a multiple shRNA approach. *Mol Ther*. 2006; 14(6): 883-92/ **[9]** Eekels JM, et al. Long-term inhibition of HIV-1 replication with RNA interference against cellular co-factors. *Antiviral Res*. 2011; 89(1): 43-53/ **[10]** Boutimah F, et al. Antiviral strategies combining antiretroviral drugs with RNAi-mediated attack on HIV-1 and cellulars co-factors. *Antiviral Res*. 2013; 98(1): 121-9/ **[11]** Leonard JN, et al. HIV evades RNA interference directed at TAR by a novel compensatory mechanism. *Cell Host Microbe*. 2008; 4(5): 484-94/ **[12]** Wolkwicz et al. Gene therapy progress and prospects: Novel gene therapy approaches for AIDS. Gene Ther. 2005; 12(6): 467-76./ **[13]** Lamothe et al. Current develoments and future prospects for HIV gene therapy using interfering RNA-based strategies. *Front Biosci*. 2000; 5: 527-55/ **[14]** http://www.who.int/en/ **[15]** Berkhout et al. HIV-1 escapte from RNAi antivirals: Yet another houdini action? *Mol Ther Nucleic Acids*. 2012; 1:e26. doi: 10.1038/mtna.2012.22./ **[16]** The International AIDS Society Scientific Working Group on HIV Cure. Towards an HIV cure: a global scientific strategy. *Nat Rev Immunol*. 2013; 12(8): 607-14./ **[17]** Calmy A, Cavassini M. News in HIV/AIDS therapy. *Rev Med Suisse*. 2012; 8(324): 130-4/