ISSN 2693-2326

A Novel Vaccine RNA-peptide against *HIV-1*: Exosomes as Carrier in Viral Progression

International Journal of Infectious Diseases and Research Research Article

*CRUZ-RODRIGUEZ L.[1,2,3,4], ZAYAS TAMAYO A.[1,2], DILSIZ N.[5] and *LAMBERT BROWN D.[1,2]

[1]*ELIDAN dynamic LLC, Miami, Florida, USA.*

[2]*ExCELab Co. Ltd., Environmental Biotechnology Department, Kingston, Jamaica.*

[3]*ELIDAN america LLC, Miami, FL, USA*

[4]*ELIDAN genome SAS, 1 av du Lycée, 77130 Montereau FY; France*

[5]*Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Medeniyet University, Istanbul, Turkey.*

***Correspondence authors**

Prof. Dr. CRUZ-RODRIGUEZ Luis
ELIDAN america LLC
Miami, FL, USA
Email : luis.cruzrguez@gmail.com
lcr@elidan-genome.com

Dr. LAMBERT BROWN David
CEO,
ExCELab Co. Ltd.
Environmental Biotechnology Department
Kingston, Jamaica.
Email : dalamja1@gmail.com

Submitted : 20 Mar 2021 ; Published : 12 Apr 2021

Abstract

Introduction of a new drug/vaccine model against HIV-1 with prophylactic and therapeutic actions, also useful, in HIV-1 rehabilitation. This RNA-peptide vaccine projects involving RNA from SARS-CoV-2, and peptides from human HIV-1 and human PARP-1 proteins. As mRNA target, we used primers miRNA repertoire and host immune factor regulation upon avian coronavirus infection in eggs. The primers were modified with poly adenine (A) target. We designed the peptides target from HIV-1 protein and PARP-1 human protein. Our analysis, according to the algorithms Cruz Rodriguez (CR) identified an RNA-peptide with theorical fusion value stability FS=80.04 cruz, EA= 97.22 ro and BA= 1.21 to treat HIV-1. Where, we are proposing, the exosomes and how these vesicles could function as carriers of our RNA-peptide molecule. In this study, we expect that major histocompatibility complex l (MHC l) bind the molecule peptide (B) generated by hydrolysis (DEVD) of molecule RNA-peptide (AB) after induction of apoptosis pathways by caspase 3 or caspase 7. Also, we expect that major histocompatibility complex ll (MHC ll) bind the molecule RNA-peptide (A) generated and recognition by appropriate T-cells at the infected cell with HIV-1.

Keywords: Vaccine, algorithms Cruz Rodriguez (CR), HIV-1, SARS-CoV-2, avian coronavirus, exosomes; RNA-peptide; Fusion Stability (FS), PARP-1, Exosome Affinity (EA), Optimal Biological Action (OBA), Biological Action (BA), major histocompatibility complex (MHC).

Introduction

Human immunodeficiency virus (*HIV*) is the retroviral agent that causes acquired immune deficiency syndrome (AIDS). The number of people living with the HIV virus has been estimated as about 38 million worldwide at the end of 2019 [1]. *HIV* belongs to the Lentivirus genus of the Retroviridae family and is currently classified into two types: *HIV-1* and *HIV-2* [2].

HIV-1 has a variety of novel genes that facilitate viral persistence and regulation of *HIV-1* replication, but this virus also usurps cellular machinery for HIV-1 replication. The *HIV-1* is a viral pathogen with establishment of a persistent infection based on the ability to integrate the proviral genome into chronically infected cells, and by the rapid evolution made possible by a high mutation rate and frequent recombination during the viral replication, particularly during gene expression and virion assembly and budding [3].

The *HIV-1* disease is especially harmful because the progressive destruction of the immune system prevents the ability of forming specific antibodies and to maintain an efficacious killer T cell activity [4].

To move from science to guidelines, more than a decade was spent debating the clinical benefits, public health benefits, client autonomy, ethical conflicts, and adherence challenges for the *HIV-1* test-and-treat strategy. 2 years after WHO recommended antiretroviral therapy (ART) initiation for all, only 66% of countries reported full implementation. Many

countries with the highest *HIV* burden, with increasing new *HIV-1* infections and related deaths, have not yet adopted or fully implemented the strategy [5].

Effective prediction of *HIV-1* peptides important significance for the biological and pharmacological functions. In this study, based on the concept of Chou's pseudo amino acid (PseAA) composition were presented to predict *HIV-1* peptides [6].

According to the algorithms CRUZ RODRIGUEZ (CR) we are proposing various methods to develop a novel vaccine against *HIV-1* [7-10]. These analyses suggest that the designed vaccine can elicit specific immune responses against virus; however, these results need experimental studies to confirm the efficacy and safety profile of the proposed vaccine structure. In order to develop peptide target we were focused in amino acid frequencies of each amino acid in *HIV-1* and *HIV-2* groups (Figure 1) [11,12].

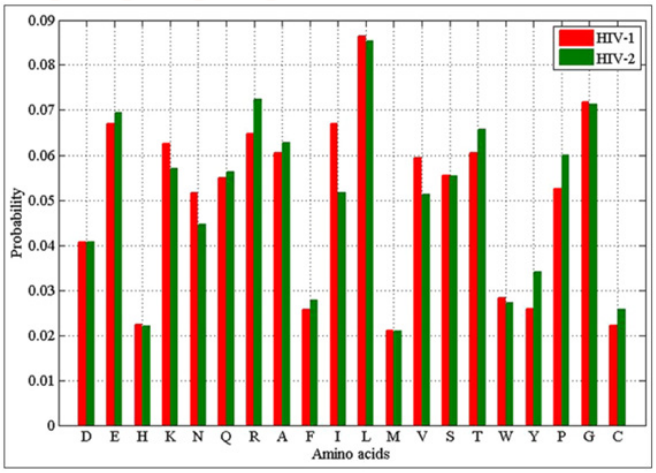

Figure 1: Amino acid frequencies of each amino acid in two HIV groups. The larger the F-score was, the more likely this feature was more discriminative. As illustrated in Figure 2 shows that Val (V) was the most discriminative feature, whereas Met (M) was the least discriminative feature, which confirmed the P-values of the Wilcoxon test for Val (V) and Met (M). They also found that most of the F-scores of 20 amino acids were low. The low F-scores of 20 amino acids were easy to understand, as most of the differences between HIV-1 proteins and HIV-2 proteins in amino acid usage were marginally or not significant [6].

Prediction of HIV-1 peptides according to ID&CR algorithms

According to the 20 amino acid compositions, 400 dipeptide compositions, 6 amino acid hydropathy compositions and 36 hydropathy dipeptide compositions were selected as the input parameters of the ID algorithm [6]. The CR algorithm was applied to examine peptide from 10 to 40 amino acids. The performance of CR algorithm for prediction of HIV-1 peptides were enumerated in Figure 2.

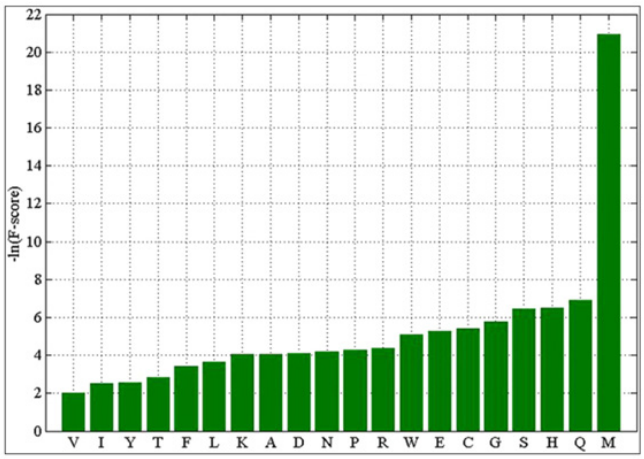

Figure 2: The F-scores of 20 amino acids. In this figure, x-axis represents the 20 amino acids, y-axis represents the-ln (F-score) [6].

Exosomes as viral carrier

Exosomes are biological nanoparticles with an average diameter of between 30 and 100 nm in size and produced by almost all cell types in the human body. Exosomes represent a hot area of research with many promising results in fighting disease with agents derived from abnormal cells. Indeed, many studies demonstrated that exosomal contents, including DNA fragments, various RNA species (coding RNAs and non-coding RNAs) and cytosolic and cell surface proteins that are important as disease predictive biomarkers for early diagnosis and determining the prognosis of patients.

These bioactive molecules are transferred from donor cells to target cells by exosome transport system, leading to reprogramming of the recipient cells. They are an excellent delivery system for anti-disease miRNAs in therapeutic instruments because of their small scale, natural products of the body cells (non-immunogenic), non-toxic characteristics and crossing the various biological barriers [13-19].

In virogenesis, cell-to-cell communication and transformation are essential: single infected cells must communicate with each other cells in order to promote its growth and, survival. It is becoming increasingly clear that exosomes derived from infected cells play a crucial role in this way of interaction/communication exchanging biomolecules which can be transferred from source cells to target recipient cells in active form (Figure 3) [8]. miRNA-peptide encapsulated by exosomes are remarkably stable compared to free RNAs and free peptides in circulation because exosomes can protect miRNA and peptide against degradation or under nonphysiological conditions [9]. The receptor protein of exosome recognized the RNA-peptide molecules according to exosome affinity between target-receptor [10-11].

By inducing inflammation and cytotoxicity, exosomes from virus-infected cells may cause tissue injury. Exosome can be isolated from a patient's fluids and after modification; it can be transferred back to the same patient with its cargo for targeted disease therapy [12]. Several clinical trials are currently under-

way around the world using engineered exosome-based in the treatment of various diseases. As a result, exosomes are highly biocompatible, with low toxicity and immunogenicity, high stability in body fluids, the ability to cross biological barriers, and the ability to deliver specific molecules to targeted cells in the treatment of diseases such as neurodegeneration, viral infection, and cancer.

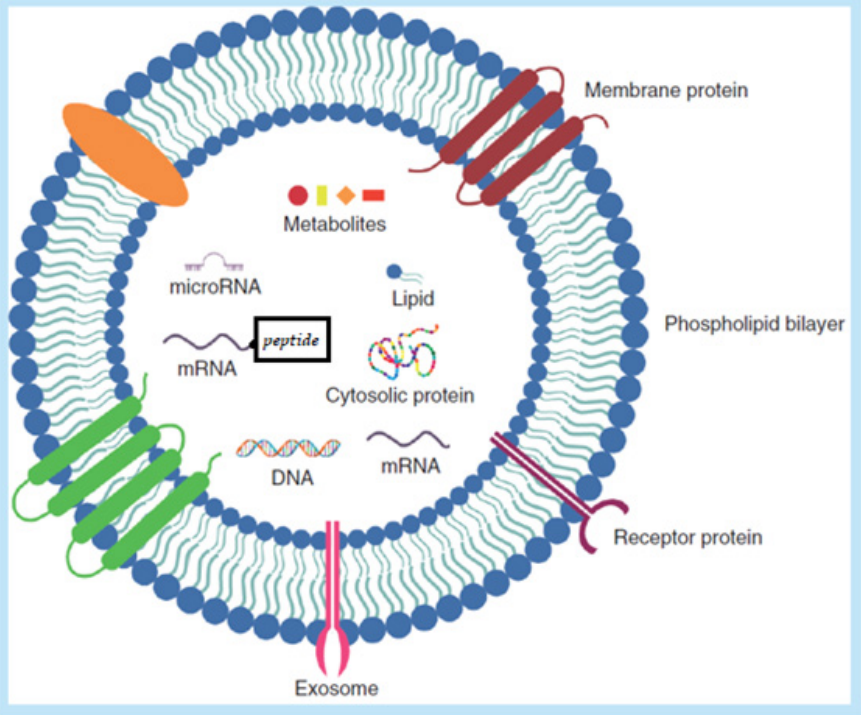

Figure 3: Modified from Dilsiz N. (2020). We are showing the exosome as carrier of RNA-peptide molecules involved in cell-to-cell communication [12].

Perspectives

The investigating of exosomes as multicomponent signaling complexes mediating cell to cell communication between both nearby and distant tissue cells is an emerging area as a novel form of communication, as well as a delivery vehicle to carry their cargo containing RNA-peptide as drugs.

Exosomes can act as not only potential biomarkers in medicine, but also as a very useful and effective 'nanovector' for delivering antiviral drugs/vaccines to target tissues with low immunogenicity and toxicity in disease therapy as compared to other antiviral drug/vaccine delivery vehicles in therapeutic applications [20-21].

Exosomes and *HIV-1*

HIV-1 and latency, the main barrier to eradicating the virus

Anti-HIV-1(human immunodeficiency virus type 1) triple therapy has reached some maturity. The combinations of molecules used represent a highly effective therapeutic approach to suppress virus replication in infected individuals and it is increasingly tolerated by patients. However, despite these successes, the triple therapy does not allow the cure [20].

Indeed, although viremia is reduced to an undetectable rate, discontinuation of therapy results in a rapid rebound in viral replication and disease progression. This rebound is explained by the persistence, in treated individuals, of viruses competent for replication, forming a reservoir of latent viruses. Latently infected cells can be defined as cells containing a provirus competent for replication, but whose genome is not expressed. The viral reservoir becomes established very early in the course of infection, before seroconversion. The major part of the reservoir of *HIV-1* is found in the mononuclear cells of the blood (peripheral blood mononuclear cell, PBMC) and in particular the CD^{4+} T lymphocytes. In these cells, *HIV-1* was able to integrate, but it is latent: it is transcriptionally inactive and does not replicate because it is no longer in a permissive environment, these cells being or becoming quiescent. Cells containing latent virus are rare (around 1 per million CD^{4+} T cells). However, the latent virus reservoir has an exceptionally long lifespan and above all is insensitive to triple therapies which only target the virus during replication.

Studies have identified many mechanisms involved in the activation of latent *HIV-1* transcription. In particular, the site of integration of the virus and the absence of transcription factors in quiescent cells strongly contribute to its latency. Activating the transcription of the latent virus in order to allow its elimination is one of the therapeutic strategies considered to eradicate *HIV-1*. It is therefore crucial to understand the mechanisms involved in the repression of *HIV-1* transcription allowing it to remain latent in the cells it has infected. Nuclear exosome factors repress reporter gene expression under the control of the *HIV-1* promoter. One of the main steps in blocking *HIV-1* transcription in latent cells is the transition step between initiation and elongation. RNA polymerase pauses after the synthesis of the first nucleotides of the viral messenger RNA, which form

a stem-loop structure called TAR (trans-activation response element). We have previously shown the involvement of the nuclear exosome in the repression of the expression of the viral genome [13].

Why HIV-1 mRNA-peptide vaccine?

1. Recent improvements in mRNA vaccines act to increase protein translation, modulate innate and adaptive immunogenicity, and improve delivery [20].
2. mRNA vaccines have elicited potent immunity against infectious disease targets in animal models of influenza virus, Zika virus, rabies virus and others, especially in recent years, using lipid-encapsulated or naked forms of sequence-optimized mRNA.
3. Diverse approaches to mRNA cancer vaccines, including dendritic cell vaccines and various types of directly injectable mRNA, have been employed in numerous cancer clinical trials, with some promising results showing antigen-specific T cell responses and prolonged disease-free survival in some cases [20].
4. Therapeutic considerations and challenges include scaling up good manufacturing practice (GMP) production, establishing regulations, further documenting safety and increasing efficacy.
5. Important future directions of research will be to compare and elucidate the immune pathways activated by various mRNA vaccine platforms, to improve current approaches based on these mechanisms and to initiate new clinical trials against additional disease targets.
6. The major histocompatibility complex (MHC) is a group of genes that encode proteins on the cell surface that have an important role in immune response.
7. The MHC class I antigen presentation pathway plays an important role in alerting the immune system to virally infected cells. MHC class I molecules are expressed on the cell surface of all nucleated cells and present peptide fragments derived MHC class I molecules bind peptides that are predominantly 8-10 amino acid in length from intracellular proteins.
8. Virus specific cytotoxic T lymphocytes (CTL) monitor cell surface MHC class I molecules for peptides derived from viral proteins and eliminate infected cells.
9. In contrast, MHC class II proteins usually accommodate peptides of 13–25 amino acid in length in their open binding groove.
10. MHC class I and II; both, their main role is in antigen presentation where MHC molecules display peptide fragments for recognition by appropriate T-cells.

Engineered exosomes for HIV-1 RNA-peptide vaccine

Exosomes modified with high levels of *HIV-1* molecules antigens (as RNA-peptide) plays an effectively recruit of anti-viral immune cells against to infected cells in collaboration with the major histocompatibility complex (MHC). It is a group of genes that encode proteins on the cell surface that have an important role in immune response. The MHC class I antigen presentation pathway plays an important role in alerting the immune system to virally infected cells. MHC class I molecules are expressed on the cell surface of all nucleated cells and present peptide fragments derived MHC class I molecules bind peptides that are predominantly 8-10 amino acid in length from intracellular proteins. Virus specific cytotoxic T lymphocytes (CTL) monitor cell surface MHC class I molecules for peptides derived from viral proteins and eliminate infected cells. In contrast, MHC class II proteins usually accommodate peptides of 13–25 amino acid in length in their open binding groove (Figure 4).

MHC class I and II; Both, their main role is in antigen presentation where MHC molecules display peptide fragments for recognition by appropriate T-cells.

The algorithm CRUZ RODRIGUEZ as tool to develop RNA-peptide *HIV-1* vaccine.

The algorithm CRUZ RODRIGUEZ (CR) is a tool that allows predicting the stability of hybrid oligonucleotide and protein molecules in their most simplified expression of cDNA/RNA and peptide. This hybrid molecule has a high affinity for exosomes, allowing its extracellular transport from cell to cell. These chimeras in cDNA-peptide or RNA-peptide constructs have a specific biological action with antiviral efficacy due to their chemical structure since they participate in the viral pathway's replication. On the other hand, they present specific antigenic structures that can involve immune responses.

The RNA target and peptide (A|B) target are molecules selected according to the three following CR parameters:

1. Fusion Stability (FS)
2. Exosome Affinity (EA)
3. Biological Action (BA)

- Optimal Biological Action (OBA); where, 0.8 < OBA < 1.3 (antiviral)

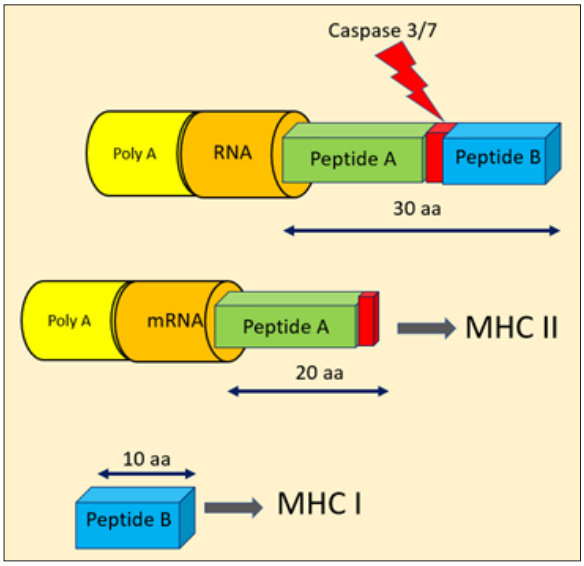

Figure 4: CRUZ RODRIGUEZ`s schema of structure of *HIV-1* RNA-peptide vaccine: We show the antigen presentation pathway for peptide B in MHC class I and the RNA-peptide A presentation in MHC class I.

How to select the peptide A

Integrase, partial [Human immunodeficiency virus 1 (*HIV-1*)]
HIV-1 Source : Human immunodeficiency virus 1

Sequence 201 amino acids (aa)
ORIGIN
WRALASDFGLPPVVAKEIIANCPKCHIRGEAIHGQ-
VDCSPGVWQMDCTHVEGKVIIVAVHVASGFIEAE-
VIPAETGQETAYFLLKLAARWPVKVIHTDNGPNFT-
SATMKAACWWTNIQHEFGIPYNPQSQGVVEAMN-
KELKSIIQQVRDQAEHLKTAVQMAVFVHNFKRK
GGIGGYTAGERLIDILASQI QTTELQKQILK

HIV-1 peptide nature: CPKCHIRGEAIHGQVD
A) HIV-1 (peptide nature +SPDE): CPKCHIRGEAIHGQSP-
DEVD

How to select the peptide B

The target human PARP-1 protein as peptide B
PARP-1 Source *Homo sapiens* (human)

Sequence 1014 aa

MAESSDKLYRVEYAKSGRASCKKCSESIPKDSLR-
MAIMVQSPMFDGKVPHWYHFSCFWKVGHSIRHPDVE-
VDGFSELRWDDQQKVKKTAEAGGVTGKGQDGIGS-
KAEKTLGDFAAEYAKSNRSTCKGCMEKIEKGQVRLSK-
KMVDPEKPQLGMIDRWYHPGCFVKNREELGFRPEY-
SASQLKGFSLLATEDKEALKKQLPGVKSEGKRKG-
DEVDGVDEVAKKKSKKEKDKDSKLEKALKAQND-
LIWNIKDELKKVCSTNDLKELLIFNKQQVPSGESAIL-
DRVADGMVFGALLPCEECSGQLVFKSDAYYCTG-
DVTAWTKCMVKTQTPNRKEWVTPKEFREISYLK-
KLKVKKQDRIFPPETSASVAATPPPSTASAPAAVNSSA-
SADKPLSNMKILTLGKLSRNKDEVKAMIEKLGGKLTG-
TANKASLCISTKKEVEKMNKKMEEVKEANIRVVSED-
FLQDVSASTKSLQELFLAHILSPWGAEVKAEPVEV-
VAPRGKSGAALSKKSKGQVKEEGINKSEKRMKLTLK-
GGAAVDPDSGLEHSAHVLEKGGKVFSATLGLVDIVK-
GTNSYYKLQLLEDDKENRYWIFRSWGRVGTVIGSNK-
LEQMPSKEDAIEHFMKLYEEKTGNAWHSKNFTKYP-
KKFYPLEIDYGQDEEAVKKLTVNPGTKSKLPKPVQD-
LIKMIFDVESMKKAMVEYEIDLQKMPLGKLSKRQIQA-
AYSILSEVQQAVSQGSSDSQILDLSNRFYTLIPHD-
FGMKKPPLLNNADSVQAKVEMLDNLLDIEVAYS-
LLRGGSDDSSKDPIDVNYEKLKTDIKVVDRDSEE-
AEIIRKYVKNTHATTHNAYDLEVIDIFKIEREGECQRYK-
PFKQLHNRRLLWHGSRTTNFAGILSQGLRIAPPEAPVT-
GYMFGKGIYFADMVSKSANYCHTSQGDPIGLILL-
GEVALGNMYELKHASHISKLPKGKHSVKGLGKTTPD-
PSANISLDGVDVPLGTGISSGVNDTSLLYNEYIVYDIA-
QVNLKYLLKLKFNFKTSLW

B) PARP-1 (peptide nature): DEVDGVDEVAKKKE

How to fusion the peptide A with peptide B

PEPTIDE TARGET: A|B
A|B) HIV-1 (peptide modified)-PARP1(peptide nature): Peptides fusioned by overlap in DEVD site: CPKCHIRGEAIH-GQSPDEVDGVDEVAKKKE

Formula: $C_{137}H_{224}N_{42}O_{47}S_2$
Using ProtParam we achieved:
User-provided sequence:
10 20 30
CPKCHIRGEA IHGQSPDEVD GVDEVAKKKE

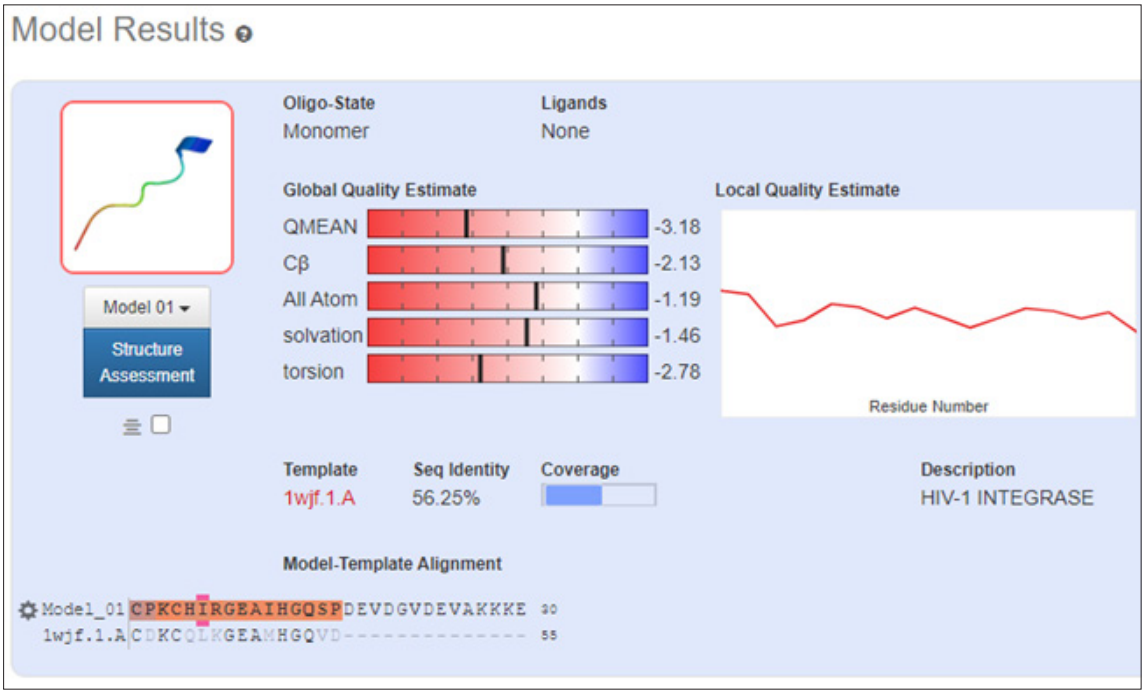

Figure 5: Description of the structure of HIV-1 peptide from Integrase protein.
https://swissmodel.expasy.org/interactive/F8ggNq/models/

Identification of RNA target and primer (poly A + mRNA)

The the RNA target was selected from cDNA primer (Biolegio, Nijmegen, and The Netherlands):

cDNA selected: 5`CTCCTAGAACTAGCATTACAGATG 3`
RNA target : 5`CUCCUAGAACUAGCAUUACAGAUG 3`
[7-10]

Primer: (poly A—mRNA)

Primer: 5` AAAAAAAAAAAAAA--CUCCUAGAACUAG-CAUUACAGAUG
Number of nucleotides: 38 nt
poly A: 5`AAAAAAAAAAAAAA
Number of nucleotides: 14 nt

mRNA: 5`CUCCUAGAACUAGCAUUACAGAUG
Number of nucleotides: 24 nt
Molecular weight (MW)=7701 Da

The sequence of *RNA*-peptide(A|B) overlap DEVD
5`AAAAAAACUCCUAGAACUAGCAUUACAGAUG—CPKCHIRGEAIHGQSPDEVDGVDEVAKKKE

Where:
peptide(A):

CPKCHIRGEAIHGQSPDEVD
Number of amino acids (aa): 20
Molecular weight (MW): 2191.42 Daltons (Da)
Theoretical pI: 5.31

peptide(B)
GVDEVAKKKE
Number of amino acids: 10 aa
Molecular weight: 1102.25 Da
Theoretical pI: 6.18

MHC interaction with peptide B and peptide A

1. peptide(B): Given the role that the MHC class I:

Antigen presentation pathway plays in the detection of tumoral cells by CTLs, we are expecting that infected cell in MHC class I, expose the peptide(B), size 10 aa from PARP-1, and active the metabolic pathways involved in elimination of HIV-1 infected cells.

2. RNA-peptide(A): Given the role that the MHC Class II:

Antigen presentation pathway plays in the detection of tumural cells by cells such as dendritic cells, mononuclear phagocytes, some endothelial, thymic epithelial cells, and B cells. These cells are important in initiating immune responses. We are expecting that tumoral cell in MHC class II expose the RNA-peptide(A), sizes 38 nt, 20 aa, and active the pathways action of immune system.

About targets:
peptide (A|B):

CPKCHIRGEAIHGQSPDEVDGVDEVAKKKE
Number of amino acids: 30
Molecular weight: 3275.66
Theoretical pI: 5.46

PEPTIDE TARGET: A|B
What is RNA-peptide vaccine against *HIV-1* infection?

We expect that MHC class I bind the molecule peptide (B) generated by hydrolysis (DEVD) of molecule *RNA*-peptide (A|B) by caspase 3 or caspase 7, and induction of apoptosis pathways. Also, expect that MHC class II bind the molecule RNA-peptide(A) generated and recognition by appropriate T-cells at HIV-1 infected cell.

Results

According to the algorithms CRUZ RODRIGUEZ (CR)
Fusion Stability (FS)
FS= a*b*c*d (cruz)

a= Size poly A / Size poly Cys
b= MW mRNA / MW peptide
c= Size peptide / Size mRNA
d= [mRNA (2*(A+ U) +3*(C+G)) / (PI peptide^2)]

a= 14/1=14
b= 7701/3275.66=2.3509
c= 30/24= 1.25
d= [(2*(8+ 6) +3*(6+4)) / (5.46^2)] = [(58)/(29.81)]=1.9456

FS= 14*2.3509*1.25*1.9456 (cruz)
FS= 80.04 cruz

Exosome Affinity (EA)
EA= FS* [(MW peptide / MW mRNA) + (Size peptide / Size primer)]
EA= (ro)

EA= 80.04 *[(3275.66/ 7701) +(30/38)] = 80.04* [(0.4253) + (0.7894)]
EA=80.04*1.2147=97.22
EA=97.22 ro

Biological Action (BA)
BA= EA / FS
BA= 97.22 / 80.04
BA=1.21 ro/cruz

Optimal Biological Action (OBA)
OBA= (ro/cruz)

value for antiviral efficacity to RNA-peptide with exosome as carrier are
0.8 < OBA < 1.3

Conclusions

Our analysis, according to the algorithms CR identified a miRNA-peptide with theorical fusion value stability *FS=80.04 cruz, EA= 97.22* ro and *BA= 1.21* to treat *HIV-1*. Where, we are proposing, the exosomes and how these vesicles could function as carriers of RNA-peptide molecule. In this study, we expect that MHC class I bind the molecule peptide (B) generated by hydrolysis (*DEVD*) of molecule RNA-peptide (AB) by caspase 3 or caspase 7; and induction of apoptosis pathways. Also, expect that MHC class II bind the molecule RNA-peptide (A) generated and recognition by appropriate T-cells at infected cell with *HIV-1*.

As a result of these findings, exosomes appear to be promising candidates for use as diagnostic biomarkers and prognostic markers for viral infections and virus-related disease. Furthermore, by transferring interfering RNA, miRNA, and therapeutic compounds, exosomes are an ideal vehicle for therapeutic applications.

In conclusion, exosomes are a novel horizon in modern therapy and open exciting new opportunities for advanced vaccines, immune-checkpoint inhibitors, antigens for adoptive cell transfer (ACT), and vaccine/drug delivery all increase therapeutic effect and cause an anti-disease response. Exosomes may be used to develop antiviral therapies including exosome-based vaccines for retrovirus-related diseases.

Future clinical research on the subject is needed to elucidate the exact role of exosomes in *HIV* virus infection and to decide the clinical application of these exosomes for infection prevention.

References

1. Estimated number of people living with HIV worldwide (2020) World Health Organization (WHO).
2. Rezaie, J., Aslan, C., Ahmadi, M., Zolbanin, N. M., Kashanchi, F., & Jafari, R. (2021) The versatile role of exosomes in human retroviral infections: from immunopathogenesis to clinical application. *Cell & Bioscience, 11*(1): 1-15.
3. Monique R Ferguson, Daniel R Rojo, Jana J von Lindern, William A O'Brien. (2002). "HIV-1 replication cycle": *Clin Lab Med. 22*(3): 611-635. doi: 10.1016/s0272-2712(02)00015-x.
4. Juan Mei, Ji Zhao. (2018)" Prediction of HIV-1 and HIV-2 proteins by using Chou's pseudo amino acid compositions and different classifiers". *Sci Rep. 5;8*(1): 2359. doi: 10.1038/s41598-018-20819-x.
5. Phanuphak N, Seekaew P, Phanuphak P (2019)" Optimising treatment in the test-and-treat strategy: what are we waiting for?". *Lancet HI: Lancet HIV. 6*(10): e715-e722. doi: 10.1016/S2352-3018(19)30236-X.
6. Mei J and Zhao J. (2018)" Prediction of HIV-1 and HIV-2 proteins by using Chou's pseudo amino acid compositions and different classifiers". *Sci Rep. 5;8*(1):2359. doi: 10.1038/s41598-018-20819-x.
7. Cruz-Rodriguez L., Ziarati P, Hochwimmer B., Kaabar M. K. A., Bin Zhao, N., Dilsiz N., Sanchez Batista L., Barea R., Pelaez Figueroa Y. (2020) "A DNA-Peptide Fusion as a Vaccine Candidate against Breast and Ovarian Cancer Metastasis": Consequent Benefit in Certain Lung Cancers. *Journal of Bioscience & Biomedical Engineering1* (3): 1-11. doi.org/10.47485/2693-2504.1021
8. Cruz-Rodriguez L, Dilsiz N, Barea R, Ziarati P, Hochwimmer B, Am ZT and Brown DL. (2021). "The Algorithms Cruz Rodriguez (CR) are Proposing A Novel Vaccine RNA Peptide against Breast, Ovarian, and Lung Cancers Disease: Exosomes as Carrier in Cancer Progression and Metastasis". *J Med – Clin Res & Rev. 5*(2): 1-16.
9. Cruz-Rodriguez L, Cruz-Rodriguez LD, Batista LS, Hochwimmer B. (2020). "Calculation of Fusion Stability of [DNA or RNA] - Peptide (FS) Algorithm "Cruz-Rodriguez" *Journal of Bioscience & Biomedical Engineering, 2*(1). doi.org/10.47485/2693-2504.1003
10. Cruz-Rodriguez L, Sanchez Batista L, Hochwimmer B, Bin Zhao and Ziarati P. (2020)."The mathematical formula to estimate the Exosome Affinity between miRNA-peptide and Exosome: ALGORITHM CRUZRODRIGUEZ (EA)", *Journal of Bioscience & Biomedical Engineering*, Volume 1, Issue 2, doi.org/10.47485/2693-2504.1012.
11. Liu B, Wang SY, Long R, Chou KC. (2017) " iRSpot-EL: identify recombination spots with an ensemble learning approach. *Bioinformatics*". 33: 35–41. doi: 10.1093/bioinformatics/btw539.
12. Dilsiz, N. (2020) Role of exosomes and exosomal microRNAs in cancer. *Future Science OA, 6*(4), FSO465. 1-15.
13. Cruz-Rodriguez L, Dilsiz N, Barea R, Ziarati P, Hochwimmer B, Am ZT and Brown DL. (2021). "The Algorithms Cruz Rodriguez (CR) are Proposing A Novel Vaccine RNA Peptide against Breast, Ovarian, and Lung Cancers Disease: Exosomes as Carrier in Cancer Progression and Metastasis". *J Med – Clin Res & Rev. 2021; 5*(2): 1-16.
14. Fernández-Lázaro, D., García Hernández, J. L., García, A. C., Córdova Martínez, A., Mielgo-Ayuso, J., & Cruz-Hernández, J. J. (2020). Liquid Biopsy as Novel Tool in Precision Medicine: Origins, Properties, *Identification and Clinical Perspective of Cancer's Biomarkers. Diagnostics, 10*(4): 215; 27.
15. Mashouri, L., Yousefi, H., Aref, A. R., mohammad Ahadi, A., Molaei, F., & Alahari, S. K. (2019). Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Molecular cancer, 18*(1): 75; 39.
16. Antonyak MA, Cerione RA. (2014) Microvesicles as mediators of intercellular communication in cancer. *Methods Mol. Biol*. 1165, 147–173: 10,14,27,38.
17. Peng, H., Ji, W., Zhao, R., Yang, J., Lu, Z., Li, Y., & Zhang, X. (2020) Exosome: a significant nano-scale drug delivery carrier. *Journal of Materials Chemistry B, 8*(34): 7591-7608.

18. Pi, Y. N., Xia, B. R., Jin, M. Z., Jin, W. L., & Lou, G. (2021) Exosomes: powerful weapon for cancer nano-immunoengineering. *Biochemical pharmacology*, 186: 114487.
19. Zhang, L., Ju, Y., Chen, S., & Ren, L. (2021) Recent Progress on Exosomes in RNA Virus Infection. *Viruses, 13*(2): 256.
20. Viceconte R, Cisneros V, Thomas DS, Spacapan F, Ventura MLF, Petriglieri C, Lopardo G. (2020)" Impact of low-grade viremia on the risk of virological failure in patients with HIV-1 infection on antiretroviral therapy". *Rev Chilena Infectol]. 37*(5): 550-554. doi: 10.4067/S0716-10182020000500550.
21. Mathieu M, Martin-Jaular L, Lavieu G, Th´ery C. (2019) Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol. 21*(1): 9.