Peptide-induced apoptosis of latently infected cells and reduction of the HIV reservoir in people living with HIV: preliminary results of a clinical trial.

Ricardo Sobhie Diaz,[1] Mauro Schechter,[1,2] Daniel Elbirt,[4] Esmira Naftali,3 Juliana T Maricato,[1] Marcella Vassao de Almeida Baptista,[1] Juliana Galinskas ,[1] Danilo Dias,[1] Alessandra Bassini,[1] , Nadya Lisovoder,[4] Yaron Ilan,[5] James R Hunter,[1] Eynat Finkelshtein,[3].

1 Laboratório de Retrovirologia, Escola Paulista de Medicina, Universidade Federal de São Paulo2 Departamento de Doenças Infecciosas e Parasitárias, Faculdade de Medicina, Universidade Federal do Rio de Janeiro 3 Code Pharma, The Netherlands (R&D based in Israel) 4 Clinical Immunology, Allergy and AIDS Center Kaplan Medical Center, Affiliated with Hadassah-Hebrew University Medical School Jerusalem, Rehovot, Israel 5 Faculty of Medicine, Hebrew University Hadassah Medical Center, and the Department of Medicine, Hadassah Medical Center Jerusalem, Israel. Disclosure: EF and EN are employees of Code Pharma; DE and YI are consultants for Code Pharma. NL is a medical advisor and a clinical CRO.

Abstract P212

Background:

Reducing the size of the HIV reservoir is essential to decrease HIV microinflammation and to achieve remission (cure) of HIV infection. One obvious way to obtain this goal is to selectively eliminate HIV-infected cells in people on antiretroviral therapy (ART). Gammora® is a 16-mer synthetic peptide based on a short sequence of the HIV-1 integrase. The peptide comprises amino acid residues 174-188 of the HIV-1 integrase enzyme, with an additional tryptophan residue at the N-terminus. In cell cultures infected with HIV, in the absence of antiretrovirals, this peptide stimulates the integration of multiple copies of HIV DNA into the chromatin of host cells to the point that it triggers their self-destruction. 1 It has been demonstrated that in cell cultures infected with HIV exposed to the association of the peptide and a protease inhibitor (PI), there is a selective eradication of infected cells, termination of the infection process, absence of HIV RNA copies, absence of p24, and most importantly, extinction of HIV DNA in infected cells upon interruption of treatment of the cell culture with antiretrovirals. 2 These results were only observed when both the peptide and the PI were used together. It was demonstrated that a caspase 3-dependent apoptosis pathway was the leading cause of the peptide-induced cell death. 1 It should be noted that although PIs have anti-apoptotic properties, they may be paradoxically pro-apoptotic when high intracellular levels occur. 3 The peptide is the only active ingredient in the formulation, which can be given intravenously (IV) or subcutaneously (SC). Imaging data indicates that the peptide readily passes through the cell membrane and enters cells. 3 Gammorra® has been tested in rodents with a single administration to determine the maximum tolerated dose (MTD), repeated increasing doses over a week, and with repeated administrations (twice a week) for 4 weeks to support the first dose in humans. Safety data from the first human pilot study and compassionate cancer treatments, which involved up to 15 administrations in a week of the peptide for up to 3 months, demonstrated that the product is safe, non-toxic, and well-tolerated. 4 Furthermore, in an open-label phase 1 trial evaluating the safety and preliminary efficacy of Gammora® in moderate COVID-19 at a dose of 20 mg SC twice daily for 10 5days, it was found to be well tolerated and safe, with minimal side effects. 5 We, therefore, conducted a pilot trial to investigate whether Gammora® plus a PI-based ART can lead to a meaningful reduction of the HIV reservoir. The rationale for the strategy is presented in Figure 1.

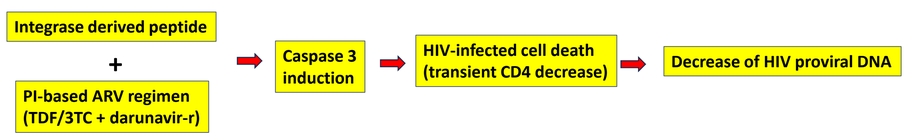

Figure 1. Rational schematics for the use of Gammora® with ART to decrease the HIV reservoir among people with HIV (PWH).

Material and Methods:

This is a pilot open-label randomized Phase II clinical trial.

Study Design

The design of the study is summarized in Figure 2. Forty ART-naïve individuals with a recent diagnosis of HIV infection were to be randomized 1:1 to:

- A lead-in period of two weeks of Gammora® 2 mL, one SC injection daily, followed by Tenofovir/3TC 300/300 mg + Darunavir 800 mg + Ritonavir 100 mg OD PLUS Gammora® 2 mL, one SC injection every other day.

OR

- Wait two weeks and start only the same antiretroviral regimen (Tenofovir/3TC 300/300 mg + Darunavir 800 mg + Ritonavir 100 mg OD) **Summary description (Figure 2)**

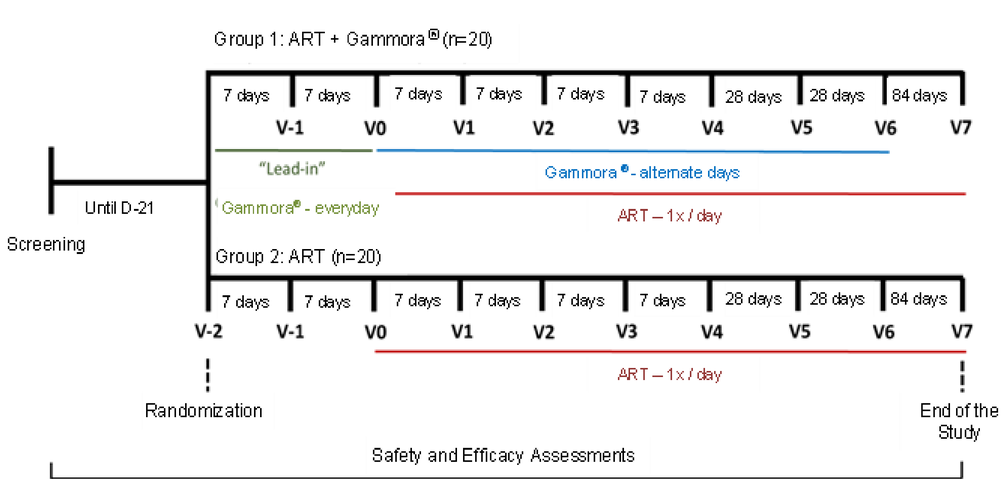

Figure 2. Schematic design of the study

Primary Objectives

•Comparison of the size of the reservoir between the two groups

•Comparisons of apoptosis markers between the two groups.

Total DNA quantitation in PBMCs: Viral DNA was measured as an estimate of the viral reservoir by published qPCR techniques[6,7,8] following in-house analyses aimed at ruling out the effect of PCR inhibitors.

Statistical Analysis: Data were analyzed by non-parametric repeated measures ANOVA following an appropriate transformation where necessary.

Apoptosis determination: Apoptosis analysis was performed using the BD Pharmingen™ PE Annexin V Apoptosis Detection Kit (BD Biosciences). 5x10^7 PBMC were stained with Anti-CD4-PECy7 (BD, Biosciences) and anti-CD3-APC (BD, Biosciences). The cells were analyzed by flow cytometry in FACS Canto II (BD Bioscience). Viable cells were represented as the double-negative population, cells undergoing apoptosis were represented by Annexin V positive and 7-AAD negative population, and the late apoptotic/dead cells were represented by Annexin V/7-AAD double positive population.

Results:

In this interim analysis, we present the results of the first 13 randomized patients, 7 in the Gammora® plus PI based ART group and 6 in the PI-based ART-only group. Herein, we present the results of the first eight weeks after antiretrovirals had been introduced for both groups. Viral load declined in all participants (Figure 3). In 4 of 7 Gammora® arm participants, there was a Log_{10} decrease in total DNA in the first eight weeks of combined therapy (Figure 4b). This was accompanied by a significant increase in early and late apoptosis markers, which started only after ART was introduced (Figure 5). The CD4 + T cell counts dynamics for both groups is depicted in Figure 6.

Conclusions:

Highly significant increases in apoptosis markers were accompanied by transient drops in CD4 counts and a one Log_{10} decline in total HIV DNA in 4 of 7 Gammora® arm participants, a magnitude of decline that would take many years to occur under normal circumstances. These results suggest that latently HIV-infected cells were being rapidly eliminated. This is because viral replication had been interrupted by ART and Gammora® plus PI induce apoptosis exclusively of HIV-infected cells. If confirmed by an ongoing, larger trial, Gammora® may help forward the path to cure HIV infection.

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO
1933

Figure 3A

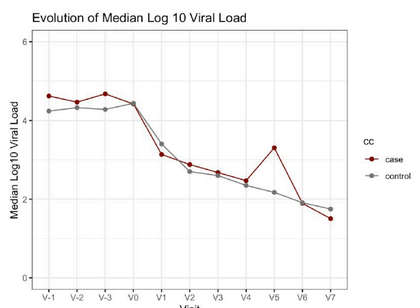

Figure 3B

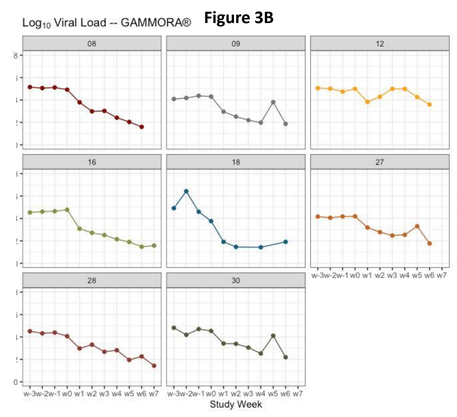

Figure 3C

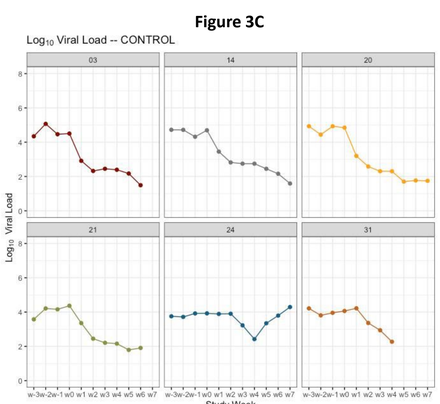

Figure 3. HIV RNA viral load over time in the Gammora-treated group (case) and the control group. PI-based ART started at week zero (W0). Lead-in period with Gammora only in the treatment group did not change the median viral load (screening, week -2 and week-1, Figure 3a). Figure 3B depicts the viral load for the individual participants of the case group, whereas Figure 3C shows the individual participants of the control group.

Figure 4A

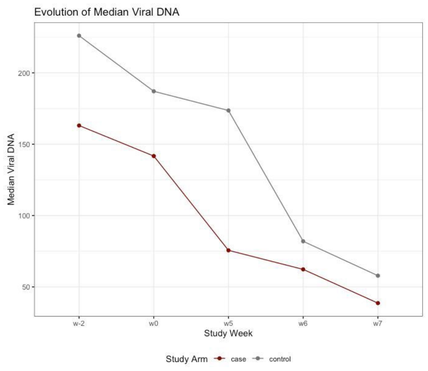

Figure 4B

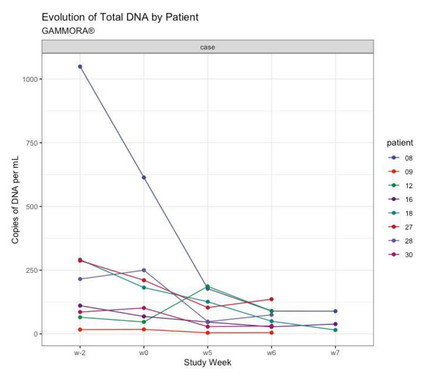

Figure 4C

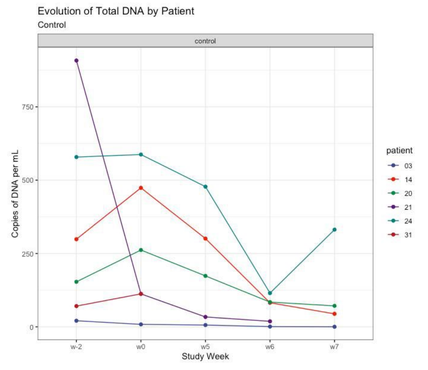

Figure 4. Total DNA dynamics from treatment and control group (Figure 4A) and for individual participants in the case (Figure 4B) and control group (Figure 4c)

Figure 5A

GATE STRATEGY

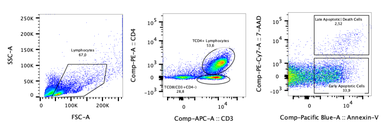

Figure 5B GROUPED ANALYSIS – CELL COUNT TCD4$^+$

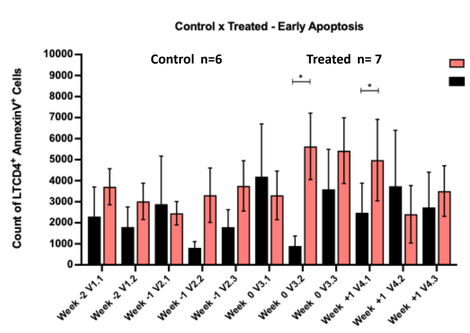

Figure 5C GROUPED ANALYSIS – CELL COUNT CELL COUNT TCD4$^+$

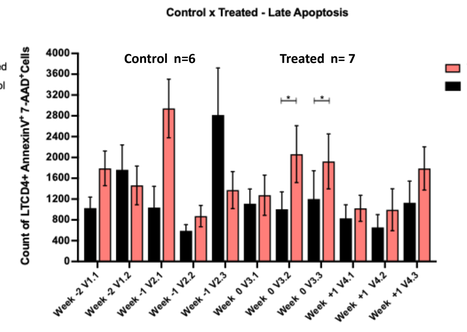

Figure 5D GROUPED ANALYSIS – CELL COUNT TCD8$^+$

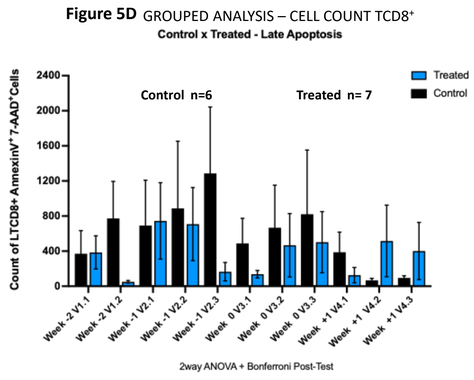

Figure 5E GROUPED ANALYSIS – CELL COUNT TCD8$^+$

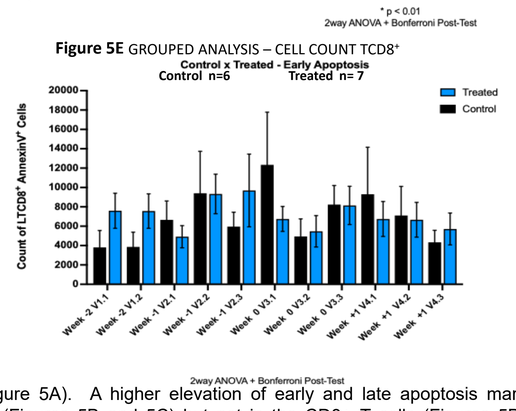

Figure 5. Cell cytometry Gate Strategy for determining early and late apoptosis (Figure 5A). A higher elevation of early and late apoptosis markers once Gammora® was associated with a PI-based ART was observed in the CD4+ T cells (Figures 5B and 5C) but not in the CD8+ T cells (Figures 5D and 5E). Exposure to Gammora before ART during the lead-in period did not show differences in apoptosis (Figures 5B and C). W-2 and W-1 refer to the lead-in period, and W-0 and W-1 to the ART period. Samples were collected thrice weekly (W-2 1, W-2 2, W-2 3, etc.).

Figure 6A

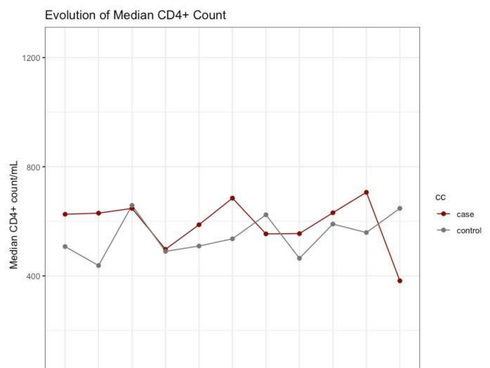

Figure 6B

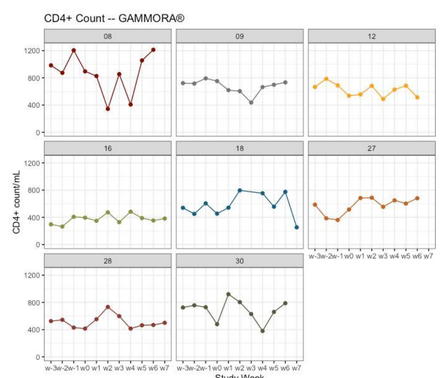

Figure 6C

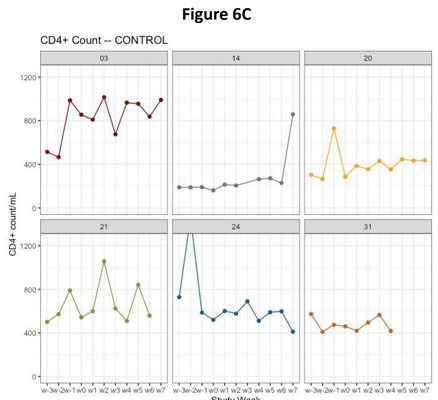

Figure 6D

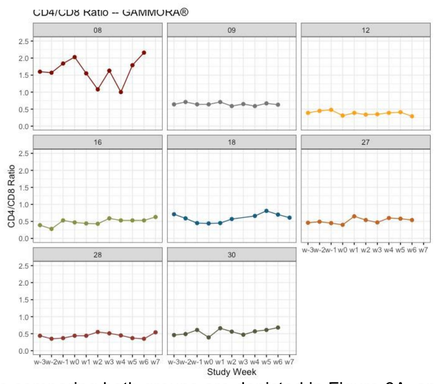

Figure 6E

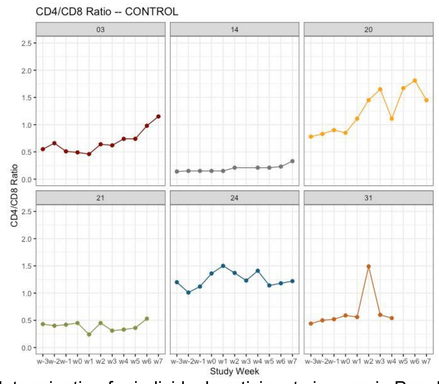

Figure 6. CD4+ T cell counts comparing both groups are depicted in Figure 6A ,and the determination for individual participants is seen in Panels B (case) and C (control). The CD4/CD8 ratios are presented in Panels D and E.

References:

1. Levin, A., Z. Hayouka, M. Helfer, R. Brack-Werner, A. Friedler and A. Loyter (2010). "Stimulation of the HIV-1 integrase enzymatic activity and cDNA integration by a peptide derived from the integrase protein." Biopolymers 93(8): 740 751.
2. Levin, A., Z. Hayouka, A. Friedler and A. Loyter (2010). "Specific eradication of HIV-1 from infected cultured cells." AIDS Res Ther 7: 31.
3. Rizza SA and Badley AD (2008). HIV Protease Inhibitors Impact on Apoptosis. Med Chem. 4(1): 75–79.
4. Shapira S, Finkelshtein E, Kazanov D, Naftali E, Stepansky I, Loyter A, Elbirt D, Hay-Levy M, Brazowski E, Bedny F, Dekel R, Hershkovitz D, Blachar A, Wolf I, Arber N. Integrase-derived peptides together with CD24-targeted lentiviral particles inhibit the growth of CD24 expressing cancer cells. Oncogene. 2021 Jun;40(22):3815-3825. doi: 10.1038/s41388-021-01779-5. Epub 2021 May 6. PMID: 33958722; PMCID: PMC8175240.
5. Kolben Y, Finkelshtein E, Naftali E, Kenig A, Kessler A, Cardoso F, Lisovoder N, Schwartz A, Elbirt D, Maayan S, Ilan Y. Codivir suppresses SARS-Cov-2 viral replication and stabilizes clinical outcome: In vitro and Phase I clinical trial results. Discoveries (Craiova). 2022 Dec 31;10(4):e158. doi: 10.15190/d.2022.17. PMID: 37457644; PMCID: PMC10348448.
6. Komninakis, S. V. et al. HIV-1 proviral DNA loads (as determined by quantitative PCR) in patients subjected to structured treatment interruption after antiretroviral therapy failure. J. Clin. Microbiol. 50, 2132–2133 (2012).
7. Buzón, M. J. et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. Nat. Med. 16, 460–465 (2010).
8. Kumar, A. M. et al. Human immunodeficiency virus type 1 in the central nervous system leads to decreased dopamine in different regions of postmortem human brains. J. Neurovirol. 15, 257–274 (2009).