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Evaluation of antiviral activity of dipeptide tryptophylglycine-amid (WG) against HIV-1 isolates harboring gp120 resistance-associated mutations

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Introduction

- A limited number of people living with HIV-1 (PLWH) develop resistance to multiple drug classes, thus new antiretrovirals with different mechanisms of action are therefore warranted.
- One promising compound is the naturally occurring dipeptide tryptophylglycine-amid (WG-am), which is enriched in the blood of untreated Elite Controllers (EC).
- Wg-am is both entry and reverse transcriptase inhibitor. In a previous study we demonstrated that it has anti-HIV-1 activity against multidrug resistant HIV-1 isolates and synergistic efficacy when combined with antiretrovirals from the NRTI, NNRTI, PI and INSTI classes (1).
- In this study we aimed to evaluate its activity against four HIV-1 isolates carrying the mutations M426L/P or S375N/T in gp120, which have emerged at viral failure in PLWH exposed to the attachment inhibitor fostemsavir, and its synergistic activity when combined with raltegravir (RAL) and tenofovir (TDF).

Materials and methods

- The amide form of tryptophylglycine had a purity of >95%.
- Viral isolates (n=4) were provided by the HIV Monitoring Laboratory (HML) of the Department of Medical Biotechnologies of the University of Siena, Italy.
- Phenotypic susceptibility to WG-am was determined through a TZM-bl cell-based assay and expressed as fold-change (FC) with respect to the reference wild type virus.
- The combinatorial activity was determined in a checkerboard assay by infecting TZM-bl cells exposed to a 6x6 drug concentration matrix in duplicate. Luminescence values were normalized to calculate the percentage of inhibition of viral replication and elaborated with the Synergy Finder tool to calculate synergy scores using ZIP model. (**figure 1**)

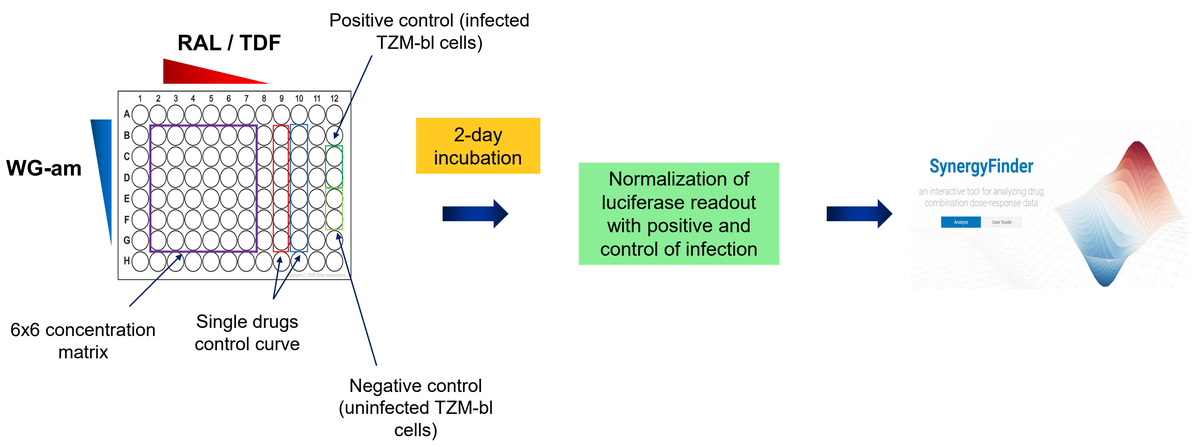

Figure 1. Workflow of the determination of the combinatorial activity of WG-am and RAL/TDF.

Virus Mutation	ARV	Ratio WG-am:ARV	Most synergistic area score	ZIP Synergy Score
M426P	RAL	1000:1	11.253	6.95
M426L	RAL	1000:1	13.021	7.85
S375T	RAL	1000:1	15.936	9.59
S375N	RAL	1000:1	19.862	12.02
M426P	TDF	100:1	5.017	1.59
M426L	TDF	100:1	3.798	0.65
S375T	TDF	100:1	6.527	2.7
S375N	TDF	100:1	7.162	3.03

Table 1. Synergy values of the combinatorial activity of WG-am and RAL or TDF.

Results

- WG-am exhibited significant inhibition of HIV-1 replication across all tested viruses, with efficacy comparable to that observed in wild-type HIV-1, which served as the control.
- FC values for site-directed mutants M426L, M426P, S375T and S375N were 0.71, 0.97, 1.42, 0.93, respectively.
- WG-am + RAL or TDF showed additive activity in all cases, except for S375N which showed synergistic activity with the combination of WG-am + RAL. (**table 1, figure 2 and figure 3**)

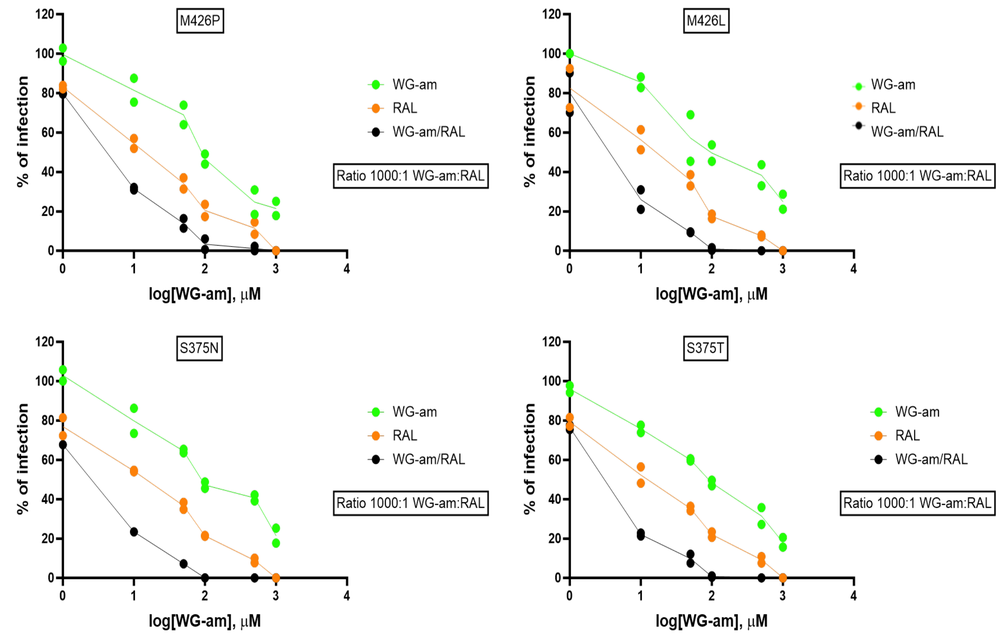

Figure 2. Comparison of the antiviral activity of WG-am, RAL and WG + RAL.

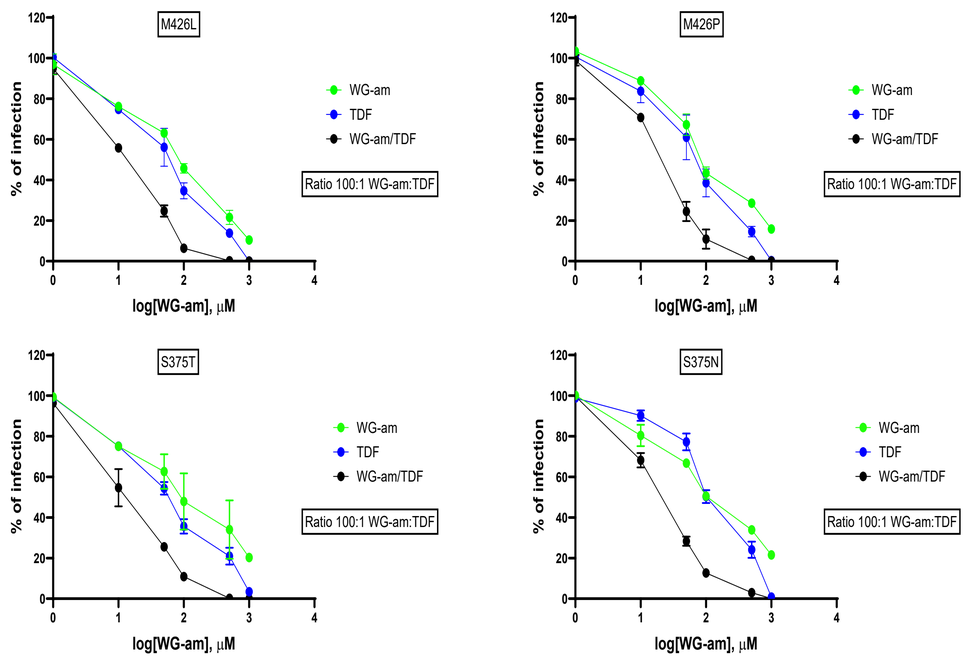

Figure 3. Comparison of the antiviral activity of WG-am, TDF and WG + TDF.

Conclusion

- The ability of WG-am to inhibit HIV-1 strains harboring these gp120 mutations underscores its potential as an antiviral agent, particularly in cases where resistance to standard therapies is a concern.
- Building on these promising findings, we are currently exploring the synergistic potential of WG-am in combination with other antiretroviral drugs, particularly the capsid inhibitor lenacapavir, to potentially enhance its therapeutic efficacy in the management of HIV-1 infection.

References
Giammarino F, Sönnerborg A, Ceña-Diez R. Broad synergistic antiviral efficacy between a novel elite controller-derived dipeptide and antiretrovirals against drug-resistant HIV-1. Front Cell Infect Microbiol. 2024 Jun 10;14:1334126. doi: 10.3389/fcimb.2024.1334126. PMID: 38915925; PMCID: PMC11194349.