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Leronlimab (PRO 140) *in vitro* activity against 4-class drug resistant HIV-1 from heavily treatment experienced subjects☆

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Dear Editor,

Most people living with HIV (PLWH) can keep HIV-1 infection under control thanks to the most widely used combinations of antiretrovirals. The key antiretroviral classes block one of the viral enzymes, including nucleoside (NRTIs) and non-nucleoside (NNRTIs) reverse transcriptase inhibitors, integrase strand transfer inhibitors (INSTIs) and protease inhibitors (PIs). However, PLWH experiencing multiple treatment failures may harbor a multidrug resistant virus population and require alternative drugs. Indeed, HIV-1 entry inhibitors such as the fusion inhibitor enfuvirtide, the C-C chemokine receptor 5 (CCR5) antagonist maraviroc and more recently the CD4 receptor binding monoclonal antibody ibalizumab and the HIV-1 gp120 attachment inhibitor fostemsavir have been approved as treatment options in the presence of extensive resistance to the main drug classes. Leronlimab (formerly PRO 140) is an investigational humanized immunoglobulin G4 monoclonal antibody targeting CCR5 and endowed with antiviral activity against HIV-1 isolates using CCR5 as a coreceptor for viral entry [1]. Leronlimab binds to a hydrophilic region in the amino-terminal domain and to the extracellular domain 2 of CCR5, thus interfering with the interaction between HIV-1 gp120 and CCR5 and preventing downstream events leading to virus entry [2]. Leronlimab expands the arsenal of host, rather than virus, targeting anti-HIV-1 agents, currently including maraviroc and ibalizumab. Although both leronlimab and maraviroc target CCR5, they bind to different regions of the coreceptor and have been shown to synergize with each other in cell culture [3]. However, similar to maraviroc, leronlimab is expected to be a valuable drug only for HIV-1 isolates entering the target cell through the CCR5 receptor (R5 viruses), thus analysis of the virus coreceptor tropism is required before treatment. Leronlimab is being studied both as a maintenance treatment for PLWH with sustained control of virus replication and as a rescue agent for PLWH harboring multidrug resistant virus. In this study, we examined for the first time the ability of leronlimab to inhibit *in vitro* pseudotyped viruses obtained from PLWH with resistance to NRTI, NNRTI, PI and INSTI. Plasma or peripheral blood mononuclear cell (PBMC) samples were obtained from the Italian PRESTIGIO Registry, a data and sample collection for the study and management of PLWH with documented genotypic resistance to NRTIs, NNRTIs and PIs plus genotypic resistance or virological failure to INSTI. PRESTIGIO has been approved by the Ethics Committee of the 40 participating clinical centers and is registered on the ClinicalTrials.gov website with the NCT04098315 identifier. All patients enrolled in the PRESTIGIO Registry provided a written informed consent for the use of anonymized biological specimens.

Coreceptor tropism determination: Phenotypic coreceptor tropism was assessed through a home-made assay based on pseudotyped viruses expressing patient derived Env protein and luciferase as reporter gene, as previously described. Briefly, U87-R5 and U87-X4 cells were infected in triplicate with pseudotyped viruses by spinoculation at 1200 xg for one hour, then the supernatant was replaced with fresh medium. After 72 h, the Relative Luminescence Units (RLU) measured in each well were elaborated with GraphPad version 6.0. Viral tropism was defined as X4 or R5 when the mean of the RLU values was at least two-fold the negative control background (uninfected cells) and the reference X4 or R5 coreceptor antagonists (AMD3100 and maraviroc, respectively) reduced RLUs of at least 0.3 log, in U87-X4 or U87-R5 cells, respectively. Tropism was classified as dual/mixed (DM) when both criteria were satisfied in both cell lines. Genotypic coreceptor tropism was also tested by both Sanger population sequencing and next generation sequencing (NGS) of the HIV-1 gp120 V3 domain followed by Geno2pheno[coreceptor] analysis available at https://coreceptor.geno2pheno.org/. Viruses were considered non-R5 by Sanger when the false positive rate (FPR) was $\leq$ 10% [4]. Viruses were considered non-R5 by NGS when $>$ 2% viral species had an FPR $\leq$ 3.5% [5]. Viral pseudoparticles were generated from 24 plasma samples from viremic PLWH, all with documented resistance to NNRTIs, NRTIs PIs and INSTIs (Supplementary Table 1). Based on Sanger, NGS and phenotypic assay, R5 virus was

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Table 1
Baseline HIV-1 RNA, $CD4^+$ T cell counts Genotypic coreceptor tropism results obtained by Sanger and NGS and leronlimab *in vitro* susceptibility for the 9 phenotypically R5 pseudoviruses.

Patient ID	HIV-1 RNA (copies/mL)	$CD4^+$ T-cells/μL	Percentage of non-R5 variants by NGS (FPR ≤3.5%)	FPR by Sanger (%)	IC_{50} (mean±SD) PRO 140	Previous maraviroc exposure	Exposure to maraviroc at current analysis
25	1104	240	32.7	4.4	0.3 ± 0.2	Yes	No
27	13,835	168	0.1	not done	1.2 ± 0.3	No	No
37	12,580	207	0.1	69.8	0.4 ± 0.3	Yes	Yes
55	17,888	326	0.0	54.7	0.7 ± 0.2	No	No
58	2986	518	0.0	51.3	0.5 ± 0.3	No	No
82	373,713	942	83.8	54.7	0.4 ± 0.3	No	No
90	84,534	201	0.6	16.4	0.8 ± 0.6	No	No
117	6718,473	34	0.1	51.2	0.3 ± 0.1	Yes	Yes

NGS, Next Generation Sequencing. FPR, False Positive Rate. IC50, Half-maximal inhibitory concentration. SD, standard deviation.

observed in 33%, 38%, and 33% of cases, respectively. Complete concordance in coreceptor tropism among the three methods was 70% while pairwise agreement was 82% for Sanger and NGS, 79% for NGS and phenotypic assay, 86% for Sanger and phenotypic assay.

Measurement of leronlimab activity in vitro: For viruses labeled as R5 by phenotypic assay, susceptibility to leronlimab was assessed by infecting U87-R5 cells with viral pseudoparticles in the presence of 5-fold dilutions of leronlimab (range 10 μM – 5.12 pM) and measuring luciferase activity as previously described [6]. Half-maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad version 6.0. The reference wild-type HIV-1 AD8 virus (AIDS Reagent Program catalog number ARP-11346) was used as the CCR5-tropic prototype. Values were reported as median (IQR) or percentage, as appropriate. The Mann-Whitney U test was used to compare leronlimab IC50 values between independent groups of samples. Statistical analyses were performed using GraphPad version 6.0. Phenotypic susceptibility to leronlimab was measured for the eight viruses with R5 phenotype (Table 1). The median IC_{50} was 0.4 (0.3–0.7) nM, comparable to the IC_{50} of the reference R5 AD8 virus (mean IC_{50} 0.7 ± 0.4 nM). Pseudoviruses from maraviroc-naïve (n = 3) and maraviroc-exposed (n = 5) subjects were slightly different (median IC_{50} 0.70 [0.45–1.0] nM *vs.* 0.35 [0.30–0.40] nM; p = 0.054). The two patients under maraviroc containing therapy at the time of sampling had leronlimab IC_{50} values of 0.4 and 0.3 nM, comparable to the median value obtained from the six individuals without maraviroc in their current regimen (median IC_{50} 0.60 [0.38–0.9] nM). A negative control was added to all the tests and we compared the inhibitory activity of leronlimab against the reference AD8 (CCR5 tropic) and NL4–3 (CXCR4 tropic) viruses (Supplementary Fig. 1).

Concluding remarks and future perspectives: In this small patient group, leronlimab maintained full activity in the presence of extensive resistance to the four main antiretroviral classes, as expected. It is also reassuring that leronlimab IC_{50} did not appear to be significantly altered by previous or current exposure to maraviroc, a small molecule HIV-1 inhibitor sharing the same target with leronlimab. In agreement with this finding, limited data were previously reported indicating good leronlimab activity against virus isolates which gained resistance to investigational small molecule CCR5 antagonists. However, there are no data available to support the lack of cross-resistance in the opposite direction, *i.e.*, maraviroc activity against isolates resistant to leronlimab. In vitro, leronlimab and maraviroc have been reported to have synergistic activity [3], further corroborating the different mechanism of action of the two drugs despite the same CCR5 target. The main limitation in the use of leronlimab as a component of salvage therapy in PLWH harboring multidrug resistant virus remains the higher prevalence of the virus population with X4-phenotype (67% in our case file). On the other hand, leronlimab may have some advantages over maraviroc as a clinically valuable CCR5 antagonist, including lower toxicity, less drug-drug interaction issues and less frequent dosing. It must be noted that leronlimab is currently being developed also as a long-acting treatment maintenance strategy in patients with durable suppression of viral replication [7], an area where the prevalence of non-R5 phenotype may have a lower impact. In addition, use of leronlimab as an immunomodulator outside the HIV-1 context is currently under investigation, leaving uncertain the future of this monoclonal antibody as a novel anti-HIV-1 agent. In the HTE subjects with MDR virus, the CCR5-tropic strains were susceptible to leronlimab and they were not significantly influenced by MVC exposure. While a substantial proportion of HTE subjects may harbor X4 or D/M viruses, leronlimab can play a key role in subjects with very limited therapeutic options and CCR5-tropic virus. In conclusion, despite the limited size of the study population, our analysis indicates that the *in vitro* susceptibility to leronlimab is not affected by extensive drug resistance and exposure to maraviroc, suggesting that leronlimab might represent a promising candidate for salvage therapy in one third of individuals included in this study with CCR5-coreceptor using viral populations.

Author contributions

Originators of project: SR, AG. Participated in research design: SR, MZ, MMS, AC. Conducted experiments: FS, MCB, LG, MMS, AG. Performed data analysis: All authors. Wrote or contributed to the writing of the manuscript: All authors.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M.M.S. has received funds for attending symposia, speaking and organizing educational activities from ViiV Health Care, Janssen-Cilag and Theratechnologies. M.Z. reports consultancy for ViiV Healthcare, Gilead Sciences, Janssen-Cilag, Theratechnologies and Merck Sharp and Dohme (MSD) and grants for his institution from ViiV Healthcare, Theratechnologies and Gilead Sciences outside the submitted work. S.R. reports honoraria for presentations and scientific advice for Merck, Sharp & Dohme, Mylan, GSK, Janssen Cilag, ViiVHealthcare, Gilead Sciences and research grants for his institution from Janssen Cilag, ViiV Healthcare, Gilead Sciences. A.C. has received consultancy payments and speaking fee from Bristol-Myers Squibb, Gilead, ViiV Health Care, Merck Sharp & Dohme, ABBvie, and Janssen-Cilag. R.G. has received travel grants from Janssen, Gilead Sciences, Viiv; grants for speakers's honoraria/educational activities from ViiV Healthcare, MSD; grants for advisory board from ViiV Healthcare. E.F. received speaker's honoraria, consultancy fees and research grants from Viiv Healthcare, Gilead Sciences, Merck, Sharp & Dohme, Janssen-Cilag and Sobi.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2022.106064.

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