Journal of Virus Eradication 6 (2020) 100002

Contents lists available at ScienceDirect

Journal of Virus Eradication

journal homepage: www.viruseradication.com

Viewpoint

HIV-1 resistance patterns to integrase inhibitors in Chilean patients with virological failure on raltegravir-containing regimens

Pablo Ferrer [a,*], Consuelo Rodriguez [a], Alicia Sciaraffia [a], Rocío Tordecilla [a], Veronica Ramos [a], Magdalena Durán [a], Carlos Beltrán [b], Alejandro Afani [a]

[a] Laboratorio de Medicina Molecular, Hospital Clínico Universidad de Chile, Santiago, Chile
[b] Grupo SIDA CHILE, Hospital Ramón Barros Luco y Universidad de Santiago de Chile, Chile

ARTICLE INFO

Keywords:
HIV
Integrase
Integrase strand transfer inhibitors (INSTIs)

ABSTRACT

In this viewpoint we would like to describe our results in terms of resistance pattern in Chilean patients with virological failure (VF) on raltegravir (RAL)-containing-regimens and highlight the need for the concomitant availability of genotypic resistance testing to integrase strand transfer inhibitors (INSTIs) introduction in antiretroviral regimens, particularly in countries in South America. Indeed we found in our study the presence of two or more primary mutations in some of the participants which is associated with cross-resistance to all INSTIs. By using timely genotyping, we could optimally manage these patients, early after detection of VF.

There are currently 45,000 patients in Chile on antiretroviral therapy (ART) in the public and private health care sectors.[1] ART is highly effective at achieving virologic suppression, immune reconstitution and to prevent onward HIV-1 transmission.[2] Raltegravir (RAL) was the first INSTI used in Chile in 2008, initially only as part of a salvage regimen in patients with virological failure (VF) (2 consecutive viral loads >1000 HIV-1 copies/mL) in association with reverse transcriptase and protease inhibitors. In 2013, its use was expanded to some treatment-naïve patients and is now one of the preferred first-line drugs.[3–5] These drugs play a key role in the management of patients with previous VF. Other INSTIs available in Chile include elvitegravir (EVG) since 2013, dolutegravir (DTG) since 2015, and bictegravir (BIC) since 2020.[6–10]

We have enrolled into an observational study (December 1, 2012 to December 31, 2013) patients on long-term salvage regimen with VF on RAL with the aim of establishing if they had developed INSTI resistance, characterize mutations and whether these conferred INSTI cross-resistance.

On December 31, 2013, Chile had 33,000 confirmed HIV-positive individuals, of whom 20,000 were on ART, among whom 1,360 used RAL as part of a salvage therapy. Our observational study included 44 HIV-1 positive participants (41 men and 3 women), with a median age of 43.4 years (range: 28–69) with VF while on a RAL-containing regimen. Overall, 90% of our study participants were rescued with RAL between 2008 and 2009 and were evaluated in 2013. The remaining 10% were treated with RAL between 2010 and 2013 (also evaluated for INSTIs genotyping test in 2013). RAL was introduced in these patients after an average of 4 therapeutic regimens (range: 2–7). All patients had had genotyping for reverse transcriptase or protease inhibitors resistance. Genotypic reports showed resistance to reverse transcriptase or protease inhibitors. The more frequent mutations were for the nucleoside reverse transcriptase inhibitors (NNRTI), M184V and K65R. The non-nucleoside reverse transcriptase inhibitors NNTRI included K103N and E138A. In terms of protease inhibitors mutations, some of the patients showed N88S, V82A, I50L and I54V mutations.

At failure of RAL-based regimen, median CD4 T lymphocyte count was 186 (range: 9–868) cells/μL and median viral load (VL) was 398,500 (range: 1200–2,700,000) HIV-1 copies/mL All were infected with subtype B, the most frequent subtype in Chile.[11] Subjects were diagnosed HIV-1 positive between 1995 and 2012 with ART initiated on average 22 months (range: 1 to 90) after diagnosis.

When performing genotyping HIV-1 RNA was automatically extracted using EasyMag (bioMérieux). Genotypic resistance test for INSTIs was conducted according to the method described by Harrigan et al.[12,13] The sequences were analysed by RECall[14] and INSTIs resistance reporting used HIVdb.[15] Results were reported as susceptible, potential low-level resistance, low, intermediate, or high level of resistance. Potential low-level resistance was considered susceptible.

We found 12/44 (27.3%) patients showing resistance to INSTIs (1 with resistance to RAL, 6 to RAL and EVG, and 5 to all INSTIs) and 32 (72.7%) remained susceptible to this class of drugs The type and

* Corresponding author. Hospital Clinico de la Universidad de Chile Jose Joaquin Aguirre, Santiago, Region Metropolitana, Chile.
E-mail addresses: pferrer40@gmail.com, pferrer@hcuch.cl (P. Ferrer).

https://doi.org/10.1016/j.jve.2020.06.002
Received 15 June 2020; Accepted 17 June 2020
Available online 25 June 2020

Table 1
INSTI mutations in 12 patients on RAL-based regimens with virological failure: prediction of resistance to RAL and other INSTIs.

Sample	Mutations	RAL	EVG	DTG	BIC
12–01	G140S, Q148H	**High-level resistance**	**High-level resistance**	**Intermediate resistance**	**Intermediate resistance**
13–03	T97A, Y143R	High-level resistance	Low-level resistance	Susceptible	Susceptible
13–05	T97A, Y143R	High-level resistance	Low-level resistance	Susceptible	Susceptible
13–08	T66I, T97A	Low-level resistance	High-level resistance	Susceptible	Susceptible
13–09	N155H	High-level resistance	High-level resistance	Susceptible	Susceptible
13–13	N155H	High-level resistance	High-level resistance	Susceptible	Susceptible
13–14	**Y143R, N155H**	**High-level resistance**	**High-level resistance**	**Low-level resistance**	**Low-level resistance**
13–16	L74 M, V151I, N155H	High-level resistance	High-level resistance	Susceptible	Susceptible
13–18	**G140S, Q148H**	**High-level resistance**	**High-level resistance**	**Intermediate resistance**	**Intermediate resistance**
13–23	Y143R	High-level resistance	Susceptible	Susceptible	Susceptible
13–24	**Q148R, E138K, V151I, S147G**	**High-level resistance**	**High-level resistance**	**High-level resistance**	**High-level resistance**
13–37	**Q148R, G140A, E138K**	**High-level resistance**	**High-level resistance**	**High-level resistance**	**High-level resistance**

INSTI: integrase inhibitor, RAL: raltegravir, EVG: elvitegravir, DTG: dolutegravir, BIC: bictegravir.

Table 2
Therapeutic regimens in patients with virological failure and resistance to INSTIs.

Patients	ART 1	ART 2	ART 3	ART 4	ART 5	ART 6	ART 7
12–01	3 TC-AZT-IDV	D4T-3 TC-IDV	D4T-3 TC-NFV	3 TC-D4T-EFV	ABC-3TC-EFV	AZT-TDF-ATZ/r	AZT-TDF-**RAL**
13–03	AZT-3TC-EFV	AZT-3TC-NVP	AZT-3TC-ATZ/r	AZT-ABC-ATV/r	AZT-ABC-ATV/r	TDF-FTC-**RAL**	MVC-ETV-AZT
13–05	TDF + FTC + ATV/r	TDF/FTC-**RAL**					
13–08	AZT/3 TC-IDV	AZT-3TC-EFV	AZT-3TC-ATZ/r	ABC-3TC-**RAL**	ABC-TDF-**RAL**-	AZT-TDF-**RAL**	
13–09	ABC/3 TC-EFV	AZT-3TC-EFV	AZT-DDI-ATZ/r	AZT-DDI-LPV/r	AZT-TDF-**RAL**	LPV/r-ETV-**DTG**	
13–13	Not information*						
13–14	AZT+3 TC	3 TC-DDI	D4T-EFV-ABC	D4T-IDV-ABC	AZT-3TC-NVP	AZT-3TC-LPV/r	ATV/r-**RAL**-ETV
13–16	AZT-3TC-EFV	DDI-3TC-EFV	DDI-3TC-**RAL**				
13–18	3 TC-AZT-IDV	D4T-DDI-EFV	DDI-D4T-LPV/r	TDF-ETV-**RAL**			
13–23	Not information*						
13–24	AZT+3 TC + LPV/r	ABC/3 TC-LPV/r	ABC-3TC-ATV/r	ABC-3TC-**RAL**			
13–37	AZT/3 TC-ATZ/r	ABC/3 TC-ATZ/r	TDF-NVP-**RAL**	AZT-DDI-LPV/r			

INSTI: integrase inhibitors; AZT: zidovudine; 3 TC: lamivudine; FTC: emtricitabine; ABC: abacavir: NVP: nevirapine; ETV: etravirine; DTG dolutegravir; ATZ/r: atazanavir/ritonavir; IDV: indinavir; TDF; tenofovir; EFV: efavirenz; D4T: stavudine; DDI: didanosine LPV/r: lopinavir/ritonavir; RAL: raltegravir; MVC: maraviroc.

frequency of the most prevalent primary and secondary INSTI mutations detected were consistent with previous reports:[16–25] Q148 H/R, Y143 R/G, N155H (9.1%), and G140 S/A (6.8%). Other primary mutations included T66I, E138K, and S147G (2.3%). The main secondary and accessory mutations detected were T97A (6.8%), L74M (2.3%), and V151I (4.5%), respectively. Table 1 shows that 12 patients were resistant to RAL, 11 had cross-resistance to EVG (25%), and 5 (11.4%) were resistant to DTG and BIC. No significant statistical differences were observed in VL at RAL failure between those with resistant or susceptible viruses ($p > 0.05$). The comparison of the median CD4 T lymphocytes counts among the patients with INSTI-susceptible and INSTI-resistant strains showed that patients with resistant virus had lower median baseline CD4 T cells than those with susceptible virus ($p < 0.05$). Patients with INSTI-resistant virus had been exposed to more ART regimens than those with INSTI-susceptible virus ($p < 0.05$). These are described on Table 2.

Our laboratory had introduced INSTI genotypic resistance testing in 2012. Historically RAL was used in our study participants as part of their rescue regimen in 2008 when there was no INSTI genotyping testing available, thereby preventing adequate assessment at the time of VF and allowing the accumulation of primary and secondary mutations which lead in some patients to the development of resistance to all presently available INSTIs.

To the best of our knowledge, this is the first report on INSTIs resistance in patients with VF to RAL in Chile. In other Latin American countries, INSTIs use has had similar or even more restrictive therapeutic indications with limited access to genotyping. Our results bring some evidence which could help clinical decisions in the region and in the countries that are starting INSTI-based therapies. We strongly advise that INSTI use should be associated with the availably of INSTI genotyping at the start of INSTI use to optimally manage patients at the time of VF.

Ethical approval

This study was approved by the University of Chile Clinical Hospital's Ethics Committee under Hospital Research Project Registry number OAIC 560/12, Certificate of Approval Number 54 (Nov/2012).

Declaration of competing interest

The authors have no competing interests to declare.

Acknowledgements

This study was supported by grants to Dr. Pablo Ferrer Campos by OAIC, University of Chile Clinical Hospital. Research Project Registry number OAIC 560/13.

We thank biochemist Liliana Barrios and Dr. Richard Harrigan from BC Centre for Excellence in HIV/AIDS, Vancouver, Canada, for providing the RECall software.

We also thank Dr. Marcelo Wolff Reyes from the University of Chile and the Arriarán Foundation for critically reviewing the manuscript and Dr. Mario Tello from the University of Santiago, Chile, for assisting

with the statistical methods.

References

1. http://www.unaids.org/en/regionscountries/countries/chile. Accessed May 2020.
2. A. Tseng, J. Seet, E.J. Phillips, The evolution of three decades of antiretroviral therapy: challenges, triumphs and the promise of the future, Br. J. Clin. Pharmacol. 79 (2014) 182–194.
3. D.J. Hazuda, P. Felock, M. Witmer, et al., Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells, Science 287 (2000) 646–650.
4. Mary Ann Liebert, Antiviral briefs. Approval recommended for raltegravir in experienced patients, AIDS Patient Care STDS 9 (2007) 702, https://doi.org/10.1089/apc.2007.9970, 5 Oct 2007.
5. K. Pandey Krishan, Raltegravir in HIV-1 infection: safety and efficacy in treatment naïve patients, Clin. Med. Rev. Therapeut. 4 (2012) 13–30.
6. F. Ceccherini-Silberstein, I. Malet, R. D'Arrigo, et al., Characterization and structural analysis of HIV-1 integrase conservation, AIDS Rev. 11 (2009) 17–29.
7. M. Métifiot, C. Marchand, K. Maddali, et al., Resistance to integrase inhibitors, Viruses 2 (2010) 1347–1366.
8. T.K. Chiu, D.R. Davies, Structure and function of HIV-1 integrase, Curr. Top. Med. Chem. 4 (2004) 965–977.
9. Q. Tang, H.Z. Lu, Latest advances in the efficacy, tolerability, and monotherapy of integrase inhibitors, BioSci. Trends 11 (2017) 490–495.
10. P. Messiaen, A.M.J. Wensing, A. Fun, et al., Clinical use of HIV integrase inhibitors: a systematic review and meta-analysis, PloS One 8 (2013), e52562.
11. P. Ferrer, M. Tello, L. Montecinos, et al., Prevalence of R5 and X4 HIV variants in antiretroviral treatment experienced patients with virologic failure, J. Clin. Virol. 60 (2014) 290–294.
12. G.Q. Lee, L.C. Swenson, A.F.Y. Poon, Prolonged and substantial discordance in prevalence of raltegravir-resistant HIV-1 in plasma versus PBMC samples revealed by 454 "Deep'' Sequencing, PloS One 7 (2012), e46181.
13. W.W. Zhang, P.K. Cheung, N. Oliveira, M.A. Robbins, P.R. Harrigan, A. Shahid, Accumulation of multiple mutations in vivo confers cross-resistance to new and existing integrase inhibitors, J. Infect. Dis. 218 (2018) 1773–1776.
14. C.K. Woods, C.J. Brumme, T.F. Liu, et al., Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool, J. Clin. Microbiol. 50 (2012) 1936–1942.
15. HIVdb program: sequence analysis, Available at: https://hivdb.stanford.edu/hivdb/by-sequences. Accessed: May 2020.
16. D. Da Silva, L. Van Wesenbeeck, D. Breilh, et al., HIV-1 resistance patterns to integrase inhibitors in antiretroviral-experienced patients with virological failure on raltegravir-containing regimens, J. Antimicrob. Chemother. 65 (2010) 1262–1269.
17. J.A. Grobler, D.J. Hazuda, Resistance to HIV integrase strand transfer inhibitors: *in vitro* findings and clinical consequences, Curr. Opin. Virol. 8 (2014) 98–103.
18. A.M. Geretti, D. Armenia, F. Ceccherini-Silberstein, Emerging patterns and implications of HIV-1 integrase inhibitor resistance, Curr. Opin. Infect. Dis. 25 (2012) 677–686.
19. K. Anstett, B. Brenner, T. Mesplede, et al., HIV drug resistance against strand transfer integrase inhibitors, Retrovirology 14 (2017) 36.
20. C. Garrido, C. De Mendoza, V. Soriano, Resistencia a los inhibidores de la integrasa, Enferm. Infecc. Microbiol. Clín. 26 (2008) 40–46.
21. J.K. Rockstroh, H. Teppler, J. Zhao, et al., Clinical efficacy of raltegravir against B and non-B subtype HIV-1 in phase III clinical studies, AIDS 25 (2011) 1365–1369.
22. J.J. Eron, D.A. Cooper, R.T. Steigbigel, Efficacy and safety of raltegravir for treatment of HIV for 5 years in the benchmrk studies: final results of two randomised, placebo-controlled trials, Lancet Infect. Dis. 13 (2013) 587–596.
23. R.T. Steigbigel, D.A. Cooper, P.N. Kumar, et al., Raltegravir with optimized background therapy for resistant HIV-1 infection, N. Engl. J. Med. 359 (2008) 339–352.
24. P. Cahn, A.L. Pozniak, H. Mingrone, et al., Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study, Lancet 382 (2013) 700–708.
25. J.M. Molina, A. LaMarca, J. Andrade-Villanueva, et al., For the Study 145 team. Efficacy and safety of once daily elvitegravir versus twice daily raltegravir in treatment-experienced patients with HIV-1 receiving a ritonavir-boosted protease inhibitor: randomised, double-blind, phase 3, non-inferiority study, Lancet Infect. Dis. 12 (2012) 27–35.