

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

HIV-1 Very Low Level Viremia is Associated with Virological Failure in HAART-treated Patients

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1539598> since 2016-12-13T22:41:49Z

Published version:

DOI:10.1089/AID.2015.0102

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is a pre-copyedited, author-produced PDF of an article accepted for publication in AIDS Research and Human Retroviruses following peer review. The version of record 2015 Oct;31(10):999-1008. doi: 10.1089/AID.2015.0102. Epub 2015 Aug 6. is available online at: <http://online.liebertpub.com/doi/10.1089/aid.2015.0102>

HIV-1 Very Low Level Viremia is Associated with Virological Failure in HAART-treated Patients.

Calcagno A^{1*}, Motta I^{1*}, Ghisetti V², Lo Re S¹, Alice T², Marinaro L¹, Milia MG², Tettoni MC¹, Trentini L¹, Orofino G³, Salassa B¹, Di Perri G¹ and Bonora S¹.

¹*Unit of Infectious Diseases, Department of Medical Sciences, University of Torino;*

²*Laboratory of Microbiology and Molecular Biology;*

³*Unit of Infectious Diseases, “Divisione A”, Ospedale Amedeo di Savoia, ASLTO2, Torino, Italy.*

**Both authors contributed equally to current work*

***Corresponding author:** Motta Ilaria

Clinica Universitaria di Malattie Infettive
Ospedale Amedeo di Savoia
C.so Svizzera 164
10149 Torino, Italy
+390114393856
Fax +390114393942
ilaria.motta@unito.it

Running Title: Undetectable HIV RNA and viral rebound

Key words: Target not detected; Cobas Taqman; Residual viremia; Raltegravir; Nevirapine.

Abstract

Objective: Aim of this study was to evaluate the impact of HIV-1 very low level viremia (<50 copies/ml) on the 2-year risk of virological failure.

Methods: A retrospective analysis including HIV-positive patients presenting two consecutive HIV RNA below 50 copies/mL (outpatient clinic in Italy, first semester of 2010) was performed. HIV RNA was measured through real time Polymerase Chain Reaction (PCR) assay CAP/CTM HIV-1 version 2.0 (detection limit: 20 copies/mL) and stratified as undetectable RNA (**“Target Not Detected”, TND**), <20 copies/mL, 20-50 copies/mL. After 96 weeks virological failure was defined as two consecutive viral loads above 50 copies/mL. Log-rank tests and a multivariate Cox proportional hazard model were used for uni- and multivariate analysis.

Results: 1055 patients (71.4% male, 87.4% Caucasian, aged 46.7 years) were included: nadir and current CD4 cell count were 203 cells/mm³ (106-292) and 554 cells/mm³ (413-713.5). HIV RNA was undetectable in 781 patients (74%), <20 copies/mL in 190 patients (18%) and 20-50 copies/mL in 84 patients (8%). Virological failure was observed in 81 patients (7.7%); at multivariate analysis detectable RNA at baseline (p=0.017), HCV infection (p=0.020), more than 3 pills in the regimen (p=0.003) and duration of HIV RNA<50 copies/mL below 2 years (p<0.001) were independently associated with virological failure. In 14 patients newly selected resistance-associated mutations were observed.

Conclusions: Undetectable HIV RNA by real-time PCR is significantly associated with a lower 2-year risk of virological failure along with **Ab** HCV-negativity, longer viral control and lower pill burden. Studies investigating the management of residual viremia under antiretroviral treatment are warranted.

Introduction

Highly Active Antiretroviral Treatment (HAART) dramatically affects HIV viral replication and immune recovery in HIV-positive patients; nevertheless low-level viremia can be detected in patients on long-term successful treatment [1,2]. The concepts of residual viremia and of very low level viremia (vLLV) has been introduced following the development of highly sensitive methods able to detect as few as one copy/mL and the widespread use of commercial tests with increasingly lower limits of detection below 50 copies/mL (40, 37 and 20 copies/mL) [3]. Although the exact source of residual HIV RNA is still debated [4], it has been associated with possible indicators of viral reservoirs size (T lymphocyte CD4⁺ cell count at nadir, pre-treatment HIV-1 RNA and quantitative cell-associated HIV proviral DNA) and with the duration of viral suppression on HAART [5-7]. Several studies suggested that the administration of NNRTIs (and specifically nevirapine) is associated with the lowest residual viremia [8,9]: the drug target (pre-integration) or the penetration into sanctuary sites have been advocated as possible explanations of this observation [10].

Although the exact impact of vLLV in the long-term management of HIV is poorly understood, two aspects have been studied. Both the presence of low-level replication and the aforementioned associated factors have been linked to higher levels of immune activation, pro-inflammatory cytokines and microbial translocation [11]. Moreover, the possible role of vLLV in predicting later virological rebounds has been investigated in a few heterogeneous studies [1,12-18]: with one exception [18] all found that having higher levels of residual HIV RNA was associated with an increased risk of further virological rebound. In the first published study [17], commercial real-time PCR with a cut off of 48 copies/mL was used, but commercial assay with lower HIV RNA cut off values are currently available in the clinical setting.

Therefore, we investigated the determinants and the virological outcome of patients with very low level viremia measured by a commercial assay with a limit of quantification of 20 copies/mL in the clinical setting.

Primary objective of this study was to evaluate the impact of very low level viremia (HIV RNA below 50 copies/mL) on the 2-year risk of virological failure in HAART-effectively treated patients. Secondary objectives were the analysis of determinants of undetectable RNA at baseline and the evaluation of virological consequences after virological failure.

Materials and Methods

Study design

A retrospective analysis was performed on all patients followed at a large outpatient clinic in Italy (Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Ospedale Amedeo di Savoia, ASLTO2) presenting two consecutive HIV RNA below 50 copies/mL in the first semester of 2010, **the more recent one was considered as baseline virale load**. Patients with at least 6 months of regular follow up were included. This retrospective study was approved by local Ethics Committee and written informed consent was obtained by patients currently in care. Data are routinely collected every three months and treatment switches were made according to clinicians' independent decision.

Virological analysis

HIV RNA was measured through the real time Polymerase Chain Reaction (PCR) assay CAP/CTM HIV-1 vs. 2.0 (CAP/CTM, Roche Molecular System, Branchburg, NJ, detection limit: 20 copies/mL of HIV-1 RNA). Patients' viremia levels were stratified according to three different intervals: undetectable RNA ("Target Not Detected", TND), <20 copies/mL, 20-50 copies/mL. The result of HIV RNA measurement below 50 copies/mL was available to clinicians. Genotypic Sensitivity Score (GSS) was calculated using cumulative genotypic resistance tests and through the Stanford algorithm [19]. GSS was considered equal to the number of administered antiretrovirals in patients with unavailable genotypic resistance tests and no history of virological failures. Genotypic resistance testing was performed in all virological failures using the standard

direct full-population sequencing from plasma samples ViroSeq HIV-1 genotyping (Abbott, IL, US). Briefly, after nucleic acid extraction with the semi-automatic NucliSENS easyMAG platform (BioMerieux, Marcy l'Etoile, F) from 1 mL of plasma, sequences of HIV-1 protease (P) and reverse transcriptase (RT) **regions** were constructed for each sample with seven different primers targeting the majority of HIV-1 P and RT genes. To enhance the sensitivity for samples with HIV-RNA <1000 copies/mL, the standard ViroSeq HIV-1 protocol was modified in that a nested PCR step was introduced. Three Amplicons generated from PCR were visualized on 1% agarose gel and sequenced on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction [20]. Sequence data were assembled and analysed with the ViroSeq HIV-1 Genotyping System software 2.7 for the identification of drug-resistance mutations. Stanford Drug Resistance Database (<http://hivdb.stanford.edu>) and the International AIDS Society, USA drug-resistance mutation list were used to interpret protease and reverse transcriptase-resistance mutations [19].

Definition of failure

Virological failure was defined as two consecutive viral loads above 50 copies/mL (at least seven days apart) or one value above 50 copies/mL in patients lost to follow up.

Statistical analysis

Normally distributed variables were described with means (\pm standard deviation, SD) and analyzed with parametric testing while not normally distributed ones were described with medians (inter-quartile ranges, IQR) and analyzed with non-parametric tests. Kaplan-Meier analysis was used to estimate the cumulative incidence of virologic failure, stratified according to 3 different exposure statuses: TND, <20 copies/mL, 20-50 copies/mL.

The impact of variables on the risk of virological **failure** was estimated through Log-rank tests and multivariate Cox proportional hazard model. This analysis was performed separately in

patients who maintained the same regimen for the 2-year observation period (“non-switchers”) as well as in all included patients: this was done with the aim of controlling for possible treatment switches guided by the presence of low-level HIV RNA. The association of determinants with undetectable baseline RNA was estimated through univariate tests (Chi-square, Mann-Whitney) and a multivariate binary logistic regression analysis. All multivariate models included variables with p values below 0.10 at univariate analysis and backward elimination (variables included in the model are detailed in the text). Data analysis was performed using SPSS software for Macintosh (version 20.0, IBM Corp.).

Results

Baseline characteristics

1055 patients were included in the study: baseline demographic, immunovirological and therapeutic characteristics are described in Table 1. Most of the patients were male (753, 71.4%), Caucasians (922, 87.4%) and median age was 46.7 years (**41.3-53.1**); co-infection with HCV was present in 295 subjects (28%). Median duration of current antiretroviral treatment (ART) and of viral load suppression were 23.7 months (14-40.6) and 39.7 months (20.3-73.1), respectively; HIV RNA was constantly below 50 copies/mL since at least 2 years in 705 (66.8%) subjects. Current CD4 cell count was 554 cells/mm³ (413-713.5); 513 (48,6%) patients had a nadir below 200 CD4/mm³.

Antiretroviral regimens were mostly (85.2%) based on the association of two NRTIs plus either a PI [433 patients, 41%: mostly atazanavir/ritonavir (220, 50,8%) followed by lopinavir/ritonavir (114, 26,3%) and darunavir/ritonavir (35, 8%)], a NNRTI [424 patients (40,2%), mostly efavirenz (238, 56,1%) and nevirapine (150, 35,4%)] or raltegravir (42 patients, 4%). Tenofovir disoproxil fumarate was the most commonly administered NRTI (751, 71,2%, in 706 patients co-formulated with emtricitabine). Most of the patients were **treatment**-experienced (940 patients, 89%):

genotypic resistance tests were available in 371 subjects (35,1%) and they were used to obtain GSS score [≥ 3 in 815 patients (77.3%)].

Baseline viral strata and associated factors

HIV RNA was undetectable in 781 patients (74%), below 20 copies/mL in 190 patients (18%) and between 20 and 50 copies/mL in 84 patients (8%).

The prevalence of baseline viral load strata according to antiretroviral regimen (all patients) and to third compound (in patients receiving 2 NRTIs plus a third drug, n=865) is depicted in Figure 1a, 1b. Paired comparisons highlighted a significantly higher TND prevalence in patients receiving NRTIs-only or NN-based regimens as compared to PI-recipients ($p=0.007$) but not to raltegravir-recipient ($p=0.75$); nevirapine recipients showed the highest prevalence (85.1%). Using ANOVA tests several significant differences were noted among regimen groups as for CD4 cell count, CD4 cell nadir, duration of HIV infection and duration of controlled viremia (for instance NNRTIs and NRTIs-only recipient had the highest duration of HIV RNA suppression).

At univariate analysis, variables associated with TND were: age (for 10 years increase, $p=0.049$), female gender ($p=0.003$), nadir CD4 above 200 cells/mm³ ($p=0.002$), duration of viral suppression above 2 years ($p=0.039$), the use of ARV regimens consisting of less than 4 pills ($p=0.03$), the use of NNRTIs or raltegravir ($p=0.007$).

At multivariate logistic regression analysis (including age, pill count and NNRTI or raltegravir use) female gender ($p=0.002$, aOR 1.68, 95%CI 1.20-2.35), nadir CD4 cell count above 200/mm³ ($p=0.002$, aOR 1.56, 95%CI 1.18-2.08) and viral suppression above 2 years ($p=0.026$, aOR 1.40, 95%CI 1.04-1.89) were independently associated with TND.

Virological outcome and associated factors

After 24 months of follow up, 102 patients were lost, but in 94 (92,2%) of them last available viral load was below 50 copies/mL. 860 subjects (81,5%) were still receiving the same baseline antiretroviral regimen (“non-switchers”); reasons for treatment switches are available in a minority of subjects only. **Virological failure was observed in 81 patients (7.7%) with median HIV-RNA of 1057 (98-25552) copies/mL; HIV-RNA at failure was between 50 and 200 copies/mL or above 200 copies/mL in 31 and 50 patients, respectively.** In the non-switcher group 23 patients presented a virological failure (11,8%); HIV-RNA was **undetectable** in 632 patients (59.9%), <20 copies/mL in 240 patients (22.7%) and 20-50 copies/mL in 182 patients (17.3%). 2-year median CD4 cell count was 572 cells/mm³ (IQR 419-736).

At univariate analysis detectable RNA at baseline (p=0.003), female gender (p=0.043), HCV infection (p=0.004), GSS score below 3 (p=0.007), more than 3 pills (p<0.001), current use of PI-based regimens (p=0.002) and duration of viral suppression below 2 years (p<0.001) were associated with virological rebound. At multivariate analysis (including gender, GSS below 3, PI use) detectable RNA at baseline (p=0.017, aOR 1.71, 95%CI 1.10-2.68), HCV infection (p=0.020, aOR 1.69, 95%CI 1.08-2.62), more than 3 pills (p=0.003, aOR 1.97, 95%CI 1.26-3.06) and duration of viral suppression below 2 years (p<0.001, aOR 2.47, 95%CI 1.58-3.86) were independently associated with virological failure. Kaplan Meier curves according to baseline factors are represented in Figure 2 (a-d).

In non-switchers the same variables were identified as independent predictor of virological failure: HCV infection (p=0.03, aOR 2.19, 95%CI 1.30-3.68), more than 3 pills (p=0.003, aOR 1.79, 95 IC 1.21-2.65), duration of viral suppression below 2 years (p<0.001, aOR 2.99, 95%CI 1.75-5.10) and detectable RNA at baseline (p=0.018, aOR 1.91, 95%CI 1.12-2.65).

We assigned an arbitrary score of 1 to the significant predictors of virological failure: a score from 0 to 4 was therefore calculated. Stratifying patients with low score (0-1) and high score (2-4) was significantly associated with the rate of virological failure during follow up (Log rank p <0.001)

(Figure 3a and 3b): the absolute risk of virological rebound was 4.5% vs. 13.7% in the low-risk and high-risk groups.

Virological outcome at rebound

Among 73 patients not lost to follow up, 23 patients (31.5%) reported a self-performed treatment interruption (Table 2). In 37 (out of 50, 74%) adherent subjects in care at the end of follow up genotype resistance testing was successful; median (and interquartile range) HIV RNA was slightly lower than in those with unsuccessful genotype test [88 copies/mL (63-440 vs. 1497 copies/mL (76-5112), Mann-Whitney p=0.10].

14 patients (28%) selected new resistance-associated mutations. Characteristics both at baseline and at virological failure are summed up in Table 3: most of newly selected mutations were in the reverse transcriptase gene (10/14) or in the integrase gene (4 out of 6 raltegravir recipients). In 2 patients (out of 2 treated with maraviroc-containing regimens) a switch to X4-tropic viruses was observed.

Discussion

Our retrospective analysis on a large sample of HIV-positive patients confirms that the undetectability of HIV RNA is associated with a lower risk of virological rebound in the following two years of follow up as compared to other two degrees of low level viremia (less than 20 copies/mL and between 20 and 50 copies/mL). Besides this virological marker a durable control of viral replication (above two years), the absence of HCV co-infection and a limited number of pills may lower the risk of virological failures. Although seven papers have been published on the subject our research may add to current knowledge the use of a low but commercially available threshold, the inclusion of patients on raltegravir-containing regimens, the analysis of other factors associated with outcome and the characterization of resistance-associated mutations at failure [1,12,14-18].

Some limitations should be highlighted: the retrospective design, the absence of a formal evaluation of adherence, the already reported **intrassay** variability in HIV RNA quantification at low viremia [21,22]. However, the use of commercial assays with high sensitivity and low limit of detection (20 copies/mL in this study) is widespread and the “target not detected” output (suggesting HIV RNA undetectability) may be relevant in the long-term management of HIV-positive patients.

The main objective of this study was to assess the impact of the level of viremia below 50 copies/mL on the risk of virological rebound after two years of follow up: 7.7% of the included subjects had a confirmed elevation of plasma viral load. We observed that patients presenting a TND at baseline had the lowest incidence of viral rebound in the follow up. The observation that patients with the lowest residual viremia had the lowest risk of subsequent virological failures has already been reported in several studies [1, 12-17]. However some differences are worthy of being mentioned. The study by Doyle and coll. is the only one using the “target not detected” output and they showed that this was the group with the best virological outcome [12]. Furthermore, difference in follow-up (from 12 to 30 months), definition of low level viremia (persistent versus non-persistent), different HIV RNA cut offs (50, 48, 40, 27, 3 copies/mL) and methodological issues are noteworthy [23-25]. The aforementioned technical differences may partially explain some of the observed differences in study outcomes: the CAP/CTM assay vs. 2.0 was associated with a better estimation of low-level viremia as compared to Abbott Real Time assay [22,26]. Two reports were not concordant with these observations: one had a short follow up and the extended study showed a roughly four-fold risk of viral failure among patients with residual viremia [15,16]. The second study by Charpentier and coll. using the CAP/CTM vs. 2.0 assay reported an association of persistent low level viremia with a higher blip ratio but not on the risk of virological rebound [18].

Other factors have been identified as being independently associated with virological rebound: HCV co-infection, a short (less than 2 years) HIV RNA suppression and the use of antiretroviral regimens containing more than three pills. Patients co-infected with HCV may have multiple

comorbidities and polypharmacy, immune-dysfunction and possibly incomplete adherence to medications [27]; however a detrimental effect of HCV-infection on the durability of HIV control has seldom being reported [28]. A prolonged control of HIV replication may be beneficial in decreasing reservoir size as well as immune activation and it has been suggested as one of the possible factors associated with virological rebound after switching to a boosted protease inhibitor regimen [29]. Despite the fact that residual viremia may persist even after seven years of **successful** antiretroviral treatment the risk of viral failure declines with the duration of suppression regardless of patients' adherence [2]. One critical issue would be measuring patients' adherence to medications and it has been recently reported [30]: even if this was not available in this study we used pill number as a surrogate marker for adherence, **although previous studies showed mixed results [31]**. It has been shown that higher degrees of compliance to medications are obtained in patients receiving easy regimens composed of few pills and possibly once a day: in this scenario the use of single tablet regimen has been associated with optimal efficacy results as well as less hospitalization and mortality [32]. **Regimens including 4 or more pills were associated with a higher risk of virological rebound. Beside worse adherence to complex therapies other reasons might explain this finding since PI-containing regimens include at least 3 pills: they are associated with worse tolerability, less forgiveness (given their short half-lives) and are usually administered to patients harboring pre-treated viruses. No difference in virological failure was observed between QD and BID treatments: despite discordant results a recent large randomized trial suggested that a twice-daily administered but excellently tolerated regimen was superior to once-daily PI-based combinations [33]**.

In the whole cohort newly selected mutations have been observed in 14 patients only (out of 50 patients with virological failure and successful viral genotype). The characteristics of the 14 patients losing future treatment options are described in Table 3 and they confirm the chance of selecting RAMs in patients treated with drugs with low (NNRTIs or raltegravir) or undefined (maraviroc) genetic barrier to resistance [34].

Combining these factors into a simple score (arbitrary assigning a 1 point to each of these variables, also taking into account the similarities in adjusted odds ratio of the multivariate model) may provide a clinical useful model. Stratifying patients according to the presence of 0 or 1 factor (low risk) or 2 or more (high-risk) factors was associated with a significant ability to discriminate patients with low or high chance of ensuing viral rebound (4.5% vs. 13.7%). **Once confirmed in prospective independent cohorts this score may be relevant for allocating patients to less frequent monitoring or less-drug regimens [35].**

These data, may suggest that the optimal target of antiretroviral treatment may be lowered (as it happened in the past with the availability of more sensitive tests) to the achievement of undetectable RNA. Nevertheless the management of low level viremia is currently unknown and it deserves prospective clinical studies. Intensification studies of conventional HAART to determine the contribution of ongoing viral replication to residual viremia have shown heterogeneous results: while Yukl et al. found a decrease of unspliced HIV RNA in the ileum (but not in plasma, PBMCs, duodenum, colon or rectum) [36] other authors found no effect [37-46].

It is noteworthy that none of these studies included the use of nevirapine: the drug has been associated with the lowest residual viremia in two studies [8,9] and with long term control of HIV viremia in a dual therapy with raltegravir [47]. Despite some unfavourable characteristics (hepatotoxicity, skin rashes, low genetic barrier to resistance) that placed nevirapine among alternative regimens in international guidelines, favourable pharmacokinetic features and tolerability may support prospective switch studies using nevirapine-containing regimens.

One of the secondary objective of this study was the analysis of the variables associated with the HIV RNA undetectability (TND) using the Taqman 2.0 test: 74% of long-term efficaciously treated HIV-positive patients presented an undetectable HIV RNA. We found that female gender, high nadir CD4 cell count and durable HIV RNA suppression were independently associated with TND.

While gender differences have not been definitively demonstrated [48,49] both CD4 nadir and durable viral replication control have been associated with low reservoir size, less residual viremia and less immune-activation [50-52]. At univariate analysis we also found that both NNRTI-based and raltegravir-based regimens had the highest prevalence of TND as compared to PI-based, NRTI-sparing and complex regimens; surprisingly 81.5% of patients taking 3 or 4 NRTIs (with no other antiretrovirals) had a **not detectable** RNA. The groups of patients with different antiretroviral regimens showed significantly differences in CD4 nadir and HIV RNA suppression and the treatment-related effect disappeared at the multivariate analysis: these baseline disparities as well as the inclusion of the number of pills (indirectly linked to patients' adherence to medication) may explain this observation. However in patients under "conventional regimens" i.e. those receiving two NRTIs plus a third drug, nevirapine and raltegravir recipients showed the highest rates of TND: while the favorable association between nevirapine use and low residual viremia has already been reported [8,9] such raltegravir effect is a novel observation. Raltegravir mechanism of action, activity in macrophages and very fast viral decay as well as the long-term excellent efficacy and tolerability may explain this finding [53]. The discovery of other possible reservoirs (such as CD4-associated) [54-56] or lymph nodes [57-59] remains essential for the goal of HIV functional cure: pharmacological properties of antiretroviral compounds may justify some of these observations (and favoring drugs with higher tissue distribution such as nevirapine) [23].

In conclusion, having an undetectable RNA, more than 2 years of HIV RNA below 50 copies/mL, being HCV-free and being treated with less than 4 pills were associated with a lower 2-year risk of virological rebound in the following two years of follow up. The majority (74%) of patients with confirmed HIV-1 RNA below 50 copies/mL present no detectable RNA using a 20 copies/mL commercially available real-time PCR assay. **Female gender, nadir CD4 cell count above**

200/mm³ and viral suppression above 2 years were independently associated with TND at baseline.

These data warrant further investigation in the context of the management of low-level viremia in HIV-positive treated patients.

Acknowledgement

This study was presented at the 14th European AIDS Conference (EACS), October 16-19, 2013, Brussels, Belgium, EU. PS7/4.

AC, SB, VG, GDP contributed to study design, data collection, interpretation of data and statistical analysis. LRS, IM contributed to data collection. AC, SB, VG, IM drafted the first version of the manuscript and finalized the manuscript. GDP contributed to study design, supervision and critical revision of the manuscript for intellectual content. All authors read and approved the final manuscript.

Funding

This work was supported by internal funding.

Transparency declaration

A.C. has received travel grants or speaker's honoraria from Abbott, Bristol-Myers Squibb (BMS), Merck Sharp & Dome (MSD) and Janssen-Cilag. S.B. has received grants, travel grants, consultancy fees from Abbott, Boehringer-Ingelheim, BMS, Gilead-Sciences, GSK, MSD, Pfizer, Janssen-Cilag. G.D.P. has received grants, travel grants, consultancy fees from Abbott, Boehringer-Ingelheim, BMS, Gilead-Sciences, GSK, MSD, Pfizer, Roche, Tibotec (Johnson & Johnson). Other

authors have nothing to declare.

References

1. Maggiolo F, Callegaro A, Cologni G, et al. Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. *J Acquir Immune Defic Syndr* 2012;**60**(5):473-82.
2. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA* 2008;**105**(10):3879-84.
3. Ryscavage P, Kelly S, Li JZ, et al. Significance and clinical management of persistent low level viremia and very low level viremia in HIV-1 infected patients. *Antimicrob Agents Chemother* 2014;**58**(7):3585-98.
4. Shen L, Siliciano RF Viral reservoirs, residual viremia, and the potential of highly active antiretroviral therapy to eradicate HIV infection. *J Allergy Clin Immunol* 2008;**122**(1):22-8.
5. Chun TW, Murray D, Justement JS, et al. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J infect dis* 2011;**204**(1):135-8.
6. Allavena C, Rodallec A, Secher S, et al. Evaluation of residual viremia and quantitation of soluble CD14 in a large cohort of HIV-infected adults on a long-term non-nucleoside reverse transcriptase inhibitor-based regimen. *J med virol* 2013;**85**(11):1878-82.

7. Pascual-Pareja JF, Martinez-Prats L, Luczkowiak J, et al. Detection of HIV-1 at between 20 and 49 copies per milliliter by the Cobas TaqMan HIV-1 v2.0 assay is associated with higher pretherapy viral load and less time on antiretroviral therapy. *J Clin Microbiol* 2010;**48**(5):1911-2.
8. Haim-Boukobza S, Morand-Joubert L, Flandre P, et al. Higher efficacy of nevirapine than efavirenz to achieve HIV-1 plasma viral load below 1 copy/mL. *AIDS* 2011;**25**(3):341-4.
9. Bonora S, Nicastrì E, Calcagno A, et al. Ultrasensitive assessment of residual HIV viraemia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional evaluation. *J Med Virol* 2009;**81**(3):400-5.
10. Fletcher CV, Staskus K, Wietgreffe SW, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc Natl Acad Sci U S A*. 2014;**111**(6):2307-12.
11. Reus S, Portilla J, Sanchez-Paya J, et al. Low-level HIV viremia is associated with microbial translocation and inflammation. *J Acquir Immune Defic Syndr* 2013;**62**(2):129-34.
12. Doyle T, Smith C, Vitiello P, et al. Plasma HIV-1 RNA detection below 50 copies/mL and risk of virologic rebound in patients receiving highly active antiretroviral therapy. *Clin Infect Dis* 2012;**54**(5):724-32.
13. Doyle T, Geretti AM. Low-level viraemia on HAART: significance and management. *Curr Opin Infect Dis* 2012;**25**(1):17-25.

14. Álvarez Estévez M, Chueca Porcuna N, Guillot Suay V, et al. Quantification of viral loads lower than 50 copies per milliliter by use of the Cobas AmpliPrep/Cobas TaqMan HIV-1 test, version 2.0, can predict the likelihood of subsequent virological rebound to >50 copies per milliliter. *J Clin Microbiol* 2013;**51**(5):1555-7.
15. Gianotti N, Galli L, Racca S, et al. Residual viraemia does not influence 1 year virological rebound in HIV-infected patients with HIV RNA persistently below 50 copies/mL. *J Antimicrob Chemother* 2012;**67**(1):213-7.
16. Gianotti N, Galli L, Salpietro S, et al. Virological rebound in human immunodeficiency virus-infected patients with or without residual viraemia: results from an extended follow-up. *Clin Microbiol Infect* 2013;**19**(12):E542-4.
17. Henrich TJ, Wood BR, Kuritzkes DR. Increased risk of virologic rebound in patients on antiviral therapy with a detectable HIV load <48 copies/mL. *PLoS One* 2012;**7**(11):e50065.
18. Charpentier C, Landman R, Laouenan C, et al. Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. *J Antimicrob Chemother* 2012;**67**(9):2231-5.
19. <http://sierra2.stanford.edu/sierra/servlet/JSierra?action=mutationsInput>, last access on 5th June 2014

20. Milia MG, Allice T, Gregori G, et al. Magnetic-silica based nucleic acid extraction for Human Immunodeficiency Virus Type-1 drug-resistance testing in low viremic patients. *J Clin Virol* 2010;**47**(1):8-12.
21. Ruelle J, Debaisieux L, Vancutsem E, et al. HIV-1 low-level viraemia assessed with 3 commercial real-time PCR assays show high variability. *BMC Infect Dis* 2012;**12**:100.
22. Wojewoda CM, Spahlinger T, Harmon ML, et al. Comparison of Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 (CAP/CTM v2.0) with other real-time PCR assays in HIV-1 monitoring and follow-up of low-level viral loads. *J Virol Methods* 2013;**187**(1):1-5.
23. Di Mascio M, Srinivasula S, Bhattacharjee A, et al. Antiretroviral tissue kinetics: In vivo imaging using positron emission tomography. *Antimicrob Agents Chemother* 2009;**53**(10):4086-95.
24. van Rensburg EJ, Tait K, Watt A, et al. Comparative evaluation of the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 version 2 test using the TaqMan 48 analyzer and the Abbott RealTime HIV-1 assay. *J Clin Microbiol* 2011;**49**:377-9.
25. Karasi JC, Dziezuk F, Quennery L, et al. High correlation between the RocheCOBAS(1) AmpliPrep/COBAS(1) TaqMan(1) HIV-1, v2.0 and the Abbott m2000 RealTime HIV-1 assays for quantification of viral load in HIV-1 B and non-B subtypes. *Clin Virol* 2011;**52**:181-6.

26. Package insert COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0

27. Marchetti G, Cozzi-Lepri A, Tincati C, et al. Immune activation and microbial translocation in liver disease progression in HIV/hepatitis co-infected patients: results from the Icona Foundation study. *BMC Infect Dis* 2014;**14**:79.

28. Bani-Sadr F, Loko MA, Pambrun E, et al. Correlates of HIV sustained viral suppression in HIV/hepatitis C virus coinfecting patients: possible role of the hepatitis C virus sustained viral response. *AIDS* 2014;**28**(8):1155-60.

29. Lambert-Niclot S, Flandre P, Valantin MA, et al. Similar evolution of cellular HIV-1 DNA level in darunavir/ritonavir monotherapy versus triple therapy in MONOI-ANRS136 trial over 96 weeks. *PLoS One* 2012;**7**(7):e41390.

30. Ammassari A. cART non-adherence together with low-level viremia is the strongest predictor of virological failure in positive treated patients. Presented at: 14th European AIDS Clinical Society (EACS); 2013, Bruxelles.

31. Atkinson MJ and Petrozzino JJ. An Evidence-Based Review of Treatment-Related Determinants of Patients' Nonadherence to HIV Medications. *AIDS Patient Care and STDs*. 2009 Nov;**23**(11): 903-914.

32. Cohen CJ, Meyers JL, Davis KL. Association between daily antiretroviral pill burden and treatment adherence, hospitalisation risk, and other healthcare utilisation and costs in a US

medicaid population with HIV. *BMJ Open* 2013 Aug 1;**3**(8).

33. Lennox JL, Landovitz RJ, Ribaud HJ, et al. Efficacy and tolerability of 3 nonnucleoside reverse transcriptase inhibitor-sparing antiretroviral regimens for treatment-naive volunteers infected with HIV-1: a randomized, controlled equivalence trial. *Ann Intern Med.* 2014 Oct 7;161(7):461-71.
34. Mackie N. Detection of HIV-1 antiretroviral resistance from patients with persistently low but detectable viraemia. *J Virol Methods* 2004;**119**(2):73–8.
35. Reekie J, Mocroft A, Sambatakou H, et al. Does less frequent routine monitoring of patients on a stable, fully suppressed cART regimen lead to an increased risk of treatment failure? *AIDS.* 2008 Nov 12;**22**(17):2381-90.
36. Yukl SA, Shergill AK, McQuaid K, et al. Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. *AIDS* 2010;**24**(16):2451-60.
37. Puertas MC, Massanella M, Llibre JM, et al. Intensification of a raltegravir-based regimen with maraviroc in early HIV-1 infection. *AIDS* 2014;**28**(3):325-34.
38. Hunt PW, Shulman NS, Hayes TL, et al. The immunologic effects of maraviroc intensification in treated HIV-infected individuals with incomplete CD4+ T-cell recovery: a randomized trial. *Blood* 2013;**121**(23):4635-46.
39. Hunt PW, Lederman MM, Deeks SG. Response: Maraviroc intensification and microbial

translocation. *Blood* 2013;**122**(13):2283-4.

40. Dinoso JB, Kim SY, Wiegand AM, et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* 2009;**106**(23):9403-8.

41. McMahon D, Jones J, Wiegand A, et al. Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. *Clin Infect Dis* 2010;**50**(6):912-9.

42. Grant PM, Palmer S, Bendavid E, et al. Switch from enfuvirtide to raltegravir in virologically suppressed HIV-1 infected patients: effects on level of residual viremia and quality of life. *J Clin Virol* 2009;**46**(4):305-8.

43. Gandhi RT, Coombs RW, Chan ES, et al. No effect of raltegravir intensification on viral replication markers in the blood of HIV-1-infected patients receiving antiretroviral therapy. *J Acquir Immune Defic Syndr* 2012;**59**(3):229-35.

44. Lam YM, McBride KL, Amin J, et al. Switching virally suppressed, treatment-experienced patients to a raltegravir-containing regimen does not alter levels of HIV-1 DNA. *PLoS One* 2012;**7**(3):e31990.

45. Gutierrez C, Diaz L, Vallejo A, et al. Intensification of antiretroviral therapy with a CCR5 antagonist in patients with chronic HIV-1 infection: effect on T cells latently infected. *PLoS One* 2011;**6**(12):e27864.

46. Yilmaz A, Verhofstede C, D'Avolio A, et al. Treatment intensification has no effect on the HIV-1 central nervous system infection in patients on suppressive antiretroviral therapy. *J Acquir Immune Defic Syndr* 2010;**55**(5):590-6.
47. Montrucchio C, Calcagno A, Lanzafame M, et al. Pharmacokinetics of the dual NRTI- and protease inhibitor-sparing regimen raltegravir plus nevirapine in HIV-1+ patients, *CROI 2013*, abstr **536**.
48. Nicastrì E, Leone S, Angeletti C, et al. Sex issues in HIV-1 infected persons during highly active antiretroviral therapy: a systematic review. *J Antimicrob Chemother* 2007;**60**(4):724-732.
49. Kuyper LM, Wood E, Montaner JS, et al. Gender differences in HIV-1 RNA rebound attributed to incomplete antiretroviral adherence among HIV infected patients in a population-based cohort. *J Acquir Immune Defic Syndr* 2004;**37**(4):1470–6.
50. Geretti AM, Smith C, Haberl A, et al. Determinants of virological failure after successful viral load suppression in first-line highly active antiretroviral therapy. *Antivir Ther* 2008;**13**:927-36.
51. Zoufaly A, Kiepe J, Hertling S, et al. Immune activation despite suppressive highly active antiretroviral therapy is associated with higher risk of viral blips in HIV-1-infected individuals. *HIV Med* 2014;**15**(8):449-57.
52. Sarmati L, Parisi SG, Montano M, et al. Nevirapine use, prolonged antiretroviral therapy and high CD4 nadir values are strongly correlated with undetectable HIV-DNA and -RNA levels

- and CD4 cell gain. *J Antimicrob Chemother* 2012;**67**(12):2932-8.
53. Scopelliti F, Pollicita M, Ceccherini-Silberstein F, et al. Comparative antiviral activity of integrase inhibitors in human monocyte-derived macrophages and lymphocytes. *Antiviral Res* 2011;**92**(2):255-61.
54. Lindkvist A, Edén A, Norström MM, et al. Reduction of the HIV-1 reservoir in resting CD4⁺ T-lymphocytes by high dosage intravenous immunoglobulin treatment: a proof-of-concept study. *AIDS Res Ther* 2009;**6**:15.
55. North TW, Higgins J, Deere JD, et al. Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *J Virol* 2010;**84**:2913-22.
56. Anderson JA, Archin NM, Ince W, et al. Clonal sequences recovered from plasma from patients with residual HIV-1 viremia and on intensified antiretroviral therapy are identical to replicating viral RNAs recovered from circulating resting CD4⁺ T cells. *J Virol* 2011;
85:5220-3.
57. Haase AT Population biology of HIV-1 infection: Viral and CD4⁺ T cell demographics and dynamics in lymphatic tissues. *Annu Rev Immunol* 1999;**17**:625-56.
58. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 1993;**362**(6418):359-62.
59. Pantaleo G, Graziosi C, Butini L, et al. Lymphoid organs function as major reservoirs for

human immunodeficiency virus. *Proc Natl Acad Sci USA* 1991;**88**(21):9838-42.