FLS SCIENCE FOSTERING, LEARNING & SHARING

FIGHT AIDS FOUNDATION

Update on Clinical Topics in Antiretroviral Therapy workshop

Hospital de Sant Pau Art Nouveau Site, Barcelona, Spain

Friday 27th and Saturday 28th April 2018

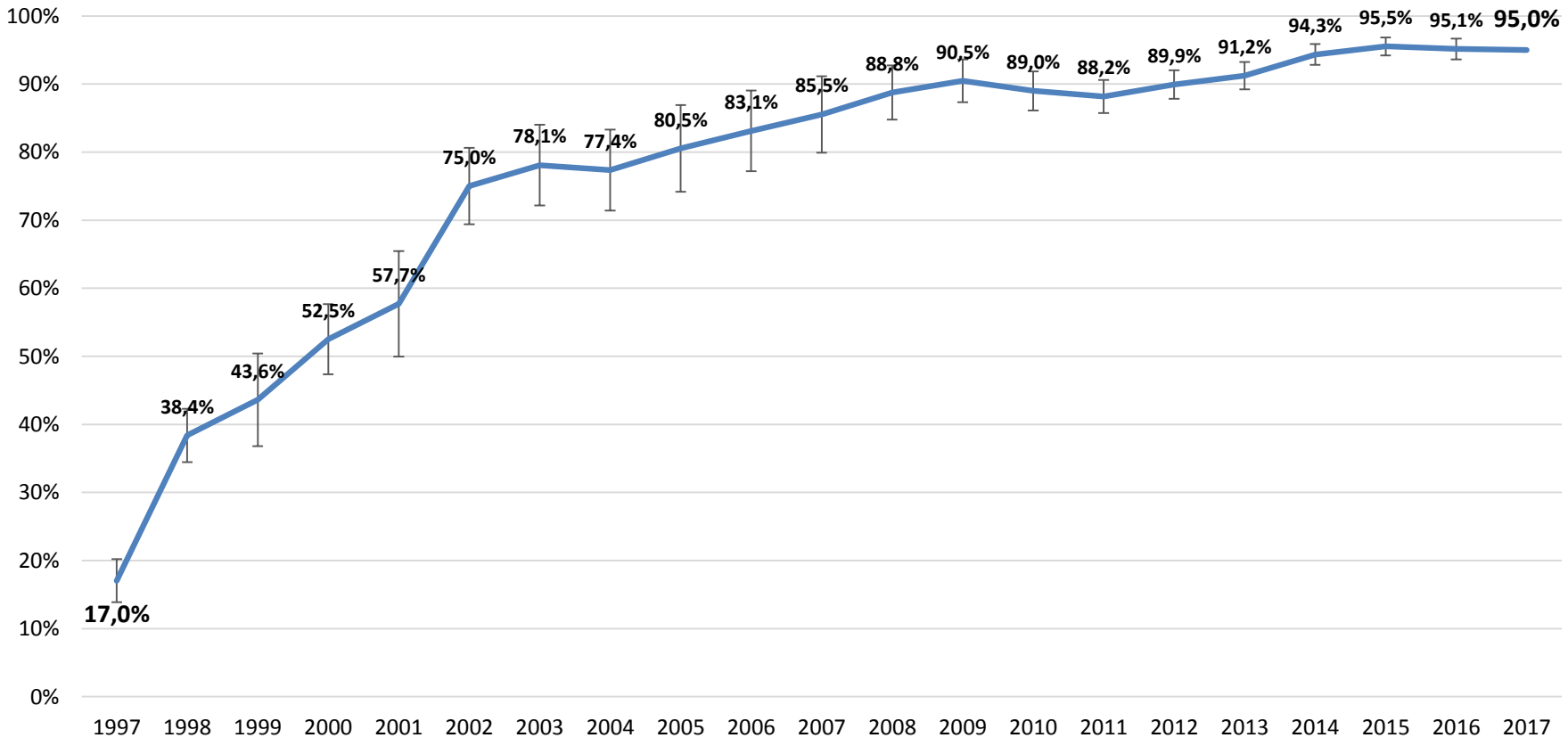
Implications from the virology field in successful ART

Carlo-Federico Perno

UNIVERSITÀ DEGLI STUDI DI MILANO

Fondazione Icona
ITALIAN COHORT NAIVE ANTIRETROVIRALS

Proportion of patients with a VL<=80 copies/mL at 12 months from starting their first ART regimen by calendar year of initiation



Jan 2018 Report

In the long-term management of HIV infection, the stable maintenance of undetectable HIV over the years is more important than the mere success of the first regimen

Definition of virologically suppressed

Clinical trials exploring switching strategies have defined suppression as an HIV-VL < 50 copies/mL for at least 6 months.

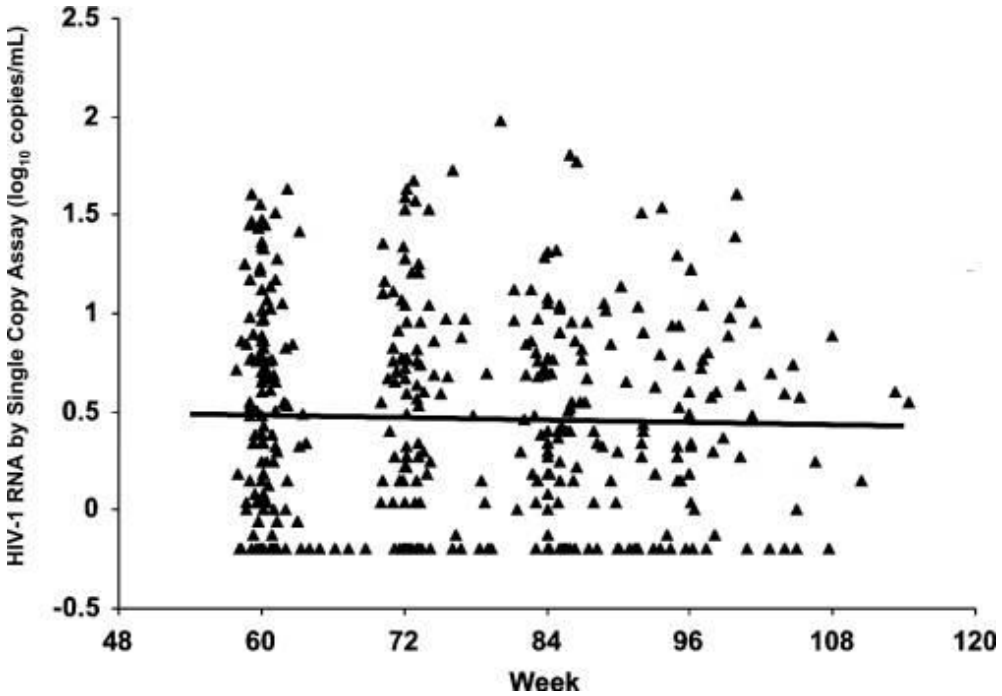
- Routine commercial assays can now accurately measure viraemia levels as low as 50 copies/mL and as high as 10 million or more copies/mL.

- Among them, the two most widely available real-time reverse transcription PCR assays have reported RNA quantification limits of 40 copies/mL (Abbot RealTime RT-PCR Assay) and 20 copies/mL (Roche COBAS Amplicor TaqMan assay version 2.0), and can qualitatively detect HIV-1 RNA below these quantification limits.

- **More sensitive research assays have reduced the lower end to much less than one copy/mL with good quantitative accuracy.**

Single copy assay results revealed that >80% of patients on initial antiretroviral therapy for 60 wks had persistent viremia of one copy/ml or more with an overall median of 3.1 (range 1-49) copies/ml.

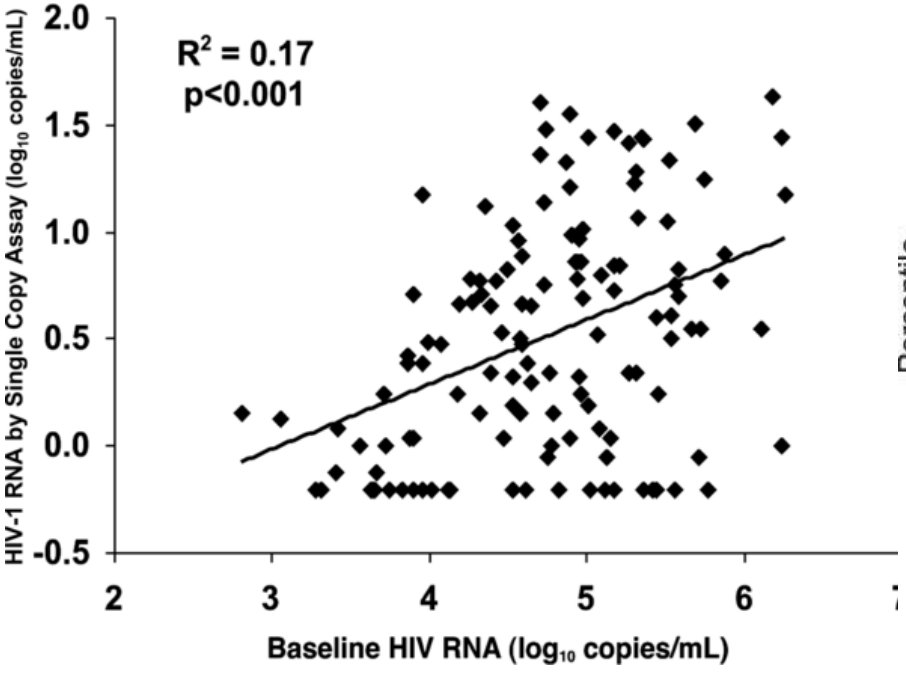
HIV-1 RNA Levels Over 50 wks of Suppressive Antiretroviral Therapy



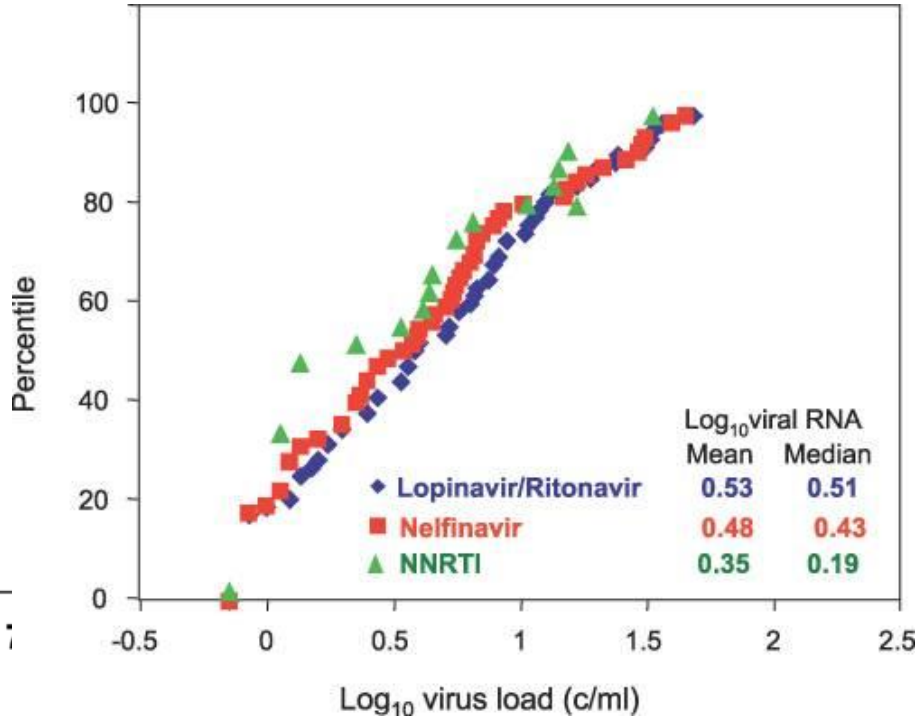
Longitudinal studies revealed no significant decline in the level of viremia between 60 and 110 wks of suppressive antiretroviral therapy. These data suggest that the persistent viremia on current antiretroviral therapy is derived, at least in part, from long-lived cells that are infected prior to initiation of therapy.

Maldarelli et al., Plos Pathog 2007

The level of viremia correlated with pretherapy plasma HIV-1 RNA but not with specific treatment.



Correlation between Pretherapy and On-Therapy Viral RNA Levels



Distribution of Plasma HIV-1 RNA Levels in Patients with Persistently Suppressed Viremia (HIV-1 RNA <50 Copies/ml from Week 24 to 60) on Standard Antiretroviral Therapy

Maldarelli et al., Plos Pathog 2007

PLOS | PATHOGENS

Several studies demonstrated that an increased risk of virological failure remains even in responder patients with very low copies of viral replication during ART.

- Lambert-Niclot S, et al. Analysis and impact of ultra sensitive viral load on virological failure in 3 protease inhibitor monotherapy trials as simplification regimen (ANRS and IMEA Studies). IAS 2017, abstract MOPEB0318.
- Gianotti N, et al., Refining criteria for selecting candidates for a safe lopinavir/ritonavir or darunavir/ritonavir monotherapy in HIV-infected virologically suppressed patients. PLoS One 2017;12(2):e0171611.
- Gianotti et al., HIV DNA loads, plasma residual viraemia and risk of virological rebound in heavily treated, virologically suppressed HIV-infected patients. Clin Microbiol Infect. 2015 21:103.e7-103.e10.
- Álvarez Estévez M, 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, 51:1555-7.
- Maggiolo F, et al. Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. JAIDS. 2012;60:473-82.
- Doyle T, et al.. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. CID 2012, 54:724-32.
- Charpentier C, et al. Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. JAC 2012.67:2231-5.
- Lambert-Niclot S, et al. Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. J Infect Dis. 2011, 204:1211-6.
- Bonora S, et al. 2009. Ultrasensitive assessment of residual HIV viremia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional study. J Med Virol. 81:400–405.

Factors influencing long-term viral suppression

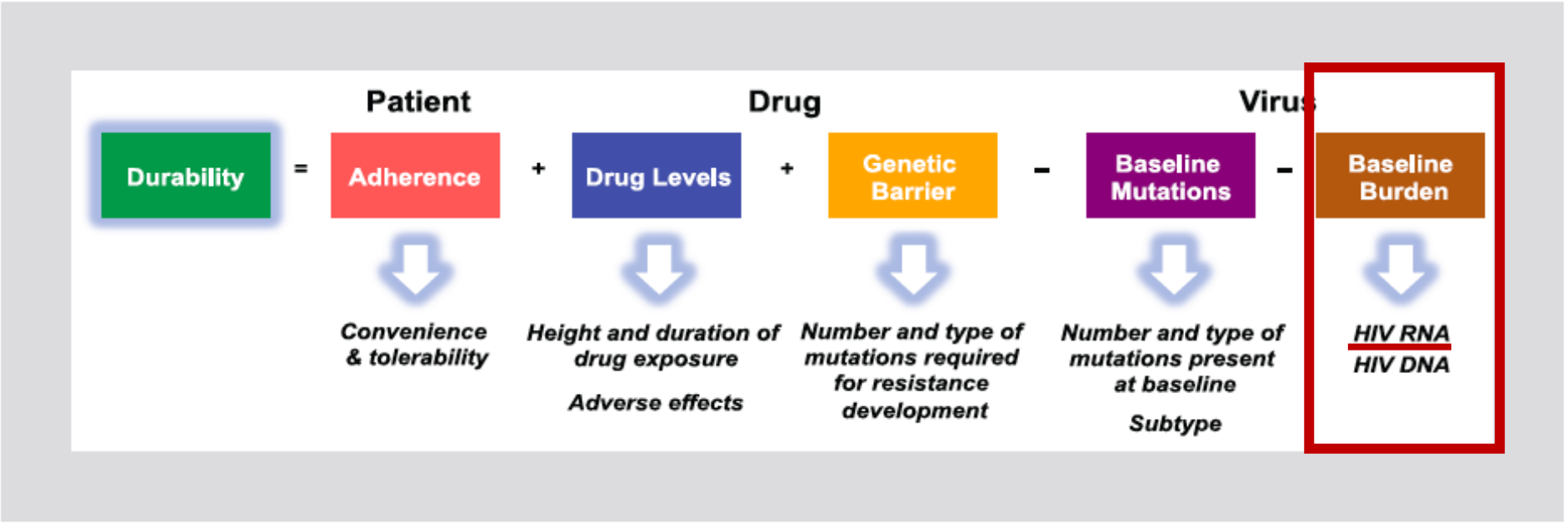
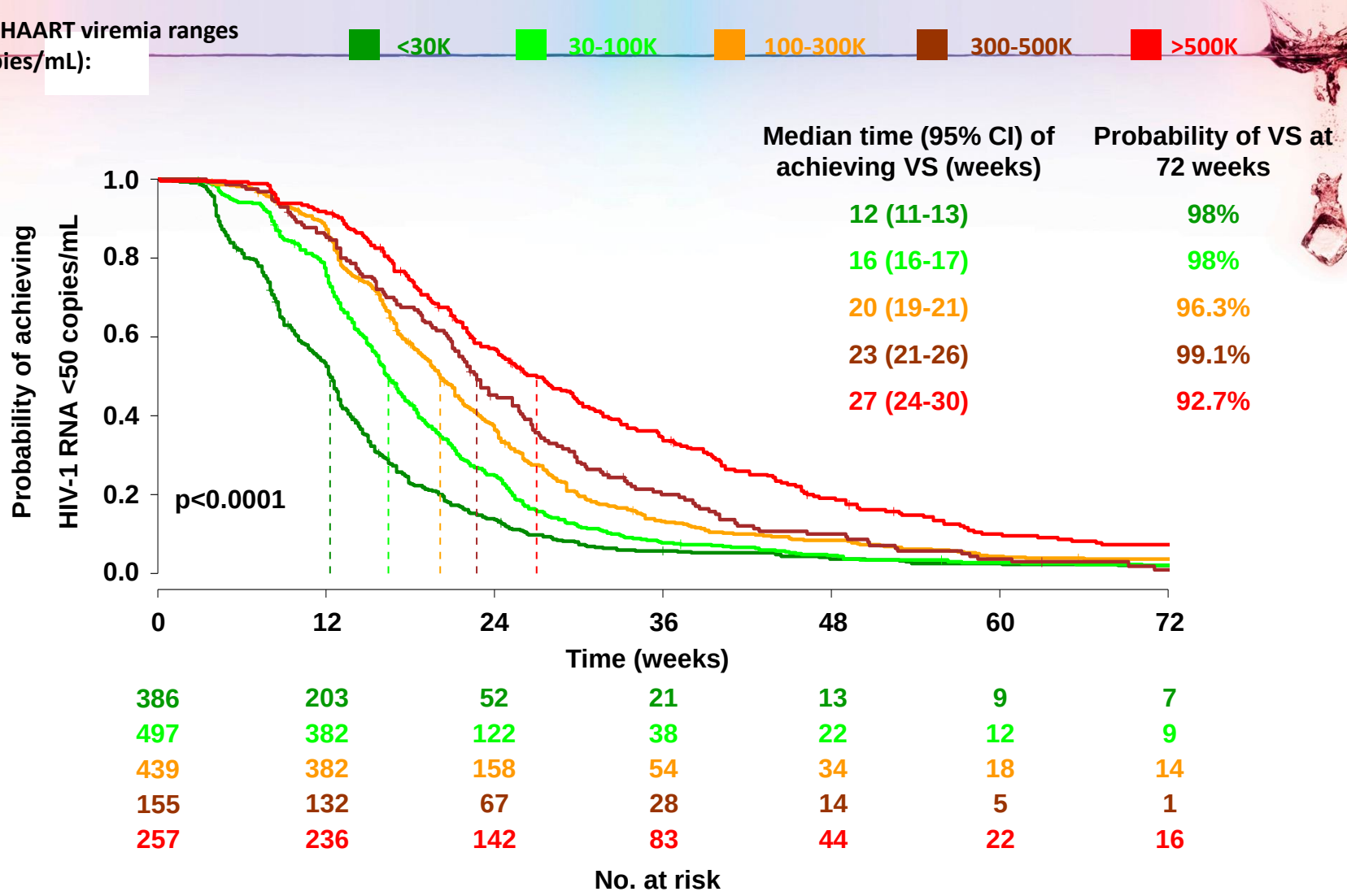


Figure 1. *Factors influencing long-term viral suppression, adapted from Massimo Andreoni personal communicaton.*

Svicher et al , Impact Study Group. AIDS Reviews 2017

Pre-HAART viremia has an impact on the achievement of virological success……..

By 72 weeks of therapy, patients having pre-HAART viremia >500,000 copies/mL showed the lowest probability of achieving VS compared to others pre-HAART viremia ranges.

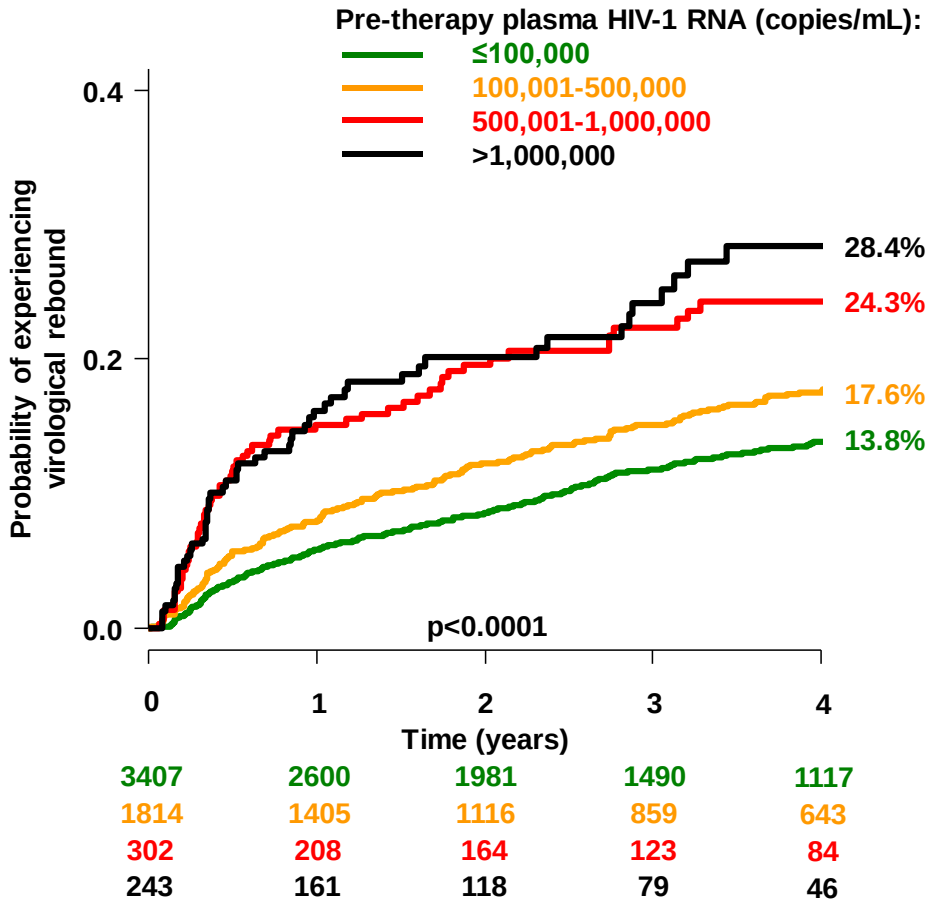


Patients (N=1,734) followed after HAART starting regardless therapy changes or interruptions. CI: confidence interval. HAART: highly active antiretroviral therapy. <30K: <30,000. 30-100K: 30,000-100,000. 100-300K: 100,000-300,000. 300-500K: 300,000-500,000. >500K: >500,000 . VS: virological success.

Santoro MM, Antiviral Therapy 2013

Pre-HAART viremia has an impact on the achievement of virological success……..
And also on its maintenance!

In patients starting a first-line regimen, high pre-cART viremia correlates with high probability of experiencing virological rebound after the achievement of virological success



Kaplan-Meier curves estimates of cumulative probability of virological rebound according to pre-HAART viremia ranges and type of first-line treatment

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Armenia et al manuscript in preparation

Factors influencing long-term viral suppression

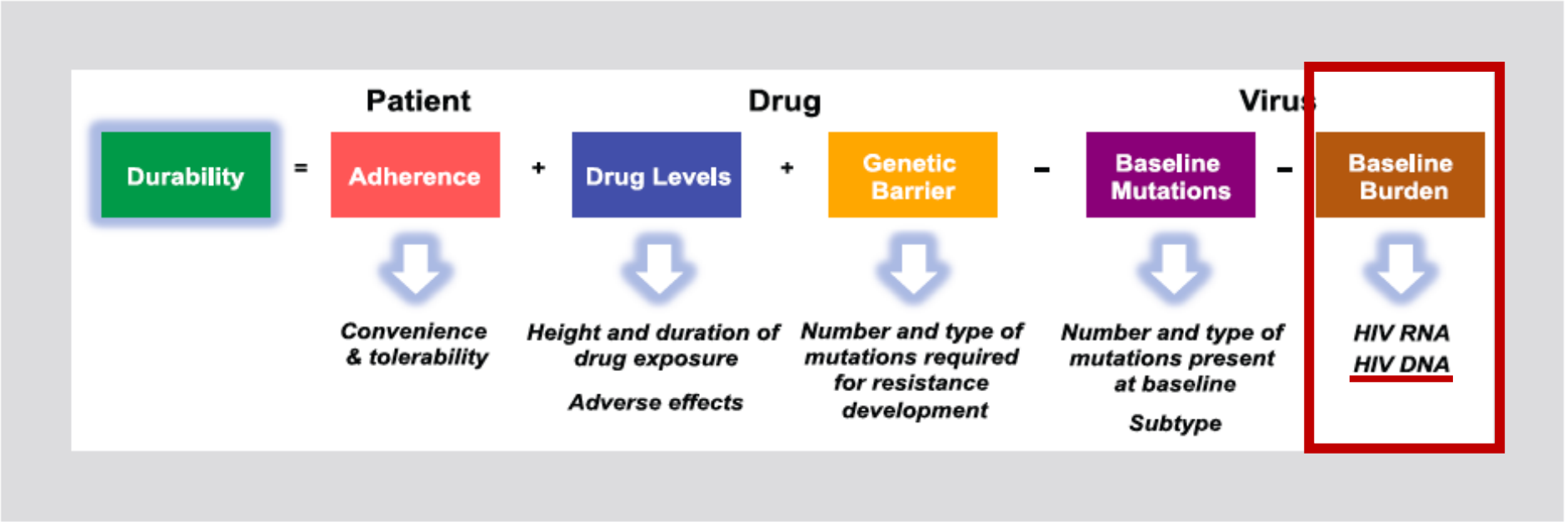


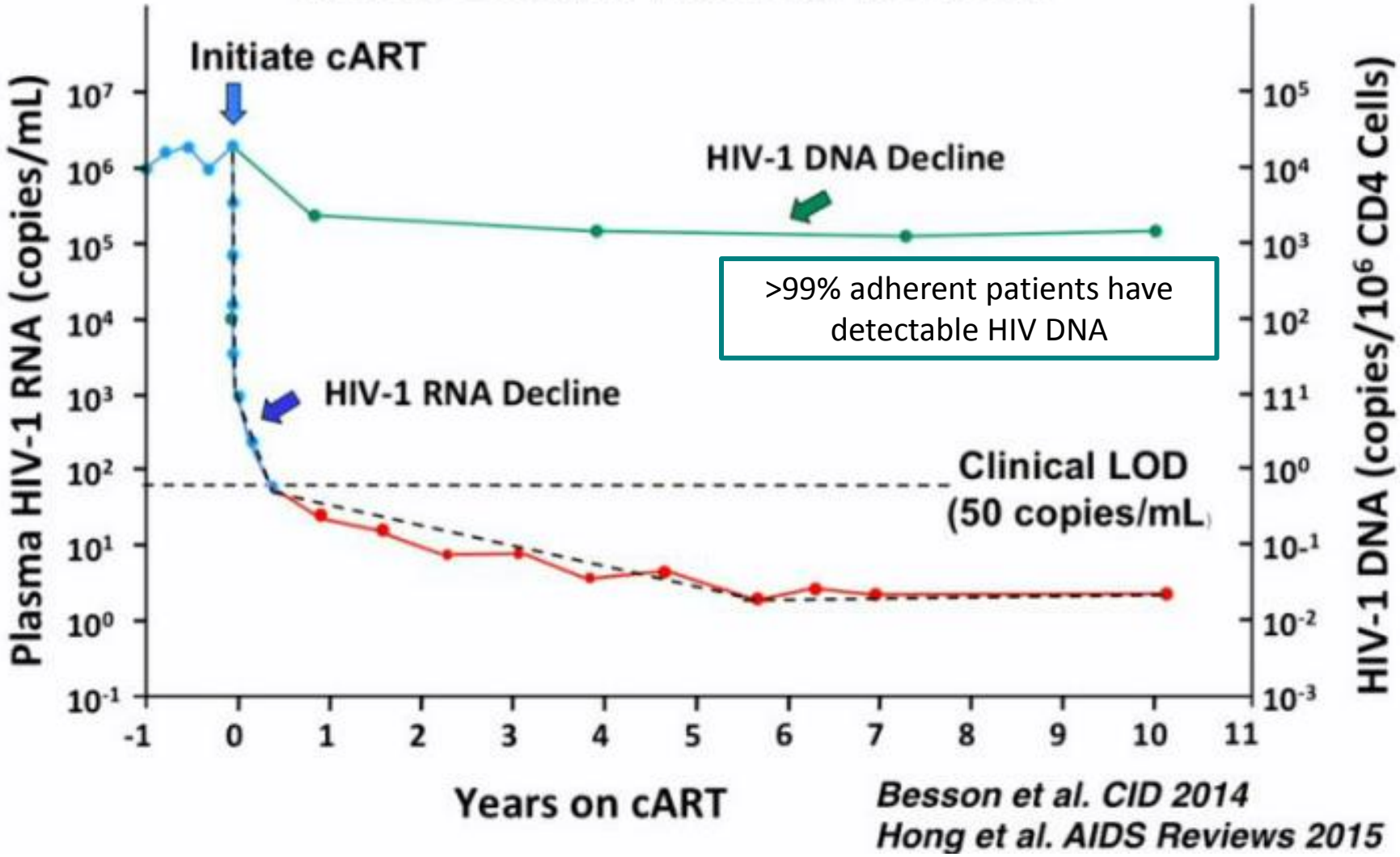
Figure 1. *Factors influencing long-term viral suppression, adapted from Massimo Andreoni personal communicaton.*

Svicher et al , Impact Study Group. AIDS Reviews 2017

HIV-DNA load correlates directly with the number of latently HIV-infected cells that constitute the viral reservoir.

Therefore, the quantification of total HIV-DNA in PBMCs provides a reliable and easy way of measuring the size of the cellular reservoirs of HIV.

Although HIV DNA Declines on Therapy, It Only Goes Down About 15-fold



#365 Session: P-D07
CROI Conference on Retroviruses and Opportunistic Infections

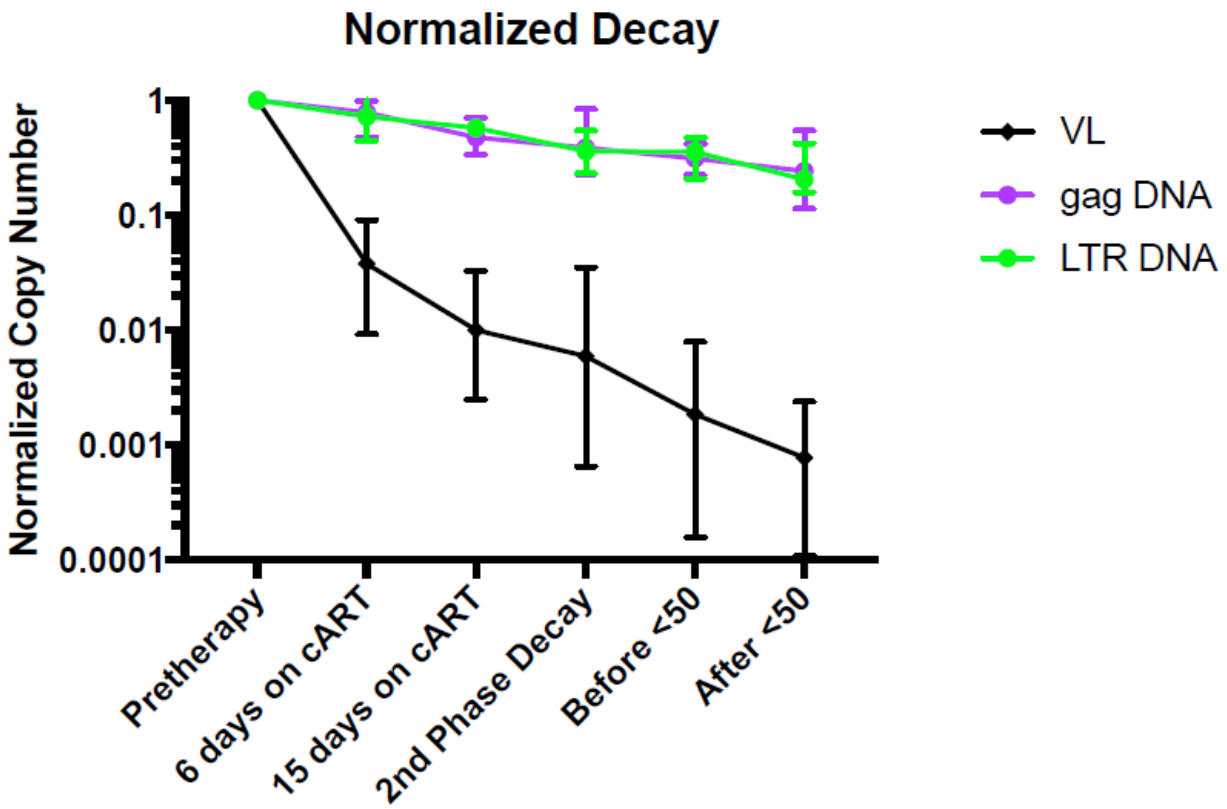
HIV Viremia is the Product of a Small Fraction of HIV Infected Cells

Elizabeth M. Anderson[1], S. Hill[1], J. Bell[3], C. Rehm[2], S. Jones[3], R. Gorelick[3], M. Polis[4], M.F. Kearney[1], J.M. Coffin[5], and F. Maldarelli[1]

[1]HIV Dynamics and Replication Program, NCI, Frederick, MD, [2]Laboratory of Immunoregulation, NIAID, Bethesda MD, [3]Leidos Biomedical Research Inc. Frederick, MD. [4]Collaborative Clinical Research Branch, NIAID, Bethesda MD, [5]Department of Molecular Biology and Microbiology, Tufts University, Boston MA

HIV Dynamics and Replication Program
NCI-Frederick

CUA



HIV DNA and Viral Load Supression

All patients had successful suppression of HIV RNA to <50cps/mL plasma by a median of 139.5 days on cART.

Overall, total HIV DNA copies/million CD4+ cells decreased an average of 4.5-fold from pre-therapy to viral suppression (75%).

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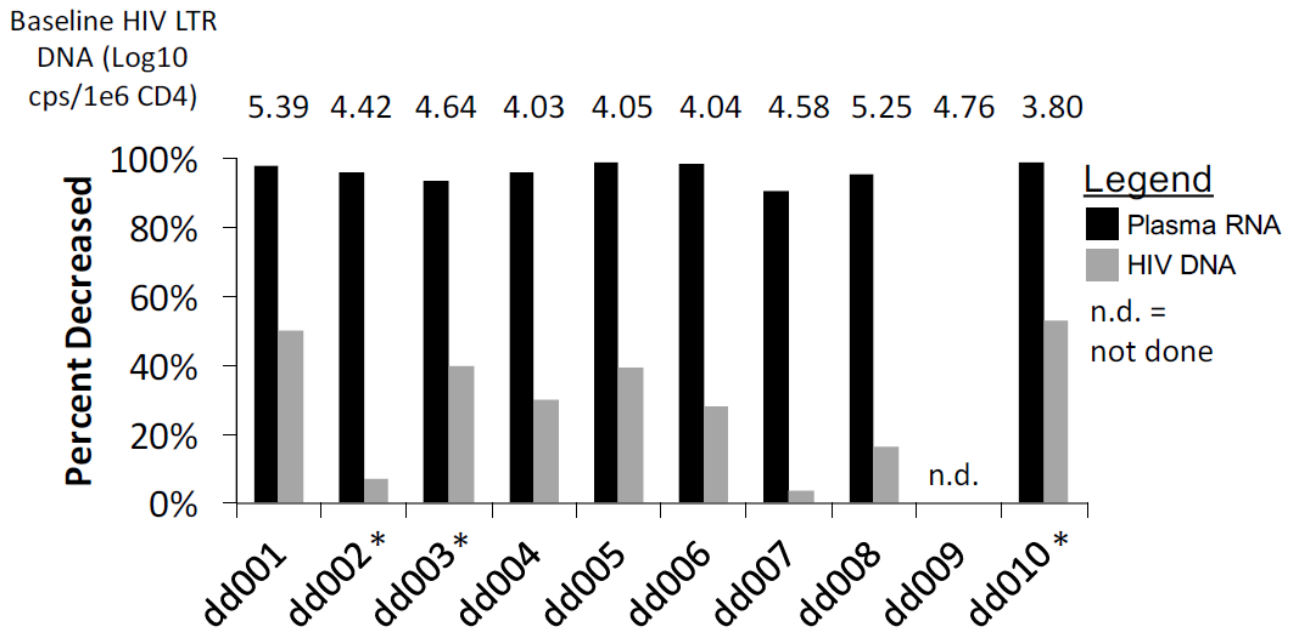
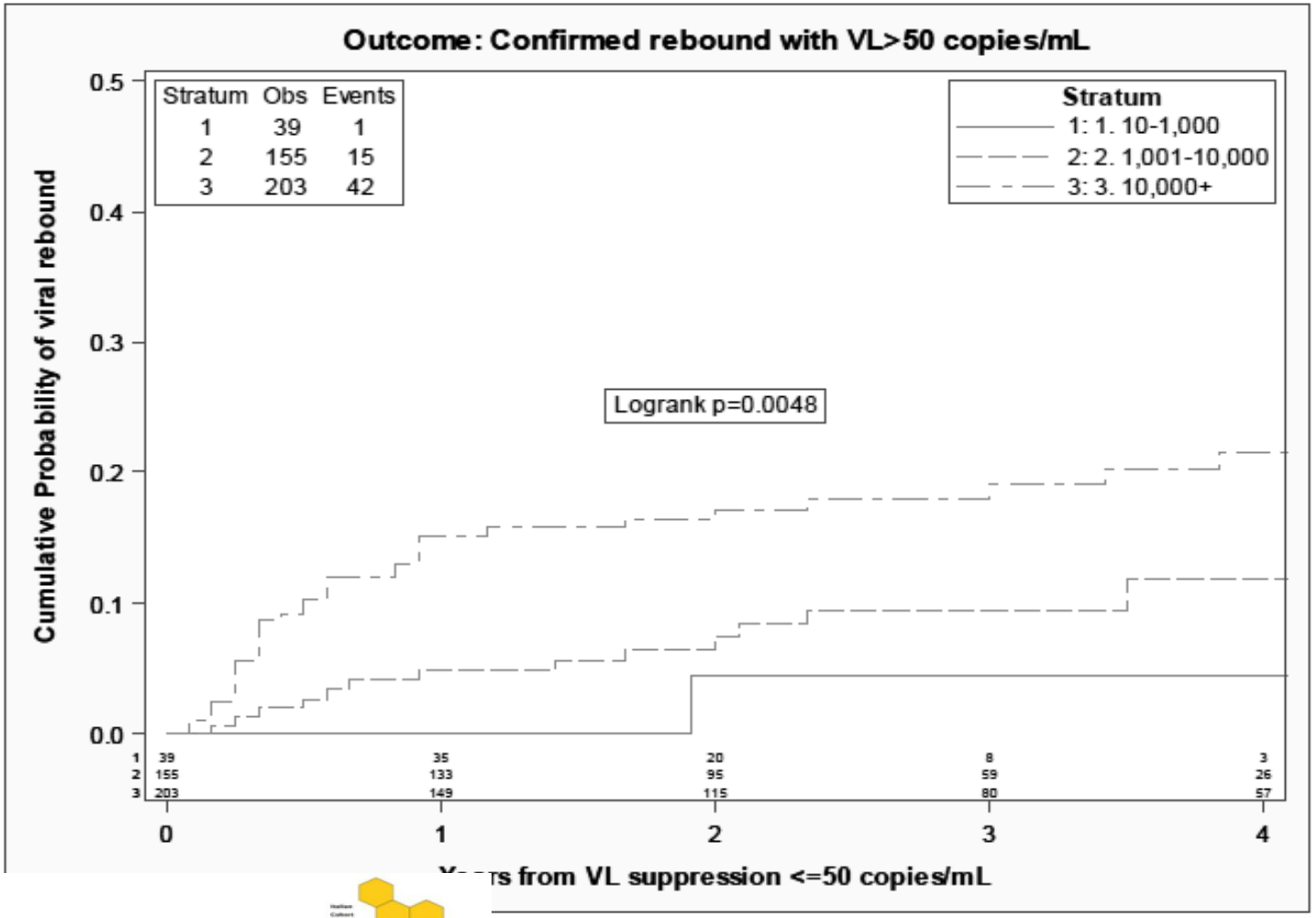


Small Fraction of HIV DNA+ PBMCs is Lost after 6 days

Strikingly, while HIV RNA in the blood declined on average 96% after 6 days on therapy, HIV DNA in the blood declined an average of only 30% (mean 491cps HIV DNA/ml; range 49-903cps/ml) indicating that the majority of viremia in the blood is produced by a small fraction of HIV infected PBMCs, suggesting that each infected cell produces a median of 104cps HIV RNA in plasma (range 21.2-8999cps/cell).

Higher levels of BL HIV DNA showed an increased probability of virological rebound after virological success



Ceccherini-Silberstein et al., CROI 2016, ICAR 2016

Various studies have shown that the level of baseline HIV-DNA can influence the maintenance of virological success under simplification therapy

J Med Virol. 2007 Jul;79(7):880-6.

Cellular HIV-1 DNA quantitation in patients during simplification therapy with protease inhibitor-sparing regimens.

Sarmati L[1], Parisi SG, Nicastri E, d'Ettorre G, Andreoni C, Dori L, Gatti F, Montano M, Buonomini AR, Boldrin C, Palù G, Vullo V, Andreoni M.

J Antimicrob Chemother. 2010 May;65(5):1005-7. doi: 10.1093/jac/dkq084. Epub 2010 Mar 18.

Impact of 48 week lopinavir/ritonavir monotherapy on blood cell-associated HIV-1-DNA in the MONARK trial.

Avettand-Fenoel V[1], Flandre P, Chaix ML, Ghosn J, Delaugerre C, Raffi F, Ngovan P, Cohen-Codar I, Delfraissy JF, Rouzioux C; MONARK Study Group.

HIV Clin Trials. 2013 May-Jun;14(3):120-6. doi: 10.1310/hct1403-120.

Long-term HIV-1 virologic control in patients on a dual NRTI regimen.

Prazuck T[1], Zucman D, Avettand-Fènoël V, Ducasse E, Bornarel D, Mille C, Rouzioux C, Hocqueloux L.

Role of Baseline HIV-1 DNA Level in Highly-Experienced Patients Receiving Raltegravir, Etravirine and Darunavir/Ritonavir Regimen (ANRS139 TRIO Trial)

PLOS ONE

2013

Charlotte Charpentier[1]*, Catherine Fagard[2,3], Céline Colin[2,3], Christine Katlama[4], Jean-Michel Molina[5], Christine Jacomet[6], Benoit Visseaux[1], Anne-Marie Taburet[7], Françoise Brun-Vézinet[1],

Virological Factors Associated With Outcome of Dual ETR/RAL Therapy (ANRS-163 Trial)

16TH EUROPEAN AIDS CONFERENCE October 25-27, 2017 Milan, Italy

PS6/4

Cathia Soulie, Lambert Assoumou, Sophie Sayon, Thuy Nguyen, Marc-Antoine Valantin, Virginie Ferre, Chakib Alloui, Brigitte Montes, Véronique Avettand-Fenoel, Constance Delaugerre, Diane Descamps, Esteban Martinez, Jacques Reynes, Gilles Peytavin, Dominique Costagliola, Christine Katlama, Vincent Calvez, Anne-Geneviève Marcelin.

FACTORS PREDICTING VIROLOGICAL FAILURE DURING DOLUTEGRAVIR MAINTENANCE MONOTHERAPY

Ingeborg Wijting, Sofie L. Rutsaert, Casper Rokx, David M. Burger, Elrozy Andrinopoulou[1], Linos Vandekerckhove, Bart Rijnders

CROI Conference on Retroviruses and Opportunistic Infections

Are we ready to test HIV DNA in clinical practice????

- Home-made technology available
- Commercial assays developed by small companies available
- Commercial assays under development by large companies as an adaptation of HIV RNA assays

Droplet Digital PCR helps improve accuracy of HIV-DNA quantification

38 Papers published from 2013 to 2016 used quantification of HIV-DNA : ddPCR ->24/38

REVIEW

Journal of Virus Eradication 2016; 2: 162–169

Diagnostic utility of droplet digital PCR for HIV reservoir quantification

Wim Trypsteen, Maja Kiselinova, Linos Vandekerckhove* and Ward De Spiegelaere

HIV Translational Research Unit, Department of Internal Medicine, Ghent University, Belgium

Digital PCR has recently been proposed as an alternative to real-time with potentially improved accuracy and precision.

In this method, each sample is divided into thousands of independent microscopic reactions prior to PCR amplification. Digital PCR provides an increased precision of four-fold to over 20-fold versus real-time, using identical quantities of clinical samples from peripheral blood.

Factors influencing long-term viral suppression

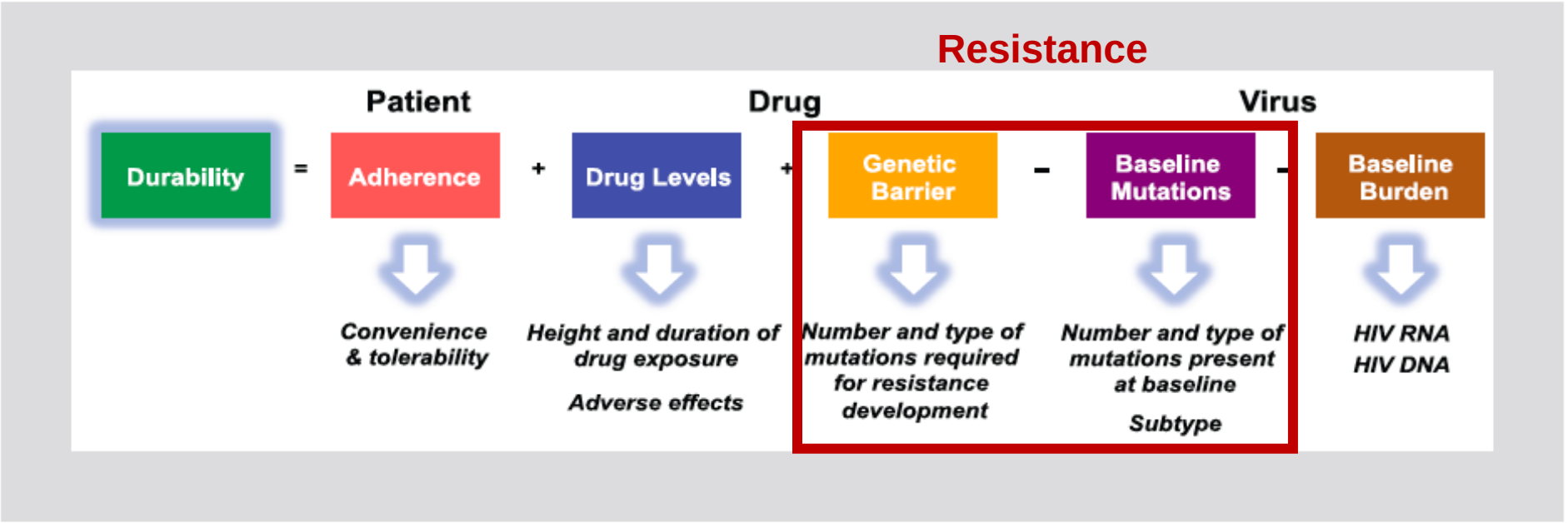
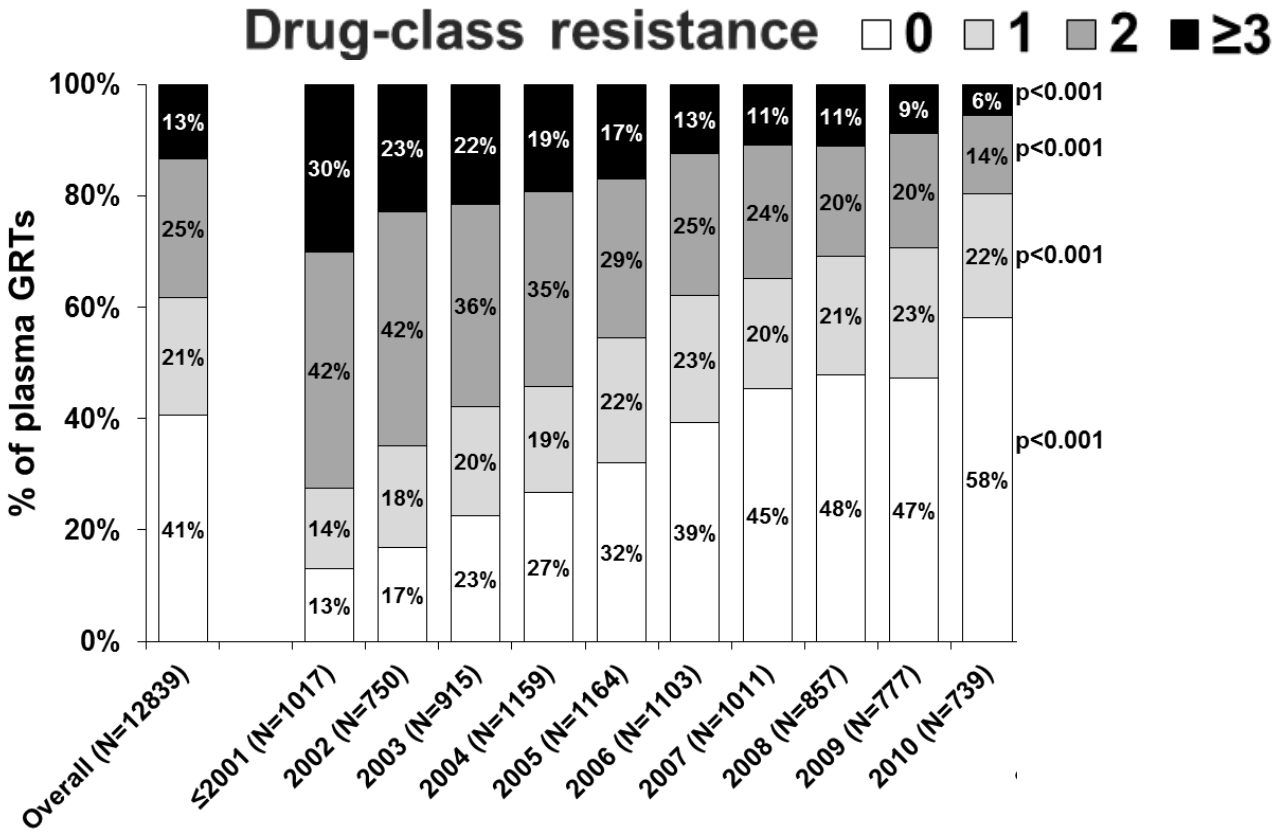


Figure 1. *Factors influencing long-term viral suppression, adapted from Massimo Andreoni personal communicaton.*

Svicher et al , Impact Study Group. AIDS Reviews 2017

Resistance at failure significantly decreased from 1999 to 2010 in conjunction with a remarkable increase of failures without resistance.

Prevalence of resistance to any drug-class among ART-experienced HIV-1 infected patients with virologic failure over the years.

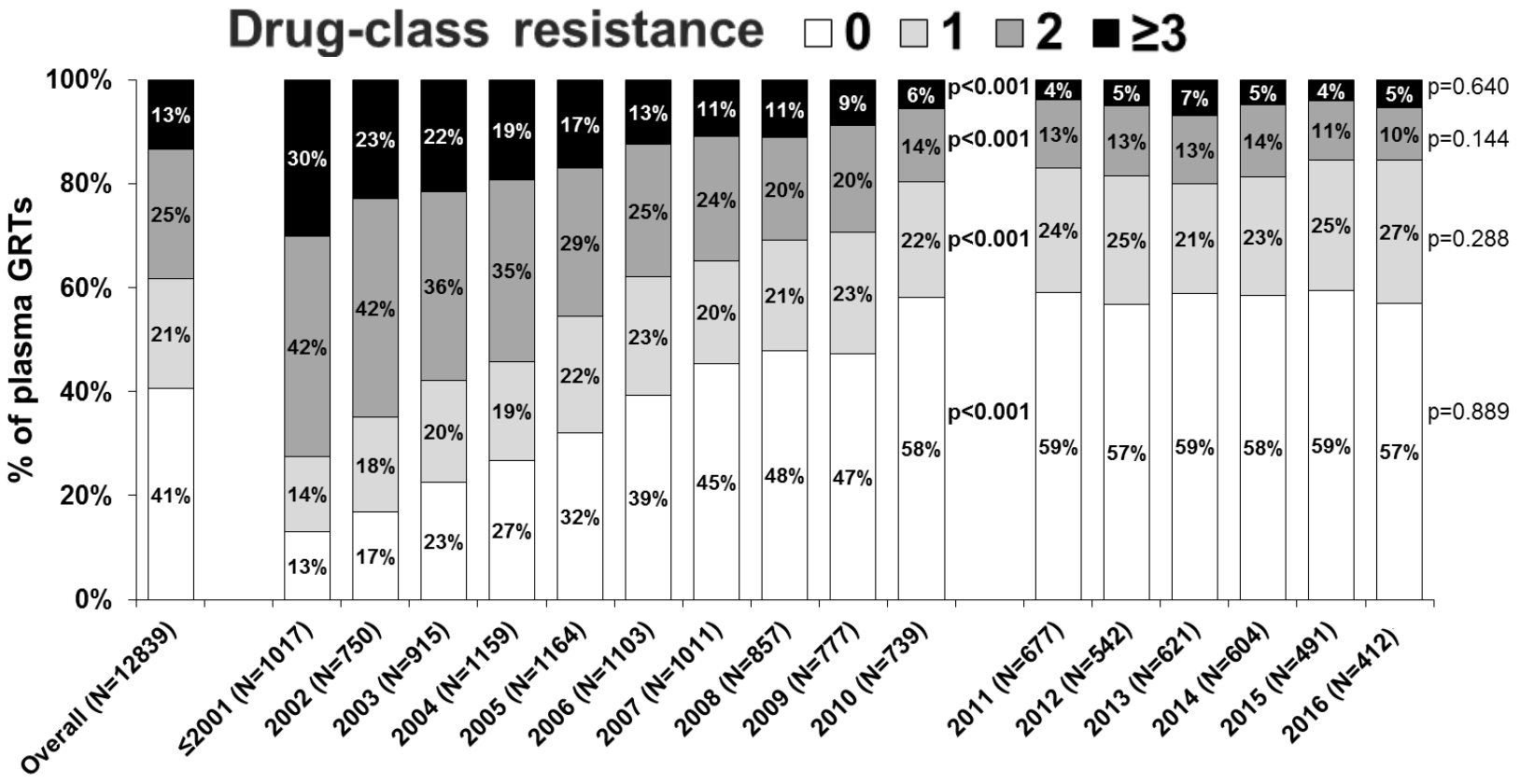


Analysis performed on 14497 sequences of protease/reverse transcriptase or integrase, from 12839 GRTs performed for routine clinical practice in ART-experienced HIV-1 infected patients (N=6147). P-values were calculated by Chi-squared test for trend; statistically significant tests ($p<0.05$) are indicated in boldface. Sequences performed from 1999 to 2001 were grouped.

Santoro MM et al., 16th EACS 2017

Beyond 2010, prevalence of resistance remained stable from 2011 to 2016.

Prevalence of resistance to any drug-class among ART-experienced HIV-1 infected patients with virologic failure over the years.



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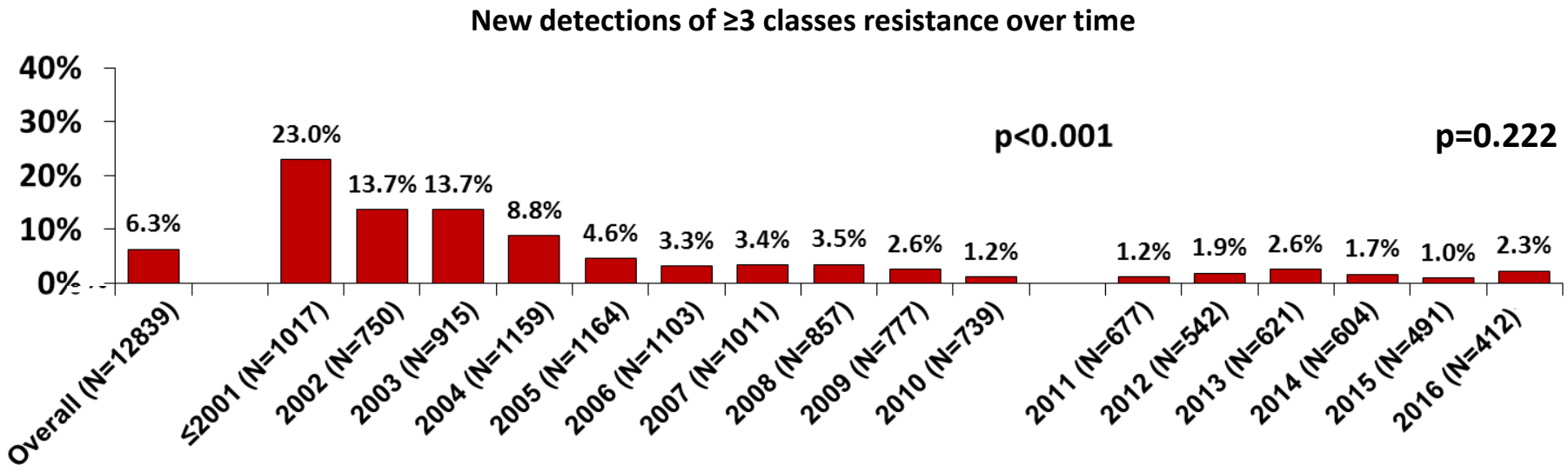
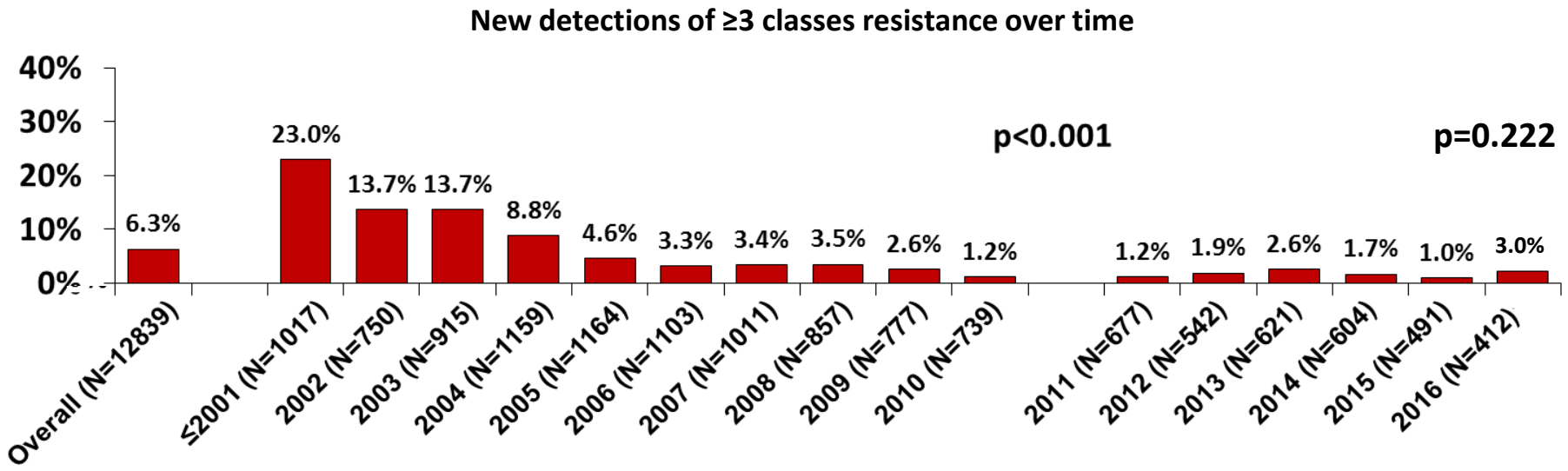
Santoro MM et al., 16th EACS 2017

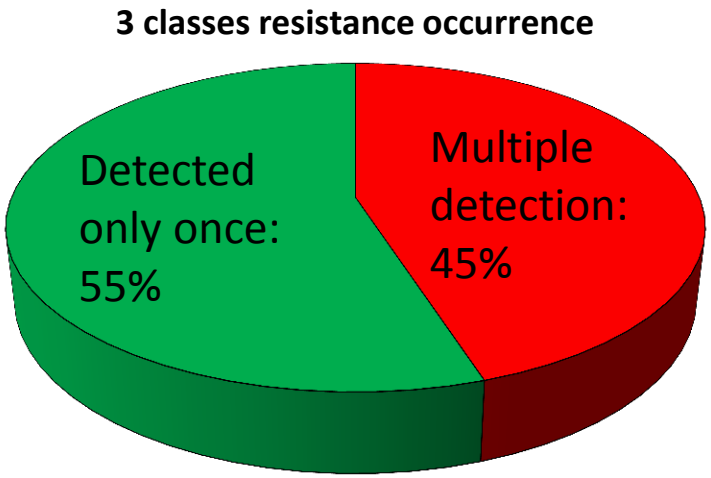
New detections of ≥3 class-resistance dramatically decreased from 23% in 1999 to 1 % in 2010 (p<0.001), and remained quite stable from 2011 up to 2016 (1% to 3%, p=0.222).



Santoro MM et al., 16th EACS 2017

Resistance to ≥3 classes was found more than once in 362/810 (45%) patients, in whom re-occurred from previous GRTs recorded around 3 years before.



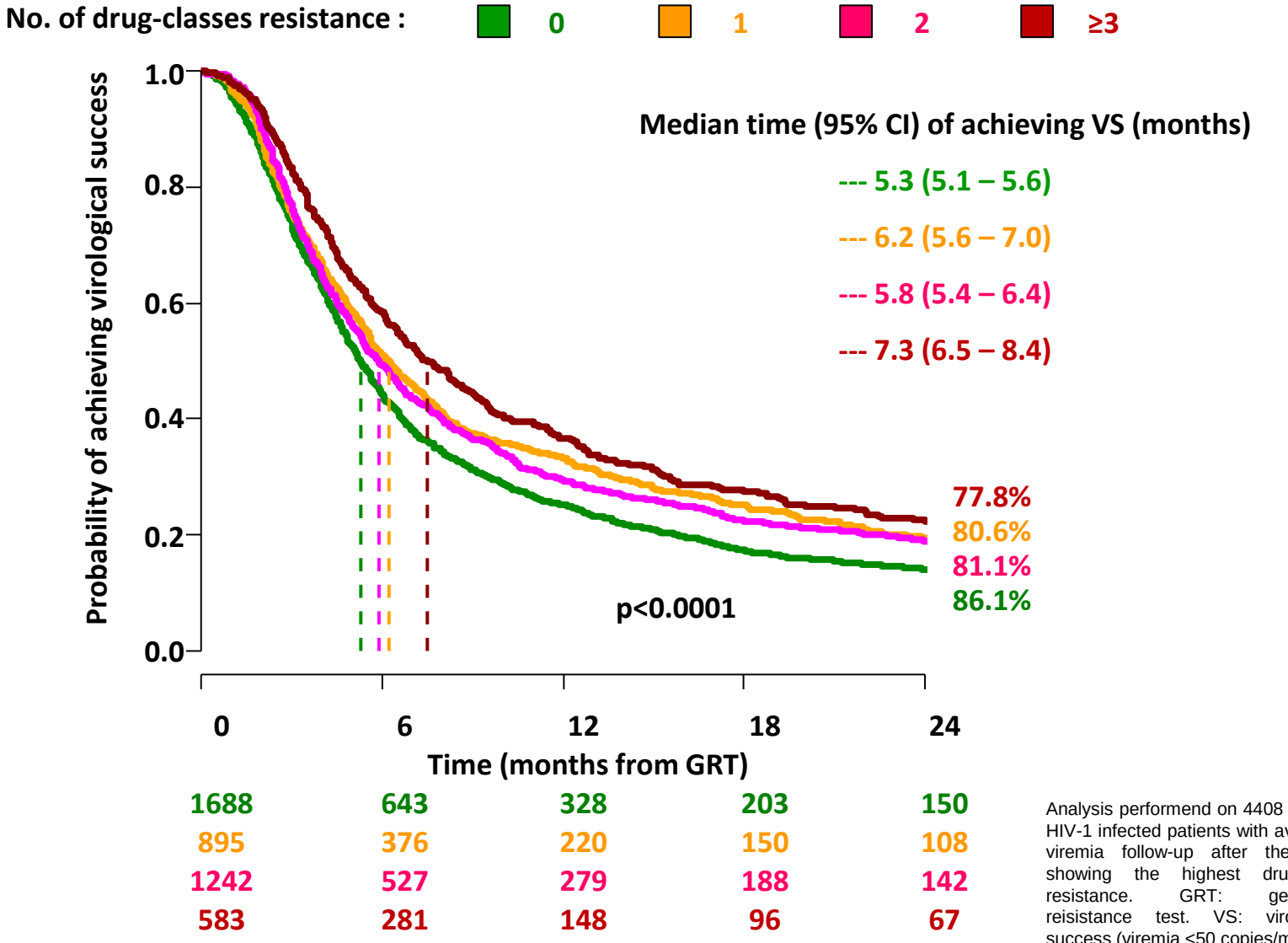


Median time of resistance re-occurrence 3.2 (1.2-5.9) years

Santoro MM et al., 16th EACS 2017

- **If properly treated, also patients infected with a multi-resistant virus can achieve virological suppression**

After the latest GRT with the highest resistance class levels, the probability of achieving virological success was significantly lower in patients having 3 drug-classes resistance compared to the others.



Analysis performend on 4408 treated HIV-1 infected patients with available viremia follow-up after the GRT showing the highest drug-class resistance. GRT: genotypic reisistance test. VS: virological success (viremia <50 copies/mL).

Armenia et al., IWHDR 2017

Long-life treatment in the management of HIV infection dictates the switch!!!

Clinicians should always review possible adverse events or tolerability issues with current antiretroviral regimens.

Just because the HIV viremia is suppressed it should not be assumed that the HIV-positive person is well adapted and tolerating the current regimen.

Therefore, therapy switch is no longer a sign of therapeutic failure. If something, it represents a major option to improve chances of long-term therapeutic success

Switch Strategies for Virologically Suppressed Persons

A complete ARV history with HIV-VL, tolerability issues and cumulative genotypic resistance history should be analysed prior to any drug switch.

Pre-existent NRTI and NNRTI resistance impacts on maintenance of virological suppression in HIV-1-infected patients who switch to a tenofovir/emtricitabine/rilpivirine single-tablet regimen

D. Armenia[1]†, D. Di Carlo[1]†, A. Calcagno[2], G. Vendemiati[2], F. Forbici[3], A. Bertoli[1], G. Berno[3], S. Carta[3], F. Continenza[3], V. Fedele[3], R. Bellagamba[4], S. Cicalini[4], A. Ammassari[4], R. Libertone[4], M. Zaccarelli[4], V. Ghisetti[2], M. Andreoni[5], F. Ceccherini-Silberstein[1], S. Bonora[2], G. Di Perri[2], A. Antinori[4], C. F. Perno[3] and M. M. Santoro[1]*

[1]Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy; [2]Division of Infectious Diseases, University of Turin, Turin, Italy; [3]Antiretroviral Drug Monitoring Laboratory, National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy; [4]Infectious Diseases Division, National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy; [5]Systems Medicine, University of Rome Tor Vergata, Rome, Italy

*Corresponding author. E-mail: santormaria@gmail.com
†D. Armenia and D. Di Carlo equally contributed to this work.

Received 3 August 2016; returned 29 August 2016; revised 28 October 2016; accepted 1 November 2016

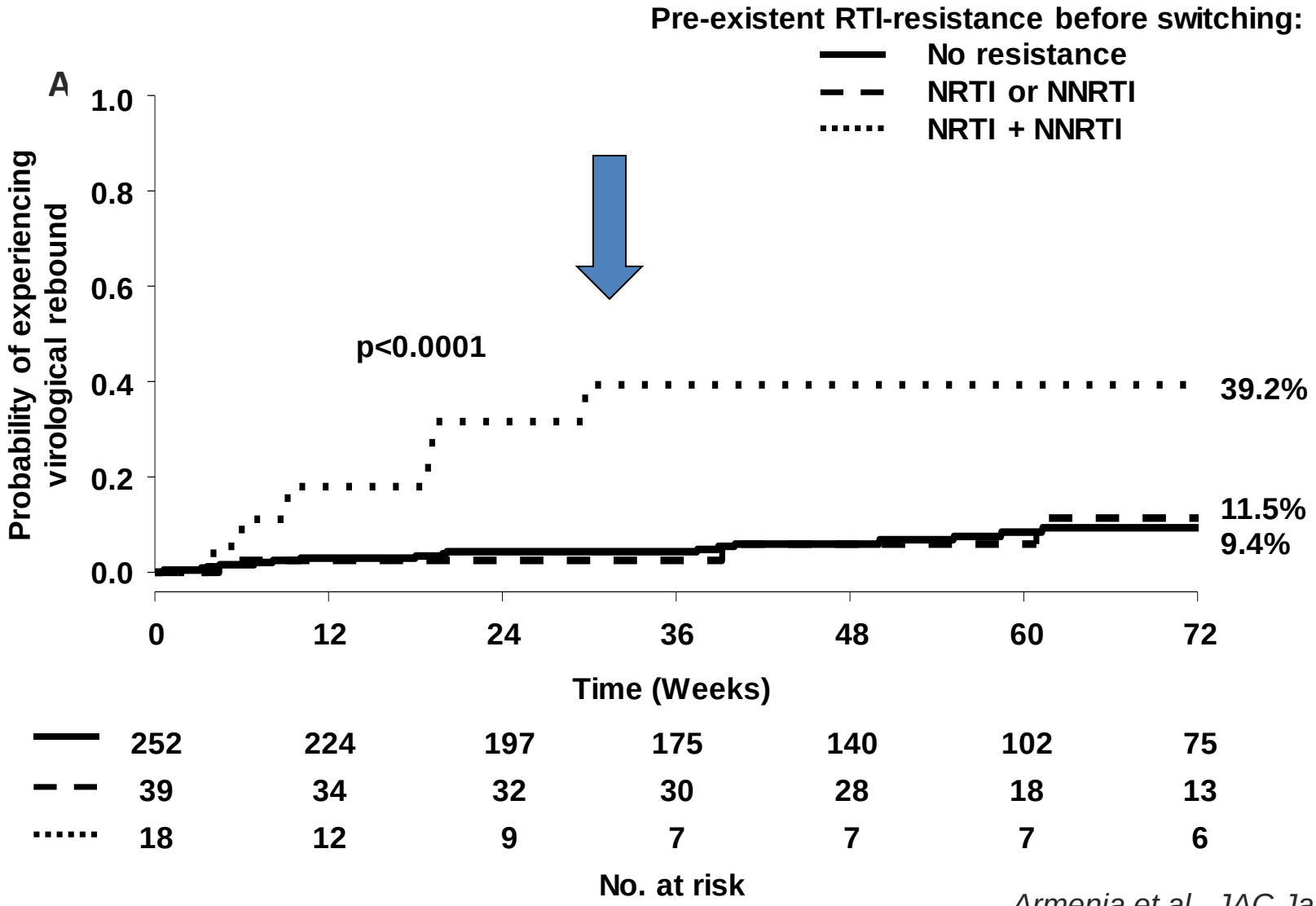
Objectives: To evaluate the maintenance of virological suppression (VS) in antiretroviral-treated HIV-1-suppressed patients switching to a tenofovir/emtricitabine/rilpivirine (TDF/FTC/RPV) single-tablet regimen, by considering pre-existent resistance (pRes).

Methods: pRes was evaluated according to resistance on all previous plasma genotypic resistance tests. Probability and predictors of virological rebound (VR) were evaluated.

Results: Three hundred and nine patients were analysed; 5.8% of them showed resistance to both NRTIs and NNRTIs, while 12.6% showed resistance to only one of these drug classes. By 72 weeks, the probability of VR was 11.3%. A higher probability of VR was found in the following groups: (i) patients with NRTI + NNRTI pRes compared with those harbouring NRTI or NNRTI pRes and with those without reverse transcriptase inhibitor pRes (39.2% versus 11.5% versus 9.4%, $P < 0.0001$); (ii) patients with a virus with full/intermediate resistance to both tenofovir/emtricitabine and rilpivirine compared with those having a virus with full/intermediate resistance to tenofovir/emtricitabine or rilpivirine and those having a virus fully susceptible to TDF/FTC/RPV (36.4% versus 17.8% versus 9.7%, $P < 0.001$); and (iii) patients with pre-therapy viraemia >500 000 copies/mL compared with those with lower viraemia levels (>500 000: 16.0%; 100 000–500 000: 9.3%; <100 000 copies/mL: 4.8%, $P = 0.009$). pRes and pre-therapy viraemia >500 000 copies/mL were independent predictors of VR by multivariable Cox regression.

Conclusions: TDF/FTC/RPV as a treatment simplification strategy shows a very high rate of VS maintenance. The presence of pRes to both NRTIs and NNRTIs and a pre-therapy viraemia >500 000 copies/mL are associated with an increased risk of VR, highlighting the need for an accurate selection of patients before simplification.

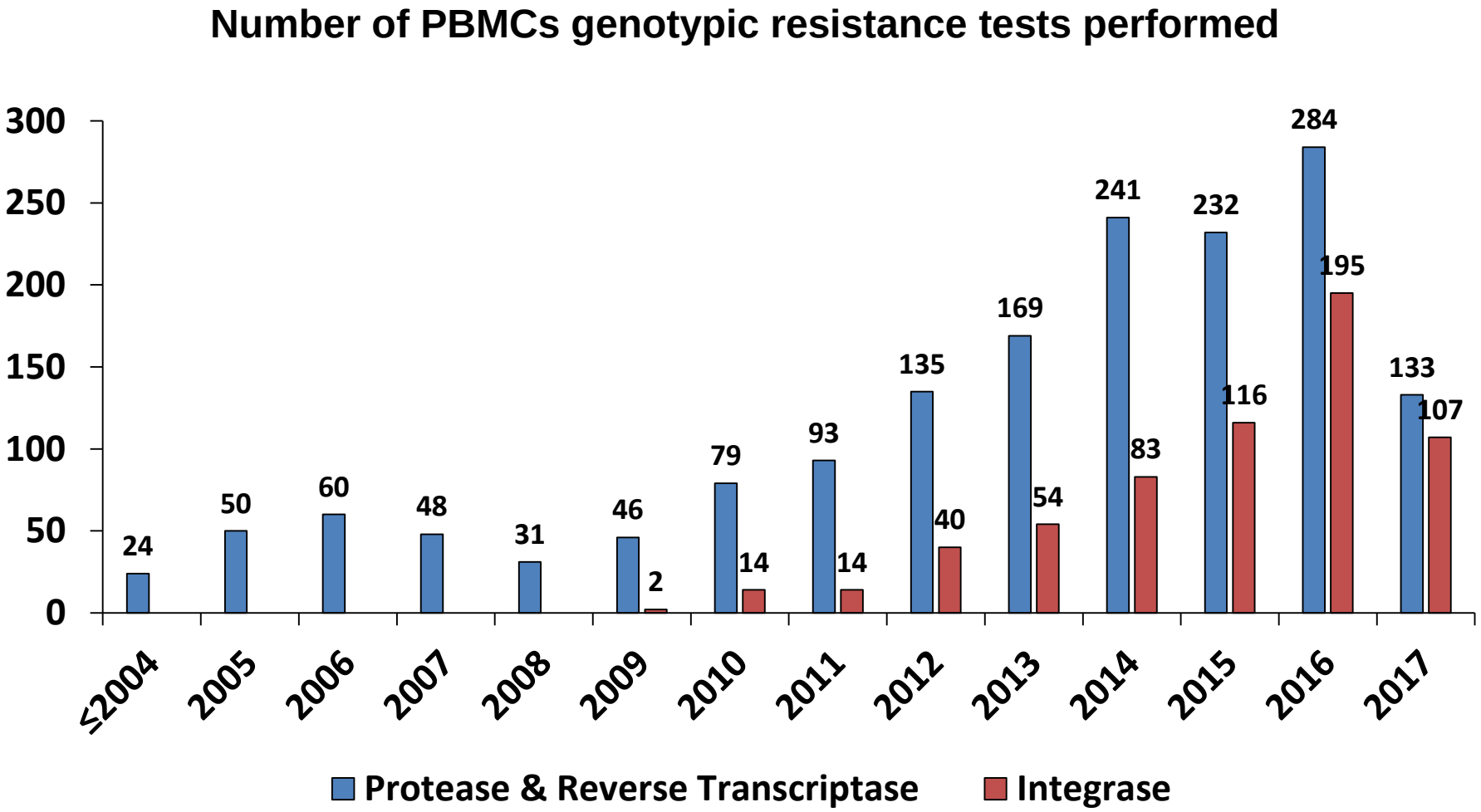
Patients with pre-existent NRTI+NNRTI resistance had a higher probability of experiencing VR compared to those harboring pre-existent NRTI or NNRTI resistance and to those without pre-existent RTI resistance.



Armenia et al., JAC Jan 2017

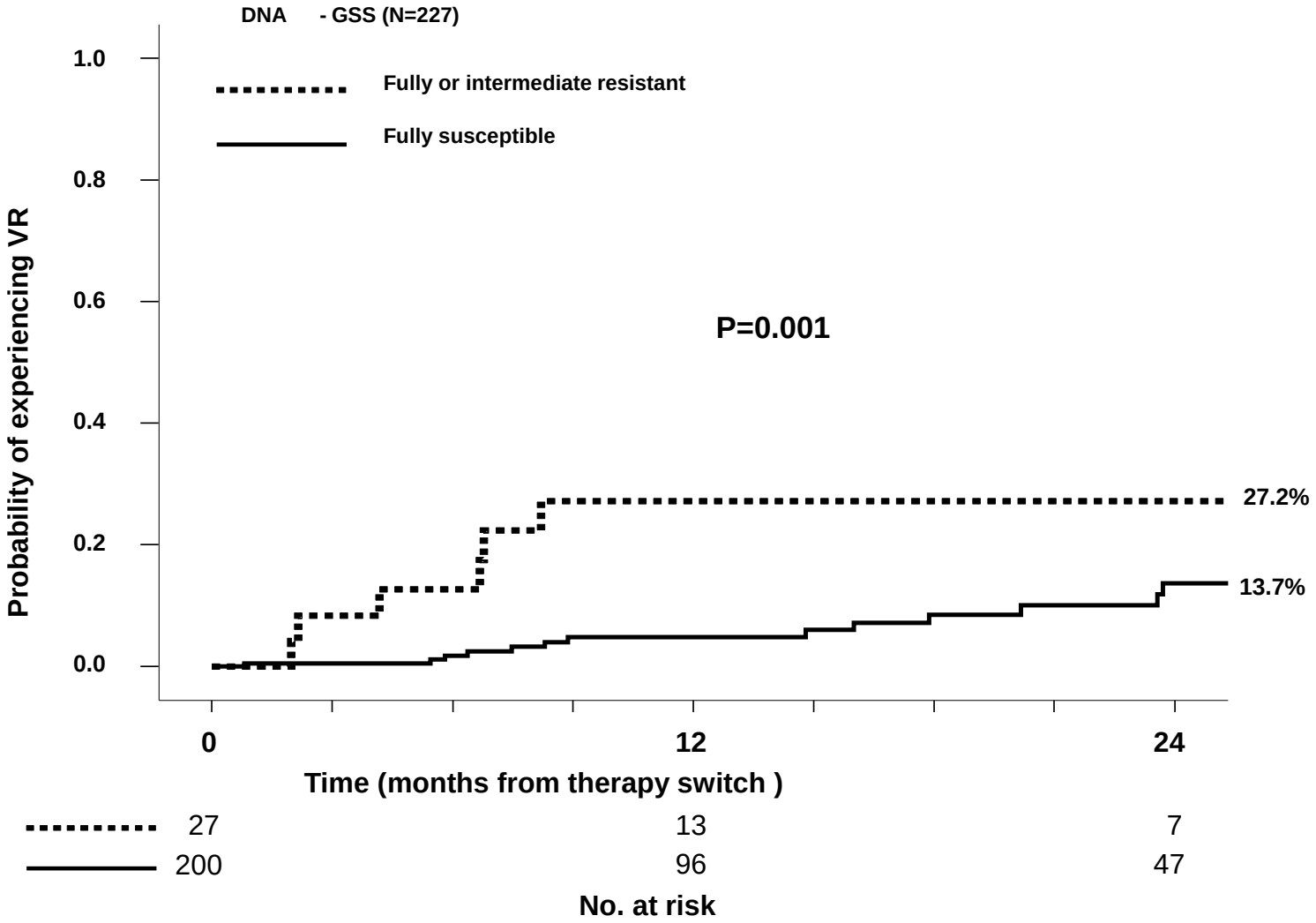
……As well as the genotypic test on HIV-DNA

Increased requests of HIV DNA GRT in clinical practice over the recent years



Armenia D unpublished data, data updated in June 2017

Virologically suppressed patients showing an intermediate or fully resistant virus at PMBC GRTs performed before a therapy switch had a higher probability of experiencing virological rebound compared to those carrying a fully susceptible virus



Armenia D et al. Journal of Clinical Virology 2018

HIV DNA Genotypic Resistance Test is a good tool for therapy optimization in both drug-naïve and drug-experienced patients

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Conclusions

- More high sensitive and specific molecular tests are fundamental for the long term monitoring of HIV infection.
- HIV infected patients often maintain detectable virological parameters not considered in the past, such as very low-level viremia and HIV-DNA.
- These parameters deserve particular attention in preventing chronic inflammation and in reducing viral reservoir.
- Resistance testing is still today an important tool to tailor a correct therapy.
- The improvement of resistance testing, finalized to detect resistance even at undetectable viremia (on DNA), allows clinicians to optimize therapy in the case of switch for treatment simplification.

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The Patients

Thank you for the attention!