New Microbiologica, **38**, 149-184, 2015

HEFFICON: HIV Effectiveness Italian Conference

Andrea Antinori[1], Massimo Andreoni[2], Carlo Federico Perno[3], Adriano Lazzarin[4], HEFFICON Study Group[#]

[1]Clinical Department, National Institute for Infectious Diseases "L Spallanzani", Rome, Italy;
[2]Infectious Disease, University of Rome Tor Vergata, Rome, Italy;
[3]Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy;
[4]Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

[#]EFFICON Study Group: Adriana Ammassari[5]; Paolo Bonfanti[6], Stefano Bonora[7], Marco Borderi[8], Maria Rosaria Capobianchi[9], Antonella Castagna[10], Massimo Clementi[11], Paola Cinque[12]; Antonella d'Arminio Monforte[13], Giovanni Di Perri[9], Massimo Galli[14], Andrea Gori[15], Paolo Maggi[16], Claudio Mastroianni[17], Cristina Mussini[18], Emanuale Nicastri[5] Massimo Puoti[19], Stefano Rusconi[20], Maria Santoro[21], Giuseppe Tambussi[10]

[5]Clinical Department, National Institute for Infectious Diseases "L. Spallanzani," Rome, Italy;
[6]Unit of Infectious Diseases, A. Manzoni Hospital, Lecco, Italy;
[7]Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy;
[8]Department of Medical and Surgical Sciences, Infectious Disease Unit, Alma Mater Studiorum, University of Bologna, Italy;
[9]Laboratory of Virology, "L. Spallanzani" National Institute for Infectious Diseases, Rome, Italy;
[10]Department of Infectious and Tropical Diseases, San Raffaele Hospital IRCSS, Milan, Italy;
[11]Laboratory of Microbiology, San Raffaele Hospital IRCSS, Milan, Italy;
[12]Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy;
[13]Clinic of Infectious and Tropical Diseases, S Paolo Hospital, University of Milan, Italy;
[14]Department of Biomedical and Clinical Science, University of Milan, Italy;
[15]Division of Infectious Diseases, Department of Internal Medicine, "San Gerardo" Hospital, University of Milan-Bicocca, Monza, Italy;
[16]Department of Infectious Diseases, University of Bari, Italy;
[17]Infectious Disease, La Sapienza University Rome, Italy;
[18]Infectious Diseases Clinics, Modena University General Hospital, Modena, Italy;
[19]Division of Infectious Diseases, Niguarda Ca' Granda Hospital, Milan, Italy;
[20]Division of Infectious Diseases, L. Sacco Hospital, University of Milan, Italy;
[21]Department of Experimental Medicine and Surgery, Tor Vergata University of Rome, Italy

SUMMARY

Since the first acquired immunodeficiency syndrome cases were reported in 1981, more than 1.5 million people have been diagnosed with Human Immunodeficiency Virus-1 in Europe, including more than 136,000 new HIV cases in 2013. Recent epidemiological data estimate an incidence of 5-10 newly diagnosed HIV infections per 100,000 population per year in Europe and an average prevalence of infection of 5.7 cases per 100,000 population. In the absence of an effective curative strategy for HIV, optimization of prevention policies and clinical management of HIV positive patients is fundamental to reduce the impact of the HIV pandemic on public health. Clinical trials represent an essential tool for translating research findings into routine clinical practice. Careful evaluation and planning of clinical trials are therefore mandatory in order to provide relevant information to clinicians. The HEFFICON Project was conceived to investigate and pinpoint methodological issues and critical points that need to be addressed in future clinical studies to increase the translation of experimental results to the real life environment.

KEY WORDS: AIDS, HIV, HAART, Integrase inhibitors.

Received January 9, 2015 *Accepted* March 24, 2015

Corresponding author
Adriano Lazzarin
San Raffaele Scientific Institute
Department of Infectious Diseases
Via Stamira D'Ancona, 20 - 20127 Milano, Italy
E-mail: adriano.lazzarin@hsr.it

INTRODUCTION

The introduction of combination antiretroviral therapy (cART) represented a medical breakthrough in the treatment of Human Immu-

nodeficiency Virus (HIV) infection, leading to effective suppression of viral replication and a dramatic increase in the life expectancy of HIV seropositive patients. Nevertheless, cART is not curative and patients need to take medications lifelong in order to control viral replication. From an epidemiological perspective, the increased longevity of HIV patients drove the formation of a more heterogeneous population of patients including individuals diagnosed at different stages of the disease and thus treated for different amounts of time. The heterogeneity of the HIV seropositive population and the wide range of clinical conditions deriving from HIV infection itself and HIV-associated co-morbidities pose great challenges in the clinical management of HIV patients. From the translational medicine perspective, these questions point to the need for clinical studies taking into account the specific characteristics of each group of patients.

Taking advantage of the EPICO methodology, the next chapters will outline the state of the art in HIV clinical practice and investigate the research questions that remain to be answered in clinical studies in specific HIV settings.

1 - EARLY TREATMENT OF PATIENTS

Coordinator A. Lazzarin

Introduction

From the etiopathogenetic point of view, HIV infection will become chronic in all cases. Today the therapeutic armamentarium of drug combinations available show 90% or better efficacy, therefore leaving no doubt over the principle that HIV infection must be treated promptly.

As a model of intervention, primary HIV infection (PHI) provides some fundamental information, which will be analysed in detail in the following chapters as a real life example of when and whether there is an indication for early treatment.

First, however rapid the diagnosis may be in the natural history of serum conversion (Feibig 1-4 status) (Fiebig *et al.,* 2003), this cannot prevent the spread of the retrovirus out of the anatomical site of the primary focus of infection (Ananworanich *et al.,* 2012; Whitney *et al.,* 2014). Only in some of the patients treated for a sufficiently long period (>5 years) does early diagnosis lead to the condition of "post-treatment élite controller" once antiretroviral treatment has been suspended (Saez-Cirion *et al.,* 2013).

Clinical trials and observational studies have not yet reached a final conclusion on the dilemma of recommending treatment of primary HIV infection (PHI) and for how long. This highlights the limitations of clinical trial evidence: insufficient numbers of treated subjects, non-homogenous treatments, and most of all, diversity in terms of levels of viremia, CD4 count, and symptoms.

When infection is not identified at onset (i.e., in almost all cases) or in those subjects where treatment cannot be deferred (AIDS and/or late - presenters =+/-40%), the decision when to start combination ART (cART) can be deduced only from the evaluation of patient cohorts followed during these years, to obtain consistent information on the risk of progression of the infection to full-blown disease based on the association with biological criteria such as CD4 and HIV-RNA (surrogate markers).

The associated progression risk factor usually considered, and still the most reliable, is the number of CD4/mm^3, which led to the standard threshold of 350 CD4/mm^3 for cART being recommended.

Since the rule of starting antiretroviral therapy <350 CD4/mm^3 was established, the threshold for early treatment naturally moved before to 500 CD4/mm^3 and today to >500 CD4/mm^3 without restriction aiming at treating patients with low immune-deficiency (Lodi *et al.,* 2011; Antinori *et al.,* 2012). In this setting, it is much more difficult to gather information leading to a clear understanding and/or assessment of risk evolution in terms of morbidity and most of all, mortality (currently >1%).

However, the 500 CD4/mm^3 threshold is already considered in those cases where co-factors of progression are present (co-morbidity or aging).

The response to this question, and to whether there is the indication for treatment for all HIV positive subjects, could come from the START study (early versus deferred treatment in subjects with >500 CD4/mm^3), whose results are expected in at least two years from now.

In the meantime, the choice to make in stable chronic asymptomatic infection showing a fairly good immune response is "personalized early treatment". This is true in those patients promising good compliance in long-life therapy and who are not threatened by long-term treatment side-effects, selecting the best cost/benefit drug combination and therapeutic strategy.

Patients with PHI

G. Tambussi, A. Gori

Evidence. The availability of potent cART for people infected with HIV led to the reduction of both progression to AIDS- and HIV-associated morbidity and mortality. According to current consensus, initiation of therapy is best based on CD4 count rather than on viral load (Sterling *et al.,* 2001).

National and international guidelines provide different recommendations for starting treatment. Over the past 10 years, the CD4 cell count threshold for ART initiation has risen steadily in parallel with the development of new therapeutic strategies, new knowledge acquisition and new presentations of HIV disease. However, these different treatment approaches can be confusing for clinicians, patients and policy-makers when they are called to define the best time to initiate therapy.

Optimizing the initiation of ART is clearly complex and must therefore be balanced between individual and broader public health needs. In particular, intense scientific discussion still surrounds the merits of initiating treatment during acute/primary HIV infection. Despite the lack of randomized clinical trials, arguments in favor of early treatment were largely fueled by the theoretical assumption that intervention at this stage of the disease may provide a unique opportunity to preserve the immune system deeply affecting the entire course of the disease.

Many concerns have been raised about ART initiation during PHI, the strongest including potential long-term toxicity, development of resistant virus strains and costs. However, recent advances in antiretroviral therapy provided well-tolerated highly effective drugs which guarantee an excellent compliance, strongly reducing the risk of resistance (Assoumou *et al.,* 2013). In addition, early treatment initiation improves survival, prevents viral reservoirs diffusion (Yerly *et al.,* 2000) and reduces the risk of HIV transmission (Cohen *et al.,* 2011b).

Others issues raised against early treatment of HIV infection are the increased ART exposure, for how long therapy should be prolonged or if, at this point, a suspension of therapy is indeed justified once this has been initiated.

Regarding the first issue, given the increased survival of people living with HIV, the decision to start early treatment occurs in a relatively narrow window of time in the life span of a person with HIV infection. A few extra years of therapy out of a total of 40 to 50 years on treatment for those living a near-normal life span represent, in our opinion, relatively minor differences in long-term exposure to treatment.

Population. The decision to treat patients with acute HIV infection is based on facts and hypothesis. However several shortcomings affect data on primary/early HIV infection treatment. Amongst others, it is difficult to identify persons at early stages of HIV infection because of limitations in diagnostic techniques and the paucity of symptoms occurring during this particular phase of the HIV infection (Branson and Stekler 2012).

Despite the aid of useful algorithms (Fiebig *et al.,* 2003), it is not always easy to differentiate PHI from an early infection. In this latter case, therapeutic intervention is likely to be initiated well after peak viremia, and extensive damage may already have been done.

Intervention. In the past year, 3 prospective, randomized, controlled studies (Fidler *et al.,* 2002; Grijsen *et al.,* 2012; Hogan *et al.,* 2012) have evaluated the potential benefits of antiretroviral treatment initiation during acute HIV-1 infection.

These studies provide evidence that greater CD4 cell recovery is achieved with earlier initiation of therapy during PHI, but the question of whether the relatively small clinical advantages of therapy outweigh the disadvantages of treatment remains unanswered.

On the other hand, these articles support the current Department of Health and Human Services and International Antiviral Society-USA guidelines for resource-rich settings, which suggest ART for nearly everyone who is HIV-infected, regardless of the stage of infection. Obviously, this recommendation is definitely

stronger to justify the decision to undertake the treatment of a primary infection.
More recently, data from the "Visconti" (Virological and Immunological Studies in Controllers after Treatment Interruption) trial, showed that some patients treated early during PHI have no signs of viral load rebounds following termination of their treatment regimens (Saez-Cirion *et al.,* 2013). This evidence may ultimately provide guidance to scientists pursuing a "functional" HIV cure, as long as treatment starts early enough.
Outcome. It is thus conceivable to think that treatment of PHI might provide additional benefits (i.e. less damage to the immune system, in particular to the gut mucosal immunity, and reducing the extent of viral reservoirs (Chun *et al.,* 2007). Finally, patients who receive diagnosis and adequate ART for enough time shortly after infection, may also have a better chance of responding to "functional cure" strategies, as suggested by the Visconti cohort.
While awaiting a "definitive" clinical study, it is ethical to consider early treatment, which can be defined as "plausible" based on our understanding of HIV biology, HIV pathogenesis, the availability of better drugs, the evidence from cohort studies, and the public health implications of viral load suppression and decreased HIV transmission.

Patients with high CD4 counts: early treatment as prevention

A. Castagna, E. Nicastri

Evidence. A recent survey of 1675 HIV clinicians from New York and Washington USA conducted from September 2010 and May 2011 suggests that only half of them prescribe HAART to HIV patients with CD4 cell strata ranging from 350 to 500 CD4/mm^3 and that in only 15% of cases is HAART started irrespective of CD4 cell value (Kurth *et al.,* 2012).
The evidence-based data on antiretroviral therapy initiation based on CD4 cell count come from Cohorts and Randomized Controlled Trials (RCT) with viro-immunological and clinical end points.
Data from patients with 350-500 CD4 cell/mm^3 are consistent in both Cohort and RCT trials with clinical and surrogate endpoints. Conversely, in patients with >500 CD4/mm^3 there are no data at all from RCT, while in the NA-Accord there is statistically significant evidence of a better clinical performance, but very few cases are collected and appear to drive this conclusion. Finally, in case of HIV serodiscordant couples (98% of them in monogamous heterosexual relationships) the HPTN 052 trial showed strong evidence of benefit of early HAART initiation irrespective of CD4 cell count: 96% reduction in HIV transmission (P <0.001) (Cohen *et al.,* 2011b). Recently, confirming data on the clinical benefit of early treatment in the 350-500 CD4/mm^3 stratum were reported in the HPTN 052 trial (Grijsen *et al.,* 2012).
Although anticipated (Attia *et al.,* 2009), this finding has catalyzed enormous interest in how ART could not only benefit the single individual, but also reduce the epidemic burden in the community
Population. Recently, a supplement of the PLOS Medicine Journal was published on the topic of treatment as prevention. Mathematical models provide a framework to assemble data on the impact that different treatment programs can have on preventing HIV infections, and several models of the epicenter of the worldwide epidemic, sub-Saharan Africa, have been used to investigate the potential impact of treatment on HIV incidence. In this collection, Eaton and coworkers *et al.* (Eaton *et al.,* 2012) presented the results of a systematic model comparison exercise in which 12 of these models were used to simulate the same sets of interventions. The model results were relatively consistent for short-term (eight-year) projections of reduction in incidence associated with treatment (i.e. 80% of individuals with<350 CD4/mm^3); the models projected that the incidence rate would be reduced by 35%-54%, compared with what it would be in the absence of any ART.
Intervention. In spite of inconsistencies between modeling assumptions and projects and real world situations, treatment as a prevention strategy has already generated major reductions in incidence in a few selected categories. The Option B+ approach of lifelong ART for all HIV-infected pregnant women, regardless of CD4 count, is an example of this policy.(Schouten *et al.,* 2011; World Health Organization 2012b). The advantages of OptionB+ include a simplification of PMTCT program;

extended protection from MTCT in future pregnancies directly from conception; a prevention benefit against sexual transmission in serodiscordant couples; likely benefit to the woman's health of earlier treatment and avoiding the risks of stopping and starting antiretrovirals; full integration between HIV center of treatment and care and antenatal care centers; and a simple message to communities: once ART is started, it is taken for life. Still, there are important programmatic, operational and clinical challenges, including service organization and delivery of antiretrovirals, cost and sustainability. But major limits in the preliminary reports from different African countries (Francesco Vairo, personal communication) on Option B+ are retention in care and the maintenance of optimal adherence to HAART.

Outcome. It is reasonable to discuss the option of an earlier therapy in patients with chronic HIV infection with good immunology status to preserve immune system deterioration and to avoid AIDS- or non-AIDS-defining events. However, at this time no unequivocal definitive clinical study claims that early treatment should be considered the standard of care. The WHO model of Option B+ in HIV-infected pregnant women could provide the long-term clinical and viro-immunological data from a clinical perspective to confirm the choice of an initial antiretroviral treatment irrespective of CD4 cell count.

2 - ADVANCED PATIENTS WITH INFECTIOUS COMORBIDITIES

Coordinator C.F. Perno

Introduction

It is estimated that across Europe about one-third of HIV-infected persons are unaware of their HIV status. One of the main consequences of this scarce knowledge of HIV status is a significant level of late diagnosis and a robust rate of virus transmission (obviously longer and stronger, whenever the duration of the infection lasts over the years in conditions of good health and consequent normal life habits, such as sexual activity) (Fisher 2008; Adler *et al.,* 2009).

HIV late-presenters can be defined as those presenting with "advanced HIV disease", that is a CD4 cell count <200 cells/mm^3 or with and an AIDS defining condition (Thompson *et al.*, 2012; World Health Organization 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b). Late presentation is dangerous in terms of individual and social health, and transverse efforts in planning common strategies at European level have been extensively carried forward: recently, European guidelines have been developed for implementing HIV testing in adults in public healthcare settings (Panel on Guidance on Indicator Condition-Guided HIV testing in Adults 2012).

Data existing so far are not sufficient to define whether there should be specific treatments of HIV infection in this stage of the disease compared to earlier stages (i.e. more aggressive therapies? Use of drugs more prone to increment CD4? Others?). Interventions are needed to clarify the main factors influencing late presentation and re-presentation in care, and which strategies to adopt to.

a) lower the impact of late presentation;
b) improve diagnosis assessment;
c) ameliorate immune-virological and clinical outcomes of late/AIDS presenters.

The complexity of the disease in patients with late diagnosis requires a rapid control of HIV replication, a quick and effective improvement of immune functions, and, whenever present, control opportunistic infections, cancer, and all other clinical conditions associated with advanced HIV disease.

For all these reasons, a wide panel of treatment options should be made available for these patients, rather than just few standardized treatments equal for everyone. All late-presenting patients should therefore undergo an individual treatment plan encompassing both antiretroviral therapy and, where necessary, treatment for opportunistic infectious, cancer and related pathologies. The choice of initial HIV treatment should reflect patient characteristics (including viroimmunological elements), the results of resistance testing and the complexity of concomitant disease(s).

With regards to the resistance testing, HIV resistance to antivirals, transmitted to naïve patients, is a phenomenon with important clinical implications, that may compromise initial antiretroviral therapy if not properly considered (Little *et al.,* 2002; Pozniak *et al.,* 2006; Borro-

to-Esoda *et al.,* 2007). Indeed, studies clearly demonstrated that the virological response to a regimen selected on the basis of results of standard genotypic testing appears to be nearly as effective as the initial treatment of a patient without transmitted drug resistance, and in any case more effective than a regimen selected without resistance testing (Palella *et al.,* 2009; Wittkop *et al.,* 2011). For the above mentioned reasons, drug resistance testing before therapy starts, both in patients with acute infection and in those chronically infected, is considered the standard of care for the management of HIV-infected individuals initiating ART (Hirsch *et al.,* 2008; Vandamme *et al.,* 2011; Tang and Shafer 2012; World Health Organization 2012c; Santoro *et al.,* 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b).

In the management of a first line regimen, particular attention should be given to naïve HIV patients starting an antiretroviral regimen with a high baseline viral load. For a long time, the level of HIV-RNA >100,000 copies/ml has been identified as the unique threshold of high baseline viral load to be considered for the evaluation of the efficacy of different drug regimens. However, this definition of "high" viral load does not distinguish among groups that may differ biologically and functionally. Indeed, modern assays are able to quantify a wide range of viral loads above such level (100,000 to 10,000,000 copies/ml). Recent studies show that patients starting their first line regimen with very high pre-HAART viremia (>500,000 copies/ml) have less probability of achieving and maintaining virological success (Armenia *et al.,* 2013; Santoro *et al.,* 2013), thus reinforcing the importance of resetting the threshold of high viremia, and of defining the different tiers of this parameter that may have clinical relevance (i.e. <100,000, 100,000-500,000, >500,000, etc.).

Taken together, these findings reinforce the indication in guidelines to consider high viremia levels an important parameter in setting both appropriate therapeutic strategies and the frequency of viral load monitoring. Patients with very high pre-HAART viremia represent a significant population that may deserve special attention. Ad hoc studies are needed to set the most appropriate strategies aimed at achieving the same high rate of therapeutic success in patients with high baseline viral load as that usually obtained in patients starting HAART at lower HIV-RNA levels.

The same concepts may apply to immunological parameters. As an example, the threshold of CD4 number may need to be further stratified, since having <10 CD4/ mm^3 may not be equal, in terms of clinical outcome and therapeutic response, to having 190 CD4/ mm^3 (both situations today generally defined as "advanced disease"). Limited data are available so far in this crucial area, particularly referring to the situation driven by recent and modern therapeutic approaches (that may have completely changed the results obtained in the past with old and less effective therapeutic regimens). Further studies are needed to define this important point.

Particular attention should also be given to patients with advanced HIV infection and neurocognitive impairment (NCI). The determination of accurate incidence of neurocognitive impairment and its progression during HIV infection remains a challenge (Mind Exchange Working 2013) because to date there is no single well-established and validated diagnostic biomarker or neuropsychological test to be used.

The central nervous system (CNS) is a major reservoir of HIV-1 (Schrager and D'Souza 1998), and therefore represents an important target for antiretroviral therapy. Different classes of antiretroviral drugs appear to penetrate the CNS to differing degrees (McGee *et al.,* 2006; Tozzi *et al.,* 2009; Winston and Garvey 2009). Data from large randomized trials are needed to confirm the impact of antiretroviral regimens with greater CNS penetration on the improvement in neurocognitive performance, while issues of potential long-term neurotoxicity require careful attention. Paradoxically, the ability of drugs to penetrate within the CNS is still challenged as a fundamental element for the success of antiviral therapy at the CNS level. Indeed, recent accumulating evidence supports either the key role of drug penetration, or the crucial importance of controlling HIV at systemic level.

The role of earlier introduction of antiretroviral therapy and CNS-penetrating regimen for the prevention of HIV-associated neurocognitive impairment in high-risk patients still requires investigation.

Finally, among the HIV-associated infectious comorbidities, both HBV and HCV infection require special attention since their natural evolution may well impact both life duration and quality. In the last 10 years, dramatic advances have been made in the treatment of HBV (Centers for Disease Control and Prevention) and HCV (World Health Organization 2012a) infection and, particularly in the case of HCV infection, unprecedented cure rates are now achievable with new drugs and regimens, while newer and even better options are being tested with efficacy expectations approaching 95% (Gane *et al.,* 2013). Thus, new drugs and newer insights into human genetics are the main factors driving a revolutionary era in the management of chronic hepatitis (Aguilar Marucco *et al.*, 2007; Ge *et al.,* 2009; Tavel *et al.,* 2010; Lange *et al.,* 2011; European Association For The Study Of The Liver 2012; Wilby *et al.,* 2012; Bertelsen; Kieran *et al.,* 2013; Zolopa *et al.,* 2013). On the side of HIV-HCV co-infected patients, efforts are needed to improve the rates of inhibition of both viruses parallel the increased attention to the pathogenetic events determining liver fibrosis, and the molecules able to limit and control this phenomenon.

Since this chapter is dedicated to advanced patients with infectious co-morbidities, we should not forget the analysis of the classical infectious pathologies associated with HIV-driven immunodeficiency. Toxoplasmosis, cytomegalovirus infection, cryptococcosis, atypical mycobacteriosis, and other opportunistic infections are still present and diagnosed in such patients, though their prevalence has dramatically dropped. Again, despite the late presentation (even today) of many HIV-infected patients, the rate of those having <50 CD4/ mm^3 (a condition favoring such opportunistic infections) is limited. At the same time, *pneumocystis jiroveci carinii* pneumonia remains a problem in our patients at the time of late diagnosis. Finally, tuberculosis, particularly in immigrants, represents a growing infection at the time of late HIV-diagnosis. Taken all together, infections are still today aggravated by a significant rate of mortality in the first weeks after diagnosis. Dropping this mortality represents a main goal of the holistic approach to HIV infection, also including the improvement of earlier diagnosis

Taken together, many challenges still remain while new ones appear in the extensive area of HIV patients with advanced infection and with infectious co-morbidities. These issues will be of even greater relevance if we consider the extended survival of HIV-infected patients and the difficulty (so far) in decreasing the rate of patients whose diagnosis of HIV infection is made at a very late stage. For these reasons, much attention must be given to new strategies aimed at approaching the problem from a different perspective, with the aim of focusing on the epidemiological aspects of late infection and co-morbidities, and the potential solutions. HEFFICON has been designed with the purpose of providing answers in this framework.

Late presenter or AIDS presenter patients

A. d'Arminio Monforte, C. Mussini

Evidence: The definition of late presentation was made by an International Consensus Conference on the basis of the need to start antiretroviral treatment or not. As a consequence, according to guidelines, a patient is considered a late presenter if the CD4 count at diagnosis is <350 cells/mm^3 (Antinori *et al.,* 2011). More recently, national and international guidelines (including WHO) bring forward the start of treatment to a CD4 value <500 cells/mm^3 (Thompson *et al.,* 2012; World Health Organization 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b). The definition of late presentation should then be modified accordingly, possibly including only symptomatic patients or subjects with a CD4 count at presentation <200 cells/ mm^3. Moreover, the cascade of care has shown that there is another population of patients presenting at a late stage of infection, i.e., those who are not engaged or not-retained in care after HIV infection diagnosis (Ndiaye *et al.,* 2009; Mocroft *et al.,* 2013b; Mugavero *et al.,* 2013). Several studies show how the cascade of care differs according to the health system (Figure 1 and Figure 2): even if in countries like Italy the rate of retention in care is higher than in the USA, the numbers are still high (Gardner *et al.,* 2011; Lazzaretti *et al.,* 2012).

One of the most important issues in HIV late presentation is how to identify subjects with HIV infection better and earlier and how to increase the rate of testing in the population at

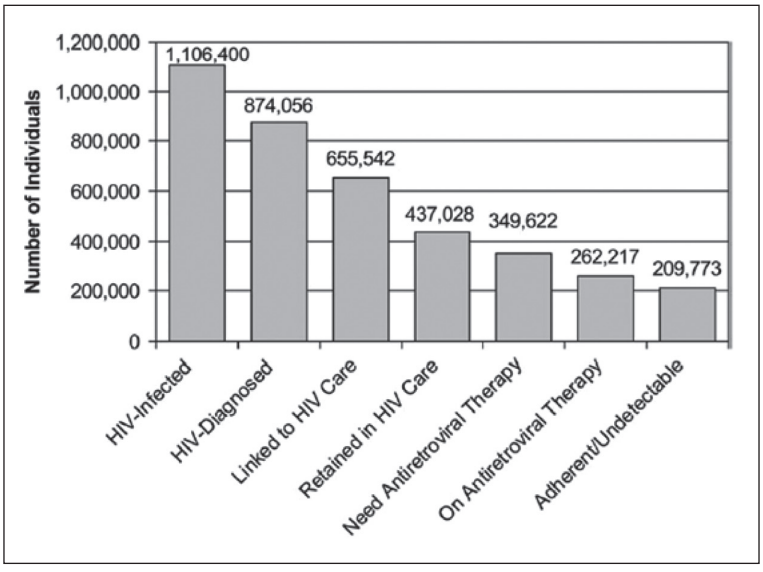

FIGURE 1 - *The cascade of care in the USA (Gardner et al. 2011).*

risk. As HIV infection spreads all over different strata of the population, there is no single behavioral hallmark of infection and virtually all age and socio-economic strata could be affected. However, higher risk subjects remain those with promiscuous sexual partnerships, independently of gender.

Late presentation is dangerous in terms of individual and social health, and transverse efforts in planning common strategies at European level have been extensively carried forward. European guidelines have recently been developed to implement HIV testing in adults in public healthcare settings (Panel on Guidance on Indicator Condition-Guided HIV testing in Adults 2012).

Basically, HIV testing is highly recommended to avoid late presentation of the disease, that may have at least three different clinical consequences:

1) Late presentation may have significant adverse implications in clinical management

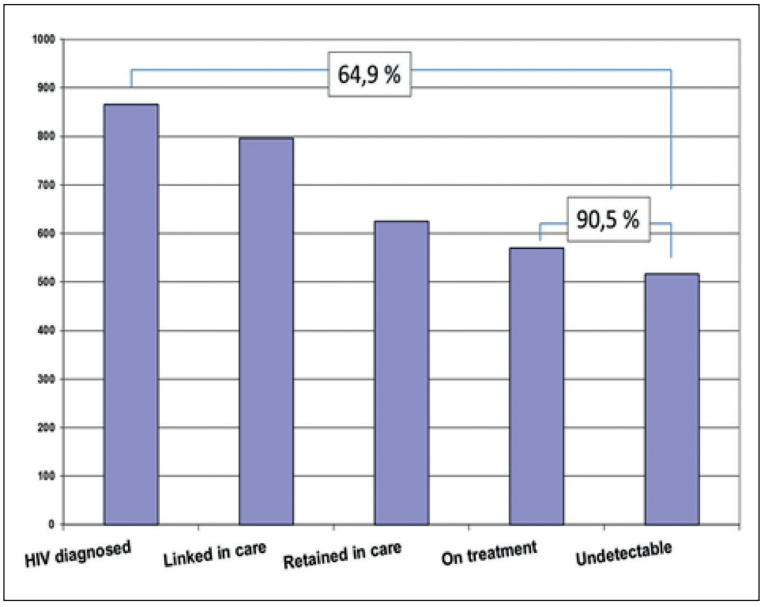

FIGURE 2 - *The Cascade of care in the Modena cohort (Lazzaretti C. et al. HIV11, Glasgow 2012, abstract P105, ref 9).*

in case of cancers (commonly present at late stages of HIV disease).
2) Until the advanced disease occurs, there is more time and chances to transmit the virus through high-risk sexual behaviors.
3) The presence of diseases known to be associated with immunosuppression that make the prognosis less favorable.

In this regard, guidelines identify a list of diseases associated with an HIV prevalence of >0.1%, and other conditions considered likely to have a HIV prevalence of >0.1%, that recommend the testing of HIV (Table 1). Therefore, in all cases in which HIV infection can be suspected (like those reported above), HIV testing is strongly recommended (Panel on Guidance on Indicator Condition-Guided HIV testing in Adults 2012).

As a consequence, there is an urgent need for education of physicians from clinical areas other than infectious diseases. They should be educated to consider HIV testing a must whenever clinical symptoms falling in their area of expertise, known to be potentially associated with HIV infection (see above), may occur. This will increase the rate and appropriateness of HIV testing.

Moreover, there still is a clear need to educate the general population on HIV symptoms and epidemiology. In a series of patients with newly diagnosed HIV infection in the UK, 76% out of 263 patients had been in contact with their general practitioner in the year prior to diagnosis, but only in 17% of them was the issue of possible HIV infection raised (Wohlgemut *et al.*, 2012). Similarly, in a ANRS survey, HIV testing was performed in a low percentage (<10-15%) of subjects with diseases considered HIV-associated, such as generalized lymphadenopathy or recurrent bacterial pneumonia (Champenois *et al.*, 2012). Existing data are not sufficient to define medical needs and the best treatment options in this population, and current guidelines do not specifically address this issue.

Population. On the basis of what was discussed above, the target population for the implementation of HIV testing also includes all patients with advanced HIV disease (i.e. those with a full-blown AIDS or with a CD4 count <200 cells/mm^3) not yet diagnosed, and, more in general, all those individuals with a high potential of transmission (see above), still unknown to be HIV positive, that may spread the infection to their sexual partners.

Intervention. Interventions are needed to:
1) clarify the main factors influencing late presentation and re-presentation in care, and which strategies to adopt to lower the impact of late presentation; ii) improve diagnosis assessment and immune-virological and clinical outcomes in this patient population.

Some possible interventions that could be useful to improve the management of late presenters:

TABLE 1 - Conditions associated with an undiagnosed HIV prevalence of >0.1 (A). 2b. Other conditions considered likely to have an undiagnosed HIV prevalence of >0.1% (adapted from HIV Indicator Conditions, (Panel on Guidance on Indicator Condition-Guided HIV testing in Adults 2012)).

Strongly recommended testing	• Sexually transmitted infections • Malignant lymphoma • Anal cancer/dysplasia • Cervical dysplasia • Herpes zoster • Hepatitis B or C (acute or chronic) • Mononucleosis-like illness • Unexplained Leukocytopenia/ thrombocytopenia lasting >4 weeks • Seborrheic dermatitis/exanthema • Invasive pneumococcal disease • Unexplained fever • Candidemia • Visceral leishmaniasis • Pregnancy (implications for the unborn child)	*Offer testing*	• Primary lung cancer • Lymphocitic meningitis • Oral hairy leukoplakia • Severe or atypical psoriasis • Guillan-Barré syndrome • Mononeuritis • Subcortical dementia • Multiple sclerosis-like disease • Peripheral neuropathy • Unexplained weight loss • Unexplained lymphoadenopathy • Unexplained oral candidiasis • Unexplained chronic diarrhea • Unexplained chronic renal impairment • Hepatitis A • Community-acquired pneumonia • Candidiasis

1) Local data collection (maybe through a questionnaire) of the causes occurring in case of late presentation of HIV, or non-engagement in care.
2) Periodical surveillance in all centers to identify patients lost to follow-up and telephone calls to try to re-engage patients in care.
3) A drug utilization study to better understand the approach to treat this population and related outcomes.
4) A systematic review to identify medical needs and the best treatment regimens according to short-term immune-virological outcomes.

Moreover, it must be taken into consideration that late/AIDS presenters should promptly start a potent, highly effective and rapid treatment, in order to reduce all consequences related to the immune-virological and clinical conditions typical of this stage of infection. In this population cluster, the major aim of treatment is to preserve organs and systemic functionality from further deterioration. This could be achieved with a therapeutic approach beyond traditional and consolidated regimens, such as administering more than 2 ARV drug classes or using/combining last generation ARV drugs.

Outcome. Expected outcome is to identify a number of HIV positive individuals at an earlier stage of infection, who thus could benefit from ART treatment and could reduce their infectious potential.

Moreover, other expected outcomes of the proposed interventions could be:

a) development of targeted local campaigns, on the basis of the obstacle to testing that will likely emerge from the questionnaire;
b) local strategies to increase retention in care;
c) identification of the best possible regimens to be tested in randomized clinical trials.

Resistance transmitted to naïve patients

C.F. Perno, M.S. Santoro

Evidence. A substantial number of studies have been performed across the world to determine HIV drug resistance transmitted to naïve patients (transmitted drug resistance, TDR) (Vercauteren *et al.*, 2009; Wheeler *et al.,* 2010; Agwu *et al.,* 2012; Castor *et al.,* 2012; Frentz *et al.,* 2012; World Health Organization 2012d). This is a phenomenon with important clinical implications that may compromise initial antiretroviral therapy if not properly considered (Little *et al.,* 2002; Pozniak *et al.,* 2006; Borroto-Esoda *et al.,* 2007; Kuritzkes *et al.,* 2008). Therefore, in the whole developed world, drug resistance testing before therapy starts both in patients with acute infection and in those chronically infected is considered the standard of care for the management of HIV-infected individuals initiating ART (Hirsch *et al.,* 2008; Vandamme *et al.,* 2011; Tang and Shafer 2012; World Health Organization 2012c).

Levels of TDR are consistent throughout the world with some differences driven by several factors including the rate of therapeutic successes in each country, and the time to start antiviral therapies (that in turn may reduce the number of patients able to transmit resistant virus). In addition, some variations over time of the rate of resistance occur, again reflecting the above-mentioned factors as well as changes in the prescription of antiretrovirals (ARVs) (Vercauteren *et al.,* 2009; Wheeler *et al.,* 2010; Agwu *et al.,* 2012; Castor *et al.,* 2012; Frentz *et al.,* 2012; World Health Organization 2012d; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b).

Data suggest that up to 10-17% of ART-naïve HIV-infected individuals living in Australia, Japan, North America and Europe are infected with a virus resistant to at least one ARV class (World Health Organization 2012d). These levels of TDR occurred early after ART was introduced in many high-income countries in the late 1990s, but have since then plateaued and, in some cases, decreased. The proportion of people achieving virological suppression increased over time, thus reducing the emergence of acquired drug resistance and its subsequent transmission.

Although lower proportions of TDR were found in Latin America (6.3%), Africa (4.7%), and Asia (4.2%) (Castor *et al.,* 2012), studies have reported prevalence rates as high as 10% in localized areas (Hamers *et al.,* 2011; Price *et al.,* 2011; Thorat *et al.,* 2011; Tshabalala *et al.,* 2011). Moreover, transmitted NNRTI gradually increased in recent years in resource-limited settings (Frentz *et al.,* 2012; Gupta *et al.,* 2012;

World Health Organization 2012d). This can be explained by the ARVs becoming more available during recent years in these continents. In this regard, HIV-1-infected individuals in countries in which ART had been available for at least 5 years were 1.7 times more likely to have TDR than those living in a country where ART had been available for less than 5 years (P<0.001) (Stadeli and Richman 2013). There is no clear evidence of increasing TDR levels to ARV classes different than NNRTI.

A variation of TDR prevalence among different HIV risk groups is found. For example, homosexual individuals are more likely than heterosexual individuals to be infected with drug-resistant HIV. One potential explanation, though not fully exhaustive, is that individuals who acquired HIV through heterosexual contact are (still) more likely to come from regions with limited access to ARVs (Vercauteren *et al.,* 2009; Frentz *et al.,* 2012).

Current American and European HIV guidelines recommend that drug resistance testing should be performed when a patient is first diagnosed with HIV-1 (Hirsch *et al.,* 2008; Vandamme *et al.,* 2011; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b). In newly diagnosed patients, a delay in testing would increase the risk that a TDR mutation would decrease in its proportion relative to the fitter wild-type revertants, and therefore no longer be detected by standard genotypic resistance testing, which cannot consistently detect variants present at levels below 20% of the plasma virus population. In patients who defer treatment, repeat genotypic resistance testing should be considered if the patient is at high risk of having been superinfected. Moreover, in patients with TDR, the test before the ART starts may supply information on the viral evolution and kinetics of disappearance of mutations.

Standard genotypic drug resistance testing in ART-naïve individuals involves testing for mutations in the protease and reverse transcriptase genes. Although transmission of integrase strand transfer inhibitor (INSTI)-resistant virus has rarely been reported, as use of INSTIs increases the potential for transmission of INSTI-resistant viruses may also increase (Cossarini *et al.,* 2011; Hurt 2011). Therefore, for naïve HIV-1 individuals whose infection with HIV carrying INSTI resistance is suspected (because the source of infection is known, as an example), genotypic resistance testing also for this drug class should be considered (Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b).

Population. The evaluation of TDR is definitely important for all HIV-1-infected subjects (both in acute or chronic infection) at their entry into care regardless of whether ART will be initiated immediately or deferred. This analysis needs to be carried out in order to characterize the virus before the therapy starts, and therefore to limit the risk of virologic failure (Little *et al.,* 2002; Pozniak *et al.,* 2006; Borroto-Esoda *et al.,* 2007). Indeed, studies clearly demonstrated that the virological response to a regimen selected on the basis of results of standard genotypic testing appears to be almost as effective as the initial treatment of a patient without TDR, and in any case more effective than a regimen selected without resistance testing (Palella *et al.,* 2009; Wittkop *et al.,* 2011; Santoro *et al.,* 2013).

Intervention. In regions where initial genotypic resistance testing is not readily available, appropriate campaigns of primary drug resistance surveillance should be performed in order to identify populations at high risk for TDR, for whom ART recommendations may need to be modified or for whom baseline resistance testing should be considered (Jordan *et al.,* 2012; Tang and Shafer 2012).

Outcome. TDR is associated with suboptimal virologic response to initial ART (Little *et al.,* 2002; Pozniak *et al.,* 2006; Borroto-Esoda *et al.,* 2007; Kuritzkes *et al.*, 2008). Therefore, data on HIV TDR (both at the population level, and at the single patient level) provide the basis for selecting correct first line regimens. TDR is also predicted to have a potentially significant impact on future HIV mortality (Bertagnolio *et al.,* 2013; Cambiano *et al.*, 2013). For the above-mentioned reasons, it is critical to remain vigilant over transmission of drug-resistant HIV, even at our time when many therapeutic options are available, and the issue of resistance seems to be no longer as relevant as it was in the past.

Naïve patients with high viral loads

S. Bonora, P. Cinque

Evidence. The level of HIV-RNA >100,000 copies/ml has been identified as the threshold of high baseline viral load (VL) and is currently considered a key point to evaluate the performance of different regimens in naïve patients. In most recent trials, virological efficacy in patients starting treatment with VL above 100,000 copies/ml was lower than in those starting below 100,000 copies/ml, with a significantly lower achievement of virological success for some drugs (e.g. lower virological response to Rilpivirine as compared to Efavirenz in ECHO and THRIVE, and of Darunavir/Ritonavir as compared to dolutegravir in FLAMINGO) (Cohen *et al.,* 2011a; Molina *et al.,* 2011; Clotet *et al.,* 2014). However, the choice of this threshold was based on methodological criteria (i.e., upper level of HIV-RNA quantification of previous assays and/or median of study populations) rather than biological or functional parameters. In the clinical setting, the question is whether 100,000 copies/ml is the real VL threshold to be considered in difficult-to-treat patients. Some trials, such as the STAR study comparing rilpivirine and efavirenz both in a single tablet regimen with tenofovir/Emtricitabine (TDF/FTC) showed that the risk of virological failure previously associated with viremia above 100,000 copies/ml is restricted to patients with very high baseline viremia, specifically above 500,000 copies/ml (Cohen *et al.,* 2013). Therefore, the current definition does not distinguish between groups that may differ biologically and functionally, while modern assays are able to quantify a wide range above such level (100,000 to 10,000,000 copies/ml). As a consequence, patients with viremia levels >100,000 copies/ml are included but poorly characterized in clinical trials. Yet, predictors of virological efficacy in this population have not been specifically studied.

Moreover, recent data suggest that achievement of VL <50 copies/ml is proportionally slower in patients starting with viremia >100,000 copies/ml (especially at viremia >500,000 copies/ml) (Santoro *et al.,* 2013), and this could also impact on the definition of virological success as the achievement of VL <50 copies/ml both at week 24 in the clinical setting or at week 48 according to time to loss of virological response (TLOVR) or snapshot analysis in trial condition. In fact, the slower achievement of VL <50 copies/ml target could impact on TLOVR, while Snapshot analysis could be affected by more frequent viral blips observed in patients with high viral loads.

In conclusion, there is a substantial lack of clinical trials specifically designed for patients with high viral load and classical endpoints of efficacy, e.g., viral load at 48 weeks might not be adequate in patients with high viral loads.

Population. Naïve HIV positive patients starting HAART with baseline viral load above 100,000 copies/ml. Patients will need to be differently grouped accordingly to CD4 cell count, demographics, comorbidities and other variables.

Intervention. A review of current literature through meta-analysis studies or design of observational or cohort studies, and, depending on feasibility, randomized clinical trials, aiming to:

- define with more accuracy "patients with high viral load", taking into consideration viremia strata above 100,000 copies/ml;
- define criteria and endpoints of virological success to use in the clinical setting according to the level of baseline viral load;
- identify predictors of virological response among subjects with viral load >100,000 copies/ml (adherence, CD4 count, gender, PK, comorbidities, etc.) not yet captured by clinical trials;
- identify new biomarkers complementary to viral load assessment and predictors of treatment responses;
- assess the impact of virological response on clinical outcomes (clinical events, mortality, immune reconstitution);
- assess the frequency of selection of resistance at failure according to viral load strata >100,000 copies/ml;
- evaluate therapeutic approaches specifically targeting difficult-to-treat patients with high viral loads such as:

1) adapted dosing or schedule of selected drugs;
2) alternative triple class regimens (e.g. combining more than 2 classes of drugs and integrase inhibitors (INI));

3) induction and maintenance strategies (e.g. initial 4-drugs regimen);
4) alternative methods of administration of potent drugs with a high genetic barrier (e.g. dolutegravir) to improve compliance and better maintain appropriate plasma drug levels (e.g. single tablet regimen with novel drug combination, long-acting injectable combinations).

Outcome. The final outcome is setting the most appropriate strategies to equal in patients with high baseline viral loads, the rate of therapeutic success usually obtained in patients starting HAART at lower HIV-RNA levels.

Patients with neurocognitive impairment
C. Mastroianni
Evidence. The global prevalence of HIV-associated neurocognitive impairment (NCI) has not changed from the pre– to the potent ART era, remaining at approximately 50% (Heaton *et al.,* 2010). In this respect, there has been a decline in HIV-associated dementia (HAD) (from 18% to <5%), whereas the prevalence of mild or asymptomatic NCI was stable or even increased to about 20-30% (Ellis *et al.,* 2007; Tozzi *et al.,* 2007). Fluctuations of NCI may occur and about 30% of patients change their neurocognitive status (improving, declining, or fluctuating) over time (Antinori *et al.,* 2007).
An accurate determination of the incidence of NCI and its progression during HIV infection remains a challenge (Mind Exchange Working 2013) because to date there is no single well-established diagnostic biomarker or neuropsychological test to be used. In particular, there is no standard validated easy-to-perform test to screen for mild NCI in all HIV-infected patients. There are insufficient data to establish the best time for follow-up assessments and the effect of treatment and preventive measures on HIV neurocognitive impairment. Finally, there are no systematic studies addressing in what extent neurocognitive impairment may be reversible or remains permanent.
Population. The identification and differential diagnosis of HIV-associated NCI in clinical practice should take into consideration all HIV-infected patients with the following evidence-supported risk factors:

a) disease factors (low nadir CD4 count, high-level of HIV replication, past CNS infections);
b) treatment factors (poor adherence, incorrect ART regimen, potential ART neurotoxicity);
c) AIDS and non-AIDS comorbidities;
d) demographic factors (aging, low socio-economic status);
e) other neurological and psychiatric disorders.

Intervention. Short and validated monitoring tools for NCI are needed for follow-up assessment of HIV-associated NCI. Cerebrospinal fluid (CSF) analysis should be performed in patients with neurological symptoms or signs but it remains to be addressed whether this is indicated in patients with asymptomatic NCI. Studies that better clarify the role of some virological tests (e.g. HIV-DNA), biomarkers of immune activation and neurological injury as well as neuroimaging or neurophysiologic assessment should be strongly encouraged. Data from large randomized trials are needed to confirm the impact of ART regimens with greater CNS penetration on the improvement in neurocognitive performance, while issues of potential long-term neurotoxicity require careful attention. Finally, the role of earlier introduction of cART and CNS-penetrating regimen for the prevention of HIV-associated NCI in high risk patients should be investigated.
Outcome. Final outcomes include the prompt identification of patients with HIV-associated NCI, correct follow-up assessment and appropriate therapeutic and preventive strategies.

Patients with HBV/HCV coinfection
G. Di Perri, M. Puoti
Evidence: Among the HIV-associated infectious comorbidities both HBV and HCV infection require special attention since their natural evolution may well impact on both life duration and quality. In the last 10 years, dramatic advances have been made in the treatment of HBV (Centers for Disease Control and Prevention) and HCV (World Health Organization 2012a) infection and, particularly in the case of HCV infection, unprecedented cure rates are now achievable with new drugs and regimens, while newer options are being tested with efficacy expectations approaching 95% (Gane *et*

al., 2013). Thus new drugs and newer insights into human genetics are the main factors driving a revolutionary era in the management of chronic hepatitis.

The treatment of HBV infection with nucleoside/nucleotide analog inhibitors of reverse transcriptase (N/NtRTIs) provide in most cases full inhibition of HBV replication together with normalization of liver enzymes levels and a substantial interruption of the chronic evolution of liver fibrosis .

In parallel with the development of TAF (alafenamide, formerly GS7340) for anti-HIV treatment, interest is also focused on having the drug available for anti-HBV therapy. TAF is a new prodrug of TDF, showing more potent anti-HIV-1 activity and higher intracellular TDF levels compared with tenofovir disoproxil fumarate, while maintaining lower plasma tenofovir exposure at 40 mg with good tolerability over 14 days of monotherapy (Markowitz *et al.,* 2014). Moreover TAF provides significantly reduced renal tubular toxicity (Sax *et al.,* 2013). Recent studies highlighted that TAF also leads to higher levels of active tenofovir in hepatocytes (liver cells) (Agarwal *et al.,* 2013) and shows an antiviral activity similar and comparable to TDF also in HBV-infected patients (6). The use of this antiviral in HIV/HCV-coinfected patients should therefore be investigated.

The two available pharmaceutical forms of pegylated interferon (PEG-IFN) are among the therapeutic weapons in both anti-HBV and HCV treatment. Currently, treatment for chronic HCV infection in HIV-positive patients is still based on interferon (IFN) and ribavirin (RBV), that are the recommended treatment for HCV genotypes 2, 3 and 4 (European AIDS Clinical Society Guidelines (EACS) 2013). It should also be taken into account that IFN in HIV positive patients could determine a reduction in CD4 T cell count of different magnitude. However, no descriptions of IFN effects on plasma HIV-RNA have ever been detailed. In an Italian experience the treatment of HCV chronic hepatitis in HIV-infected patients not taking antiretrovirals was analyzed to evaluate the effects of IFN administration on HIV-related immunovirological parameters. Further to the expected CD4 T cell count decrease, a significant drop in HIV-RNA was also seen, with median (range) HIV-RNA decline of –1.46 (2.75; –0.22), –1.43 (–2.84; 0.21), and –1.07 (–2.46; 0.62) log10 copies/ml at 2, 4 and 12 weeks, respectively (Aguilar Marucco *et al.* 2007). Although major attention should be paid to the CD4 T cell count decrease, such a significant antiretroviral effect might to some extent compensate for the transient numerical decrease of CD4 T cells (Tavel *et al.,* 2010).

Like antiretroviral treatment, anti-HCV therapy is now also based on three-drug regimens, as the validated PEG-IFN/RBV backbone has been successfully associated with a third antiviral drug, either Boceprevir (BOC) or Telaprevir (TLV), with cure rates above 70% in HCV genotype 1 (Kieran *et al.,* 2013). HCV direct-acting antiviral agents (DAA)-based therapy with either BOC or TLV is now the new standard of treatment in HCV genotype 1 infection in HIV-positive persons where available.

Both these new DAAs are substrates of isoenzyme CYP3A, on which they exert an inhibitory effect. This translates into some defined drug-drug interactions, also including antiretrovirals, and caution must be observed in case of co-administration with drugs sharing this metabolic pathway (Wilby *et al.,* 2012; Bertelsen 2013).

IFN-free regimens can also theoretically be used in HIV-positive patients coinfected with HCV and are expected to achieve high SVR rates, though further investigation is needed (European Association For The Study Of The Liver 2012).

The study of human genetics is providing important new information on both the spontaneous outcome of untreated HCV infection as well as the likelihood of treatment response to anti-HCV treatment. Interleukin 28 polymorphisms (IL28B) and additional genetic variations in regions coding for factors included in the process of activation/enhancement of cellular immune response (e.g. vitamin D promoter) are shedding light on potentially pre-classifying patients before any specific anti-HCV regimen is chosen (Ge *et al.,* 2009; Lange *et al.,* 2011). These findings are thus paving the way to individualized therapy, with possible achievements in terms of lower rates of untoward effects as well as a reduction in drug expenditure.

Regardless of the therapeutic regimen, a unique window of opportunity to get rid of evolving

HCV infection is the treatment of acute infection. Cure rates may be as high as 75% with just PEG-IFN even in patients being infected with HCV-genotype 1 (De Rosa *et al.,* 2006). No definite recommendations have so far been released on how to manage acute HCV infection (spontaneous clearance is also an occurrence taking place with significant frequency), but according to few published case series the chance of sustained virological response (SVR) is on the average higher than in case of chronic infection, and this also applies to HIV-HCV-coinfected patients.

Among the drugs approved against HIV infection, the potential anti-fibrotic effects of maraviroc (MVC), a CCR5-specific HIV entry inhibitor, is currently exploited. In a comparative Italian study on HIV-HCV patients MVC was added as 4th drug to a successful antiretroviral regimen consisting of ritonavir-boosted atazanavir and TDF/FTC, while controls remained on the original regimen. Improvement in liver stiffness was seen in the study arm (Nasta *et al.,* 2011). MVC has been also found to reduce chronic inflammation and immune activation, atherogenesis and hepatocellular carcinoma (Sauzullo *et al.,* 2010; Ochoa-Callejero *et al.,* 2013). Should any of these properties be confirmed to play a clinically relevant role, MVC might become a "multitask" drug to be considered in chronic infections.

Population: All naïve HIV-positive individuals who are coinfected with HBV and/or HCV. These patients warrant aggressive treatment against both HIV and hepatitis viruses to reduce the rate of liver-specific disease and death.

Intervention: A review of current literature through meta-analysis studies or design of observational or cohort studies, and, depending on feasibility, randomized clinical trials, aiming to:

- Evaluate the efficacy and safety of TAF in HIV/HBV-coinfected patients.
- Evaluate the HBV genetic background in modulating the virological response to TAF. In this regard, it is already known that a high degree of HBV genetic variability can lead to the emergence of viral strains with a natural resistance to tenofovir in HIV/HBV-coinfected patients, even if at low-level (Lada *et al.,* 2012). Thus, HBV-genotypic testing can help identifying patients at risk of disease progression and thus patients who need an early and highly effective treatment.
- Evaluate the potential drug-drug interactions between DAAs and antiretrovirals and mechanisms related to the reduction of antiviral activity determined by these interactions in HIV/HCV-coinfected patients.
- Assess the best treatment choice including the novel anti-HCV drugs used with or without IFN/RBV.
- Evaluate molecules among the drugs used against HIV infection able to limit the pathogenetic events determining liver fibrosis. In this frame, more studies are required to confirm the potential anti-fibrotic role of MVC.
- Evaluate the role of other human polymorphisms (a part from IL28) in the control of HCV infection in the context of HIV infection.

Outcome: The final outcome is setting the most appropriate strategies to improve quality of life and reduce the rate of liver specific-disease and death in HIV-positive patients coinfected with HBV or HCV.

3 - PATIENTS WITH NON-INFECTIOUS COMORBIDITIES

Coordinator A. Antinori

Introduction

Following the impact of cART on the disease natural history and considering prolonging survival, AIDS-defining conditions are increasingly rare in patients receiving cART and presenting persistent suppressed HIV viremia (Mocroft *et al.,* 2013a). By contrast, the list of HIV-associated non-AIDS conditions as non-infectious comorbidities is growing (High *et al.,* 2012). Several of these conditions are strongly associated with advancing age and chronic inflammation, and include cardiovascular disease, non-infectious cancers, osteopenia and osteoporosis, liver disease, renal disease and neurocognitive impairment (Sulkowski and Thomas 2003; Lucas *et al.,* 2007; Silverberg *et al.,* 2009; Freiberg *et al.,* 2011; Womack *et al.,* 2011; Silverberg *et al.,* 2012).

Evidence from randomized and observational studies indicates that both HIV infection and ART may influence morbidity and mortality

through effects on inflammation, treatment-related toxicities (such as renal, bone, and effects on cardiovascular risk disease), complex interactions with other chronic viral infections (as CMV infection), and non-infectious comorbidities like those typically associated with increased aging (Deeks *et al.,* 2013). For all these reasons, HIV-infected persons exhibit an excess burden of co-morbid conditions and the premature onset of clinical symptoms and syndromes, very close to those typically associated with advanced aging, multimorbidity, and polypharmacy (High *et al.,* 2012).

Moreover, it is well known that chronic inflammation plays a central role in the pathogenesis of untreated and ART-treated HIV infection. In fact, HIV replication directly contributes to T cell activation (Papagno *et al.,* 2004). Moreover, the HIV-mediated breakdown in the gut mucosa produces chronic exposure to gut microbial products like lipopolysaccharide (LPS) that may be a recognized factor driving inflammation (Brenchley *et al.,* 2006). Finally, dysfunctional immunoregulatory factors may contribute to sustain a picture of persistent inflammation. The chronic inflammatory environment may cause fibrosis in lymphoid tissues, with CD4 T cell regenerative failure and persistent chronic dysfunction and disease (Zeng *et al.,* 2012). cART only partially reverses many of these proinflammatory pathways, but the effect is still largely incomplete, and inflammation may indefinitely persist.

It is well known that monoctye turnover and activation could be directly linked to HIV pathogenesis. Soluble CD14 (sCD14) and CD163, recognized monocyte activation markers, were found elevated in HIV disease and were both predictive of increased morbidity and mortality in the HIV-infected population (Burdo *et al.,* 2011). sCD14 can bind LPS and deliver it to vascular endothelial cells, promoting their activation by LPS. CD163, the hemoglobin scavenger receptor, is expressed on the monocytes surface, particularly those that are more inflammatory (CD14+CD16+). CD163 is released as a soluble form (sCD163) in response to a number of inflammatory signals. Activated (CD14+CD16+) monocytes are frequently elevated in untreated and treated HIV disease, and proinflammatory CD16+ monocytes may be related to an increased risk of coronary artery calcium progression. All these observations support the hypothesis that chronic activation of innate immunity may contribute to enhance morbidity and mortality in HIV-infected adults even successfully treated by antiretroviral drugs. Persistent levels of ongoing HIV replication during ART exposure may impact immune activation and chronic inflammatory activation pathway, and progressively increase the risk of comorbidities even in ART-treated individuals.

Chronic upregulation of these immunologic pathways may cause disease. It has been assessed that monocyte and macrophage related inflammation pathways are crucial for the pathogenesis of atherosclerosis even in the general population. In the HIV-infected population, chronic enhanced inflammation, altered blood flow dynamics, circulating bacterial products, proatherogenic lipids (related to toxic prolonged effects of antiretroviral drugs exposure), and other factors associated with HIV infection can cause endothelium damage and upregulation of adhesion factors. Monocytes are recruited in blood vessel walls and may contribute to the formation of atherosclerotic plaques.

Further, HIV infection may causes a hypercoagulable state, strongly related to chronic inflammation and enhancing the risk of comorbid conditions. The role of hypercoagulability as a cause of morbidity and mortality in HIV-infected individuals was recognized after demonstration that D-dimers strongly correlate with all-cause mortality (Kuller *et al.,* 2008). Several studies confirmed the association of D-dimers with mortality, occurrence of cardiovascular disease (Duprez *et al.,* 2012), as well as venous thromboembolic disease (Musselwhite *et al.,* 2011). The model of evidence on hypercoagulation as a direct cause of morbidity, already recognized in the general population, may be transferred to the HIV disease setting. From a pathogenetic point of view, HIV persistent replication, even at a low-level, may affect hypercoagulation and induce an increase in D-dimer levels. The rate of HIV replication could correlate with D-dimer levels in untreated disease (Calmy *et al.,* 2009), and several studies demonstrated that starting cART may be associated with a reduction in levels of D-dimer (Palella *et al.,* 2010), although this reduction effect during

treatment is not sufficient to reverse D-dimer amounts to preinfection levels, as suggested by comparative measurements in non-infected controls. Intensification studies with an additional potent antiretroviral drug (e.g. INSTI) in the context of a successful cART regimen may decrease ongoing HIV replication and produce a decrease of D-dimer levels (Hatano *et al.,* 2013a). Finally, chronic inflammation related to untreated or persistently low-level replication of infection may also be related to enhanced coagulation status, as demonstrated by the observation that more elevated circulating levels of sCD14 and sCD163 are associated with increased D-dimer levels (Funderburg *et al.,* 2010).

In the HIV setting, advances in knowledge could be driven by the use of biomarkers. Several biomarkers have been suggested as predictive of an increased comorbidities burden or organ diseases in the HIV population. Nevertheless, the degree to which these biomarkers really have prognostic characteristics in HIV-infected people is still largely unknown. This is also true considering the problems in identifying cohorts of behaviorally and demographically similar infected and uninfected adults. Many biomarker studies reported to date have included untreated and treated HIV individuals, despite the fact that HIV replication is known to be a major determinant of inflammation, immune function, and overall health. Unfortunately, even those studies that focused on long-term treated individuals rarely controlled for the degree of viral suppression. Further, behavioral aspects may affect both biomarker behavior and the risk of non-AIDS morbidity and mortality, and this may be a further confounder of study design and results. A priority in the development of biomarkers and clinical prognostic indices (such as the Veterans Aging Cohort Study Index (VACS)) are to identify those biomarkers indicative of residual disease (in terms of immune activation, immunologic dysfunction and chronic inflammation), to characterize the link between chronic inflammation and persistent immunodeficiency during treatment, and to analyze the size effect of both modifiable risk factors for non-infectious comorbidities and chronic HIV infection.

Randomized clinical trials will need to test the increased approaches to aging and comorbidities in the HIV-infected population. The studies should focus on management strategies or individual interventions and involve a combination of behavioral and therapeutic approaches. ART initiation trials may test the impact of health interventions, and the effectiveness of these interventions related to the rate of patient adherence to ART. Innovative methods of performing trial design may allocate the HIV population to standard care versus medical management guided by the VACS Risk Index or frailty phenotype, and address signs of cardiovascular, liver, renal, and bone injuries. Further, proof-of-concept small intensive trials should be used to identify interventions that reduce HIV-associated inflammation and/or immune activation in order to progressively move into larger studies characterized by clinical endpoints focused on morbidity occurrence and survival.

Patients with chronic inflammation and cardiovascular risk: role of biomarkers

P. Maggi, M. Galli

Evidence. HIV infection is associated with numerous co-morbidities, whose incidence increases with patient age, allowed by the success of antiretroviral therapy. Therapy itself is considered to be involved in the pathogenesis of some of these, leading to an increased and earlier incidence than in the general population. HIV infection can be considered a typical example of a chronic inflammatory disease, characterized by a persistent and aberrant activation of the immune system induced by HIV as well as a wide range of non-HIV-related stimulations. An early loss of gut mucosal integrity, the pro-inflammatory cytokine milieu, co-infections and the progressive disruption of lymph node architecture contribute to determine a permanent activation of both the innate and adaptive immune systems. CD4 T cell loss, induced by providing an additional substrate for viral infection in the form of activated CD4 T cells and by priming non-infected 'bystander' CD4 T cells for death by apoptosis, is one of the major consequences of such persistent immune activation (Ipp and Zemlin 2013).

Chronic inflammation is a major cause of cancer and aging and is involved in the pathogen-

esis of a wide variety of age-related diseases, such as diabetes, cardiovascular and autoimmune disorders (Khansari *et al.,* 2009).
Immunity deregulation and chronic inflammation can be considered key driving forces also for non-HIV-related complications such as cardiovascular, kidney and bone, liver, lung, CNS disease and cancer. It is debated whether the incidence of some non-HIV-related co-morbidities is anticipated or simply enhanced or both. However, the incidence of some aging-related disorders is reported to increase despite a good response to ART.
Population. In a scenario where therapy should be continued for the entire lifespan duration of each patient, the whole population of people with HIV infection should be submitted to a careful clinical screening and laboratory monitoring designed to assess the individual risk of developing comorbidities associated with the persistence of a chronic inflammatory state. Although this indication can be applied to the entire HIV positive population, advanced naïve patients, people over 50 years and, for some pathological conditions, menopausal women are considered by many experts as groups deserving special attention. Particular consideration is also given to those with or at risk of coinfections such as HBV, HCV, HPV. The importance of the use of biomarkers of inflammation is discussed and investigated in this context.
For these reasons, the elaboration of algorithms based also on inflammation biomarkers as well as the identification of cost-effective markers of inflammation to be used to predict the risk of inflammation-related comorbidities represent hot topics for current and future research.
Intervention. The candidate biomarkers currently under investigation to improve the assessment of the cardiovascular risk in HIV patients include CRP, IL-6, VCAM, ICAM, VWF, D-dimer and ADMA.
A possible challenge complicating the use of such biomarkers is the different behavior shown by some of them (e.g., IL-6, sCD14) in HIV disease and in the general population (Neuhaus *et al.* 2010). The association of an increased level of inflammation biomarkers and the subsequent morbidity/mortality is sometimes stronger in HIV-infected compared to HIV-uninfected individuals. For example, the adjusted odds ratios of mortality associated with abnormal levels of sCD14, IL-6 and D-dimer observed in the SMART study (Kuller *et al.,* 2008) were significantly higher than those observed in the general population, but the risks associated with increased CRP levels were much lower than might be expected given the IL-6 data.
Nevertheless, the role of IL-6 and D-dimer levels as powerful predictors of non-AIDS comorbidities and death was recently confirmed combining the data of 3 large clinical trials (SMART, ESPRIT, and SILCAAT) (Grund *et al.,* 2013). The authors also proposed an equation including both parameters which is more predictive of clinical events and death than each of them considered separately. This equation should be validated in prospective studies and could be used to design clinical trials aimed at reducing chronic inflammation.
IL-6, D-dimer and sCD14 (markers of inflammation, coagulation and monocyte activation respectively) were also strongly related with the VACS index calculated in patients on cART (Justice *et al.,* 2012). The VACS index (Tate *et al.,* 2013) is a combined prognostic score for predicting the survival of HIV-infected patients, based on CD4 cell count, HIV-RNA, HBV, HCV status, estimated glomerular filtration rate (eGFR) and FIB-4 (platelets, ALT, AST, Age). It also incorporates multifaceted HIV effects, multimorbidity, and toxicity. Although VACS does not include inflammatory markers, it is considered a reliable proxy of inflammatory status and is currently used in several studies.
Recent evidence supports a role of asymmetric dimethylarginine (ADMA) as a valuable predictor of vascular events. ADMA is an endogenous inhibitor of endothelial nitric oxide (NO) synthase, an endogenous anti-atherogenic molecule. Any condition that will reduce endothelial NO production may therefore promote atherosclerosis. In the general population, ADMA levels were reported to predict cardiovascular events and mortality (Boger 2003).
Studies in HIV-infected patients showed that elevated levels of ADMA are associated with low CD4 count and high viral load (Baker *et al.,* 2012; Parikh *et al.,* 2013). Moreover, ART initi-

ation leads to a decline in ADMA levels, which are also related to the degree of inflammation and coagulation parameters. This finding suggests a role of ADMA upregulation in contributing to premature vascular disease in HIV-infected individuals.
The role of ART and the reduction of HIV replication in the containment of the inflammatory response are also proved by the reduction of the serum level of several other surrogate markers of inflammation. On the other hand, some of these biomarkers do not seem to be significantly influenced by a short-term treatment. This happened, for example, in a longitudinal study conducted in advanced naïve HIV patients, showing high base-line levels of ICAM-1, VCAM-1, IL-6, D-dimer and hsCRP. After three months on ART, D-dimer levels were significantly decreased, while ICAM/VCAM were further increased and remained stable throughout the 12-month follow-up (Bellacosa *et al.,* 2012). In a combined analysis from WIHS and MACS, the initiation of effective ART was associated with a decline in IL-6 and D-dimer, but an apparent increase in C-reactive protein (CRP) (Palella *et al.,* 2010). These findings confirm the possible persistence of an 'inflammatory background' despite the virologic success of ART, and suggest that in different phases of HIV infection there are different chances to limit inflammation and inflammation-related comorbidities by ART.
The different behavior of the biomarkers of inflammation might also relate to coinfection with other viruses, such as HCV. In the Study of Fat Redistribution and Metabolic Change in HIV Infection (FRAM), CRP was considerably lower in HIV and HCV-coinfected individuals than in either controls or in HIV-monoinfected patients (Tien *et al.,* 2010).
In the general population, the predictive gain obtained adding the evaluation of inflammation biomarkers to conventional risk factors is still minimal (Melander *et al.,* 2009). Nevertheless, some biomarkers are widely utilized in predicting cardiovascular risk. A body of evidence demonstrated that high sensitivity C-reactive protein (hsCRP) levels are an independent predictor of future myocardial infarction and stroke among apparently healthy men and women (Ridker *et al.,* 1997; Tracy *et al.,* 1997; Koenig *et al.,* 1999; Danesh *et al.,* 2000; Ridker *et al.,* 2000), and the addition of CRP testing to standard lipid screening seems to provide an improved method to determine vascular risk (Ridker *et al.,* 1998; Ridker *et al.,* 2000).
In a recent study in HIV patients, high hsCRP was associated with cardiovascular disease risk, independently from traditional cardiovascular risk factors. HIV replication, the type of ART and high IL-6 and P-selectin levels were also independently associated with increased CVD risk, although the association was weaker than for hsCRP (De Luca *et al.* 2013).
Outcome. In conclusion, HIV-infection is responsible of persistent inflammation which can also continue in ART-treated patients with stable viral suppression.
Chronic inflammation, in turn, is a major cause of non-AIDS comorbidities and aging processes. The use of inflammatory biomarkers in clinical practice could open future perspectives to predict, and to prevent, the risk of non-HIV-related comorbidities, especially cardiovascular events.
However, further studies are warranted to better clarify their role in the different phases of HIV disease, and their gains over conventional risk factors in predicting the risk of development of non-HIV-related comorbidities.

Patients with renal damage.
Safety endpoints
P. Bonfanti
Evidence. Renal function abnormalities are present in a large percentage of the HIV-infected population. Patients with HIV infection may develop renal damage associated with HIV infection itself. The risk is increased in patients with coinfections of HCV or HBV, diabetes, or hypertension (Ross 2014). The widespread employment of highly active antiretroviral therapy has reduced the prevalence of HIV-associated nephropathy and the evolution towards end-stage renal disease. Despite this protective effect on the kidney, some antiretrovirals have been associated with renal toxicity, particularly tenofovir and less prevalently the protease inhibitors (Calza 2012; Ross 2014; Yombi *et al.,* 2014).
Conflicting data on the renal safety of tenofovir are reported in the literature. Few tenofo-

vir discontinuations due to renal toxicity have been reported in large clinical trials. On the other hand, cohort studies have shown a higher incidence of nephrotoxicity in patients receiving tenofovir, especially when coadministered with boosted protease inhibitors (PIs). Drug accumulation within proximal renal tubules, leading to mitochondrial damage, is the main mechanism by which tenofovir causes renal toxicity (Tourret *et al.,* 2013).

Some data gaps still remain, particularly on:

- the reversibility of renal damage;
- the role of subclinical tubular dysfunction in predicting the subsequent development of chronic kidney disease in patients taking antiretroviral drugs;
- the correct assessment method for renal toxicity: since the damage caused by tenofovir is tubular, the glomerular filtration rate is not the best marker for renal damage;
- the lack of clear knowledge of the relationship between polymorphisms in genes associated with tubular transporters and clinical outcome.

The introduction of new antiretroviral agents such as cobicistat and dolutegravir that potentially affect renal function means that filling these data gaps is a matter of urgency.

Population. All HIV naïve patients should be screened to identify risk factors for developing renal damage before initiation of ARV treatment.

All treated patients, particularly those taking nephrotoxic drugs, should not only be monitored with the GFR estimation but also using tubular damage markers.

Intervention. In order to properly evaluate patients taking a tenofovir-containing regimen or new drugs (cobicistat, dolutegravir), the correct evaluation of proximal tubular nephropathy needs to be identified and standardized. We need to know which markers to use, how many markers should be considered, and whether or not we should introduce new markers in clinical settings.

We also need to improve our understanding of the role of genetic markers associated with tubular damage.

Outcome. The prevention and identification of renal toxicity associated with antiretroviral drugs.

Patients with metabolic or bone damage. Safety endpoints

M. Borderi

Evidence. There is a lack of information on the long-term effect of cART on bone metabolism, and on the interactions between cART and other risk factors. Few studies have addressed the effect of treatment with vitamin D and PTH, and clinical trials do not distinguish between osteoporosis and osteomalacia, while data on vertebral fracture risk in HIV patients are still lacking. Published studies do not properly analyze the risk of bone toxicity in women and men separately and in patients with different duration of HIV disease.

To date, we do not know what laboratory assessment tests are best for screening, what other screening modalities [i.e. quantitative ultrasound (QUS), (Prinapori *et al.,* 2013)] should be considered, how diagnostic assessments for bone disease should be interpreted, how frequently monitoring should be performed, how ART should be managed in ART naïve and in experienced patients at risk of bone disease, and what ART should be selected in naïve and experienced patients to preserve bone mineral density.

Population. All HIV patients, naïve and experienced, should be screened to identify the risk for fragility fracture, and all patients with bone disease should be monitored before, following initiation and during ARV treatment. There could well be special therapeutic considerations in special populations (i.e. aging subjects).

Intervention. We need to identify concomitant risk factors and their specific role in promoting bone loss; to assess the risk in special populations (i.e. does cART-associated increased bone turnover translate into accelerated bone loss and rising fracture rates in postmenopausal women?); to better define hyperparathyroidism role on tenofovir-related bone loss; to have correct tools assessing osteomalacia (it would be useful to determine reliable markers able to differentiate between osteoporosis and osteomalacia, since the treatment differs); finally, we need to determine the role of vitamin D deficiency in patients with normal PTH levels. We do not know which patients at risk for fragility fracture should be treated, at what threshold treatment should be initiated, what are the recommend-

ed approaches for the treatments of patients at risk for fragility fracture, and if they differ in HIV-infected versus non-HIV-infected populations (reference to vitamin D supplementation, calcium supplementation, bisphosphonates).
Outcome. Final outcomes are to preserve bone mineral density and reduce fragility fractures.

4 - EXPERIENCED PATIENTS WITH RESIDUAL VIREMIA OR WITH LOW-LEVEL VIREMIA

Coordinator M. Andreoni

Introduction

The aim of ART is to reduce the replication of HIV under the limits determined for tests used in clinical practice (<50 HIV-RNA copies/ml). A favourable clinical evolution with an increase in CD4 lymphocytes regularly occurs in patients who present this result. However, low-levels of viral replication persist in most patients undergoing therapy.
Two hypotheses may explain the origin of low viremic levels in patients undergoing antiretroviral treatment: residual viremia may be the result of a virus actively replicating because it is uncontrolled by the therapy currently in operation or it may represent virus reactivation by latently infected cells. Active replication of the virus is confirmed by the evolution of the virus in some patients undergoing treatment in which there are new mutations and notable stability in the HIV-DNA levels in infected cells (Buzon *et al.,* 2011). On the other hand, other studies have shown that intensification of the therapy using various pharmaceutical drugs is not able to change the levels of residual viremia demonstrating that an actively replicating virus is not being dealt with in these cases (Dinoso *et al.,* 2009; Yukl *et al.*, 2010b; Gandhi *et al.*, 2012; Negredo *et al.,* 2013).
It has been well known since 1995 that there is a rapid reduction of viremia at the start of ART. This reflects the short half-life of the free virus in the plasma and that of activated T CD4 lymphocytes that represent the cells that produce most of the virus found in the plasma.
In fact, various studies have shown that when a patient starts a highly effective therapy plasma HIV-RNA levels are rapidly and exponentially reduced. However, it is important to remember that all of the antiretroviral pharmaceutical drugs used prevent the infection of new cells without blocking the production of viruses by cells with previously integrated virus. The rate of virus level reduction produced by cells infected again has been measured and found to be similar in all patients unrelated to the stage of disease. This rate of reduction of new virus production has been used to calculate that the half-life of activated T CD4 lymphocytes is only 1-2 days and that the rapid initial reduction of the virus in the plasma is due to the turnover of activated CD4, which live for approximately one day.
The start of therapy in this first phase is followed by a second phase with a slower reduction in virus levels due to infected cells producing viruses with a longer half-life, the macrophages. In most patients, viremia in this second phase falls below the determinable levels (<50 copies/ml HIV-1 RNA in plasma). Eradication of this cellular component needs at least 2-3 years of continuous highly effective therapy.
However, the opportunity to eradicate the infection has been completely frustrated by the discovery of a third population of infected cells with an even longer half-life. In fact, HIV can infect various types of immune cells in the initial phase of infection such as monocytes, macrophages, NK cells, and T lymphocytes (especially unactivated CD4 cells) in addition to other non-immunising cells in which the virus maintains its latent form integrating with the cellular genome and preserving its potential to replicate. The cell reservoirs are stable through time with either no replication or a reduced frequency of replication. Furthermore, these cells would be less susceptible to the effect of the pharmaceutical drugs and the action of the immune system consequently favoring the persistence of the virus.
This cell population is the actual viral reservoir. It is mainly composed of resting T CD4 lymphocytes that in normal physiology represent most of the T lymphocytes that may be naïve and consequently still not responding to extraneous or memory antigens and so have already participated in an immune response. When resting lymphocytes meet an antigen, they are activated and divide generating cellular electricity ac-

tivated by the same antigenic specificity. Many of these activated cells die at the end of the immune response while others return to a resting state like memory cells. These cells persist for a long time and their decay is related to future immunological responses against specific antigens. Therefore, HIV can infect some lymphocytes which, after their activation, return to resting state like memory cells with an integrated untranscribed viral genome.
Consequently, should they be activated, the cell reservoirs produce new viral particles. The T memory cells with the integrated viral genome are verifiable even though not numerous in the order of one every unactivated 10^6-10^7 CD4 cell in both patients with acute infection and those with chronic and persistent infection independent of the antiretroviral therapy. Each cell reservoir preserves the virus through its potential to replicate itself exponentially. Furthermore, precisely because of their function as memory cells, these cells have a very long half-life of approximately 5 months, which increases in patients undergoing HAART therapy. In particular, a study of patients with viremia <50 copies/ml treated for more than 7 years calculated a half-life for these cells of 44.2 months, evaluating that for one eradication of HIV from the latent reservoir, an effective therapy of approximately 73 years would be necessary (Finzi *et al.,* 1999).
The existence of this stable latent reservoir leads to the idea that there is a third phase of viral decay: a small number of latently infected cells are activated each day and produce virus that is not able to infect new cells but is released into the plasma at low-levels resulting in residual viremia. This third phase cannot be demonstrated using the classic tests involving dosage of the viremia because the levels are too low and can either be found using highly sensitive methods or in the form of blips in some cases.
Some studies have found that the reservoir cells producing viral particles can express small quantities of viral RNA and produce a limited number of virions and not only do this when activated but also when dormant (Swiggard *et al.,* 2004; Pace *et al.,* 2012). Consequently, the virus is cultivatable in most infected subjects at any stage of disease including during highly suppressive therapy. This phenomenon is related to the fact that the virus integrates itself near the genes with the greatest transcriptional activity. It is important to remember that none of the antiretroviral pharmaceutical drugs currently available or being developed is able to act on HIV DNA that is already integrated.
In conclusion, the presence of residual viremia in patients undergoing highly active therapy may be due to both a virus produced by latently infected resting cells that have been activated and by a virus produced by cells infected again in which the infection has not been controlled by antiretroviral therapy. In the light of this consideration only a reduction in the number of copies of HIV-DNA through the course of time is able to demonstrate that the therapy activated is not only able to control the replication of free virus in plasma but also controls virus released into cells.
From the clinical point of view, it is critical to understand that if very low-levels of replication are sufficient for new mutations of pharmacological resistance to develop in individual patients, the therapy will consequently fail. It has been demonstrated that the viruses that replicate themselves at such low-levels can also produce clones with individual mutations of resistance but these are unlikely to generate viral particles with multiple mutations of resistance. Certainly, the higher the levels of residual viremia, the greater the risk of the therapy failing. Understanding this phenomenon justifies the assumption that ART should always provide for the use of pharmaceutical drugs for which resistance only occurs in the presence of multiple mutations.
Lastly, further studies should help clarify whether or not low-levels of virus replication are capable of maintaining a state of immunoactivation and hence inflammation both of which underlie chronic degenerative pathologies and neoplasia frequently found in patients effectively treated using antiretroviral therapy.

Patients with suppressed or residual viremia

M.R. Capobianchi, A. Ammassari

The main goals of cART are the achievement of "complete virologic suppression" for as long as possible, together with the improvement of quality of life, the restoration and maintenance of the immune system and the prevention of

HIV transmission. According to current guidelines (European AIDS Clinical Society (EACS) 2014; HIV/AIDS Italian Expert Panel 2014; Panel on Antiretroviral Guidelines for Adults and Adolescents 2014b), "complete virologic suppression" is achieved if plasma VL drops to levels below 50 copies/ml, conventionally assumed as the lower limit of detection of the molecular tests currently available, at 6 months after start of cART. In clinics nowadays, the use of powerful therapeutic regimens and the adoption of genotype-driven antiretroviral combinations allow "complete virologic suppression" to be achieved in most treated patients: around 65-85% (Gill *et al.,* 2010; Moore and Bartlett 2011). Continuing cART administration is necessarily lifelong, also when "complete virologic suppression" is reached and is stable for a long time, because the eradication of HIV infection cannot be achieved with currently available antiretroviral regimens due to viral persistence (Deeks *et al.,* 2013). In fact, in the very early phase of HIV infection a "pool" of latently infected cells called a reservoir is established with a long half-life and releasing minimal amounts of viral particles also in the presence of effective therapy and prolonged suppression of viremia. Resting memory CD4 T-lymphocytes represent the main component of the reservoir, but other cell types hosting the virus genome in an integrated form also contribute to HIV persistence (Chun *et al.,* 1995; Pierson *et al.,* 2000; Zhu *et al.,* 2002; Sundstrom *et al.,* 2007; Rozera *et al.,* 2009; McNamara and Collins 2011; Palmer *et al.,* 2011; Watters *et al.,* 2013; Abreu *et al.,* 2014). In the absence of therapy, active viral replication continuously refills the reservoir. In the presence of cART, the amount of free virus in the blood falls to levels undetectable with the currently available diagnostic tests, but the virus persists as provirus, integrated into the genome of the cells that can survive for years in a resting state. When these cells receive a stimulation signal, they transit to a metabolically active state, resulting in the production and release of viral particles that infect new target cells. Therefore, even when viremia is reduced to levels <50 copies/ml, minimal amounts of virus are always produced and released in the bloodstream replenishing residual viremia (RV) (Van Lint *et al.,* 2013).

Two models have been suggested on the origin of RV during effective cART (Shen and Siliciano 2008). According to the first model, RV is the result of virus release from latently infected CD4 T-cells, stochastically activated following specific antigenic stimulation. Under these conditions, RV is unable to originate new replication cycles, due to the presence of antiretroviral agents. However, recent studies have shown that in many patients RV is dominated by viral clones that are profoundly underrepresented in resting CD4 T cells from the peripheral blood, suggesting an additional source of residual viremia (Brennan *et al.,* 2009; Sahu *et al.,* 2009). The other model suggests that RV is the consequence of a reduced concentration of antiretroviral drugs in particular compartments, named HIV sanctuaries (lymphoid organs, central nervous system, genital tract, renal epithelium), where the virus continues to replicate at low-levels and/or undergoes cell-to-cell spread. Several lines of evidence indicate the two models as not mutually exclusive and probably coexist in different proportions during time (Joos *et al.,* 2008; Shen and Siliciano 2008; Sahu *et al.,* 2009; Yukl *et al.,* 2010a; Anderson *et al.,* 2011; Gandhi *et al.,* 2012).

Interestingly, several studies and clinical trials investigating cART intensification through addition of another antiretroviral, such as Raltegravir or Maraviroc, to a virologically effective cART have not been able to demonstrate reduction or elimination of RV (McMahon *et al.,* 2010; Hatano *et al.,* 2011; Llibre *et al.,* 2012; Hatano *et al.*, 2013a; Puertas *et al.,* 2014). Nevertheless, in some effectively treated patients a rapid and transient increase in the level of 2-LTR circles was found, indicating that cART intensification and optimization may impact on residual VL replication (Hatano *et al.,* 2013a) and a perhaps limited effect on CD4 T cell reconstitution has been suggested (Negredo *et al.,* 2013; Rusconi *et al.,* 2013).

Given these facts and thanks to technological improvements, several studies have been started, aimed at answering a new clinical question: should patients who have a very low, but "detectable" plasma VL be considered treatment failures or doomed to fail? Innovative issues/needs are challenging the scientific community, such as to establish a new cut-off threshold val-

ue of VL to define therapeutic success, to interpret and ascribe a precise clinical significance to low-levels of viral load, and to design better therapeutic management strategies for patients with low-levels of plasma VL.

Various tests have been developed to quantify RV (Amendola *et al.,* 2011; Glaubitz *et al.,* 2011; van Rensburg *et al.,* 2011; Yukl *et al.,* 2011; Taylor *et al.,* 2013) and the presence of RV in virologically suppressed cART-treated individuals, even after many years, has been widely described (Palmisano *et al.,* 2005; Palmer *et al.,* 2008; Maggiolo *et al.,* 2012). Based on published studies, approximately 39-60% of HIV-positive cART-treated individuals with viremia below 50 copies/ml have RV, while in 40-71% of cases plasma VL is below 3 copies/ml(Doyle *et al.,* 2012; Gianotti *et al.,* 2012; Maggiolo *et al.,* 2012). The levels of RV in virologically suppressed individuals ranges from 1-5 to 20-30 copies/ml (average about 3 copies/ml) and fluctuates around a set-point value for long time (Havlir *et al.,* 2003; Siliciano *et al.,* 2003).

The clinical significance of the RV level is currently the objective of numerous studies and should further be investigated, particularly by looking at the association with therapeutic failure, such as virologic and immunologic outcome, and immune activation. At the same time, factors predictive of RV have to be described in detail in order to allow for identification of the most vulnerable patient population.

In several observational studies, RV has been shown to increase the risk of subsequent virologic rebound (i.e. >50 copies/ml, >400 copies/ml)(Widdrington *et al.,* 2011; Doyle *et al.,* 2012; Gianotti *et al.,* 2012; Henrich *et al.,* 2012; Maggiolo *et al.,* 2012), virologic rebound followed by a cART change (Doyle *et al.,* 2012), and lower CD4 cell recovery (Widdrington *et al.,* 2011; Gianotti *et al.,* 2012; Henrich *et al.,* 2012). However, besides the impact of a low-level virologic set-point on virologic rebound, adherence behavior may also play an important role. Although the interplay of these variables has not been extensively investigated, it seems reasonable to affirm that patients with higher RV self-reporting non-adherence behaviors are the most vulnerable patient population with the highest rebound rates when compared to those having undetectable RV or with detectable RV and optimal adherence to cART.

The relationship between persistency of RV and immune activation as well as chronic inflammation is controversial and still under investigation. Some studies found increased T-cell activation (Mavigner *et al.,* 2009) and higher blood levels of soluble immune activation markers (Ostrowski *et al.,* 2008) in cART-treated patients with RV when compared to those without. Other researchers documented a correlation of plasma RV with the size of the CD4 T cell viral reservoir, but not with markers of immune activation, suggesting that reactivation of the latent viral reservoir may not be the sole source of residual plasma viremia (Ostrowski *et al.,* 2008). Notably, Hatano *et al.,* found that even "elite controllers" with pre-cART plasma HIV RNA levels <40 copies/ml had a statistically significant decrease in ultrasensitive plasma HIV RNA levels after initiation of ART together with a statistically significant decrease in HIV antibody levels and a reduction in immune activation confirming that some HIV replication persists in controllers and contributes to a chronic inflammatory state (Hatano *et al.,* 2009; Chun *et al.,* 2013; Hatano *et al.,* 2013b).

With regard to factors influencing RV, baseline levels of viremia before starting cART (Chun *et al.,* 2011), duration of virologic suppression (Maggiolo *et al.,* 2012; Allavena *et al.,* 2013), and deepness of HIV RNA suppression (Maggiolo *et al.,* 2012) all seem important factors affecting the extent of RV. Female gender and younger age probably favor "non-detectable" RV (Allavena *et al.,* 2013). Fewer previous regimens (Allavena *et al.,* 2013), non-nucleosidic reverse transcriptase inhibitor based cART (Bonora *et al.,* 2009; Haim-Boukobza *et al.,* 2011; Maggiolo *et al.,* 2012) and composition of the therapeutic regimen can influence RV levels due to the different genetic power of antiretrovirals and their ability to cooperate with each other (Shen *et al.,* 2008; Bonora *et al.,* 2009; Haim-Boukobza *et al.,* 2011). The levels of RV correlate directly with both the amount of circulating CD4 T-lymphocytes and with the proviral DNA load before the beginning of ART (50). Today, however, it is not yet possible to establish a precise relationship between these

two virological parameters, and in addition the size of the HIV reservoir is probably profoundly underestimated (at least 50 times) (Siliciano and Siliciano 2013).
Further research is needed to address the issue of RV and specific clinical and virologic situations. In particular, the role of coinfection with hepatitis viruses as well as with CMV and EBV on RV levels still needs to be assessed. It is also possible that patients having clinical involvement of HIV sanctuaries, such as the central nervous system, may have higher RV because of continuing viral replication due to lower antiretroviral action in this anatomical district. Moreover, the time needed to obtain low-levels of RV (i.e. <3 cp/ml) is currently under investigation and may be substantially different from the 6 months suggested by HIV guidelines for achieving treatment success (HIV RNA <50 cp/ml). Finally, the relationship between HIV RNA and HIV DNA decay curves needs to be explored.
In conclusion, the significance of RV is at present one of the most interesting research topics in the field of HIV for developing new therapeutic strategies aimed at eradicating HIV or at finding a functional cure for the infection. In the past, maximal attention was paid to the power of antiretroviral regimens and the patient's medication adherence only when viremia levels in plasma resulted detectable and quantifiable. Now, with more sophisticated tools to detect and measure HIV viremia, therapeutic interventions to attack the virus directly in the reservoir may need to include RV as a marker of incomplete viral suppression.

Patients with low-level viremia
M. Clementi, S. Rusconi
Evidence. Evidence confirming the importance of suppressing HIV-1 viremia comes from the large body of work describing the immunological response to antiretroviral therapy. Indeed, therapy for antiretroviral naïve HIV-1-infected subjects may differ from that for drug-experienced individuals, and its kinetics, including 4 reduction steps, are well characterized (Palmer *et al.,* 2011; Doyle *et al.,* 2012). Various HIV therapeutic guidelines (from DHHS to EACS, including national ones) state that the goal of antiretroviral therapy is the optimum suppression of viral replication, which is generally defined as a viral load persistently below the level of detection (<20-75 copies/ml), which depends on the assay used. From this point of view, it is important to emphasize the elusiveness of the definition of "low viremia level" since it has been used with various nuances both in and outside clinical trials, with HIV-RNA cut-offs ranging from 1 to 400 copies/ml (Doyle and Geretti 2012; Doyle *et al.,* 2012).
The goal in the therapeutic armamentarium of reaching a persistent viral suppression in the peripheral plasma is balanced by the concept that a significant drop in HIV-1 burden will also occur in reservoirs, either tissutal or cellular. Different drug classes can act in different ways and some, namely integrase inhibitors (INI) and also ritonavir-boosted protease inhibitors (PI/r), can cause a rapid drop in HIV-1-RNA particularly in the first phase of viral decay (Murray *et al.,* 2007).
Nevertheless, sensitivity tests confirmed the persistence of HIV-1-RNA load in approximately 80% of subjects who received a suppressive HAART for more than 7 years. Interruption of antiretroviral treatment results in a dramatic rise of viral replication, which derives from a viral replication that is not completely controlled. Reduced adherence, even during a successful antiretroviral regimen, can inexorably lead to sustained ongoing viral replication consequently leading to the emergence of drug resistance and to virological failure.
During early antiretroviral treatment, a viremia decline of <1 log at week 4 is significantly correlated with an increased risk of virological failure. Low-level viremia may persist despite long-term virological success. This is due to the continuous production of viral particles by HIV-1-infected cellular reservoirs. The clinical consequences of this residual viremia can be dangerous for the clinical progression of HIV-infected patients (Dornadula *et al.,* 2001).
Where does this low-level HIV-1 replication originate? There are several sources for this increasingly controlled viral burden, including maintenance of a transcriptionally silent (latent) HIV-1 genome in long-lived, resting target cells such as CD4 memory T cells, proliferation of the latently infected cells, inadequate anti-HIV-1 clearance mechanisms, and ongoing

de novo infection of susceptible target cells through continued viral replication.
Analysis of HIV-1-RNA results in preceding years can predict the persistence of detectable levels of plasma viremia. A recent study has demonstrated that there is a significant difference in the virologic outcome in three levels of plasma viremia:, always under 1 copy/ml, between 1 and 50 copies/ml, and never fully suppressed. So it is clear that control of virus replication is a dynamic process and that the present virus comes from one that was previously uncontrolled.
Population. The analysis of low-level viremia is definitely important for HIV-1-infected subjects in different clinical and therapeutic settings. This analysis needs to be carried out to compare significant differences between baseline and further time-points.
The conditions that may be considered include acute infection, chronic infection, and in the case of virologic success, therapeutic strategy of induction-maintenance, PI/r monotherapy and salvage therapy.
PI/r monotherapy is particularly interesting in the light of more recent PI/r. Two clinical trials using darunavir (DRV)/r have been reported: MONET and MONOI. Analysis of HIV-1 RNA <5 copies/ml at week 144 in the MONET study (observed data analysis) found 83.3% and 80.4% in the standard and DRV/r therapy arms respectively The proportion of subjects enrolled with HIV-1 RNA <1 copy/ml at week 144 in the MONET study was 66% and 63% in the triple and monotherapy arms respectively (Lambert-Niclot *et al.,* 2011).
There an increasing patient population receiving different anti-HIV-1 compounds belonging to the class of integrase inhibitors (INI).
At the time of writing, Raltegravir (RAL) is the only INI that is fully licensed by both the FDA and EMA. This compound has been studied in several clinical trials including BENCHMRK trials (Steigbigel *et al.,* 2010) in drug-experienced subjects with virological failure. Due to the excellent safety profile and potency of RAL, its use has been extended (July 2009) to drug-naïve individuals - e.g. STARTMRK trial (Lennox *et al.,* 2010).
Several trials of Elvitegravir (EVG) have been completed but it is still early in its clinical use. The drug is licensed in the United States in a single tablet regimen (Stribild) and has received a favorable opinion from the EMA.
dolutegravir (DTG) is the newest INI, which has had very convincing results. DTG was tested in INI-failing patients in the Viking-3 trial (Nichols *et al.,* 2013), in drug-naïve subjects as in the Spring-2 & Single trials (Curtis *et al.,* 2013), and more recently in drug-experienced INI-naïve patients in the Sailing trial (Cahn *et al.,* 2013).
Intervention. Several laboratory parameters such as inflammatory biomarkers and indicators of immune-activation can be investigated: hsPCR, IL-6, D-dimer, CD38+DR+, CD14/LPS, and annexin-V. HIV-1 DNA quantification. Although HIV-1 DNA quantification in PBMCs and other cell sources has been reported, quantification of the various forms of HIV-1 DNA within cells currently remains a research goal. The various forms of HIV-1 DNA include integrated HIV-1 DNA, linear unintegrated HIV-1 DNA, and 1 or 2-LTR circles. HIV-1 DNA forms that can be quantified include total HIV-1 DNA, 2-LTR circles, and integrated HIV-1 DNA, although methods for the latter DNA form are still being optimized.
Outcome. Low-level viremia is caused by the continuous production of viral particles in HIV-1-infected cellular reservoirs despite anti-retroviral therapy. This condition reflects an incomplete block of viral replication by treatment and exposes the patient to the selection of multi-drug-resistant viral variants. From this point of view, low-level viremia can be considered an at-risk condition, and the clinical consequences of this residual viremia can be dangerous for the clinical progression of HIV-1-infected patients (Dornadula *et al.,* 2001).

CONCLUSION

Evolution of HIV clinical practice is strictly dependent on the development of new approaches able to fill the present gap between scientific evidence and real life environment. In recent years, levels of awareness on the possible issues affecting clinical trials due to incorrect definition, duration of observation and patient enrollment have been raised. This position paper identifies critical parameters and population

characteristics that need to be taken in account for the design of future clinical studies. New and more accurate methodologies and outcome measurements of efficacy and safety will play a fundamental role in addressing these questions.
List of Abbreviations: HIV: human immunodeficiency virus; AIDS: acquired immunodeficiency syndrome; PHI: primary HIV infection; cART: combination antiretroviral therapy; ART: antiretroviral therapy; RTC: randomized controlled trials; TDR: transmitted drug resistance; VL: viral load; TLOVR: time to loss of virological response; NCI: neurocognitive impairment; CNS: central nervous system; IFN: interferon; RBV: ribavirin; INI: integrase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; PI: protease inhibitors; RV: residual viremia; DAA: direct-acting antiviral agents; VACS: veterans aging cohort study index.

Author contributions (Working Groups): Early treatment of patients: A. Lazzarin (Coordinator); Patients with PHI: G. Tambussi, A. Gori; Patients with high CD4 count: early treatment as prevention: A. Castagna, E. Nicastri; Advanced patients with infectious comorbidities: C.F. Perno (Coordinator); Late/AIDS presenter patients: A. d'Arminio Monforte, C. Mussini; Resistance transmitted to naïve patients: C.F. Perno, M. Santoro; Naïve patients with high viral loads: S. Bonora, P. Cinque; Patients with neurocognitive impairment: C. Mastroianni; Patients with HBV/HCV coinfection: G. Di Perri, M. Puoti; Patients with non-infectious comorbidities: A. Antinori (Coordinator); Patients with chronic inflammation and cardiovascular risk. Role of biomarkers: P. Maggi, M. Galli; Patients with renal damage. Safety endpoints: P. Bonfanti; Patients with metabolic or bone damage. Safety endpoints: M. Borderi; Experienced patients residual viremia or with low-level viremia: M. Andreoni (Coordinator); Patients with suppressed or residual viremia: M.R. Capobianchi, A. Ammassari; Patients with low-level viremia: M. Clementi, S. Rusconi.

ACKNOWLEDGEMENTS
This work was supported by ViiV Healthcare Italy. We thank Dr Elisa De Crignis for technical editing of the manuscript.

REFERENCES

Abreu C.M., Price S.L., Shirk E.N., Cunha R.D., Pianowski L.F., et al. (2014). Dual role of novel ingenol derivatives from Euphorbia tirucalli in HIV replication: inhibition of de novo infection and activation of viral LTR. *PLoS One.* **9,** e97257.

Adler A., Mounier-Jack S., Coker R.J. (2009). Late diagnosis of HIV in Europe: definitional and public health challenges. *AIDS Care.* **21**, 284-293.

Agarwal K., Fung S.K., Nguyen T.T., Cheng W., Sicard E., et al. (2013). Twenty-eight day safety and efficacy of tenofovir alafenamide (TAF) fumarate in chronic hepatitis B (CHB) patients AASLD, Washington DC.

Aguilar Marucco D., Veronese L., de Requena D.G., Bonora S., Calcagno A., et al. (2007). "Antiretroviral activity of pegylated interferon alfa-2a in patients co-infected with HIV/hepatitis C virus. *J. Antimicrob. Chemother*. **59** (3), 565-568.

Agwu A.L., Bethel, Hightow-Weidman J., Sleasman J.W., Wilson C.M., et al. (2012). Substantial multiclass transmitted drug resistance and drug-relevant polymorphisms among treatment-naive behaviorally HIV-infected youth. *AIDS Patient Care STD.*S **26**, 193-196.

Allavena C., Rodallec A., Secher S., Reliquet V., Baffoin S., et al. (2013). 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.* **85**, 1878-1882.

Amendola A., Bloisi M., Marsella P., Sabatini R., Bibbo, et al. (2011). Standardization and performance evaluation of "modified" and "ultrasensiti-ve" versions of the Abbott RealTime HIV-1 assay, adapted to quantify minimal residual viremia. *J. Clin. Virol.* **52**, 17-22.

Ananworanich J., Schuetz A., Vandergeeten C., Sereti I., de Souza M., et al. (2012). Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. *PLoS One.* **7**, e33948.

Anderson J.A., Archin N.M., Ince W., Parker D., Wiegand A., et al. (2011). 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.* **85**, 5220-5223.

Antinori A., Arendt G., Becker J.T., Brew B.J., Byrd D.A., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology.* **69**, 1789-1799.

Antinori A., Coenen T., Costagiola D., Dedes N., Ellefson M., et al. (2011). Late presentation of HIV infection: a consensus definition. *HIV Med.* **12**, 61-64.

Antinori A., Marcotullio S., Ammassari A., Andreoni M., Angarano G., et al. (2012). Italian guidelines for the use of antiretroviral agents and the diagnostic-clinical management of HIV-1 infected persons. Update 2011. *New Microbiol.* **35**, 113-159.

Armenia D., Santoro M.M., Cozzi-Lepri A., Calcagno A., Forbici F., et al. (2013). Impact of pre-HAART viral load on virological rebound in HIV-1 infected patients starting their first line Regimen. 1th European Meeting on HIV & Hepatitis, Rome, Italy.

Assoumou L., Descamps D., Yerly S., Dos Santos G., Marcelin A.G., et al. (2013). Prevalence of HIV-1 drug resistance in treated patients with viral load >50 copies/mL in 2009: a French nationwide study. *J. Antimicrob. Chemother.* **68**, 1400-1405.

Attia S., Egger M., Muller M., Zwahlen M., Low N. (2009). Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *Aids*. **23**, 1397-1404.

Baker J.V., Neuhaus J., Duprez D., Freiberg M., Bernardino J.I., et al. (2012). HIV replication, inflammation, and the effect of starting antiretroviral therapy on plasma asymmetric dimethylarginine, a novel marker of endothelial dysfunction. *J. Acquir. Immune Defic. Syndr.* **60**, 128-134.

Bellacosa C., Maggi P., Leone A., Altizio S., Ladisa N., et al. (2012). Carotid intima media thickness changes, endothelial activation and inflammatory markers in advanced naïve HIV patients starting antiretroviral therapy. 11th International Congress on Drug Therapy in HIV Infection, Glasgow, Journal of the International AIDS Society 2012.

Bertagnolio S., Perno C.F., Vella S., Pillay D. (2013). The Impact of HIV Drug Resistance on the Selection of First- and Second-Line ART in Resource-Limited Settings. *J. Infect. Dis.* **207** (Suppl. 2), S45-48.

Bertelsen K. (2013). Effect of Protein Binding on Darunavir PK with and without Telaprevir. 14th International Workshop on Clinical Pharmacology of HIV Therapy, Amsterdam, Reviews in Antiviral Therapy & Infectious Diseases.

Boger R.H. (2003). The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. *Cardiovasc. Res.* **59**, 824-833.

Bonora S., Nicastri E., Calcagno A., Gonzalez de Requena D., D'Ettorre G., et al. (2009). Ultrasensitive assessment of residual HIV viraemia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional evaluation. *J. Med. Virol*. **81**, 400-405.

Borroto-Esoda K., Waters J.M., Bae A.S., Harris J.L., Hinkle J.E., et al. (2007). Baseline genotype as a predictor of virological failure to emtricitabine or stavudine in combination with didanosine and efavirenz. *AIDS Res. Hum. Retroviruses*. **23**, 988-995.

Branson B.M., Stekler J.D. (2012). Detection of acute HIV infection: we can't close the window. *J. Infect. Dis.* **205**, 521-524.

Brenchley J.M., Price D.A., Schacker T.W., Asher T.E., Silvestri G., et al. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12**, 1365-1371.

Brennan T.P., Woods J.O., Sedaghat A.R., Siliciano J.D., Siliciano R.F., et al. (2009). Analysis of human immunodeficiency virus type 1 viremia and provirus in resting CD4+ T cells reveals a novel source of residual viremia in patients on antiretroviral therapy. *J. Virol.* **83**, 8470-8481.

Burdo T.H., Lo J., Abbara S., Wei J., DeLelys M.E., et al. (2011). Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J. Infect. Dis.* **204**, 1227-1236.

Buzon M.J., Codoner F.M., Frost S.D., Pou C., Puertas M.C., et al. (2011). Deep molecular characterization of HIV-1 dynamics under suppressive HAART. *PLoS Pathog.* **7**, e1002314.

Cahn P., Pozniak A.L., Mingrone H., Brites C., Andrade-Villanueva J.F., et al. (2013). 7th IAS Conference on HIV pathogenesis, treatment and prevention, Kuala Lumpur, Malaysia.

Calmy A., Gayet-Ageron A., Montecucco F., Nguyen A., Mach F., et al. (2009). HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *Aids*. **23**, 929-939.

Calza L. (2012). Renal toxicity associated with antiretroviral therapy. *HIV Clin. Trials*. **13**, 189-211.

Cambiano V., Bertagnolio S., Jordan M.R., Lundgren J.D., Phillips A. (2013). Transmission of drug resistant HIV and its potential impact on mortality and treatment outcomes in resource-limited settings. *J. Infect. Dis.* **207** (Suppl. 2), S57-62.

Castor D., Low A., Evering T., Karmon S., Davis B., et al. (2012). Transmitted drug resistance and phylogenetic relationships among acute and early HIV-1-infected individuals in New York City. *J. Acquir. Immune Defic. Syndr.* **61**, 1-8.

Centers for Disease Control and Prevention. "Hepatitis B FAQs for Health Professionals." from http://www.cdc.gov/hepatitis/HBV/HBVfaq.htm.

Champenois K., Cousien A., Cuzin L., Le Vu S., Deuffic-Burban S., et al. (2012). Missed opportunities for HIV testing in newly diagnosed HIV-infected patients in France. 19th Conference on Retroviruses and Opportunistic Infections (CROI, Seattle.

Chun T.W., Finzi D., Margolick J., Chadwick K., Schwartz D., et al. (1995). In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat. Med.* **1**, 1284-1290.

Chun T.W., Justement J.S., Moir S., Hallahan C.W., Maenza J., et al. (2007). Decay of the HIV reservoir in patients receiving antiretroviral therapy

for extended periods: implications for eradication of virus. *J. Infect. Dis.* **195**, 1762-1764.

Chun T.W., Murray D., Justement J.S., Hallahan C.W., Moir S., et al. (2011). Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J. Infect. Dis.* **204**, 135-138.

Chun T.W., Shawn Justement J., Murray D., Kim C.J., Blazkova J., et al. (2013). Effect of antiretroviral therapy on HIV reservoirs in elite controllers. *J. Infect. Dis.* **208**, 1443-1447.

Clotet B., Feinberg J., van Lunzen J., Khuong-Josses M.A., Antinori A., et al. (2014). Once-daily dolutegravir versus darunavir plus ritonavir in antiretroviral-naive adults with HIV-1 infection (FLAMINGO): 48 week results from the randomised open-label phase 3b study. *Lancet*. **383**, 2222-2231.

Cohen C., Wohl D., Arribas J., Henry K., Bloch M., et al. (2013). STaR Study: Single-Tablet Regimen Rilpivirine/Emtricitabine/tenofovir DF Has Non-Inferior Efficacy Compared to Efavirenz/Emtricitabine/tenofovir DF and Improves Patient Reported Outcomes. 7th IAS Conference on HIV Pathogenesis, Treatment, and Prevention, Kuala Lumpur, Malaysia.

Cohen C.J., Andrade-Villanueva J., Clotet B., Fourie J., Johnson M.A., et al. (2011a). Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. *Lancet*. **378**, 229-237.

Cohen M.S., Chen Y.Q., McCauley M., Gamble T., Hosseinipour M.C., et al. (2011b). Prevention of HIV-1 infection with early antiretroviral therapy. *N. Engl. J. Med.* **365**, 493-505.

Cossarini F., Boeri E., Canducci F., Salpietro S., Bigoloni A., et al. (2011). Integrase and fusion inhibitors transmitted drug resistance in naive patients with recent diagnosis of HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* **56**, e51-54.

Curtis L.D., Min S., Nichols G., Wynne B., Stainsby C., et al. (2013). Once-daily dolutegravir (DTG; S/GSK1349572) has a renal safety profile comparable to raltegravir (RAL) and efavirenz in antiretroviral (ART)-naïve adults: 48 week results from SPRING-2 (ING113086) and SINGLE (ING114467). 7th IAS Conference on HIV pathogenesis, treatment and prevention, Kuala Lumpur, Malaysia.

Danesh J., Whincup P., Walker M., Lennon L., Thomson A., et al. (2000). "ow grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ.* **321**, 199-204.

De Luca A., de Gaetano Donati K., Colafigli M., Cozzi-Lepri A., De Curtis A., et al. (2013). The association of high-sensitivity c-reactive protein and other biomarkers with cardiovascular disease in patients treated for HIV: a nested case-control study. *BMC Infect. Dis.* **13**, 414.

De Rosa F.G., Bargiacchi O., Audagnotto S., Garazzino S., Cariti G., et al. (2006). The early HCV RNA dynamics in patients with acute hepatitis C treated with pegylated interferon-alpha2b. *Antivir. Ther.* **11**, 165-171.

Deeks S.G., Lewin S.R., Havlir D.V. (2013). The end of AIDS: HIV infection as a chronic disease. *Lancet*. **382**, 1525-1533.

Dinoso J.B., Kim S.Y., Wiegand A.M., Palmer S.E., Gange S.J., et al. (2009). Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA.* **106**, 9403-9408.

Dornadula G., Nunnari G., Vanella M., Roman J., Babinchak T., et al. (2001). Human immunodeficiency virus type 1-infected persons with residual disease and virus reservoirs on suppressive highly active antiretroviral therapy can be stratified into relevant virologic and immunologic subgroups. *J. Infect. Dis*. **183**, 1682-1687.

Doyle T., Geretti A.M. (2012). Low-level viraemia on HAART: significance and management. *Curr. Opin. Infect. Dis.* **25**, 17-25.

Doyle T., Smith C., Vitiello P., Cambiano V., Johnson M., et al. (2012). Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. *Clin. Infect. Dis.* **54**, 724-732.

Duprez D.A., Neuhaus J., Kuller L.H., Tracy R., Belloso W., et al. (2012). Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS One.* **7**, e44454.

Eaton J.W., Johnson L.F., Salomon J.A., Barnighausen T., Bendavid E., et al. (2012). HIV treatment as prevention: systematic comparison of mathematical models of the potential impact of antiretroviral therapy on HIV incidence in South Africa. *PLoS Med.* **9**, e1001245.

Ellis R., Langford D., Masliah E. (2007). HIV and antiretroviral therapy in the brain: neuronal injury and repair. *Nat. Rev. Neurosci.* **8**, 33-44.

European AIDS Clinical Society (EACS). (2014, November 2014). EACS Treatment Guidelines Version 7.1." from http://www.eacsociety.org/files/guidelines_english_71_141204.pdf.

European AIDS Clinical Society Guidelines (EACS). (2013). Guidelines for the Clinical Management and Treatment of HIV infected adults in Europe. Version 7.0. from http://www.eacsociety.org/Portals/0/Guidelines_Online_131014.pdf,.

European Association For The Study Of The Liver (2012). EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J. Hepatol.* **57**, 167-185.

Fidler S., Oxenius A., Brady M., Clarke J., Cropley I., et al. (2002). Virological and immunological effects of short-course antiretroviral therapy in primary HIV infection. *Aids.* **16**, 2049-2054.

Fiebig E.W., Wright D.J., Rawal B.D., Garrett P.E., Schumacher R.T., et al. (2003). Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *Aids.* **17**, 1871-1879.

Finzi D., Blankson J., Siliciano J.D., Margolick J.B., Chadwick K., et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* **5**, 512-517.

Fisher M. (2008). Late diagnosis of HIV infection: major consequences and missed opportunities. *Curr. Opin. Infect. Dis.* **21**, 1-3.

Freiberg M.S., McGinnis K., Butt A., Goetz M.B. Brown S., et al. (2011). HIV is associated with clinically confirmed myocardial infarction after adjustment for smoking and other risk factors conference on retroviruses and opportunistic infections, Boston.

Frentz D., Boucher C.A., van de Vijver D.A. (2012). Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world. *AIDS Rev.* **14**, 17-27.

Funderburg N.T., Mayne E., Sieg S.F., Asaad R., Jiang W., et al. (2010). Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood.* **115**, 161-167.

Gandhi R.T., Coombs R.W., Chan E.S., Bosch R.J., Zheng L., et al. (2012). 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.* **59**, 229-235.

Gane E.J., Stedman C.A., Hyland R.H., Ding X., Pang P.S., et al. (2013). ELECTRON: 100% SVR rate for once-daily sofosbuvir plus ledipasvir plus ribavirin given for 12 weeks in treatment-naïve and previously treated patients with HCV GT 1 patients. 20th Conference on retroviruses and opportunistic infections (CROI), Atlanta.

Gardner E.M., McLees M.P., Steiner J.F., Del Rio C., Burman W.J. (2011). The spectrum of engagement in HIV care and its relevance to test-and-treat strategies for prevention of HIV infection. *Clin. Infect. Dis.* **52**, 793-800.

Ge D., J. Fellay, Thompson A.J., Simon J.S., Shianna K.V., et al. (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature.* **461**, 399-401.

Gianotti N., Galli L., Racca S., Salpietro S., Cossarini F., et al. (2012). Residual viraemia does not influence 1 year virological rebound in HIV-infected patients with HIV RNA persistently below 50 copies/mL. *J. Antimicrob. Chemother.* **67** (1), 213-217.

Gill V.S., Lima V.D. Zhang W., Wynhoven B., Yip B., et al. (2010). Improved virological outcomes in British Columbia concomitant with decreasing incidence of HIV type 1 drug resistance detection. *Clin. Infect. Dis.* **50**, 98-105.

Glaubitz J., Sizmann D., Simon C.O., Hoffmann K.S., Drogan D., et al. (2011). Accuracy to 2nd International HIV-1 RNA WHO Standard: assessment of three generations of quantitative HIV-1 RNA nucleic acid amplification tests. *J. Clin. Virol.* **50**, 119-124.

Grijsen M.L., Steingrover R., Wit F.W., Jurriaans S., Verbon A., et al. (2012). No treatment versus 24 or 60 weeks of antiretroviral treatment during primary HIV infection: the randomized Primo-SHM trial. *PLoS Med.* **9**, e1001196.

Grund B., Baker J., Deeks S.G., Wolfson J., Wentworth D., et al. (2013). Combined effect of interleukin-6 and D-dimer on the risk of serious non-AIDS conditions: data from 3 prospective cohorts. 20th Conference on Retroviruses and Opportunistic Infections, Atlanta.

Gupta R.K., Jordan M.R., Sultan B.J., Hill A., Davis D.H., et al. (2012). Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet.* **380**, 1250-1258.

Haim-Boukobza S., Morand-Joubert L., Flandre P., Valin N., Fourati S., et al. (2011). Higher efficacy of nevirapine than efavirenz to achieve HIV-1 plasma viral load below 1 copy/ml. *Aids.* **25**, 341-344.

Hamers R.L., Wallis C.L., Kityo C., Siwale M., Mandaliya K., et al. (2011). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. *Lancet Infect. Dis.* **11**, 750-759.

Hatano H., Delwart E.L., Norris P.J., Lee T.H., Dunn-Williams J., et al. (2009). Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. *J. Virol.* **83**, 329-335.

Hatano H., Hayes T.L., Dahl V., Sinclair E., Lee T.H., et al. (2011). A randomized, controlled trial of raltegravir intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4+ T cell response. *J. Infect. Dis.* **203**, 960-968.

Hatano H., Strain M.C., Scherzer R., Bacchetti P., Wentworth D., et al. (2013a). Increase in 2-long terminal repeat circles and decrease in D-dimer after raltegravir intensification in patients with treated HIV infection: a randomized, placebo-controlled trial. *J. Infect. Dis.* **208**, 1436-1442.

Hatano H., Yukl S.A., Ferre A.L., Graf E.H., Somsouk M., et al. (2013b). Prospective antiretroviral treatment of asymptomatic, HIV-1 infected controllers. *PLoS Pathog*. **9**, e1003691.

Havlir D.V., Strain M.C., Clerici M., Ignacio C., Trabattoni D., et al. (2003). Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. *J. Virol.* **77**, 11212-11219.

Heaton R.K., Clifford D.B., Franklin D.R. Jr., Woods S.P., Ake C., et al. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. **75** (23), 2087-2096.

Henrich T.J., Wood B.R., Kuritzkes D.R. (2012). Increased risk of virologic rebound in patients on antiviral therapy with a detectable HIV load <48 copies/mL. *PLoS One*. **7**, e50065.

High K.P., Brennan-Ing M., Clifford D.B., Cohen M.H., Currier J., et al. (2012). HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. *J. Acquir. Immune Defic. Syndr.* **60**, (Suppl. 1): S1-18.

Hirsch M.S., Gunthard H.F., Schapiro J.M., Brun-Vezinet F., Clotet B., et al. (2008). Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. *Clin. Infect. Dis.* **47**, 266-285.

HIV/AIDS Italian Expert Panel. (2014, December 18). Linee Guida Italiane sull'utilizzo dei farmaci antiretrovirali e sulla gestione diagnostico-clinica delle persone con infezione da HIV-1. from http://www.salute.gov.it/imgs/C_17_pubblicazioni_2261_allegato.pdf.

Hogan, C.M., Degruttola V., Sun X., Fiscus S.A., Del Rio C., et al. (2012). The setpoint study (ACTG A5217): effect of immediate versus deferred antiretroviral therapy on virologic set point in recently HIV-1-infected individuals. *J. Infect. Dis.* **205**, 87-96.

Hurt C.B. (2011). Transmitted resistance to HIV integrase strand-transfer inhibitors: right on schedule. *Antivir. Ther.* **16**, 137-140.

Ipp H., Zemlin A. (2013). The paradox of the immune response in HIV infection: when inflammation becomes harmful. *Clin. Chim. Acta.* **416**, 96-99.

Joos B., Fischer M., Kuster H., Pillai S.K., Wong J.K., et al. (2008). HIV rebounds from latently infected cells, rather than from continuing low-level replication. *Proc. Natl. Acad. Sci. USA.* **105**, 16725-16730.

Jordan M.R., Bennett D.E., Wainberg M.A., Havlir D., Hammer S., et al. (2012). Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004-2011. *Clin. Infect. Dis.* **54**, (Suppl. 4), S245-249.

Justice A.C., Freiberg M.S., Tracy R., Kuller L., Tate J.P., et al. (2012). Does an index composed of clinical data reflect effects of inflammation, coagulation, and monocyte activation on mortality among those aging with HIV? *Clin. Infect. Dis*. **54**, 984-994.

Khansari N., Shakiba Y., Mahmoudi M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Pat. Inflamm. Allergy Drug Discov.* **3**, 73-80.

Kieran J., Schmitz S., O'Leary A., Walsh C., Bergin C., et al. (2013). The relative efficacy of boceprevir and telaprevir in the treatment of hepatitis C virus genotype 1. *Clin. Infect. Dis.* **56**, 228-235.

Koenig W., Sund M., Frohlich M., Fischer H.G., Lowel H., et al. (1999). C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation.* **99**, 237-242.

Kuller L.H., Tracy R., Belloso W., De Wit S., Drummond F., et al. (2008). Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med.* **5**, e203.

Kuritzkes D.R., Lalama C.M., Ribaudo H.J., Marcial M., Meyer W.A., 3rd, et al. (2008). Preexisting resistance to nonnucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment-naive HIV-1-infected subjects. *J. Infect. Dis.* **197**, 867-870.

Kurth A.E., Mayer K., Beauchamp G., McKinstry L., Farrior J., et al. (2012). Clinician practices and attitudes regarding early antiretroviral therapy in the United States. *J. Acquir. Immune Defic. Syndr.* **61**, e65-69.

Lada O., Gervais A., Branger M., Peytavin G., Roquebert B., et al. (2012). Quasispecies analysis and in vitro susceptibility of HBV strains isolated from HIV-HBV-coinfected patients with delayed response to tenofovir. *Antivir. Ther.* **17**, 61-70.

Lambert-Niclot S., Flandre P., Valantin M.A., Peytavin G., Duvivier C., et al. (2011). Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. *J. Infect. Dis.* **204**, 1211-1216.

Lange C.M., Bojunga J., Ramos-Lopez E., von Wagner M., Hassler A., et al. (2011). Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J. Hepatol.* **54**, 887-893.

Lazzaretti C., Borghi V., Franceschini E., Guaraldi G., Mussini C. (2012). Engagment and retention

in care of patients diagnosed with HIV infection and enrolled in the Modena HIV Surveillance Cohort. 11th International Congress on Drug Therapy in HIV Infection, Journal of the International AIDS Society 2012.

Lennox J.L., Dejesus E., Berger D.S., Lazzarin A., Pollard R.B., et al. (2010). Raltegravir versus Efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy, durability, subgroup, safety, and metabolic analyses. *J. Acquir. Immune Defic. Syndr.* **55**, 39-48.

Little S.J., Holte S., Routy J.P., Daar E.S., Markowitz M., et al. (2002). Antiretroviral-drug resistance among patients recently infected with HIV. *N. Engl. J. Med.* **347**, 385-394.

Llibre J.M., Buzon M.J., Massanella M., Esteve A., Dahl V., et al. (2012). Treatment intensification with raltegravir in subjects with sustained HIV-1 viraemia suppression: a randomized 48-week study. *Antivir. Ther.* **17**, 355-364.

Lodi S., Phillips A., Touloum G., Geskus R., Meyer L., et al. (2011). Time from human immunodeficiency virus seroconversion to reaching CD4+ cell count thresholds <200, <350, and 500 Cells/mm(3): assessment of need following changes in treatment guidelines. *Clin. Infect. Dis.* **53**, 817-825.

Lucas G.M., Mehta S.H., Atta M.G., Kirk G.D., Galai N., et al. (2007). End-stage renal disease and chronic kidney disease in a cohort of African-American HIV-infected and at-risk HIV-seronegative participants followed between 1988 and 2004. *Aids.* **21**, 2435-2443.

Maggiolo F., Callegaro A., Cologni G., Bernardini C., Velenti D., et al. (2012). Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. *J. Acquir. Immune Defic. Syndr.* **60**, 473-482.

Markowitz M., Zolop A., Squires K., Ruane P., Coakley D., et al. (2014). Phase I/II study of the pharmacokinetics, safety and antiretroviral activity of tenofovir alafenamide, a new prodrug of the HIV reverse transcriptase inhibitor tenofovir, in HIV-infected adults. *J. Antimicrob. Chemother.* **69**, 1362-1369.

Mavigner M., Delobel P., Cazabat M., Dubois M., L'Faqihi-Olive F.E., et al. (2009). HIV-1 residual viremia correlates with persistent T-cell activation in poor immunological responders to combination antiretroviral therapy. *PLoS One.* **4**, e7658.

McGee B., Smith N., Aweeka F. (2006). "HIV pharmacology: barriers to the eradication of HIV from the CNS. *HIV Clin Trials.* **7**, 142-153.

McMahon D., Jones J., Wiegand A., Gange S.J., Kearney M., et al. (2010). 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.* **50**, 912-919.

McNamara L.A., Collins K.L. (2011). Hematopoietic stem/precursor cells as HIV reservoirs. *Curr Opin HIV/AIDS.* **6**, 43-48.

Melander O., Newton-Cheh C., Almgren P., Hedblad B., Berglund G., et al. (2009). Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. *JAMA.* **302**, 49-57.

Mind Exchange Working G. (2013). Assessment, diagnosis, and treatment of HIV-associated neurocognitive disorder: a consensus report of the mind exchange program. *Clin. Infect. Dis.* **56**, 1004-1017.

Mocroft A., Furrer H.J., Miro J.M., Reiss P., Mussini C., et al. (2013a). The incidence of AIDS-defining illnesses at a current CD4 count >/= 200 cells/muL in the post-combination antiretroviral therapy era. *Clin. Infect. Dis.* **57**, 1038-1047.

Mocroft A., Lundgren J.D., Sabin M.L., Monforte A., Brockmeyer N., et al. (2013b). Risk factors and outcomes for late presentation for HIV-positive persons in Europe: results from the Collaboration of Observational HIV Epidemiological Research Europe Study (COHERE). *PLoS Med.* **10**, e1001510.

Molina J.M., Cahn P., Grinsztejn B., Lazzarin A., Mills A., et al. (2011). Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. *Lancet.* **378**, 238-246.

Moore R.D., Bartlett J.G. (2011). Dramatic decline in the HIV-1 RNA level over calendar time in a large urban HIV practice. *Clin. Infect. Dis.* **53**, 600-604.

Mugavero M.J., Amico K.R., Horn T., Thompson M.A. (2013). The state of engagement in HIV care in the United States: from cascade to continuum to control. *Clin. Infect. Dis.* **57**, 1164-1171.

Murray J.M., Emery S., Kelleher A.D., Law M., Chen J., et al. (2007). Antiretroviral therapy with the integrase inhibitor raltegravir alters decay kinetics of HIV, significantly reducing the second phase. *Aids.* **21**, 2315-2321.

Musselwhite L.W., Sheikh V., Norton T.D., Rupert A., Porter B.O., et al. (2011). Markers of endothelial dysfunction, coagulation and tissue fibrosis independently predict venous thromboembolism in HIV. *Aids.* **25**, 787-795.

Nasta P., Gatti F., Borghi F., Amadasi S., Chiari E., et al. (2011). Maraviroc (MVC) reduces liver stiffness (LS) in HIV-hepatitis C (HCV) co-infected patients. 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome.

Ndiaye B., Ould-Kaci K., Salleron J., Bataille P., Bonnevie F., et al. (2009). Incidence rate and risk

factors for loss to follow-up in HIV-infected patients from five French clinical centres in Northern France - January 1997 to December 2006. *Antivir. Ther.* **14**, 567-575.

Negredo E., Massanella M., Puertas M.C., Buzon M.J., Puig J., et al. (2013). Early but limited effects of raltegravir intensification on CD4 T cell reconstitution in HIV-infected patients with an immunodiscordant response to antiretroviral therapy. *J. Antimicrob. Chemother.* **68**, 2358-2362.

Neuhaus J., Jacobs D.R. Jr., Baker J.V., Calmy A., Duprez D., et al. (2010). Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J. Infect. Dis.* **201**, 1788-1795.

Nichols G., Lazzarin A., Maggiolo F., Penco G., Wright D., et al. (2013). Phase 3 assessment of dolutegravir (DTG) 50 mg twice daily in HIV-1-infected subjects with raltegravir (RAL) and/or elvitegravir (EVG) resistance in VIKING-3: week 24 results of all 183 patients enrolled. 7th IAS Conference on HIV pathogenesis, treatment and prevention, Kuala Lumpur, Malaysia.

Ochoa-Callejero L., Perez-Martinez L., Rubio-Mediavilla S., Oteo J.A., Martinez A., et al. (2013). Maraviroc, a CCR5 antagonist, prevents development of hepatocellular carcinoma in a mouse model. *PLoS One.* **8**, e53992.

Ostrowski S.R., Katzenstein T.L., Pedersen B.K., Gerstoft J., Ullum H. (2008). Residual viraemia in HIV-1-infected patients with plasma viral load <or=20 copies/ml is associated with increased blood levels of soluble immune activation markers. *Scand. J. Immunol.* **68**, 652-660.

Pace M.J., Graf E.H., Agosto L.M., Mexas A.M., Male F., et al. (2012). Directly infected resting CD4+T cells can produce HIV Gag without spreading infection in a model of HIV latency. *PLoS Pathog.* **8**, e1002818.

Palella F.J. Jr., Armon C., Buchac K., Cole S.R., Chmiel J.S., et al. (2009). The association of HIV susceptibility testing with survival among HIV-infected patients receiving antiretroviral therapy: a cohort study. *Ann. Intern. Med.* **151**, 73-84.

Palella F.J. Jr., Gange S.J., Benning L., Jacobson L., Kaplan R.C., et al. (2010). Inflammatory biomarkers and abacavir use in the Women's Interagency HIV Study and the Multicenter AIDS Cohort Study. *Aids.* **24**, 1657-1665.

Palmer S., Josefsson L., Coffin J.M. (2011). HIV reservoirs and the possibility of a cure for HIV infection. *J. Intern. Med.* **270**, 550-560.

Palmer S., F. Maldarelli, A. Wiegand, B. Bernstein, G.J. Hanna, et al. (2008). Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci.* USA. **105**, 3879-3884.

Palmisano L., Giuliano M., Nicastri E., Pirillo M.F., Andreotti M., et al. (2005). Residual viraemia in subjects with chronic HIV infection and viral load < 50 copies/ml: the impact of highly active antiretroviral therapy. *Aids.* **19**, 1843-1847.

Panel on Antiretroviral Guidelines for Adults and Adolescents. (2014a, November 13, 2014). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 2014, from http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.

Panel on Antiretroviral Guidelines for Adults and Adolescents. (2014b, November 13, 2014). "Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents." from http://aidsinfo.nih.gov/contentfiles/adultandadolescentgl003093.pdf.

Panel on Guidance on Indicator Condition-Guided HIV testing in Adults. (2012). HIV indicator conditions: guidance for implementing HIV testing in adults in health care settings. from http://hiveurope.eu/Portals/0/Documents/Guidance.pdf.pdf.

Papagno L., Spina C.A., Marchant A., Salio M., Rufer N., et al. (2004). Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. *PLoS Biol.* **2**, E20.

Parikh R.V., Scherzer R., Grunfeld C., Nitta E.M., Leone A., et al. (2013). Elevated levels of asymmetric dimethylarginine are associated with lower CD4+ count and higher viral load in HIV-infected individuals. *Atherosclerosis.* **229**, 246-252.

Pierson T., McArthur J., Siliciano R.F. (2000). Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu. Rev. Immunol.* **18**, 665-708.

Pozniak A.L., Gallant J.E., DeJesus E., Arribas J.R., Gazzard B., et al. (2006). Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes--a 96-week analysis. *J. Acquir. Immune Defic. Syndr.* **43**, 535-540.

Price M.A., Wallis C.L., Lakhi S., Karita E., Kamali A., et al. (2011). Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. *AIDS Res. Hum. Retroviruses*. **27** (1), 5-12.

Prinapori R., Rosso R., Di Biagio A., Nicolini L., Giacobbe D.R., et al. (2013). Phalangeal quantitative ultrasound: cheaper methods for screening and follow-up of bone pathologies in HIV-infected women? *New Microbiol.* **36**, 345-351.

Puertas M.C., Massanella M., Llibre J.M., Ballestero M., Buzon M.J., et al. (2014). Intensification of a raltegravir-based regimen with maraviroc in early HIV-1 infection. *Aids.* **28**, 325-334.

Ridker P.M., Cushman M., Stampfer M.J., Tracy R.P.,

Hennekens C.H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N. Engl. J. Med.* **336**, 973-979.

Ridker P.M., Glynn R.J., Hennekens C.H. (1998). C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation.* **97**, 2007-2011.

Ridker P.M., Hennekens C.H., Buring J.E., Rifai N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.* **342**, 836-843.

Ross M.J. (2014). Advances in the pathogenesis of HIV-associated kidney diseases. *Kidney Int.* **86**, 266-274.

Rozera G., Abbate I., Bruselles A., Vlassi C., D'Offizi G., et al. (2009). Massively parallel pyrosequencing highlights minority variants in the HIV-1 env quasispecies deriving from lymphomonocyte sub-populations. *Retrovirology.* **6**, 15.

Rusconi S., Vitiello P., Adorni F., Colella E., Foca E., et al. (2013). Maraviroc as intensification strategy in HIV-1 positive patients with deficient immunological response: an Italian randomized clinical trial. *PLoS One.* **8**(11): e80157.

Saez-Cirion A., Bacchus C., Hocqueloux L., Avettand-Fenoel V., Girault I., et al. (2013). Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog.* **9**, e1003211.

Sahu G.K., D. Paar, S.D. Frost, M.M. Smith, S. Weaver, et al. (2009). Low-level plasma HIVs in patients on prolonged suppressive highly active antiretroviral therapy are produced mostly by cells other than CD4 T-cells. *J. Med. Virol.* **81**, 9-15.

Santoro M.M., Armenia D., Alteri C., Flandre P., Calcagno A., et al. (2013). Impact of pre-therapy viral load on virological response to modern first-line HAART. *Antivir. Ther.* **18**, 867-876.

Sauzullo I., Lichtner M., Mengoni F. (2010). Effect in vitro of CCR5 antagonists on innate immune system: maraviroc inhibits the migration of neutrophils, macrophages, and DC. 17th conference on retroviruses and opportunistic infections 2010, San Francisco.

Sax P., Brar I., Elion R., Zolopa A., Ortiz R., et al. (2013). 48 Week Study of tenofovir Alafenamide (TAF) vs. tenofovir Disoproxil Fumarate (TDF), Each in a Single Tablet Regimen (STR) with Elvitegravir, cobicistat, and Emtricitabine [E/C/F/TAF vs. E/C/F/TDF] for Initial HIV Treatment. 53rd ICAAC, Denver.

Schouten E.J., Jahn A., Midiani D., Makombe S.D., Mnthambala A., et al. (2011). Prevention of mother-to-child transmission of HIV and the health-related millennium development goals: time for a public health approach. *Lancet.* **378**, 282-284.

Schrager L.K., D'Souza M.P. (1998). Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. *JAMA.* **280** (1), 67-71.

Shen L., Peterson S., Sedaghat A.R., McMahon M.A., Callender M., et al. (2008). Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. *Nat. Med.* **14**, 762-766.

Shen L., Siliciano R.F. (2008). Viral reservoirs, residual viremia, and the potential of highly active antiretroviral therapy to eradicate HIV infection. *J. Allergy Clin. Immunol.* **122**, 22-28.

Siliciano J.D., Kajdas J., Finzi D., Quinn T.C., Chadwick K., et al. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat. Med.* **9**, 727-728.

Siliciano J.D., Siliciano R.F. (2013). HIV-1 eradication strategies: design and assessment. *Curr. Opin. HIV/AIDS.* **8**, 318-325.

Silverberg M.J., Chao C., Leyden W.A., Xu L., Tang B., et al. (2009). HIV infection and the risk of cancers with and without a known infectious cause. *Aids.* **23**, 2337-2345.

Silverberg M.J., Lau B., Jing Y., D'Souza G., Engels E.A., et al. (2012). Risk of anal cancer in HIV-infected patients and HIV-infected controls in North America. 12th International Conference on malignancies in AIDS and other acquired immunodeficiencie, Bethesda, MD.

Stadeli K.M., Richman D.D. (2013). Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. *Antivir. Ther.* **18**, 115-123.

Steigbigel R.T., Cooper D.A., Teppler H., Eron J.J., Gatell J.M., et al. (2010). Long-term efficacy and safety of Raltegravir combined with optimized background therapy in treatment-experienced patients with drug-resistant HIV infection: week 96 results of the BENCHMRK 1 and 2 Phase III trials. *Clin. Infect. Dis.* **50**, 605-612.

Sterling T.R., Chaisson R.E., Moore R.D. (2001). HIV-1 RNA, CD4 T-lymphocytes, and clinical response to highly active antiretroviral therapy. *Aids.* **15**, 2251-2257.

Sulkowski M.S., Thomas D.L. (2003). Hepatitis C in the HIV-Infected Person. *Ann. Intern. Med.* **138**, 197-207.

Sundstrom J.B., Ellis J.E., Hair G.A., Kirshenbaum A.S., Metcalfe D.D., et al. (2007). Human tissue mast cells are an inducible reservoir of persistent HIV infection. *Blood.* **109**, 5293-5300.

Swiggard W.J., O'Doherty U., McGain D., Jeyakumar D., Malim M.H. (2004). Long HIV type 1 reverse transcripts can accumulate stably within resting CD4+ T cells while short ones are degraded. *AIDS Res. Hum. Retroviruses.* **20**, 285-295.

Tang M.W., R.W. Shafer. (2012). HIV-1 antiretroviral

resistance: scientific principles and clinical applications. *Drugs*. **72**, e1-25.

Tate J.P., Justice A.C., Hughes M.D., Bonnet F., Reiss P., et al. (2013). An internationally generalizable risk index for mortality after one year of antiretroviral therapy. *Aids*. **27**, 563-572.

Tavel J.A., Huang C.Y., Shen J., Metcalf J.A., Dewar R., et al. (2010). Interferon-alpha produces significant decreases in HIV load. *J. Interferon. Cytokine Res*. **30**, 461-464.

Taylor N., Grabmeier-Pfistershammer K., Egle A., Greil R., Rieger A., et al. (2013). Cobas ampliprep/cobas TaqMan HIV-1 v2.0 assay: consequences at the cohort level. *PLoS One*. **8**, e74024.

Thompson M.A., Aberg J.A., Hoy J.F., Telenti A., Benson C., et al. (2012). Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA*. **308**, 387-402.

Thorat S.R., Chaturbhuj D.N., Hingankar N.K., Chandrasekhar V., Koppada R., et al. (2011). Surveillance of transmitted HIV type 1 drug resistance among HIV type 1-positive women attending an antenatal clinic in Kakinada, India. *AIDS Res. Hum. Retroviruses*. **27**, 1291-1297.

Tien P.C., Choi A.I., Zolopa A.R., Benson C., Tracy R., et al. (2010). Inflammation and mortality in HIV-infected adults: analysis of the FRAM study cohort. *J. Acquir. Immune Defic. Syndr.* **55**, 316-322.

Tourret J., Deray G., Isnard-Bagnis C. (2013). Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? *J. Am. Soc. Nephrol.* **24**, 1519-1527.

Tozzi V., Balestra P., Bellagamba R., Corpolongo A., Salvatori M.F., et al. (2007). Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: prevalence and risk factors. *J. Acquir. Immune Defic. Syndr.* **45**, 174-182.

Tozzi V., Balestra P., Salvatori M.F., Vlassi C., Liuzzi G., et al. (2009). Changes in cognition during antiretroviral therapy: comparison of 2 different ranking systems to measure antiretroviral drug efficacy on HIV-associated neurocognitive disorders. *J. Acquir. Immune Defic. Syndr.* **52**, 56-63.

Tracy R.P., Lemaitre R.N., Psaty B.M., Ives D.G., Evans R.W., et al. (1997). Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the cardiovascular health study and the rural health promotion project. *Arterioscler. Thromb. Vasc. Biol.* **17**, 1121-1127.

Tshabalala M., Manasa J., Zijenah L.S., Rusakaniko S., Kadzirange G., et al. (2011). Surveillance of transmitted antiretroviral drug resistance among HIV-1 infected women attending antenatal clinics in Chitungwiza, Zimbabwe. *PLoS One*. **6**, e21241.

Van Lint C., Bouchat S., Marcello A. (2013). HIV-1 transcription and latency: an update. *Retrovirology*. **10**, 67.

van Rensburg E.J., Tait K., Watt A., Schall R. (2011). 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.* **49**, 377-379.

Vandamme A.M., Camacho R.J., Ceccherini-Silberstein F., de Luca A., Palmisano L., et al. (2011). European recommendations for the clinical use of HIV drug resistance testing: 2011 update. *AIDS Rev.* **13**, 77-108.

Vercauteren J., Wensing A.M., van de Vijver D.A., Albert J., Balotta C., et al. (2009). Transmission of drug-resistant HIV-1 is stabilizing in Europe. *J. Infect. Dis.* **200**, 1503-1508.

Watters S.A., Mlcochova P., Gupta R.K. (2013). Macrophages: the neglected barrier to eradication. *Curr. Opin. Infect. Dis.* **26**, 561-566.

Wheeler W.H., Ziebell R.A., Zabina H., Pieniazek D., Prejean J., et al. (2010). Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. *Aids*. **24**, 1203-1212.

Whitney J.B., Hill A.L., Sanisetty S., Penaloza-MacMaster P., Liu J., et al. (2014). Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature*. **512**, 74-77.

Widdrington J., Payne B., Medhi M., Valappil M., Schmid M.L. (2011). The significance of very low-level viraemia detected by sensitive viral load assays in HIV infected patients on HAART. *J. Infect.* **62**, 87-92.

Wilby K.J., Greanya E.D., Ford J.A., Yoshida E.M., Partovi N. (2012). A review of drug interactions with boceprevir and telaprevir: implications for HIV and transplant patients. *Ann. Hepatol.* **11**, 179-185.

Winston A., Garvey L. (2009). Clinical implications of antiretroviral pharmacokinetics and pharmacodynamics in the CNS. *HIV Therapy*. **3**, 7.

Wittkop L., Gunthard H.F., de Wolf F., Dunn D., Cozzi-Lepri A., et al. (2011). Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. *Lancet Infect. Dis.* **1**, 363-371.

Wohlgemut J., Lawes T., Laing R.B. (2012). Trends in missed presentations and late HIV diagnosis in a UK teaching hospital: a retrospective comparative cohort study. *BMC Infect. Dis.* **12**, 72.

Womack J.A., Goulet J.L., Gibert C., Brandt C., Chang C.C., et al. (2011). Increased risk of fragility fractures among HIV infected compared to uninfected male veterans. *PLoS One*. **6**, e17217.

World Health Organization. (2012a, April 2014). Hepatitis C Fact sheet N. 164, from http://www.who.int/mediacentre/factsheets/fs164/en/.

World Health Organization. (2012b). Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants.

World Health Organization. (2012c). WHO global strategy for the surveillance and monitoring of HIV drug resistance 2012.

World Health Organization. (2012d). WHO HIV drug resistance report 2012. from http://www.who.int/hiv/pub/drugresistance/report2012/en/.

World Health Organization. (2013). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. from http://hiveurope.eu/Portals/0/Guidance/2012-014_CHIP_losark-UK_v2.pdf.

Yerly S., Kaiser L., Perneger T.V., Cone R.W., Opravil M., et al. (2000). Time of initiation of antiretroviral therapy: impact on HIV-1 viraemia. The Swiss HIV Cohort Study. *Aids*. **14**, 243-249.

Yombi J.C., Pozniak A., Boffito M., Jones R., Khoo S., et al. (2014). Antiretrovirals and the kidney in current clinical practice: renal pharmacokinetics, alterations of renal function and renal toxicity. *Aids*. **28**, 621-632.

Yukl S.A., Gianella S., Sinclair E., Epling L., Li Q., et al. (2010a). Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. *J. Infect. Dis.* **202**, 1553-1561.

Yukl S.A., Li P., Fujimoto K., Lampiris H., Lu C.M., et al. (2011). Modification of the Abbott RealTime assay for detection of HIV-1 plasma RNA viral loads less than one copy per milliliter. *J. Virol. Methods*. **175**, 261-265.

Yukl S.A., Shergill A.K., McQuaid K., Gianella S., Lampiris H., et al. (2010b). 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*. **24**, 2451-2460.

Zeng M., Southern P.J., Reilly C.S., Beilman G.J., Chipman J.G., et al. (2012). Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. *PLoS Pathog.* **8**, e1002437.

Zhu T., Muthui D., Holte S., Nickle D., Feng F., et al. (2002). Evidence for human immunodeficiency virus type 1 replication in vivo in CD14(+) monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. *J. Virol.* **76**, 707-716.

Zolopa A., Ortiz R., Sax P., Brar I., Elion R., et al. (2013). Comparative study of tenofovir alafenamide vs tenofovir disoproxil fumarate, each with elvitegravir, cobicistat, and emtricitabine, for HIV treatment. 20th Conference on retroviruses and opportunistic infections, Atlanta.