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An update on integrase inhibitors: new opportunities for a personalized therapy? The NEXTAIM Project

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SUMMARY

Thanks to the development of antiretroviral agents to control HIV replication, HIV infection has turned from a fatal disease into a treatable chronic infection. The present work collects the opinions of several experts on the efficacy and safety of recently approved second generation of integrase inhibitors and, in particular, on the role of this new class of drugs in antiretroviral therapy.

The availability of new therapeutic options represents an opportunity to ameliorate the efficacy of cART in controlling HIV replication also within viral reservoirs. The personalization of the treatment driven mainly by the management of comorbidities, HIV-HCV co-infections and aging, will be easier with antiretroviral drugs without drug-drug interactions and with a better toxicity and tolerability profile.

Future assessment of economic impact for the introduction of new innovative drugs in the field of antiretroviral therapy will likely need some degree of adjustment of the evaluation criteria of costs and benefit which are currently based almost exclusively on morbidity and mortality.

KEY WORDS: HIV, Integrase Inhibitors, HAART, PEP, PrEP.

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INTRODUCTION

Adriano Lazzarin

After two decades of major achievements in HIV/AIDS care the goal of defeating HIV/AIDS is at a crucial juncture, both in terms of its immediate trajectory and its sustainability.

The development of an effective preventive vaccine and a cure remain the ultimate goals in the HIV field, however, given the current technical and scientific challenges, they are now considered long term objectives in the global health and development agendas.

Therefore, besides placing a sustained long term effort in pursuing these objectives, the scientific community must focus on the current needs in care and treatment of infected people.

In these regard, improvement of the profile of tolerability and efficacy of the available antiretroviral compounds is of pivotal importance in order to guarantee a *life-long* accessibility to the triple drug combinations that constitute modern ART to each HIV infected patient.

New drugs, including the long-acting antiretroviral formulations now in clinical development, could be considered as potential tools only in the frame of a *life-long* perspective for HIV therapy.

The NEXTAIM project is intended to provide a state-of-the-art review of the most recent therapeutic options, starting from the concept that the availability of a considerable number of different cART regimens including integrase inhibitors, showing superiority of the performances in comparison of the current standard of care, represents an unmissible opportunity to redraw the plan of action of HIV treatment.

Thanks to the considerable efficacy of cART, so far enormous gains have been made in controlling HIV replication, saving million of people from progression of HIV infection and AIDS related illness and death.

Therefore the next aim of antiretroviral therapy must be the capitalization of the innovations offered by new drugs to increase the efficacy in advanced naïve patients and the long term control of ongoing HIV replication in chronic patients with a reduced risk of toxicity and tolerability. In contrast to the current protocols for the evaluation of effectiveness of treatment options which are mostly based on the performances of the drugs in terms of reduction of morbidity and mortality, this project underlines the importance of an exhaustive evaluation of the actual welfare gain and cost-benefit ratios of cART regimens.

NEW OPTIONS FOR PRE - AND POST-EXPOSURE PROPHYLAXIS

Vincenzo Puro, Gabriella De Carli

Despite major advances in antiretroviral treatment, each year around 4000 new HIV-1 infections occur in Italy (Camoni *et al.* 2014), and 2 million new HIV-1 infections are estimated worldwide (UNAIDS 2013).

To succeed in achieving a significant reduction in HIV-1 incidence, the application of multiple prevention measures, i.e. combination HIV-1 prevention, is recommended, including biomedical interventions aimed at HIV-1 uninfected individuals in which antiretroviral drugs (ARVs) are provided as post-exposure (PEP) or pre-exposure (PrEP) prophylaxis (Marrazzo *et al.* 2014). In addition, PEP is a well consolidated measure for healthcare workers reporting an at-risk occupational exposure to HIV (Puro *et al.* 2004; Kuhar *et al.* 2013).

We here describe available findings for the potential role of HIV integrase inhibitors when used as PEP and PrEP.

Post-Exposure Prophylaxis

Animal models and observational human studies have established the biological plausibility of preventing HIV acquisition by prior use of nucleoside and nucleotide reverse transcriptase inhibitors (NRTI) (Tsai *et al.* 1995; Cardo *et al.* 1997; Van Rompay *et al.* 2001), as well as protease inhibitors (PI) (Bourry *et al.* 2009) and integrase strand transfer inhibitors (INSTI) (Dobard *et al.* 2014). Occupational guidelines were updated by the US Public Health Service in 2013, which advocate for using a three-drug regimen for 28 days regardless of the severity of exposure (Kuhar *et al.* 2013) preferring tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) with raltegravir (RAL) based on improved tolerability of these newer ARVs. Indeed, previous regimens including co-formulated NRTI and PI resulted highly affected by adverse events and inability to tol-

TABLE 1 - *Completion rates of various post exposure prophylaxis regimens over past 10 years. (Modified, from Jain S et al., 2014).*

Regimen (28 days)	*Study*	*Enrollment (N)*	*Exposure*	*Interruptions due to side effects*	*Completion rate*
AZT/3TC+TDF	Burty *et al.*, 2010	171	Occupational and sexual	12.9%	81.5%
AZT/3TC+PI (mostly NFV)	Rabaud *et al.*, 2001	79	Occupational and sexual	10.1%	64.5% (76% of whom with side effects)
	Mayer *et al.*, 2008	119	Sexual	N.R.	38.8%
	Winston *et al.*, 2005	225	Sexual	5.8%	68%
AZT/3TC+ FPV/RTV	Burty *et al.*, 2008	46	Occupational and sexual	13.0%	47.8% (54.5% of whom with side effects)
AZT/3TC+ATZ unboosted	Diaz-Brito *et al.*, 2012	98	Occupational and sexual	17.3%	64.3%
AZT/3TC+ LPV/RTV	Rabaud *et al.*, 2005	121	Occupational and sexual	16.5%;	64.5% (48.7% of whom with side effects)
	Diaz-Brito *et al.*, 2012	102	Occupational and sexual	15.7%;	63.7%
	IRAPEP, 2014	1806	Occupational and sexual	13.6%	77.4%
TDF/3TC+ ATZ/RTV	Burty *et al.*, 2010	152	Occupational and sexual	14.5%	79.0%
TDF/FTC+ LPV/RTV	Tosini *et al.*, 2010	188	Occupational and sexual	11.7%;	88.3%, (42.2% of whom with side effects)
	IRAPEP, 2014	639	Occupational and sexual	12.8%	76.8%
TDF/FTC+ RAL	Mayer *et al.*, 2012	100	Sexual	None	84%
	McAllister *et al.*, 2014	86	Sexual (MSM)	None	92% (9% of whom with side effects)
	IRAPEP, 2014	165	Occupational and sexual	1.8%	84.8% (29% of whom with side effects)

AZT: zidovudine; 3TC: lamivudine; TDF: tenofovir; PI: protease inhibitor; NFV: nelfinavir; FPV: fosamprenavir; RTV: ritonavir; ATZ: atazanavir; LPV: lopinavir; IRAPEP: Italian Registry of Antiretroviral Post Exposure Prophylaxis; FTC: emtricitabine; RAL: raltegravir; MSM: men who have sex with men.

erate the medication currently prescribed: in 10 PEP studies published since 2001, and including also data from the Italian Registry of Antiretroviral Post Exposure Prophylaxis up to 2014, 14% of 3231 treated individuals failed to complete the 28-day course because of adverse events, compared to 0.8% of 351 receiving TDF/FTC and RAL (Table 1). Also switching to RAL-based PEP regimen seems to be associated with a decrease in reported side effects (Annandale *et al.* 2012).

Apart from significantly more favorable tolerability, all three drugs in the recommended regimen (TDF, FTC and RAL) act before viral integration with cellular DNA, providing a theoretical advantage in preventing establishment of HIV infection. This regimen can be given in pregnancy (FDA pregnancy category: TDF and FTC: class B; RAL: class C).

Other features that make RAL an attractive candidate for PEP are its potency, ease of use (low pill burden, small tablets, and dosing independently of food), short time from administration to maximum plasma concentration (T_{max}), and low potential for drug interactions. The latter feature is the basis of the recommendation to use RAL in non-occupational PEP (NPEP) regimens started after sexual assault,

and whenever there is the need for providing emergency contraception with levonorgestrel (HIV/AIDS Italian Expert Panel 2014). Indeed, efavirenz (EFV) and ritonavir-boosted PIs are associated with decreased levels of contraceptive hormones that could compromise contraceptive effectiveness requiring an increased progestin dose (Robinson *et al.* 2012), while RAL is not known to affect the activity of any hepatic cytochrome isoenzymes, and a randomized two-arm crossover study failed to demonstrate clinically significant interactions between RAL and a triphasic combined oral contraceptive (Anderson *et al.* 2011).

Finally, RAL has a rapid absorption and a favorable pharmacokinetic profile with a median time to peak plasma concentration in healthy volunteers ranging from 0.5 to 1.3 h (Iwamoto *et al.* 2008; Brainard *et al.* 2011). As RAL targets an earlier stage of the virus life cycle than reverse transcriptase inhibitors, it can inhibit infection of primary CD4 T cells and macrophages when added at later post-infection time points, and thus the length of time after exposure to virus in which the drug can still prevent infection may be extended. The resulting, several hours' difference may be critical in the context of PEP (Marsden *et al.* 2012).

Recently revised PEP guidelines now recommend similar protocols whether exposures were occupational or non-occupational, including victims of sexual assault. World Health Organization (WHO) recommends that a three-drug low pill burden regimen is preferable, favoring TDF/FTC plus RAL or ritonavir-boosted darunavir (DRV/r) (World Health Organization 2014), while the New York State Department of Health AIDS Institute's Medical Care Criteria Committee now recommends TDF/FTC plus either RAL or dolutegravir (DTG) as the preferred initial PEP regimen because of its excellent tolerability, proven potency in established HIV infection, and ease of administration (NYSDOH 2014).

DTG has been more recently approved, and earlier studies have demonstrated that it is well tolerated and has a much higher barrier to resistance than RAL (Hightower *et al.* 2011; Kobayashi *et al.* 2011), a characteristic which would be ideal for high-risk exposures to highly treatment-experienced individuals. In addition, it can be administered once daily and does not require metabolic boosting. A clinical trial (NCT02211690) to assess tolerability of, and adherence to, DTG with TDF/FTC for HIV NPEP, is currently recruiting participants.

A recent review discussing NPEP trial data and management in details (Jain and Mayer 2014), suggests that several newer agents, such as the integrase strand inhibitors elvitegravir (EVG) and DTG, may be useful for PEP when combined with TDF/FTC, but require further study. Tolerability studies in HIV-1 uninfected individuals and long-term clinical experience are needed to guide whether these agents or other new compounds should be considered for routine use in NPEP.

Pre-Exposure prophylaxis

Clinical trials of oral PrEP have focused testing on regimens of TDF with or without FTC. However, TDF may be associated with toxicities (renal, bone) and FTC may select for drug resistance. Moreover, effectiveness is limited by low adherence (McMahon *et al.*, 2014). Thus, further agents that might serve as alternatives to TDF/FTC for HIV-1 prevention are needed. ARV drug formulations that require infrequent dosing may increase adherence and thus PrEP effectiveness.

Optimal PrEP agent(s), as defined by the Division of AIDS of the NIAID-NIH, would be safe and tolerable, penetrate and protect against HIV-1 infection in target tissues, be long-lasting with convenient dosing, have a unique resistance profile or a high barrier to resistance, have few or no drug-drug interactions, and be affordable, easy to use and implement. In addition, antiretrovirals that are not used commonly for HIV-1 treatment would be more attractive for use as PrEP agents.

In this regard, RAL is generally safe and well tolerated, and has few drug-drug interactions; its concentrations in vaginal secretions approximate those in blood, with a half-life about twice as long, while in gut-associated lymphoid tissue these are 1,5-7 fold higher (Jones *et al.*, 2009; Patterson *et al.*, 2012); and it has been assessed in a humanized mouse model, demonstrating efficacy as PrEP (Neff *et al.*, 2010). On the other side, it requires twice-daily dosing, has a low genetic barrier to resistance, and is

used commonly in HIV-1 treatment regimens. Due to these limitations, no current clinical studies of RAL PrEP are planned (Abraham and Gulick 2012).
A new, long-acting, investigational HIV-1 integrase inhibitor, Cabotegravir (GSK1265744) is in Phase II clinical development and seems a promising drug although integrase inhibitors are used commonly in HIV treatment. Cabotegravir is an INSTI and structural analogue of dolutegravir with potent anti-HIV-1 activity, a half-life of about 40 h when dosed orally, and a low propensity for drug interactions (Ford *et al.*, 2013). Monthly injections of the experimental drug, Cabotegravir LA, at plasma concentrations achievable with quarterly injections in humans, protected all six macaques from repeated attempts to infect the animals with intravaginal inoculations of SHIV twice a week for up to 11 weeks. (Radzio *et al.*, 2015). In a second study, where depot medroxyprogesterone acetate, which promotes viral transmission vaginally, was added, GSK744 LA treatment protected six of eight female rhesus macaques against three high-dose SHIV challenges (Andrews *et al.*, 2015).
In both studies, all controls receiving placebo were infected. These data support advancement of GSK744 LA as a potential PrEP candidate for women. Such a long-lasting drug would indeed help overcome one of the major problems with current medications that attempt to protect against HIV-1 infection - the ability of people to take their medication on a daily basis. A Phase III clinical trial program is expected to be launched soon.

Conclusions
For both PEP and PrEP, the ideal ARV should prevent the infection of cells when exposed to HIV-1, or at least abort the virus replication cycle acting before viral integration with cellular DNA, providing a theoretical advantage in preventing establishment of HIV-1 infection rather than limiting infection.
Before integration, the HIV-1 genome is labile and decays with a half-life of approximately 1 day (Zhou *et al.*, 2005), while once it has integrated, it can persist for the life of the infected cell (Finzi *et al.*, 1999). Therefore, the best opportunity for preventing a persistent infection is to abort the replication cycle of HIV-1 before it can integrate (Marsden *et al.*, 2012).
For both PEP and PrEP, other drug characteristics critical when selecting the appropriate ARVs are safety, tolerability, adequate penetration into target tissues, convenient dosing, and few potential drug-drug interactions. Safety is the most important quality of a PEP and PrEP agent, due to the fact that these preventive drugs are being targeted for use by HIV-1 uninfected individuals, while tolerability strongly impacts on the individual adherence to the proposed regimen, therefore affecting its potential efficacy.
As for PEP, currently available integrase inhibitors have many of the characteristics of an ideal agent and are currently recommended in first line regimens, while for PrEP, new agents in this class are being developed which show promising results and might represent a new option in HIV prevention.

EVIDENCES SUPPORTING THE USE OF INTEGRASE INHIBITORS FOR EARLY TREATMENT IN PHI

Giuseppe Tambussi, Silvia Nozza

The role of combination antiretroviral therapy (cART) in the management of Primary HIV-1 infection (PHI) remains controversial; current guidelines strongly recommend ART in symptomatic patients (HIV/AIDS Italian Expert Panel 2014). They generally are classified as Fiebig III-V stages.
In chronic HIV-1 infection cART reduces mortality and morbidity, but these data were non clear in PHI, due to small and proof of concept studies. The SPARTAC study is the first multicentric randomised trial that enrolled 366 participants; it demonstrated that a 48-week course of cART in patients with PHI delayed disease progression (Spartac Trial Investigators *et al.*, 2013). cART cannot cure the infection and stopping cART has been associated with risk (Saez-Cirion *et al.*, 2013). However, 14 HIV-1 patients has been identificated as post treatment controllers (PTCs), the viremia remained controlled for several years after the interruption of prolonged cART initiated during the primary infection (Strategies for Management

of Antiretroviral Therapy Study Group *et al.*, 2006). Moreover, the incidence of viral control after the interruption of early antiretroviral therapy was higher among the PTCs than has been reported for spontaneous control. These results show that early and prolonged cART may allow some individuals with a rather unfavorable background to achieve long-term infection control and may have important implications in the search for a functional HIV-1 cure.
There is not a recommended regimen in PHI treatment, but PIs based therapy were generally preferred due to higher genetic barrier. However, the implication of HIV-1 DNA in disease progression and viremia post treatment control made INSTIs an attractive option. SPARTAC trial explored associations between HIV-1 DNA and immunological and virological markers of clinical rebound, including viral rebound in those interrupting therapy. HIV-1 DNA was more predictive of disease progression than HIV-1 plasma viral load and, at treatment interruption, predicted time to plasma virus rebound. Fast decline of HIV-1 RNA associated with INSTIs usage is important in PHI, that generally is characterised by high viral load levels, for resolution of symptomatic stage and for implications in transmission.

Conclusions
There are not study of triple standard therapy with INSTIs and NRTIs in PHI treatment, but results in clinical trials in patients' subset with HIV-1 RNA viral load above 100,000 copies/mL are good; most important limitation is the resistance test, often not available when therapy is started. There are not registered INSTIs mutations in naïve patients, another motivation for the use of this class.

IMMUNOLOGICAL ASPECTS

Andrea Gori, Stefano Rusconi

The main objective of antiretroviral therapy was initially limited to the prevention of the clinical manifestations of AIDS, thus reducing the HIV/AIDS mortality. This objective has been largely achieved, due to enormous progress in the pharmaceutical arena (Guihot *et al.* 2010). Nevertheless, it is clear that the management of HIV-1 infection is rapidly changing and the results it is aimed nowadays are very different from before. The virologic control of HIV-1 cannot be considered the unique scope of antiretrovirals with a long-life therapy (Autran *et al.* 1997).
The new approach to HIV-1 disease includes non-AIDS morbidities, with a particular emphasis on cardiovascular pathologies, neurocognitive impairment, bone deterioration, and cancer. Despite a long-lasting control of HIV replication, we still detect elevated levels of inflammation and, less so, T cell immune-activation (Tenorio *et al.* 2014) and these altered parameters are somehow connected with the lack of immune reconstitution and the onset of non-AIDS morbidities. This process resembles what is seen in the physiological immunosenescence (Deeks 2011).
Our armamentarium is composed of several classes of antiretroviral drugs, so it is fundamental to understand the role of these compounds in regulating the immune system, thus preventing those pathologies that are related to its unbalance.

Pathogenesis of immune-reconstitution: the cross-link among HIV-1 disease, CD4 T lymphocytes and HIV-1 RNA
The progressive lost of CD4 T lymphocytes is one of the peculiar characteristics of HIV-1 disease. Although HIV-1 is targeted to the destruction of CD4 T lymphocytes, the pathogenetic pathway that causes their decrease is made up by a network of complex mechanisms (Gougeon and Montagnier 1999; Hunt *et al.* 2003; Bandera *et al.* 2010). Killing of CD4 T lymphocytes is mediated directly by the viral cytopathic effect and indirectly by various actions - e.g. apoptosis - that are stimulated by increased levels of immune-activation (Gougeon and Montagnier 1999; Hunt *et al.* 2003; Bandera *et al.* 2010). On the other side, the consequent homeostatic activity tries to counteract the HIV-1 mediated effect via the increased production of bone marrow progenitor cells (such as CD34 cells) and the cellular differentiation in the thymus (Isgro *et al.* 2008).
All of this, notwithstanding the fact that these two body districts are severely compromised by HIV-1 in the early phases of infection, together with the gastro-intestinal tract (Brenchley *et*

al. 2004). Thus, HIV-1 is able to compromise CD4 T naïve lymphocyte production through the interaction in the bone marrow and in the thymus, or in what is left behind. As a consequence, the deficit in the turnover of naïve CD4 T lymphocytes and of mature memory CD4 T lymphocytes is accelerated by the antigen-specific clonal expansion and the antigen-independent homeostatic defect. This malevolent network brings to an almost complete deprivation of the CD4 T cell compartment, both organic and functional.

In the absence of antiretroviral treatment, the progression of HIV disease is strictly related to HIV-1 RNA levels but its course is a consequence of other surrogate markers, such as HIV-1 DNA, residual viremia, and immune-activation (Hunt *et al.* 2003). All these parameters have been demonstrated to be connected to the mechanisms that are involved in the lack of immune response in HIV-1 infected patients who receive antiretrovirals and present an optimal virological response.

cART contribution

After starting an antiretroviral combination regimen, the usual observation is represented by a decrease in HIV-1 viral load and an increase in CD4 T lymphocytes. This last aspect is related to the removal of the block that precludes the immune-reconstitution. These complex mechanisms provide different kinetics according to the cell type that is involved. The CD4 T cell compartment is going towards an immune-reconstitution passing through two main stages. The first one, after the cART beginning and the consequent reduction in HIV-1 viremia, is constituted by a rapid increase of memory CD4 T lymphocytes, which is independent from a homeostatic mechanism of repopulating naïve CD4 T cells.

This early phase is mainly caused by redistribution processes from the lymphoid organs where cART reduced the load of HIV-1 replication and thus inflammation. This stage is characterized by a reduction in the pro-inflammatory cytokines and chemokines, such as TNF-α, IL-6 e MCP-1.

At the same time, there is an increase in the number of CD8 T lymphocytes, NK cells and B cells in the peripheral blood, also reflecting a non-specific mechanism of cellular redistribution induced by the reduction of viral replication. Next, the second phase of immune-reconstitution that occurs over several years is implemented and this the result of the regeneration of the population of naïve CD4 T lymphocytes, which restores the diversity of the T cell receptor (TCR) repertoire. The immune processes involving the reconstitution of naïve lymphocytes characteristically take place more slowly and involve cell phenotypes significantly more involved in the functional recovery of the correct T cell homeostasis. Of note, the compartment of the central memory CD4 T lymphocytes, particularly susceptible to HIV-1 infection during the course of the disease, seems to play a key role in the successful restoration of both numerical and mostly functional mechanisms of immune reconstitution after initiation of cART (Autran *et al.* 1997; Hunt *et al.* 2003).

In this regard, there is still some controversy on the role of cART for the proper reconstitution of the damaged CD4 T cell compartment, which had been infected with HIV-1. Some studies have reported, in some clinical conditions, failure in the reconstitution of CD4 T lymphocytes in the long term, showing a plateau in the process of immune-reconstitution after 4-5 years of cART. This phenomenon seems to reflect a variety of factors, including age, CD4 T cell counts before the start of cART (CD4 T cell nadir) and intermittent HIV-1 replication. It was also demonstrated that hyperactivation of CD4 T cell lymphocytes can persist even after obtaining virologic suppression mediated by cART and may have a significant adverse effect on the recovery of CD4 T lymphocytes during therapy. In fact, despite the drop of viral replication in plasma, subjects with a modest immune reconstitution maintain high levels of CD4 T lymphocytes activation, which is com(Cahn *et al.* 2013) parable to that observed in subjects who do not show viral suppression. These data, as well as highlighting the possible pathogenic role of immune-activation on the recovery of CD4 T lymphocytes, raise the issue of the real cause of the persistent lymphocyte activation. The residual viral replication, the persistent antigenic stimulation mediated by microbial translocation, and viral packaging

have been proposed as possible mechanisms underlying the persistent lymphocyte activation during cART (Hunt *et al.* 2003; Marchetti *et al.* 2006; Moore and Keruly 2007; Bandera *et al.* 2010).

Role of integrase inhibitors

The fastest dynamics of virologic suppression induced by integrase inhibitors may also affect the immune recovery to a higher degree. The hypothesis is that integrase inhibitors may extinguish the kinetics of viral replication faster and to a greater extent and are able to impact on the pathogenetic mechanisms at the basis of the processes of immune reconstitution in a more effective than the other antiretrovirals. This is particularly interesting in regards to the mechanism of T lymphocyte homeostasis, which allows a more complete recovery of the immune function.

Integrase inhibitors may be able to interact significantly on two essential mechanisms: immune-activation and immune-proliferation, as they are closely related to the mechanisms underlying the phenomena of immune exhaustion and the depletion of CD4 T lymphocytes related to HIV-1 replication. These effects are mirrored by the CD4 T lymphocytes gain and the improvement in the quality of immune system as seen in both naïve and drug-experienced patients (Cahn *et al.* 2013; Rockstroh *et al.* 2013).

VIROLOGICAL ASPECTS AND RESISTANCE

Maria Mercedes Santoro, Massimo Clementi, Carlo Federico Perno

Resistance profile of INSTIs

NSTIs potently inhibit HIV-1 and HIV-2 replication by blocking the strand-transfer reaction that catalyzes integration of HIV DNA into the host genome (Geretti *et al.*, 2012). Despite their high potency and tolerability, the first generation INSTIs RAL and EVG offer a low-to-moderate genetic barrier to resistance, depending on the background drug-regimen (Geretti *et al.*, 2012).

RAL failure is associated with integrase mutations in at least 3 distinct, but non-exclusive, genetic pathways defined by 2 or more mutations including a primary mutation among Q148H/K/R, N155H or Y143C/H/R and at least one additional minor mutation (Canducci *et al.*, 2010). Minor mutations described in the Q148H/K/R pathway include L74M, E138A/K, or G140S. The most common pathway is Q148H+G140S, which also confers the greatest loss of drug susceptibility. Minor mutations described in the N155H pathway include L74M, E92Q, T97A, Y143H, G163K/R, V151I, or D232N (Wensing *et al.*, 2014). N155H mutants tend to predominate early in the course of RAL failure, but are gradually replaced by viruses with higher resistance, often bearing mutations Q140S+Q148H/K/R, with continuing RAL treatment (Wensing *et al.*, 2014). Y143C/R is often associated with the polymorphic mutation T97A. This combination strongly reduces the susceptibility to RAL and rescues the catalytic defect due to the Y143C/R mutation (Reigadas *et al.*, 2011).

The following EVG mutations have been observed in INSTI-naïve and experienced patients in whom therapy is failing: T66A/K/I, E92Q/G, T97A, F121Y, S147G, Q148H/K/R, N155H (Wensing *et al.*, 2014). In the phase III studies GS-US-236-0102, patients failing a first line regimen containing EVG/cobicistat (COBI)/FTC/TDF fixed dose combination, the rate of resistance development was 2.9% (White *et al.*, 2015). Despite this very low prevalence of resistance emerged at failure, in EVG arm all patients with primary genotypic resistance-associated mutations to INSTI (mostly E92Q) showed also the M184I/V mutation. Differently, in the EFV arm, even if the prevalence of resistance was slightly higher, only around 30% of patients showed resistance to both non-nucleoside reverse transcriptase inhibitors (NNRTI) and NRTI class (White *et al.*, 2015). Thus a clear linkage of integrase resistance mutations and M184V/I was observed in this study as well as in other studies evaluating RAL resistance such as STARTMRK and QDMRK (Eron *et al.*, 2011; Rockstroh *et al.*, 2013).

RAL and EVG share cross-resistance: resistant strains emerge rapidly during in vitro passage experiments, and in a short-medium period in patients experiencing virological failure. For this reason, the sequential use of these two IN-

STIs (in either order) may be problematic.
Differently, DTG has limited cross-resistance to RAL and EVG in vitro, and offers the promise of an improved genetic barrier to resistance (Geretti *et al.*, 2012). Indeed, this INSTI maintains its activity in vitro against viruses with Q148 single mutants or against viruses with Y143 or N155 signature mutations regardless of RAL-associated secondary mutations (Canducci *et al.*, 2011). On the other hand, cross-resistance studies with RAL- and EVG-resistant viruses indicate that Q148H and G140S in combination with mutations L74I/M, E92Q, T97A, E138A/K, G140A, or N155H are associated with 5-fold to 20-fold reduced DTG susceptibility (Geretti *et al.*, 2012) and reduced virologic suppression under DTG treatment (Eron *et al.*, 2013a; Raffi *et al.*, 2013b; Castagna *et al.*, 2014). Indeed, in treatment experienced INSTI-resistant patients, the response rates (both at day 8-10 and week 24 of DTG-treatment) were closely related to the presence of Q148 plus ≥2 secondary mutations (among L74I, E138A/K/T and G140A/C/S) at baseline (Eron *et al.*, 2013a; Castagna *et al.*, 2014). These findings suggest that in RAL failing subjects, the presence of only few and specific INSTI resistance mutations confer reduced susceptibility to DTG.
Beyond the mutation already associated with resistance to first line INSTIs, DTG can select the specific R263K mutation with other specific mutations such as H51Y, G118R and F121Y in vivo and in vitro (Malet *et al.*, 2014; Mesplede and Wainberg 2014). However, these mutations strongly diminishes both viral replication capacity as well as the enzymatic activity of the integrase, impairing the ability of the viruses harbouring these mutations to acquire further resistance(Oliveira *et al.*, 2014).
This phenomenon might explain the fact that DTG, when used as part of first-line therapy, is the only HIV-1 drug that has not selected for resistance mutations in the clinic. Indeed, in individuals failing a first line regimen containing DTG, no INSTI, NRTI or PI mutations have been found (Raffi *et al.*, 2013a; Mesplede and Wainberg 2014).
Moreover, in pluri-treated individuals failing DTG as first INSTI, the proportion of subjects with evidence of INSTI resistance is significantly lower if they were treated with DTG (50 mg once daily, QD) than those failing to RAL (400 mg twice daily, BD) (Cahn *et al.*, 2013).
A potential role of integrase and reverse transcriptase mutations on susceptibility to INSTIs was recently described. Indeed, a synergistic effect of NNRTI resistance mutation K103N or E138K with INSTI resistance mutations G140S/Q148H on susceptibility to EFV and/or RAL was demonstrated (Hu and Kuritzkes 2014).

Efficacy of INSTIs

A crucial characteristic of the INSTI activity in vivo is the prompt virological response. Indeed, a rapid virological suppression was observed in patients treated with co-formulated EVG/COBI/FTC/TDF versus co-formulated EFV/FTC/tenofovir or co-formulated FTC/TDF and ritonavir-boosted atazanavir (ATV/r) (DeJesus *et al.*, 2012; Sax *et al.*, 2012). Again, in DTG Clinical Trial Program, a rapid and significant virological suppression has been observed with DTG vs DRV/r and EFV (Walmsley *et al.*, 2013; Clotet *et al.*, 2014).Similar results were previously reported when RAL was compared with EFV (Lennox *et al.*, 2009), and fully confirmed by clinical practice. Two recent systematic reviews and meta-analysis showed a significant clinical benefit in favour of INSTIs in combination with two NRTIs in HIV-1 individuals starting a first-line regimen in comparison to other third drug classes (Messiaen *et al.*, 2013; Lee *et al.*, 2014). In particular, the meta-analysis by Lee *et al.*, supported the superiority of all 3 INSTIs as third drug options compared to NNRTIs (Lee *et al.*, 2014). In the ACTG 5257 (aiming to compare the efficacy and safety of three non-nucleoside-sparing regimens in US antiretroviral-naïve adults with a HIV-1 RNA viral load >1,000 copies) first-line RAL proved superior to ATV/r or DRV/r (all taken with TDF/FTC) after 96 weeks of treatment in an endpoint combining virologic efficacy and safety. Findings from SPRING-2, SINGLE and FLAMINGO studies supported the use of DTG in adult treatment-naïve HIV-1-infected subjects (Raffi *et al.*, 2013a; Raffi *et al.*, 2013b; Walmsley *et al.*, 2013; Clotet *et al.*, 2014). A non-inferiority of DTG (50 mg QD) to RAL (400 mg BD) was overall demonstrated both at week 48 and week 96 (SPRING-2) (Raffi *et al.*, 2013a; Raffi *et al.*, 2013b). In particular, a superiority of

DTG to RAL at 96 weeks was demonstrated for baseline HIV-1 RNA viral load >100,000 copies/mL. A superiority of DTG plus abacavir (ABC)/lamivudine (3TC) compared to the single tablet regimen EFV/TDF/FTC was demonstrated at week 48, 96 and 144 (SINGLE study) (Walmsley *et al.*, 2013; Walmsley *et al.*, 2015). A superiority of DTG (50 mg QD) to DRV/r (800/100 mg QD) at 48 and 96 weeks of treatment was also demonstrated (FLAMINGO study) (Clotet *et al.*, 2014; Molina *et al.*, 2015).

Overall, these results imply similar efficacy within the INSTI class, a position since adopted by the Department of Health and Human Services in October 2013 and the International Antiviral Society - USA in July 2014, when all three INSTIs became listed as 'Preferred' options in combination with 2 NRTIs (DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents 2014; Gunthard *et al.*, 2014).

Novel treatment strategies at first line failure, such as the combination of INSTI with a PI (sparing the NRTIs) have been also explored. A large trial evaluating this concept (NEAT-001) showed that RAL boosted DRV is non-inferior at week 96 compared to TDF/FTC/DRV/r and may represent a treatment option for patients with CD4 T cell counts higher than 200 cells/μL (Raffi *et al.*, 2014). However, this RAL plus DRV/r was less effective in patients starting with a high baseline HIV-1 RNA viral load (>100,000 copies/mL).

Use of INSTI is beneficial even in treatment-experienced patients with virological failure (Messiaen *et al.*, 2013). In treatment-experienced INSTI-naïve patients, EVG QD was non-inferior to RAL BD and showed durable long-term efficacy (Elion *et al.*, 2013), while a superiority of DTG to RAL was demonstrated at week 48 (Cahn *et al.*, 2013). In highly treatment-experienced patients harbouring RAL- and/or EVG resistant viruses, the administration of DTG 50 mg BD is effective; a reduction of response is associated with the baseline presence of Q148 plus at least two resistance associated mutations (Castagna *et al.*, 2014; Akil *et al.*, 2015).

Recent evidence demonstrated that EVG, COBI, FTC in combination with 2 NRTIs might be a suitable alternative in virologically suppressed patients under a NNRTI based regimen (Pozniak *et al.*, 2014). However, in successfully treated patients with a history of therapy failure, switching a high genetic barrier drug towards an INSTI is not supported by the meta-analyses (Messiaen *et al.*, 2013). The results indicate that when switching virologically suppressed patients, individual patient management is needed to assess history of treatment failure, available resistance profiles and duration of the current suppressive regimens in order to perform a safe switch.

Conclusions

RAL, EVG and DTG appear equivalent in regard to their efficacy in therapy of both naïve and treatment-experienced HIV-1 individuals. A recently published network meta-analysis, that can provide estimates of relative efficacy for treatments not directly studied in head to-head randomized controlled trials, suggests that DTG is also favorable or comparable to other commonly used third agents (ATV/r, LPV/r, RPV, and EVG/c) (Patel *et al.*, 2014). However, substantial differences are found in regard to HIV-1 drug resistance. Indeed, because of the low/moderate genetic barrier of RAL and EVG, to avoid the resistance development, particular attention should be deserved on adherence levels and on background drugs used in both cART-naïve and cART-experienced patients treated with these INSTIs. The broad cross-resistance profile between RAL and EVG precludes their sequential use in individuals failing either of them.

Differently, in patients who have previously failed to RAL or EVG, even though the presence of Q148 with at least two additional INSTI mutations can decrease their response rate, DTG BD administration may warrant a residual efficacy. Moreover, the emergence of novel resistance under DTG treatment is a rare event that select viruses less prone to accumulate further resistance because of their low replication capacity.

Indeed, in treatment naïve patients, DTG is the only antiretroviral for which no emergent resistance has been detected. These unique properties of DTG may have relevance for public health strategies aimed at limiting or stopping the spread of HIV-1.

EVIDENCES SUPPORTING THE USE OF INTEGRASE INHIBITORS FOR FIRST LINE THERAPY

Antonella d'Arminio Monforte, Franco Maggiolo

The beginning of the cART era traces back to 1996 in Vancouver, where the two events which would have constituted the first two milestones of HIV therapy took place.

The first milestone was the the publication of the clinical studies involving protease inhibitors, whereas the second was the introduction of a quantitative method to measure plasma HIV-RNA, the diagnostic tool which allowed to shorten the time necessary for drug development of the future ARVs.

Despite having a precise date of birth, cART evolved gradually, led by a progressive optimization of the overall aims, endpoints, therapeutic regimens, and patient management.

The current aim of cART is extending the life span of the patients while at the same time preserving the quality of their lifes, i.e. a life without disease as well as without therapy side effects.

So far, cART is made by the combination of three ARVs, although this concept is currently debated. Despite little changes in the cART setup over the last two decades, significant progresses have been made in drug formulation, delivery and combination. Initially, the regimens were extremely demanding for the patients who needed to take up to 12 tablets per day. The request for multiple doses was mostly related to the short plasmatic half life of the majority of the molecules. It was also for this reason that high doses of ARVs were administered to the patients, increasing the likelihood of adverse events. To limit the onset of adverse effects and maximize the absorption of the active compounds, dietary restrictions and limitations to the intake of food and liquids were often mandatory. Over the time, the number of tablets, the number of daily doses as well as dietary restrictions have been significantly reduced. Among all the factors determining this evolution, three have played a major role. First, the introduction of new molecules and new classes of drugs, whose development was specifically targeted at improving farmacokinetic properties and absorption rate and at extending the half-lives of the active molecules. This was the case for the NNRTIs, which were introduced at the beginning of the cART era, and for the most recent PIs: Second, the intuition that modulation of drug metabolism could improve efficacy, tolerability and overall convenience of specific ARVs. Based on this notion, new drugs were developed to be used as boosters, i.e. drugs which influence the metabolism and excretion of other farmaceutic compounds by regulating the hepatic microsomal system. Finally, the commitment of the pharmaceutical industry which led to a constant evolution in terms of pharmacological techniques.

All together, these processes allowed to evolve new therapies with increasing efficacy, as demonstrated by the increase in the number of positive outcomes in clinical trials over the time. The initial goal of therapy optimization has been improvement of drug efficacy, however, in a later time the focus has been moved to the parallel improvement of tolerability since coupling these two characteristics guarantees better clinical outcomes. Integrase inhibitors, the most recent class of drugs introduced in HIV therapy, can be considered an example of this new line in drug development as shown by the recent drug registration studies.

Currently two new molecules belonging to INSTI class have been licensed: EVG (as single tablet regimen contained EVG/COBI/FTC/TDF) and DTG. The following paragraphs will describe the clinical trials which supported the registration of these new drugs.

The study 103 (DeJesus *et al.*, 2012) is a randomized double blind phase III clinical trial (1:1), aimed at evaluating efficacy, safety and tolerability of EVG 150 mg/COBI 150 mg/FTC 200 mg/TDF 300 mg (n=353) compared with ATV/r (300 mg ATV, 100 mg RTV) combined with FTC 200 mg/TDF 300 mg (n=355) in adult patients naive to therapy with HIV RNA >5000 copies/mL. The primary endpoint of the Study 103 was suppression of viral replication (HIV RNA <50 copies/mL) at 48 weeks of treatment, according to the FDA approved snapshot algorithm.

At baseline, average HIV RNA and CD4 T cell count were 4.88 log10 copies/mL and 364 cells/μL, respectively, in the EVG arm, whereas patients included in the ATV/r arm showed 4.86

log10 copies/mL HIV RNA and a CD4 T cell count of 375 cells/µL. Considering both arms together, 41% of the patients showed baseline plasma HIV RNA above 100000 copies/mL, and 13% had a baseline CD4 T cell count equal or lower than 200 cells/µL. CD4 T cell counts increased at similar rates for both treatment arms with an average increase of 207 cells/µL in the EVG arm and of 211 cells/µL in the ATV/r arm. The rate of virological failure was 5% for both therapeutic schemes. Common adverse events (at least 10% of the patients) for both treatment arms were: diarrhoea, nausea, upper respiratory tract infections, headache, fatigue and ocular jaundice. Common adverse events were reported at similar rates between the two treatment arms, with the exception of scleral jaundice which was more frequently observed in the EVG arm compared to the ATV/r arm (1% and 14%, respectively).

The Study 102 (Sax *et al.*, 2012) is a a randomized double blind phase III clinical trial (1:1), aimed at evaluating efficacy, safety and tolerability of the combination EVG/COBI/FTC/TDF (n=348) vs EFV 600 mg/FTC 200 mg/TDF 300 mg (n=352). Enrolled patients were adults naive to treatment with a plasma HIV RNA concentration of 5000 copies/mL or more. The primary endpoint of the Study 102 was suppression of viral replication (HIV RNA <50 copies/mL) at 48 weeks of treatment, according to the FDA approved snapshot algorithm.

At baseline, patients included in the EVG arm had an average plasma HIV-1 RNA of 4.75 log10 copies/mL and an average CD4 T cell count of 391 cells/µL. In EFV treated group, average plasma HIV-1 RNA was 4.78 copies/mL and average CD4 T cell count was 382 cells/µL. Overall 33% of patients had plasma HIV-1 RNA above 100.000 copies/mL, and 13% of patients had a CD4 T cell count equal or below 200 cells/µL. The overall virological failure rate was comparable (7%) in the two arms of treatment. Among the patients with plasma HIV RNA above 100.000 copies/mL at baseline, 84% of patients in the EVG arm and 82% of patients in the EFV arm achieved virological suppression. The average CD4 T cell count increase was 239 cells/µL among EVG treated patients and 206 cell/µL among EFV treated patients (p>0,009).

During the study, 4% of patients included in the EVG arm and 5% of patients included in the EFV arm stopped treatment due to adverse events. The adverse events which were more frequently reported, for both treatment arms, were: diarrhoea, nausea, and rash. EVG treated patients reported less frequently neuropsychiatric adverse effects including abnormal dreams (15% of the EVG treated patients vs 27% of the EFV treated patients), dizziness (7% vs 24%), and insomnia (9% vs 14%). The average increase in total cholesterol and LDL cholesterol was lower among EVG treated patients (+10 mg/dl total cholesterol; +10 mg/dl LDL cholesterol) compared to EFV treated patients (+19 mg/dl total cholesterol e +17 mg/dl LDL cholesterol) (p=0.001). The average increase in serum creatinine levels was 0,14 mg/dl in the EVG arm e a 0,01 mg/dl in the EFV arm.

The clinical evaluation of the more recent integrase inhibitor, DTG, included a comparison of the new drug with existing drug combinations including RAL or with EFV and DRV based drug regimens.

The SPRING study (Raffi *et al.*, 2013a) is a non-inferiority study, aimed at comparing the efficacy and safety of a single daily 50 mg dose of DTG with 400 mg twice daily dose of RAL, both administered in combination with two NRTIs. The 411 HIV patients naïve to treatment included in the study were randomly assigned to one of the two arms. The primary endpoint was the proportion of participants with HIV-1 RNA less than 50 copies per mL at 48 weeks according to the FDA approved snapshot algorithm. Primary endpoint, i.e. non inferiority of DTG compared to RAL, has been met. After 48 weeks of treatment 88% and 85% of patients showed complete virological suppression in the DTG and RAL arm, respectively. At week 96, 81% patients in the DTG group and 76% in the RAL group had HIV-1 RNA less than 50 copies per mL confirming non-inferiority (Raffi *et al.*, 2013a)

Tolerability was similar between treatment groups, with few adverse events leading to discontinuation (2% in each group). Approximately 10% of study participants in each group reported nausea attributable to the treatment; whereas other adverse events were reported in less than 5% of the patients enrolled in both treatment arms.

SINGLE is a double blind, multicenter randomized clinical trial comparing the safety and efficacy of DTG at a dose of 50 mg plus ABC/3TC once daily with the fixed dose combination EFV/tenofovir/emtricitabine (Walmsley *et al.*, 2013). Treatments were administered for 48 weeks in adult HIV-1 infected patients naïve to treatment. 833 patients were enrolled and randomly assigned to treatment, (414 treated with DTG based combination and 419 with control regimen). The median age of the patients enrolled in the study was 35 years, majority were man (84%), white, with a median CD4 T cell count was 338 cells/µL. After 48 weeks of treatment, 364 (88%) of patients in the DTG group achieved virological suppression (primary endpoint of the study according to the FDA Snapshot algorithm) compared to 338 (81%) in the control group meeting the criterion for DTG based combination superiority (p=0.003). No significant differences in virological response were observed between the two regimens. Moreover, virological response remained similar across subgroups after stratification for baseline viral load (threshold 100.000 copies/mL plasma HIV-1 RNA) and CD4 T cell count (threshold 200 cells/µL). Within the subgroup with a viremia below 100.000 copies/mL, 90% of patients in the DTG arm achieved virological suppression compared with 83% of patients in the EFV arm, whereas within the subgroup with a viremia above 100.000 copies/mL the percentage of patients achieving plasma HIV-1 RNA load <50 was 83% and 76% in the DTG and EFV arm, respectively. Overall patients showed a more favourable response in the DTG group compared with the EFV group, both within the subgroup with a baseline CD4 T cell count higher than 200/µL (89% vs 81%) as well as within the subgroup with a baseline CD 4 T cells count lower than 200 cells/µL (79% vs 77%), although these differences did not reach the level of statistical significance. Moreover, DTG treated patients achieved virological suppression more rapidly than EFV treated patients (28 days vs 84 days) and the regimen was associated to a significantly higher CD4 T cell increase (267 cells/µL in the DTG arm vs 208 cells/µL in the EFV arm; p<0.001).

Adverse events rate was slightly different between treatment groups, abnormal dreams, rash and dizziness were more common in the EFV group, whereas insomnia was reported more frequently among participants included in the DTG group. The number of patients who discontinued therapy owing to adverse events was lower in the DTG group than in the EFV group (10/414 patients [2%] in the DTG arm vs 42/419 [10%] in the EFV arm); of note, the higher tolerability of the DTG regimen contributed to its more favourable outcome that was significantly confirmed at week 144 where 71% of patients in DTG regimen vs 63% in EFV regimen maintained viral load suppression (p=0.01) (Walmsley *et al.*, 2015).

FLAMINGO (Clotet *et al.*, 2014; Molina *et al.*, 2014b) is a randomized study, comparing efficacy and tolerability of DTG or DRV/r combined with investigator-selected backbone NRTIs. HIV-1-positive naïve to antiretroviral therapy adults with HIV-1 RNA ≥1000 copies/mL and no evidence of viral resistance were enrolled in the study. Participants were stratified according to plasma HIV-1 RNA levels (100,000 copies/mL). The primary endpoint was the proportion of patients achieving HIV-1 RNA lower than 50 copies/mL (FDA snapshot and a non inferiority margin of -12%) by week 48. A total of 488 adults were randomized among the two groups, 243 in the DTG arm and 245 in the DRV/r arm. Of them, 86% and 79% completed the 96 weeks of treatment in the DTG group and DRV/r group, respectively. 7% of patients treated with DTG and 12% of patients treated with DRV/r discontinued therapy before reaching 48 weeks. Snapshot analysis at 48 and 96 weeks showed a significant virological response in favour of DTG arm compared with the DRV/r arm (90% vs 83% [p=0.025] at week 48; 80% vs 68% [p=0.002] at week 96) (Clotet *et al.*, 2014; Molina *et al.*, 2015) PIs. This difference was maintained across HIV-RNA subgroups and was independent of the backbone NRTIs (ABC/3TC or TDF/FTC).

Less than 1% of the patients included in the study reported sever adverse events. Of note, levels of LDL cholesterol were higher among participants receiving DRV/r, whereas serum creatinine levels were higher in the DTG arm. None of the patients experiencing virological failure had treatment-related resistance to study drugs.

Overall, new INSTIs recently introduced in clini-

cal practice proved to be efficient in suppressing viral load in naive patients, supporting their use in patients with high levels of HIV-1 replication. Moreover, due to the rapidity in inducing virological response, these new drugs might be particularly useful in patients identified at seroconversion. Finally, new INSTIs were well tolerated and showed no relevant pharmacological interactions supporting their use in patients under treatment for HIV-1 associated comorbidities. Taken together, these observations, promote the use of INSTIs as a component of first line cART. Given the simplicity and convenience of current cART regimens (1 or 2 pills/day) the inclusion of INSTIs appears to be a feasible resource for treatment of patients naive to antiretroviral therapy. Moreover, in the case of DTG clinical trials, no mutations have been reported, neither class specific nor involving other drugs included in the regimes. This makes DTG a valid alternative to regimens including PIs, in particular in subjects with high viremia. Given the absence of cross resistance between PIs and both INSTIs as well as NNRTIs, PIs could be used as a second line therapy in patients who fail the treatment with INSTIs and NNRTI.

ROLE OF INTEGRASE INHIBITORS IN THE MANAGEMENT OF VIROLOGICAL FAILURE

Antonella Castagna, Andrea De Luca

In recent years, thanks to the improved efficacy of current antiretroviral treatment regimens, virological failures occur less frequently in clinical practice.

However virological failures may sill occur in patients treated with different lines of antiretroviral therapy and different combinations of drug classes.

The use of integrase inhibitors after virological failure in patients naïve to integrase inhibitors

Several seminal studies have analyzed the role of the integrase inhibitors in this particular setting (Table 2). The Benchmrk studies were two identical Phase 3 randomized trials comparing the efficacy of RAL 400 mg bid versus placebo in addition to an optimized background therapy (OBT) in 699 patients with documented resistance to at least one drug in each of three historical classes of antiretroviral drugs.

TABLE 2 - *Efficacy of integrase inhibitors (INI)-based regimens in treatment failing INI-naïve individuals RCT, randomized controlled trial; RTG, raltegravir; EVG, elvitegravir; DTG, dolutegravir; OBT, optimized background therapy; VL, viral load.*

Study	*Type*	*INI type*	*Accompanying drugs*	*Number*	*48 weeks <50 cp/mL*	*Longer term virologic responses*	*Reference*
Benchmrk	RCT	RTG	OBT	462	62.1%	156w 51% VL<50	(Steigbigel *et al.* 2010; Eron *et al.* 2013)
Imaz	Prospective single arm	RTG	DRV/r, ETV	32	94% (24w)	-	(Imaz *et al.* 2009)
TRIO	Prospective single arm	RTG	DRV/r, ETV+ OBT	103	86%	96w 88% VL<50	(Yazdanpanah *et al.* 2009)
145	RCT	RTG	PI/r + 3rd agent	351	58%	96w 45% VL<50	(Elion *et al.* 2013)
145	RCT	EVG	PI/r + 3rd agent	351	59%	96w 47.6% VL<50	(Elion *et al.* 2013)
Nozza	Prospective single arm	RTG	MVC, ETV	28	92% (OT)	4 years 96% VL<50	(Nozza *et al.* 2010)
Capetti	Retrospective	RTG	Any	333		4-years 77% VL<50	(Capetti *et al.* 2014)
SAILING	RCT	RTG	OBT	361	64%		(Cahn *et al.* 2011)
SAILING	RCT	DTG	OBT	354	71%		(Cahn *et al.* 2011)

The OBT was decided based on the genotypic resistance results and DRV at a dose of 600 mg bid with ritonavir was permitted. At 48 weeks, in the combined analysis of the two studies, 62.1% of patients on RAL as compared with 32.9% on placebo obtained virological success (Steigbigel *et al.* 2008).

Results could be observed throughout the whole range of efficacy of the OBT, estimated based on the genotypic susceptibility score (GSS, i.e. the number of active drugs based on the interpretation of the results of resistance genotyping (Cooper *et al.* 2008)). In particular, HIV-1 RNA <50 copies/mL was achieved at 48 weeks with an OBT with a GSS=0 in 45% or RAL and 3% of placebo recipients, showing a high efficacy of RAL even in the context of a functional monotherapy. However the best efficacy results were obtained when RAL could be supported by active and potent companion drugs. Indeed, with an OBT GSS=2 77% of RAL recipients (versus 62% of placebo) achieved virological suppression. Moreover, in patients who were using enfuvirtide (ENF) or DRV for the first time, HIV-1 RNA <50 copies/mL was achieved in 89% of RAL recipients and 68% of placebo recipients. At 48 weeks, genotypic resistance mutations to RAL were selected in 68% of patients with virological failure: 75% of these had two or more resistance-associated mutations.

Results of the studies were confirmed in the long term by a follow-up of 5 years, after the studies were unblinded at 3 years and all patients were offered to continue open-label RAL (Eron *et al.* 2013b). The studies demonstrated the long term efficacy on integrase inhibitors as a new drug class in this setting and the good tolerability profile of RAL, even in the long term.

RAL was also studied in combination with DRV/r and etravirine (ETR) in two independent non-controlled studies in patients with multidrug resistant virus (Imaz *et al.* 2009).

In the first study, 32 DRV- and integrase inhibitors-naïve patients with 3-class resistant virus were enrolled. At week 24, 94% of patients achieved HIV1-RNA less than 50 copies/mL. In the second trial 103 patients who were naïve to the three drugs and had a history of virologic failure with NNRTI, > or =3 primary PI and NRTI mutations, and < or =3 DRV and NNRTI mutations. In addition 87% of patients received OBT that included NRTIs or ENF. At week 48, 86% had an HIV RNA level <50 copies/mL. Only 1 patient discontinued the investigational antiretroviral regimen, because of an adverse event (Yazdanpanah *et al.* 2009). At week 96, 88% achieved durable virologic response (<50 copies/mL).

RAL was also studied in a PI-sparing, NRTI-sparing regimen in 28 triple-class experienced HIV-1-infected patients harbouring R5 virus, who received maraviroc (MVC), RAL and ETR (Nozza *et al.* 2010). By on-treatment analysis, 26 (92%) had less than 50 copies HIV-RNA/mL at week 48. The median 48 week increase in CD4 T cell counts was 267 cells/μl. The regimen was well tolerated and its efficacy was confirmed in the long term.

In a cohort of 333 ART-failing patients initiating RAL in the clinical practice, 77% of patients had undetectable viral load at 4 years based on an intention to treat analysis (Capetti *et al.* 2014), but only 32 patients (9.6%) had true viral failure.

EVG once daily was compared to RAL twice daily in a 96-week, double-blind, randomized phase 3 study, in 702 treatment-experienced adults receiving a fully active, ritonavir-boosted PI plus a third agent (Elion *et al.* 2013). The proportion of subjects randomized to EVG that achieved and maintained HIV-1 RNA <50 copies/mL through week 96 was 47.6% compared with 45.0% for RAL. Both regimens were well tolerated, with comparable rates of adverse events.

Finally, DTG 50 mg once daily was compared to RAL 400 mg twice daily in a 48 week, phase 3, randomised, double-blind, non-inferiority study. 715 viremic patients with resistance to two or more classes of antiretrovirals, with 1-2 fully active background antiretrovirals were randomized 1:1 to receive one of the two integrase inhibitors (Cahn *et al.* 2013). At week 48, 71% patients on DTG had HIV-1 RNA < 50 copies/mL versus 64% on RAL: superiority of DTG versus RAL was then concluded.

Significantly fewer patients had virological failure with treatment-emergent integrase-inhibitor resistance on DTG (4 vs 17 patients). Adverse event frequencies were similar across groups, including safety events leading to discontinuation.

Use of DTG in failing HIV infected subjects with genotipic evidence of INIs resistance

Resistance to INSTIs is observed with increasing frequency in treatment-failing subjects both in America and in Europe. Results from specimens sent for clinical decision making to the referral US laboratory over a 4 year period (2009-2012) indicated that among 3294 sequences from 3012 patients, 471 patients had viruses with ≥1 RAL or EVG resistance mutation (15.6%); Q148 and N155 pathways were equally represented, Q148 rarely occurred without accessory mutations.

High-level DTG resistance was predicted in 12% of patients with RAL- or EVG-resistant viruses (Hurt *et al.* 2014). In Brazil, analysis of blood samples from 92 HIV-infected individuals with confirmed virological failure using RAL with optimized background showed that 32 of them (35%) showed resistance to DTG, in most cases associated with the combination of Q148H/R/K with G140S/A mutations (Cavalcanti Jde *et al.* 2015).

In France, among 502 treatment-experienced patients failing a RAL- containing regimen the most frequent mutations observed were N155H/S (19.1%), Q148G/H/K/R (15.4%) and Y143C/G/H/R/S (6.7%). At RAL failure, viruses were considered as fully susceptible to all INSTIs in 61.0% of cases, whilst 38.6% were considered as resistant to RAL, 34.9% to EVG and 13.9% to DTG (Fourati *et al.* 2015). Similar data were reported in a study conducted in Italy where the prevalence of RAL resistance mutations was analysed in 156 sequences from a subgroup of 120 patients failing RAL: 54 (36.4%) of the samples had at least one primary RAL mutation (Armenia *et al.* 2015).

There are currently three published randomized studies examining the use of DTG in failing HIV-1 infected subjects with evidence of INSTIs resistance.

VIKING is an open-label, single-arm trial, designed to evaluate the safety and efficacy of DTG in treatment-experienced subjects with genotypic evidence of RAL resistance. other antiretroviral drug classes (Eron *et al.* 2013a). Initially patients were treated with DTG 50 mg daily. The protocol was then amended to include a second cohort (DTG 50 mg twice daily) due to low viral load response observed in some patients. Patients in cohort two were also required to have one fully active antiretroviral agent in the optimized background regimen. The study consisted of a 10 day functional monotherapy where DTG was used in place of RAL in the failing regimen. On day 11, the background regimen was optimized based on resistance data. Fifty-one patients were enrolled with 27 patients in cohort one and 24 patients in cohort two.

All of the subjects had advanced HIV-1 disease, with a median CD4 T cell counts of about 200 cells/μL. The primary endpoint at day 11 was a reduction in plasma viral load of >0.7 $\log_{10}$ copies/mL from baseline or a viral load <400 copies/mL. Seventy-eight percent (21/27) of patients in cohort one and 96% (23/24) in cohort two achieved the primary outcome. At week 24, 11/27 (41%) patients in cohort one and 18/24 (75%) patients in cohort two achieved a viral load <50 copies/mL. The median cell/μL increase in CD4 T cells was 54 in cohort one and 60 in cohort two. The once- and twice-daily DTG regimens were both well-tolerated with low rates of adverse events, the most frequent of which was mild-to-moderate diarrhoea. None of the serious adverse events reported was judged as related to DTG.

VIKING-3 is a single-arm, open-label phase III study, conducted at 65 sites in US, Canada and Europe, in which 183 highly treatment-experienced adults with INSTI-resistant virus received DTG 50 mg BID while continuing their failing regimen (without RAL or EVG) through day 7, after which the regimen was optimized with at least 1 fully active drug (Castagna *et al.* 2014). Patients had to have plasma viral load >500 copies/mL and at least one active antiretroviral available for the background regimen. Patients on etravirine were only included if it was coadministered with LPV/r or DRV/ r. Median age was 48 years, 77% male, 71% white race. Baseline viral load was 4.38 $\log_{10}$ copies/mL, CD4 140 cells/μL, HCV coinfection rate was 14%. Seventy-three percent had baseline genotypic and/or phenotypic INSTIs resistance; the remaining 27% had historic evidence only. At baseline median fold-change to RAL was 47.5 while the median DTG fold-change was 1.29. Seventy-three percent of patients had >3 NRTI major mutations, 70% had >2 major PI mutations, and 59% had >2 NNRTI major mu-

tations. Primary efficacy endpoints were the mean change from baseline in plasma HIV-1 RNA at day 8 and the proportion of subjects with HIV-1 RNA <50 copies/mL at week 24. Mean change in HIV-1 RNA at day 8 was -1.43 $\log_{10}$ c/mL, and 69% of subjects achieved <50 copies/mL at week 24.

The median change in CD4 T cell count at week 24 was 61 cells/μL. Despite the advanced population included in this study, DTG 50 mg BID had a low (3%) discontinuation rate due to adverse events. Virological response was low among patients with a DTG fold-change >10 and those harbouring Q148+ >2 mutations, while the group without Q148 mutation had the highest response rates.

Activity of the background regimen did not significantly influence day 8 or week 24 response. In multivariate analysis, baseline INSTIs resistance and baseline viral load independently predicted week 24 response. For each two-fold increase in DTG fold-change, a 63% lower chance of achieving viral load <50 copies/mL was found. For every 10 fold increase in baseline viral load, the probability of achieving viral load <50 copies/mL was 80% lower. At week 48, 116/183 (63%) patients maintained a viral load <50 copies/mL; among subjects without Q148 mutation 71% maintained <50 copies/mL

VIKING-4 is a Phase III randomized, double-blind study including a placebo-controlled 7-day monotherapy phase to demonstrate that short-term antiviral activity was attributable to DTG (Akil *et al.* 2015). Thirthy adults with INSTIs-resistant virus, receiving at screening RAL o EVG were randomized to DTG 50 mg twice daily or placebo while continuing their failing regimen without INI for 7 days. At day 8, all subjects switched to open-label DTG 50 mg twice daily and optimized background therapy. The primary end point was change from baseline in plasma HIV-1 RNA at day 8. Adjusted mean change in HIV-1 RNA at day 8 was -1.06 $\log_{10}$ copies/mL for the DTG arm and 0.10 $\log_{10}$ copies/mL for the placebo arm (treatment difference -1.16 $\log_{10}$ copies/mL [-1.52-0.80]; p=0.001). Overall, 47% and 57% of subjects had plasma HIV-1 RNA <50 and <400 copies/mL at week 24, and 40% and 53% at week 48, respectively. No discontinuations due to drug-related adverse events occurred in the study.

Preliminary results documented that optimized DTG-containing antiretroviral regimens provide a substantial initial efficacy rate for salvage therapy even in heavily antiretroviral-experienced HIV-2 infected subjects with virus harboring resistance to first-generation integrase inhibitors (Descamps *et al.* 2015).

Many *in vitro* and *in vivo* data have shown that DTG has a higher barrier to resistance compared to the first-generation INSTIs. With the expanding use of integrase inhibitors in clinical practice, a prompt determination of integrase GRT is essential for the optimal management of

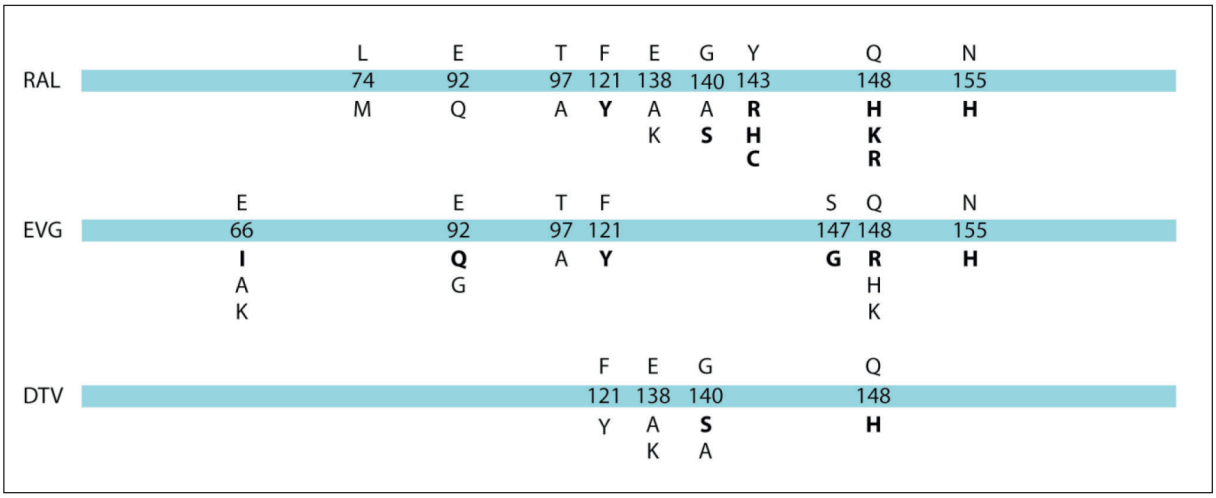

FIGURE 1 - *Mutations in the integrase gene associated with resistance to integrase inhibitors (RAL, raltegravir; EVG, elvitegravir; DTG, dolutegravir. Adapted from Wensing et al., Top. Antivir. Med. 2014).*

patients failing RAL or EVG. Changes in viraemia during virological failure may indicate the evolution of RAL resistance and may predict the emergence of secondary mutations that are associated with a decrease in DTG susceptibility.

Early discontinuation of RAL or EVG from failing regimens may therefore favour subsequent salvage with DTG. HIV-1 variants containing a combination of resistance mutation at position 143, 148 and 155 exhibit reduced susceptibility to DTG (Figure 1); viruses harbouring mutations belonging to the 148+155 escape pathway are less susceptible and exhibit similar or greater replicative capacity than viruses harbouring mutations belonging to the 143+148 or 143+155 pathways (Frantzell *et al.* 2015).

More data are needed to better understand how to guide treatment in subjects harbouring viruses with Q-148+ additional integrase mutations. More data are needed to explore clinical implications associated with the substantial further decrease in viral replicative capacity observed in presence of R263K+M184I/V in comparison with the single substitution alone (Singhroy *et al.* 2015). In the meantime, clear-cut results obtained in VIKING studies support a large use of DTG 50 mg twice daily in subjects with evidence of INSTIs resistance.

MANAGEMENT OF COMORBIDITIES: FOCUS ON KIDNEY AND BONE

Massimo Galli, Andrea Giacomelli, Marco Borderi

Impact on kidney function

The strongest limitation of current cART is that neither HIV eradication, nor a functional cure, but only a treatment-dependent blockage of HIV replication can be obtained. Consequently, cART must be taken life-long. This scenario implies to take into account patients' compliance, the side effects of the different drugs and drug combinations used and the possible interactions between the antiretroviral drugs and other concomitant medication for eventual co-morbidities. Therefore the long term positive effects of cART generally exceed drug related side effects and metabolic toxicities (Palella 1998; Antiretroviral Therapy Cohort Collaboration 2008).

Among the five classes of HIV drugs, the INSTIs, since the introduction of RAL in 2008, showed a strong antiviral efficacy combined with a good safety profile and few drug to drug interactions (Messiaen *et al.* 2013; Liedtke *et al.* 2014). Since the approval of RAL, two new different INSTIs has been added to this class. EVG, which is a first generation INSTI, is licensed in some countries as single-agent tablet (Vitekta®), that can be used in combination with ritonavir boosted protease inhibitors (Deeks 2014) or in other drug combinations including a ritonavir boost. Currently, EVG is mainly used in a single-tablet combined regimen containing fixed doses of TDF/FTC/COBI (EVG/COBI/TDF/FTC (Stribild®) (Perry 2014). COBI, the enhancer used in this fixed combination, is a potent cytochrome P450 3A inhibitor and also inhibits some renal tubular membrane transporters such as organic cation transporter 2 (OCT2), Multidrug and Toxin Extrusion Protein (MATE)-2-K and most of all MATE-1 (Gutierrez *et al.* 2014). The last INSTI approved is DTG (Tivicay®) which is the first drug of a second generation INSTI, having some new characteristics: a favourable pharmacokinetic profile with a prolonged half-life, reduced drug to drug interaction and a different resistance patterns with a significant activity against HIV-1 strain cross-resistant to RAL or EVG (Messiaen *et al.* 2013; Fantauzzi and Mezzaroma 2014).

Despite a global good safety profile of the INSTI class, some concern related to *renal toxicities* appeared during clinical trials in particular regarding the fixed dose formulation of EVG including COBI, and DTG (Curtis *et al.* 2013; Elion *et al.* 2013; Raffi *et al.* 2013a; Raffi *et al.* 2013b).

The review of the safety data of two phase three trials, the GS102 and the GS103 studies, showed modest increase in serum creatinine and a decrease in estimated creatinine clearance by week 2 of treatment with EVG/COBI/FTC/TDF, that persists through week 48 and over. The mean ± standard deviation changes in serum creatinine after 48 weeks of treatment were 0.14 mg/dL±0.13 mg/dL in the EVG/COBI/FTC/TDF arm, 0.01mg/dL ±0.12 mg/dL in the EFV/FTC/TDF arm, and 0.09 mg/dL ±0.13 mg/

dL in the ATV/r plus FTC/TDF arm. Furthermore, 8 (1.1%) subjects in the EVG/COBI/FTC/TDF arms and 1 (0.1%) subject in the combined comparator arms discontinued the study drug due to a renal adverse event. Four (0.6%) of the 8 subjects who discontinued EVG/COBI/FTC/TDF due to a renal adverse event also met the FDA criteria for proximal renal tubular dysfunction. The remaining four subjects discontinued study drug due to a renal adverse event but did not meet the FDA criteria for proximal renal tubular dysfunction. None of the subjects in the comparator arms has evidenced of proximal renal tubular dysfunction leading to study drug discontinuation. In the four EVG/COBI/FTC/TDF treated subjects who met the FDA criteria of proximal renal tubular dysfunction, the laboratory abnormalities improved but did not completely resolve upon discontinuation of EVG/COBI/FTC/TDF.

The reason for the increased frequency of proximal tubular dysfunction in the EVG/COBI/FTC/TDF group versus the comparator arms (both of which also included TDF) remains undefined (DeJesus *et al.* 2012; Sax *et al.* 2012). It is well known that EVG/COBI/TDF/FTC produce an early increment of serum creatinine with reduced estimated glomerular filtration rate (eGFR) and this arises early during treatment and remains stable over time. This is explained by COBI inhibition of MATE1, which is one of proximal tubular proteins that is involved in creatinine secretion. This can explain why a reduction of eGFR can be observed without a decrement of actual GFR (eGFR) during treatment with EVG/COBI/TDF/FTC (Manzardo 2014; Arya 2013).

As another drug in this formulation, TDF, is described to have nephrotoxic effects, especially on proximal tubule, FDA decided that a change from baseline of serum creatinine of 0.4 mg/dL could discriminate between the two different nephrotoxic process (De Jesus *et al.* 2012; Sax *et al.* 2012). From this observation derived some recommendations when EVG/COBI/TDF/FTC is prescribed.

In particular, eGFR, urine glucose and urine proteins should be assessed in all the candidates to this regimen before starting it. Moreover, EVG/COBI/TDF/FTC combination is not recommended in patients with an eGFR <70 mL/min, and the eGFR, urine glucose, urine proteins and serum phosphate should be routinely monitored in all patients for an early detection of proximal tubular dysfunction (Arya *et al.* 2013; Manzardo and Gatell 2014). However, these renal abnormalities could not be observed when ritonavir is used as pharmacokinetic enhancer of EVG instead of COBI.

DTG, the most recent INSTI drug introduced in common practice, notably inhibits *in vitro* OCT2 with IC50=1.9μM. This value is 4-fold lower than the C_{max} of DTG, so a potential interaction exists for compounds that undergo renal secretion via OCT2 such as serum creatinine (Reese *et al.* 2013). The *in vitro* studies could explain the mild 10-14% decrease in eGFR observed in patients receiving DTG.

However, this OCT2 inhibition, similarly to MATE-1 inhibition induced by COBI, is mild and reversible and data from clinical trials confirmed that the changes in serum creatinine were not related to a real renal blood flow reduction or impairment of eGFR. OCT2 is inhibited at DTG concentrations below the peak reached in clinical trials.

However, in SPRING-2 study, 48 weeks of DTG treatment did not significantly impact renal function (Raffi *et al.* 2013b). Patients receiving DTG in this trial had a small mean increase in serum creatinine level (grade 1-2) that appears during the first two weeks of treatment and remains stable through week 96 (Raffi *et al.* 2013a). Similarly, the recent results of FLAMINGO showed an increment in serum creatinine of 0.1-0.2 mg/dL in patients taking DTG.

The creatinine elevation in patients taking DTG is similar in this study to that due to COBI, as mentioned before, with a rise in serum creatinine level in the first 2-4 weeks of treatment (Clotet *et al.* 2014).

These data were confirmed at week 96 (Molina *et al.* 2014b). Interestingly, no differences in adverse event related discontinuation have been observed in the two arms of the VIKING-3 study, with a 3% discontinuation in patients where DTG was taken at double dose (50 mg BID), that was similar to the discontinuation rate seen in INSTI-naïve patients receiving DTG 50 mg once a day (Eron *et al.* 2013a; Castagna *et al.* 2014; Clotet *et al.* 2014).

In conclusion INSTIs show a good tolerabili-

ty and safety profile combined with absence of significant drug to drug interactions.
However, some precaution should be adopted regarding renal function before prescribing EVG in the fixed formulation with COBI.
It is recommended to check eGFR at baseline when using EVG/COBI/TDF/FTC and avoid use of this compound when eGFR is <70 mL/min.
A strictly monitoring of kidney function with a novel set point of eGFR after one month from starting EVG/COBI/TDF/FTC is recommended.
It is also mandatory monitoring tubular function by using urine glucose, urine proteins and serum phosphate at baseline and then routinely. These procedures are addressed to allow the discrimination of an increment of eGFR due to impaired creatinine secretion or a more concerning onset of proximal renal tubular dysfunction due to other drug used in the regimen.
Nevertheless, a serum creatinine change from baseline ≥0.4 mg/dL on consecutive visits is considered an acceptable threshold for targeting subjects with potentially serious renal adverse events who need an enhanced renal monitoring. The same procedure is suggested for DTG only when it is administered in association with TDF or other potentially nephrotoxic drugs.

Impact on bone

Decreased bone mineral density (BMD) is a growing concern for HIV-1 infected patients. The incidence of osteopenia/osteoporosis and fragility fractures is higher in these patients compared to the general population. Although HIV-1 patients frequently have more classical risk factors for low BMD, both HIV-1 infection and cART may also play a potential role, cause randomized clinical trials showed a decline in BMD by 2-6% over 48-96 weeks. Previous classes of ARVs, such as boosted protease inhibitors (PI/r) and reverse transcriptase inhibitors, have been implicated in altering BMD. Recently, INSTI based regimens not containing N(N)RTIs (N(N)RTI sparing regimens) or PI/r (PI sparing regimens) has gained a wide attention, since these combinations can avoid bone N(N)RTI/PI toxicity.
Actually, we have BMD data on INSTI-based regimens both in naïve (1) and in experienced (2) patients.

1. TNF and PI/r use is consistently associated with BMD loss in naïve-subjects, so a nucleos(t)ide- or PI/r-sparing regimen would ameliorate BMD loss associated with ARV therapy initiation. In naïve subjects, there are three BMD studies on INSTI plus FTC/TDF (a), and three BMD studies on INSTI plus PI/r (b).

(a)

- GS-US-292-0102 (Sax *et al.* 2014) is a phase 2, randomized, double-blind, double-dummy, multicenter, active-controlled study. Antiretroviral naïve adults with HIV-1 RNA ≥5,000 copies/mL and CD4 T cell count ≥50 cells/μL were randomized 2:1 to receive a single-tablet regimen of EVG/COBI/FTC/tenofovir alafenamide (TAF) or EVG/COBI/FTC/TDF, plus placebo. Over 48 weeks. EVG/COBI/FTC/TDF group had changes in bone mineral density for hip -2.39% and spine -3.37%.
- A5260s (Brown *et al.* 2014) is a limited site Bone Substudy of a large randomized clinical trial comparing TDF/FTC plus ATV/r, DRV/r, or RAL (ACTG A5257) to determine whether BMD changes over 96 weeks after ART initiation differ in HIV-infected persons starting ATV/r, DRV/r, or RAL.
  At the hip and the spine, the mean percentage BMD changes over 96 weeks were not different in the PI arms (Hip: ATV/r -3.9% vs DRV/r -3.4%, p=0.36; Spine: ATV/r -4.0% vs DRV/r -3.6%, p=0.42). At the hip and the spine, the loss of BMD was greater in the combined PI arms than the RAL arm (Hip -3.7% vs -2.4%, p=0.005; Spine: -3.8% vs -1.8%, p<0.001).
- GS103 (Clumeck *et al.* 2014) is a randomized, double-blind, active-controlled phase 3 international trial on EVG/COBI/FTC/TDF compared with ATV/r plus FTC/TDF, and demonstrated that EVG/COBI was associated with smaller decreases in BMD over 144 weeks vs. ATV/r: Hip: -2.83% vs -3.77% (p=0.23); Spine: -1.43% vs -3.68% (p=0.018).

(b)

- PROGRESS (Reynes *et al.* 2013) is a randomized, open-label, 96-week pilot study comparing a regimen of lopinavir/ritonavir (LPV/r) 400/100 mg twice daily in combination with either RAL 400 mg twice daily or

tenofovir/FTC (TDF/FTC) 300/200 mg once daily in ARV-naïve adults.
The LPV/r+TDF/FTC group had a statistically significant (p<0.001) mean percent decrease from baseline to week 96 in total bone mineral density, which was significantly different from the mean percent change in the LPV/r+RAL group (-2.48% vs +0.68%, p<0.001).

- In the RADAR study (Bedimo *et al.* 2014), antiretroviral-naıve HIV-infected patients were randomized to receive either RAL (n=42) or TDF/FTC (n=43), each with DRV/r. Changes in subtotal BMD to week 48 were: +9.2 with RAL vs.-7 g/cm² with TDF/FTC (p=0.002).
- NEAT (Bernardino *et al.* 2014) is a phase III, randomised, open-label, multicenter, parallel-group, non-inferiority trial in 78 sites in 15 European countries on HIV-1 ART-näive ≥18 years subjects with HIV-1 RNA >1,000 copies/mL, CD4 T cell count ≤500 cells/μL, HBs Ag negative, no major IAS-USA RAM, randomised 1:1 to DRV+r 800+100 mg QD + RAL 400 mg BID or DRV+r 800+100 mg QD + TDF/FTC FDC QD over 96 weeks. In lumbar spine BMD, mean % change (95% CI) was: DRV/r + RAL -0.43 (-1.51, 0.65) vs. DRV/r + TDF/FTC -2.8 (-4.0, -1.6); Mean difference (95% CI); -2.37 (-4.0, -0.74); p=0.0054. In hip BMD, mean % change (95% CI) was: DRV/r + RAL -1.74 (-2.96, -0.52) vs DRV/r + TDF/FTC -5.99 (-9.18, -2.80); Mean difference (95% CI); -4.25 (-7.92, -0.58); p=0.025.

2. Tenofovir and PI/r use is consistently associated with BMD loss during cART, so a switch to INSTI-based regimens would prevent or ameliorate BMD loss associated with ARV therapy. In experienced patients, there are two BMD pre-emptive switch studies on INSTI plus PI/r, one BMD pre-emptive switch study on INSTI plus FTC/TDF (c), and one BMD reactive switch study on INSTI plus PI/r (d).

(a)

- KITE (Ofotokun *et al.* 2012) is a 48 week single-center, open-label pilot study in which 60 HIV-infected adults with plasma HIV-1 RNA <50 copies/mL on standard HAART were randomized (2:1) to LPV/r 400/100 mg BID + RAL 400 mg BID switch (LPV-r/RAL arm) or to continue on standard HAART. No difference between treatments arms over time was significant for total BMD (p=0. 50), pelvis BMD (p=0.56), or spine BMD (p=0.72).
- SECOND-LINE (Haskelberg *et al.* 2014) is a 96-weeks phase IV multi-centre, open-label, randomised controlled trial in HIV-infected adults who have virologically failed first-line ART consisting of an NNRTI + 2NRTIs randomised to RAL + LPV/r or conventional 2-3 NRTIs + LPV/r second-line therapy. At week 96, the mean (standard deviation) percentage change from baseline in total hip BMD in the NRTI+LPV/r group was -4.1% (5.9%) versus -2.2% (4.5%) in the RAL + LPV/r group (adjusted treatment difference -1.9%; 95% confidence interval: -3.4% to -0.4%; p=0.012).
  For lumbar spine, the mean (SD) percentage BMD change over 96 weeks in the NtRTI+LPV/r group was -4.9% (4.9%) compared to -3.5% (5.0%) in the RAL + LPV/r group (adjusted treatment difference -1.9%; 95% CI -3.3% to -0.5%; p=0.009).
- SPIRAL-LIP (Curran *et al.* 2012) is a study in which patients were randomized (1:1) to continue with the PI/r-based regimen or switch to RAL, maintaining the rest of the treatment unchanged. Total BMD [0.01 (0 to 0.02) g/cm², p=0.002], total hip BMD [0.01 (0 to 0.03) g/cm², p=0.015] and total hip T score [0.12 (-0.05 to 0.21) SD, p=0.004] significantly increased with RAL, with no significant changes within the PI/r group. Differences between treatment groups were significant in femoral neck BMD [0.01 (-0.02 to 0.02) g/cm², p=0.032] and T score [0.01 (-0.18 to 0.18) SD, p=0.016].

(b)

- TROP (Bloch *et al.* 2014) is a multicentre, open–label, non-randomized study in HIV-infected, osteopenic or osteoporotic adults receiving TDF and a PI/r who switch from TDF to RAL. Mean % Change from Baseline [95% CI] over 48 weeks are: Spine 3.0 [1.9, 4.0] p<0.0001, Left total hip 2.5 [1.6, 3.3] p<0.0001, Left femoral neck 2.1 [0.9, 3.2] p 0.0011, Right total hip 2.7 [1.9, 3.5] p<0.0001, Right femoral neck 2.3 [1.2, 3.5] p<0.0001.

3. First results regarding DTG on bone markers have been recently presented (Tebas *et al.*, 2013). In the SINGLE trial, conducted in treatment-naive patients to evaluate efficacy and safety of DTG plus Abacavir/Lamivudine (ABC/3TC) vs. Tenofovir/Emtricitabine/Efavirenz (EFV/TDF/FTC), markers of bone turnover (bone-specific alkaline phosphatase, C-terminal telopeptide type 1 collagen, osteocalcin, and procollagen type 1 N-propeptide) and Vitamin D have been measured at baseline (BL) and at 48 weeks. DTG + ABC/3TC had superior virologic outcomes to TDF/FTC/EFV through 48 weeks and is associated with smaller changes in bone turnover markers. Indeed, after 48 Weeks, significantly greater changes from BL have been observed for all bone markers (Bone-specific alkaline phosphatase $p<0.001$; C-terminal telopeptide for type 1 collagen $p<0.001$; Osteocalcin $p<0.001$; Procollagen type 1 N-propeptide $p<0.001$) in subjects receiving TDF/FTC/EFV, indicating more active bone turnover when compared to changes seen in subjects receiving DTG + ABC/3TC. These differences may correlate with known TDF-associated changes in BMD over time and further study of the potential advantages of a DTG+ABC/3TC regimen appear warranted.

In conclusion, INSTI based regimen (plus FTC/TDF or PI/r) showed improvements in BMD, both in naïve and in experienced patients. Although these findings should be confirmed with larger studies with longer follow-up, INSTI might be considered as a well tolerated treatment option in certain patients, especially in the HIV-infected aging population, because of its potential beneficial bone effects.

AGING PATIENTS: CLINICAL ISSUES AND THERAPEUTIC CHALLENGES

Giovanni Guaraldi; Andrea Calcagno

In recent years, the number of elderly persons living with HIV-1 has increased (Center for disease control) as the result of both the availability of effective antiretroviral therapy, which has reduced AIDS-related mortality, and newly diagnosed infections occurring in older adults. It is projected that by 2015, more than half of all HIV- infected people in the United States will be over the age of 50 (US department of health). The overlapping epidemics between HIV-1 and aging, changes profoundly the clinical picture of HIV-1 disease. Non-infectious chronic morbidities (NICM) (Shah *et al.*, 2002; Justice 2006; Sackoff *et al.*, 2006; Weber *et al.*, 2006; Phillips *et al.*, 2008; Vance *et al.*, 2011) that typically are highly prevalent in older persons, are often superimposed to chronic pathology, murk the clinical presentation, interfere with the treatment scheme and contribute to disability and mortality. Since cardiovascular disease (CVD), hypertension (Htn), bone fractures, renal failure, diabetes mellitus (DM), neurocognitive disorders, cancer and other chronic conditions, aggregating in Poly-pathology syndromes, have a higher prevalence in HIV-1 infected patients in comparison to the general population, it has been suggested that HIV-1 in the post-cART era has become a syndrome of "accentuated aging process" (Guaraldi *et al.*, 2009; Mandalia *et al.*, 2010), on the contrary it is still a matter of concern if these condition occur at an early age describing the so call "premature aging process (Althoff *et al.*, 2014).

Neither the "accentuated" of the "premature" aging conceptualization discriminate what is the relative contribution of the host, of the HIV virus and of the toxicities of antiretroviral therapy in the development of HIV Associated Non AIDS (HANA) conditions, but in the context of availability of less toxic and more potent cART, the host contribution appear to lead clinical risk.

Host risk can be conceptualized with the definition of "frailty": a physiologic state of increased vulnerability to stressors, which increases with

age but rather represent a true measure of biological aging, that results from decreased physiologic reserves, and deregulation of multiple physiological systems. In the early stages of this process, frailty may be clinically silent. However, when the losses of reserve reach an aggregate threshold that leads to serious vulnerability, the syndrome may become detectable by looking at clinical, functional and biological markers. Understanding and detecting frailty at an early stage is important because frailty is not only the description of a functional status, but also the predictor of future clinical event and overall mortality.

Multiple measures exist to identify and measure frailty. Some are based on clinical judgment or a single item (eg, walking speed), but most scales assess multiple domains of age-related health and grade frailty by counting the number of deficits individuals have acquired (Theou *et al.*, 2014).

One commonly used scale, based on the frailty phenotype (Rockwood *et al.*, 2010), identifies frailty by the presence of 3 deficits out of 5 specific measures: self-reported unintentional weight loss >10 lbs or recorded weight loss ≥5% in a year, measured slow walking speed, measured weak grip strength, self-reported exhaustion (3-4 days per week or most of the time), and low activity/energy ex- penditure (assessed by Minnesota Leisure Time Questionnaire) (Womack *et al.*, 2013).

Another commonly used scale, the "frailty index," counts the number of deficits individuals have accumulated out of various health measures and presents them as a proportion (Vigouroux *et al.*, 2014). In contrast to the phenotypic approach, any measure can be included in a frailty index if it is generally related to age and poor health, and if the group of items covers multiple physiological systems. When at least 30 items are included, the proportion of deficits accumulated appears more informative than the specific nature of those deficits. Though the effect of each individual deficit may be small, their cumulative effects can be large.

So far frailty, which defines age as an health condition, has not been mention in any HIV-1 guideline to make recommendation regarding the screening and the treatment of people aging with HIV-1.

Nevertheless most guidelines, EACS guideline in particular, recommend extensive screening for HANA condition, with particular regards to patients aged more than 50 years, which may in fact help to preserve not only organ function but also integrity of the homeostatic mechanisms which regulate interaction between different apparatus and reduce multimorbidity onset.

In the absence of any cART studies which have analysed frailty as a clinical outcome we need to refer to the available data which describe the impact of cART regimens on traditional biomarkers of functional organ reserve. We may in fact assume that if we preserve integrity of the functional organ reserve we are reducing the risk for frailty.

The availability of the new HIV drug class of INSTI represent a valuable option for the treatment of HIV infection, not only for the potency and rapidity in viral replication decay but rather for the unique tolerability and potential impact in the capacity to contrast mechanisms of residual viral burden and secondary reduce the burden of chronic inflammation. This is the case of the afore mentioned SPIRAL study in which the switch out from a boosted PI based regimen to RAL was able to significantly reduce several inflammatory biomarkers associated with cardiovascular diseases including IL6 and hrCRP (Martinez *et al.*, 2010).

Participants in SPRING-1 receiving DTG had favourable mean changes in plasma lipids from baseline compared with those receiving EFV; a 29-fold greater increase in LDL cholesterol was observed in the EFV arm, and mean triglyceride levels fell in the DTG arm (van Lunzen *et al.*, 2012).

DTG has also been compared to boosted DRV in the FLAMINGO study whose results through 96 weeks have been recently published (Molina *et al.*, 2015). The study reported interesting metabolic data showing a significant reduction in the total cholesterol/HDL cholesterol ratio which represented the more stringent metabolic parameter in relation to cardiovascular risk. Moreover a higher number of grade 2 or higher fasting LDL lab abnormalities was shown by Week 96 in the DRV/r arm (22%) vs the DTG arm (7%), P<0.001 (pre-specified LOCF analysis) (Molina *et al.*, 2014a).

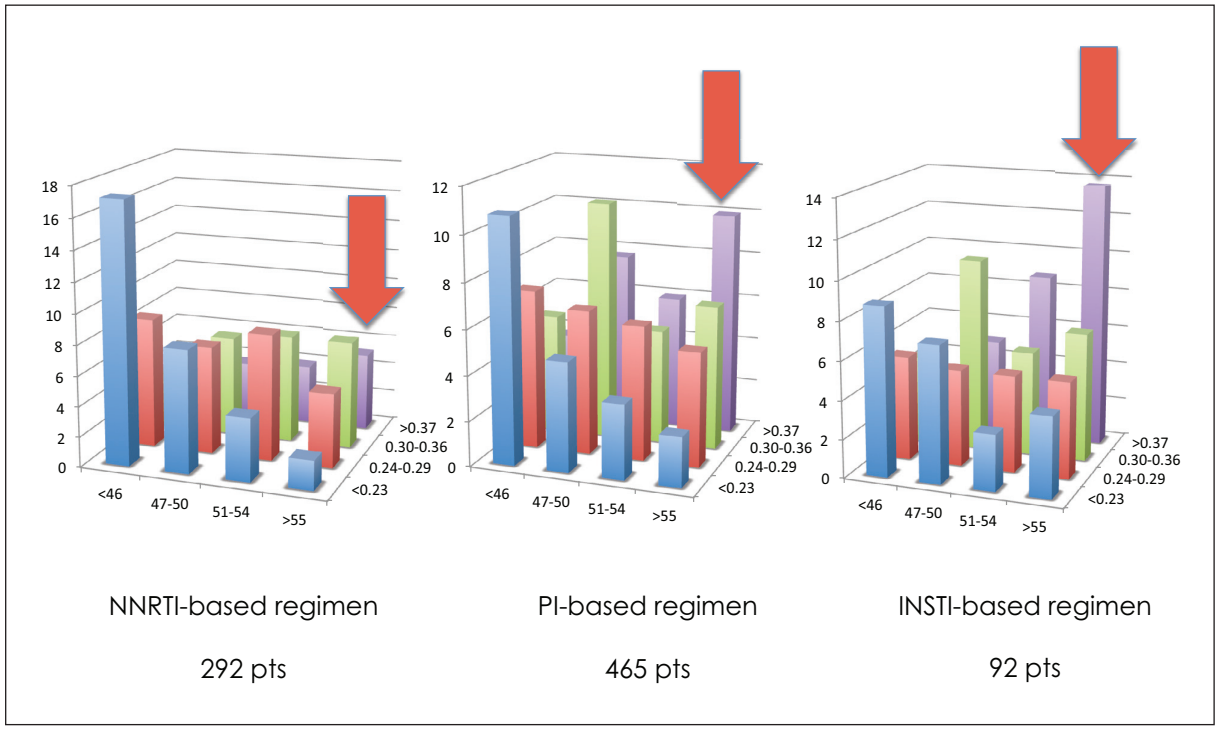

FIGURE 2 - *Age and frailty spectrum in different ARV drug classes current exposure.*

Particularly interesting are the post hoc sub-analyses that have analysed the clinical outcome in population stratified by age group or by functional organ impairment at baseline. This is the case of Study 102 and 103, week 144 Age analyses which depict a non statistical difference in median change from baseline in TC, HDL, LDL and TG in patients aged 50 years old or more when compared Stribild© respectively to Atripla© or ATV/r + Truvada© (Gazzard *et al.*, 2014).

The two major studies on Stribild© in experienced patients: strategy PI and strategy NNRTI recently presented data with regards to renal safety in patients included in the study with baseline e-GFR between 79 and 90 ml/min. A non significant difference in e-GFR reduction was shown in patient starting Stribild© with grade 2 GFR impairment. (Reeves *et al.*, 2014).

Our group have recently presents at the 5th workshop on HIV&aging a retrospective study in which we sought to describe patterns of cART use in relation to age and frailty quantified via 37-item frailty index (FI), based on the cumulative deficits model. FI variables excluded markers of HIV severity or immune depletion. FI was calculated as the proportion of health deficits present, and retrospectively assigned for each visit. ARV regimens included 2NRTI backbones plus either: PI (41%); integrase inhibitor (only RAL was available) 22%, or NNRTI (37%).

Figure 2 shows in each drug class the distribution of patients in relation of age quartiles (x-axis) or frailty index quartiles (z-axis) (Guaraldi *et al.*, 2014). The distribution of patients reflect the choice of clinicians which preferentially select INSTI based regimen in frailest patients regardless their age. What we have learned so far regarding INSTI in the setting of frail patients is promising and this drug classes has the potential to represent a "game changer" in the treatment of aging patients. We support that future randomized clinical trials with INSTI may consider frailty index as a clinical endpoint to prove the value of this drug regimen in prevention and reversibility of frailty in people aging with HIV-1.

POLYPATHOLOGY AND POLYPHARMACY

Giovanni Di Perri, Stefano Bonora

The use of highly active antiretroviral therapy has significantly reduced morbidity and mortality, thus increasing life expectancy of HIV-1 infected individuals, transforming HIV-1 infection into a chronic disease.

A major focus of antiretroviral research has been simplification of cART therapy. Many low-pill burden, once daily regimens are now available, improving adherence and making treatment-success achievable for many more patients. However, as HIV-1 patients live-longer, they may develop chronic diseases of aging, many of which occur with increased frequency compared to the general population. Treatment of these illnesses complicates care by increasing pill burden, creating drug-drug interactions and may jeopardize the gains achieved by cART simplification. For many patients overall pill burden has not significantly changed due to the non-HIV-1 medication pill burden (Krentz *et al.* 2012). As a consequence of both HIV and comorbid disease burden, polypharmacy and medication-related problems are emerging as an important challenge facing older HIV-infected adults.

Polypharmacy was defined as intake of 5 or more non antiretroviral compounds. Interplay between polypharmacy and anti-HIV-1 drugs has not been yet fully investigated as potential impact on adherence, safety and potential for drug-drug interactions.

Data gaps could be identify in 3 main fields. The first is the impact on global tolerability of therapy: the question is whether and to what extent polypharmacy and antiretroviral drugs could have additional or overlapping effect in terms of toxicity and tolerability. One of the few data available is the report of Veterans Cohort on polypharmacy in HIV-1 infected and uninfected people (Edelman *et al.* 2013). An analysis of mortality adjusted for gender, race/ethnicity and VACS index score showed an increasing hazard of death in those prescribed 3 or more medications, reaching about 2-fold for HIV-1 infected patients prescribed 5 or more medications. The association of polypharmacy with increased mortality is concerning but the cause is unclear. Most likely, greater mortality is simply due to more severe underlying comorbid illnesses. However, it is possible that patients receiving polypharmacy have more toxicity and/or lower treatment efficacy resulting from drug-drug interactions or reduced adherence. Availability of antiretrovirals with very few long-term tolerability issues, as integrase inhibitors, could decrease such risk over time. However more clinical data should be obtained from cohort studies.

The second main data gap is impact on adherence. While adherence of antiretrovirals has been fully investigated in last decades, scarse data are available on reciprocal effect between HIV and non-HIV drugs on adherence (Elzi *et al.* 2010; Juday *et al.* 2011). Impact of comorbidities on HIV-1 medication persistence was retrospectively evaluated in a US database (Maiese *et al.* 2012). This analysis of healthcare and pharmacy claims data including 3,057 patients found a relationship between increasing number of comorbidities and discontinuation of cART. Patients with 1, 2, and 3 or more comorbidities had a 6% (p=0.528), 28% (p=0.014), and 31% (p=0.02), respectively, higher risk of cART discontinuation compared with patients with no comorbidities. While multiple factors may be at play, polypharmacy may contribute to these findings. No study, however, have so far investigated the effect of schedule and number of non-HIV-1 drugs on adherence of anti-HIV-1 medications, and viceversa. Such impact could theoretically different in newly discovered HIV-1 positive patients starting HIV-1 drugs while already taking since long time a number of non-HIV medications, as compared to subjects in follow up who add an increase number of concomitant compounds to a stable HIV-1 regimen.

The third point is potential for drug-drug interaction. Many studies, after the first report by the Swiss Cohort (Marzolini *et al.* 2011), focused on an high risk of drug-drug interactions in HIV-1 positive patients administered with concomitant medications. Most of the drugs, in fact, share the same metabolic pathways of antiretrovirals, namely CYP3A4/5-based metabolism. For instance, a study reviewed the prevalence and risk factors for clinically significant drug interactions with cART, and it was found that those subjects aged >42 years with more

than three comorbidities and a treatment plan consisting of a PI were at an independently increased risk of a clinically significant drug interaction (Miller *et al.* 2007). Therefore, use of antiretrovirals with a metabolism not primarily involving cytochrome P450 is warranted in these patients. In any case, no interaction data of a number of drugs are available (e.g anticonvulsants or antiarrhythmic), and clinical significance of a known interaction is not easy to evaluate in the single patient. Most cohort studies including clinical follow up of patients with polypharmacy and high risk of drug-drug interaction are requested. Moreover, clinicians must to be aware of the impact of unpredictable drug-drug interaction in the clinical setting. A very recent example from anti-HCV treatment (FDA), where FDA is warning that serious slowing of the heart rate can occur when the antiarrhythmic drug amiodarone is taken together with sofosbuvir taken in combination with another direct acting antiviral for the treatment of HCV infection.

In conclusion, patients with HIV-1 infection have several comorbidities requiring multiple pharmacotherapies that can increase their risk of polypharmacy and related adverse events. However, little is known about the impact of aging on medication use in HIV-1 infected older individuals, the potential for interactions with cART and administered medications, and the impact of this on therapy tolerability and virological response with aging. Reducing pill burden, careful titration of medications, and increasing awareness of common DDIs can prevent coadministration of potentially harmful combinations and reduce unnecessary polypharmacy-related adverse events in this population.

OPPORTUNITIES FOR INTEGRASE INHIBITOR THERAPY IN SPECIAL PATIENT POPULATIONS

Cristina Mussini, Antonio Di Biagio

Given the potency and the relative safety of the INSTIs in initial trials and in clinical practice, there has been considerable interest in exploring their role in different group of HIV-1 infected patients.

Integrase inhibitors in HIV-infected patients with high viral load

Data from randomized trials and observational studies have shown that patients with high viral load are among those most difficult to treat. Indeed, the higher baseline HIV-1 RNA is a marker of treatment failure (Santoro *et al.*, 2013). The drug-class of integrase inhibitors, characterized by a rapid viral decay resulted in a good option for these patients since has reached good virological results either compared to EFV or to protease inhibitors. In the SINGLE study the overall difference in treatment response in favour of DTG was also observed among patients with HIV RNA >100,000 copies/mL as well as those with HIV RNA ≤100,000 copies/mL (Walmsley *et al.*, 2013). The SINGLE study results are consistent with rates in STARTMRK (Lennox *et al.*, 2009). Both regimen showed virologic success also in patients with baseline HIV-RNA >100,000 copies/mL. In the SAILING Study, DTG performed better than RAL in patients experienced with HIV-RNA >50,000 copies/mL (Cahn *et al.*, 2013). Moreover, in the Flamingo Study patients in DTG-based regimens had a better response rate among people starting treatment with a viral load above 100,000 copies (Clotet *et al.*, 2014). Also the new single tablet regimen containing EVG/COBI/FTC/TDF showed better response rate than EFV/FTC/TDF and FTC/TDF and ATV/r in patients with baseline HIV-1 RNA >100,000 copies/mL (DeJesus *et al.*, 2012; Zolopa *et al.*, 2013).

These data are confirmed in clinical practice, indeed, a recent Italian cohort study showed that an INSTIs based regimen allows obtaining a higher probability of success in a group of patients with high viral loads at baseline (INSTI vs.boosted PI HR 3.23; P<0.001) (Di Biagio *et al.*, 2014). In the ACTG 5257, a comparative, randomized, clinical trial, first-line RAL proved superior to PI/r (ATV/r and DRV/r) in an endpoint combining virologic efficacy and safety. The analysis involved 1809 eligible patients, 30% of participants had a pretreatment HIV-RNA >100,000 copies/mL (Lennox *et al.*, 2014).

Integrase inhibitors in advance naïve patients

Among patients who may benefit from an INSTI-containing regimen we could include those

with a low CD4 T cell count at diagnosis. Indeed, some trials have shown an increase in CD4 T cell count in subjects receiving this drug class, which could be clinically relevant in this group of patients. In the SINGLE Study the authors observed a statistically superior response in favours of DTG plus ABC/3TC in change from baseline in CD4 T cell counts at week 48 (267 cell/μL vs 208 cells/μL; p<0.001).
In the STARTMRK study the mean CD4 T cell count at randomization was 219 (SD 124) cells/μL and the mean changes from baseline CD4 T cell count were 332 and 295 cell/μL in the RAL and efavirenz arms, respectively (Lennox *et al.* 2010). In the SAILING Study the CD4+ cell count at baseline in the DTG arm was 204.5 cells/μL (88-368), while in the RAL arm was 193.0 cells/μL (96-365). CD4 T cell counts increased from baseline to week 48 in both groups (mean change 162 [SD 151] cells/μL in the DTG group; 153 [144] cells/μL on RAL). In the FLAMINGO the median CD4 T cell count gain measured 210 in both treatment groups.
In the ACTG 5257, at baseline 30% of participants in either group had CD4 T cell count <200 cells/μL, and the mean change in CD4 T cell count from baseline was: 256 (240-271) cells/μL in DRV/r arm; 284 (269-300) cells/μL in ATV/r arm and 288 (272-304) cells/μL in RAL arm (Lennox *et al.* 2014).

Integrase inhibitors in women of childbearing age

RAL and DTG do not have any drug-drug interactions with oral contraceptive, while, since EVG/COBI are metabolized by CYP3A and COBI is also to a minor extent by CYP2D6, there could be significant interactions with hormonal contraceptives. RAL and DTG may be given with estrogen/progestin oral contraceptives (Anderson *et al.* 2011).

Integrase inhibitor use in pregnancy

Integrase inhibitors, as all other antiretrovirals, are not licensed for the use in pregnancy and to date data are limited. RAL is classified as an FDA pregnancy category C, DTG and EVG are classified as category B (DHHS Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2014).
Nevertheless, despite the absence of any antiretroviral drug in class A, a suppressive antiretroviral regimen is mandatory not only for the health of the mother, but even more importantly to avoid transmission of HIV-1 infection to the baby. Indeed, the use of integrase inhibitors is particularly important in those who arrive at HIV-1 diagnosis very close to the delivery, since it is fundamental the rapid decay of viremia in the attempt to avoid peri-partum HIV-1 transmission (Westling *et al.* 2012; Cha *et al.* 2013; De Hoffer *et al.* 2013; Taylor *et al.* 2013; DHHS Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2014).

Paediatric and adolescents patients

The impressive antiviral potency, the convenience of dosage and the excellent tolerability profile make integrase inhibitor an attractive option for children and adolescent younger than 16 years of age, even if, at present, data are limited. RAL is FDA-approved for use in infants and children aged ≥4 weeks and weight ≥3 kg. Current paediatric approval and dosing recommendations are based upon evaluations in 122 patients aged ≥4 weeks to 18 years enrolled in IMPAACT P1066.
DTG is FDA-approved in combination with other antiretroviral drugs for children aged 12 years and older, weighing at least 40 kg, and who are treatment-naïve or treatment-experienced and INSTI-naïve.
A clinical trial, IMPAACT P1093, is an ongoing open-label trial of HIV-infected children with the plan to enrol down to age 4 weeks. FDA approval of DTG down to age 12 years was based on data from 23 treatment-experienced, INSTI-naïve adolescents. Neither EVG nor TDF/FTC/EVG/COBI is FDA-approved for use in children aged <18 years (DHHS Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children 2014).
Gaps Still present:

1. To evaluate their efficacy in late presenter patients.
2. To evaluate in cohort study if there is any peculiar advantage of this drug class concerning hard clinical end-points.
3. To evaluate if the rapid viral decay could have an impact on chronic inflammation.

HIV/HCV-COINFECTED PATIENTS

Massimo Puoti; Raffaele Bruno; Valentina Zuccaro

Antiretroviral treatment for HIV-1-infected treatment naïve and -experienced patients has substantially improved over the past 10 years with the approval of several new drugs from existing classes, such as protease inhibitors (e.g., DRV), and novel classes, such as chemokine inhibitors (CCR5) (e.g., maraviroc), and, in particular, integrase inhibitors (RAL, DTG and EVG/COBI/FTC/TDF single-tablet regimen [Stribild©]). As a result, life expectancy has improved, approaching that of uninfected individuals, based on a recent analysis of 4,600 asymptomatic treatment-naïve HIV-infected patients in high-income countries (Mocroft *et al.* 2003). The increased longevity, however, also necessitates consideration of the increasing incidence of HANA conditions, commonly associated with advancing age and chronic inflammation, including liver disease, among other comorbidities (Puoti *et al.* 2012).

A multi-target clinical strategy has been proposed to decrease liver-related morbidity and mortality in people living with HIV-1; this strategy should include: hepatitis B vaccination and optimization of dual anti-HIV-1and anti-HBV therapy, screening for and proactive treatment of alcohol abuse, optimization of cART, management of insulin resistance, prompt diagnosis and treatment of glucose intolerance and diabetes (Puoti *et al.* 2013a). However, since HCV coinfection is the most important cause of liver disease in HIV-1 infected population, a "screen and treat" strategy for HCV coinfection is, at least in theory, the most effective and relevant measure to decrease liver-related mortality among HIV-1 infected patients. In fact, overall mortality in HIV/HCV co-infected patients from the VA cohort has not significantly decreased from 1996 (pre-cART era) to 2009, because HIV-1 related mortality has been replaced by HCV-related mortality (Puoti *et al.* 2013b).

In addition to its impact on liver-related morbidity and mortality, HCV co-infection in HIV-1 infected individuals has been independently associated with an increased incidence of renal failure and pathological hip. There are also controversial data about an unfavourable impact of HCV co-infection on progression of HIV-1 infection towards AIDS and on CD4 T cell recovery after cART.

These discordant results could be due to confounders associated with HCV coinfection (history of injection drugs use, low socioeconomic status, African American ethnicity), thus the worsening role of HCV co-infection on HIV disease evolution, and response to anti-HIV treatment is still debated (Puoti *et al.* 2013b).

Characteristics of the "Ideal anti HIV therapy in HCV co-infected patients"

For these reasons the ideal antiretroviral drug for HIV HCV co-infected patients should be:

- Effective on HIV disease to counteract worsening impact of HCV coinfection.
- Kidney and bone friendly with anti HIV activity in CNS.
- Not associated with insulin resistance, lipodystrophy and dyslipidemia.
- Without significant metabolic impact and hepatotoxicity in HIV+.
- Without drug drug interactions with anti HCV drugs.
- Safe in patients with End Stage Liver Disease.

The efficacy on HIV-1 disease and the impact on metabolic, renal and bone related comorbidities of integrase inhibitors have been illustrated in other reviews.

This chapter will be focused on hepatotoxicity, drug-drug interactions with anti HCV drugs and pK and safety in patients with end stage liver diseases of integrase inhibitors.

Hepatic Safety of Integrase Inhibitors in HCV co-infected patients

Of the integrase inhibitors in clinical development, currently available data concerning the use of INSTIs in HCV co-infected patients derive from clinical trials. In treatment naive patients, protocol 021 of the STARTMRK study, 7% of patients enrolled in this trial were either hepatitis B or C co-infected. Grade 3 or 4 laboratory abnormalities occurred at similar frequencies within the EFV based arm and the RAL based arm with regard to ALT, AST, or alkaline phosphatase or total bilirubin elevations Data on the hepatic safety in hepatitis co-in-

fected patients is available at present from the phase III studies. Patients with chronic active hepatitis B and/or hepatitis C co-infection were permitted to enroll provided that the liver function tests, i.e. AST, ALT and alkaline phosphatase, did not exceed five times the upper limit of normal. Overall 113 of 699 patients were either co-infected with hepatitis B or hepatitis C or both (16.2%). In general, the safety profile of RAL in patients with hepatitis B and/or hepatitis C co-infection was similar to that in patients without hepatitis B and/or hepatitis C co-infection. Liver related laboratory abnormalities occurred at similar frequency within RAL and optimized background treatment arms. As expected, in hepatitis co-infected patients elevations of AST, ALT or total bilirubin were more often found, in 31%, 31%, and 16% of patients, respectively, as compared to 9%, 8%, and 8% of all other RAL-treated subjects. Two recent investigator initiated studies from Spain and the UK have also reviewed the hepatic safety of RAL in HCV co-infected patients. A single center study from Spain evaluated 218 HIV-positive patients who had received RAL as part of HAART. Apart from being part of a rescue regimen, RAL was prescribed in 59% of patients because of poor tolerance or side effects with an otherwise virologically suppressive regimen. Ninety-two (42%) were HCV positive. In this study any grade of ALT elevation was observed in 8% of HIV and 25% of HCV/HIV co-infected patients ($p<0.001$). Only three ALT elevations grade 3 or 4 were observed among HCV-positive patients, however all were considered not related to RAL. Among HCV-negative patients no grade 3 or 4 ALT elevation was observed. In a UK study 13 patients co-infected with HIV and HCV were switched from a PI (n=7), NNRTI (n=4), or NRTI only (n=2) to a RAL based cART. The mean ALT at baseline and three months was 247 U/l and 176 U/l, respectively. Eight patients experienced sustained improvements in the ALT at three months, though the reduction in mean ALT from baseline to three months after commencing RAL was not statistically significant ($p=0.1$). Across DTG clinical program, the percentage of participants with hepatitis B or C co-infection ranged from 7% to 11% in the three studies conducted in treatment-naive patients wherease it was 13 % in treatment-experienced trial (Min *et al.*, 2014). The incidence of aspartate aminotransferase and alanine aminotransferase elevations in individuals with HBV/HCV infection and treated with DTG was lower than in RAL- and EFV/TDF/FTC-treated ART-naive individuals but higher than in the DRV/r-treated group. The incidence of liver chemistry toxicities for subjects without hepatitis virus infection was similar in the DTG, RAL, and EFV groups, but lower in the DRV+ RTV group.

ART-experienced individuals coinfected with HBV and/or HCV who received DTG were noted to have more frequent liver enzyme elevations. Grade 3 to 4 elevations in total bilirubin were invariably associated with atazanavir administration in both DTG and RAL treatment arms (Curtis *et al.*, 2014).

In a small group of HCV co-infected subjects, switch from PI/r or NNRTI in combination with FTC/TDF to Stribild© was safe. At baseline, some subjects previously on EFV or ritonavir boosted PI had elevated ALT and/or AST levels. No grade 3 or 4 ALT and/or AST elevations or evidence of drug induced liver injury in the STB group up to week 48.

In summary integrase inhibitors appears to have a favorable hepatic safety profile and increased rates of ALT elevations among hepatitis co-infected patients are similar to those observed with other recent new drugs in other clinical trials and cohorts (Vogel and Nelson 2009).

Integrase inhibitors and anti HCV Therapy

Indications for HCV treatment in HCV/HIV co-infected persons are identical to those in patients with HCV mono-infection. Regardless of the respective costs of these options, IFN-free regimens are the most attractive options when available in HCV mono-infected and in HIV/HCV co-infected patients. The same IFN-free treatment regimens can be used in HIV-co-infected patients as in patients without HIV-1 infection, as the virological results of therapy are identical. The use of COBI-based regimens, EFV, delavirdine, ETR, nevirapine, RTV, and any HIV protease inhibitor, boosted or not by ritonavir, is not recommended in HIV-1 infected patients receiving simeprevir. The daily daclatasvir dose should be adjusted to 30 mg

daily in HIV-1 infected patients receiving ATV/r and to 90 mg daily in those receiving EFV. No drug-drug interaction has been reported between sofosbuvir and antiretroviral drugs. The fixed-dose combination of sofosbuvir and ledipasvir can be used with all antiretrovirals. However, this regimen should not be used with the combination of tenofovir/FTC with ATV/r, DRV/r, LPV/r or EVG/COBI when possible, or used with caution with frequent renal monitoring. The combination of ritonavir-boosted paritaprevir, ombitasvir and dasabuvir should not be used with EFV, ETR or nevirapine and rilpivirine should be used cautiously with repeat ECG monitoring. ATV and DRV should be taken without ritonavir and other protease inhibitors are contraindicated. EVG/COBI should not be used with this regimen because of the additional boosting effect. As far as concomitant use of HIV drugs and FDA-approved HCV drugs for treatment of HCV in HIV-Infected Adults, INSTIs seem to show less significant drug-drug interactions with any HCV drugs compared to other ARV classes although, within INSTIs, RAL and DTG offer a better profile than EVG, that is co formulated with cobicistat (DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents 2014).

Integrase inhibitors and End Stage Liver disease

Several ARVs have been evaluated in the setting of liver impairment to evaluate potential changes in PK and safety.

For ritonavir boosted PIs, for a given degree of impairment, the exposure changes vary considerably depending on the PI. Boosted-DRV exposures are unchanged with mild impairment, while the AUCtau and concentrations at 12 h after dosing (C12 h) of boosted tipranavir are 30% and 84% higher, respectively.

In patients with moderate hepatic impairment, DRV AUCtau values were 20% higher than those in matched controls, despite 2-fold higher RTV Cmin levels, indicating the independent effects of liver impairment on the PK of these agents.

No clinically relevant changes in RAL, DTG or EVG or COBI PK were observed following multiple-dose administration in subjects with moderate liver impairment versus matched control subjects and the study treatments were well tolerated; these findings can be extrapolated to mild hepatic impairment.

Accordingly, RAL, DTG and EVG, as an individual agent or as a component of EVG/COBI/FTC/TDF, may be administered without dose adjustment to HIV-1 infected patients with moderate or mild hepatic impairment.

There are no data on boosted EVG in subjects with severe impairment. RAL plasma levels are increased in HIV/HCV co-infected patients with advanced liver cirrhosis (Child-Pugh C). Despite the higher exposure, RAL was safe and well tolerated (Hernandez-Novoa *et al.* 2014).

Conclusions

New antiretroviral agents like the integrase inhibitors offer the opportunity to maintain or promote CD4 T cell increase and a prompt and durable HIV-1 suppression, to treat HIV-1 with a minimal impact on renal function, metabolic disturbances and bone metabolism with minimal hepatotoxicity and a potential for administration in End Stage Liver Disease.

In addition they offer the opportunity of modify cART for the duration of anti-HCV therapy to circumvent interactions with ribavirin, or the new anti HCV drugs and especially with regimen including anti HCV protease inhibitors or ledipasvir.

Thus RAL, EVG and DTG, may constitute a relevant treatment options in the setting of HIV-1 and HCV co-infection.

TREATMENT INTENSIFICATION WITH INTEGRASE INHIBITORS AND THE CNS RESERVOIR

Andrea Antinori, Paola Cinque

HIV-1 integrates into the host genome, and CD4 T cells can harbour latent, replication competent HIV-1 DNA for several years. This latent reservoir may be resistant to therapy, resulting in lifelong infection. Despite the sustained suppression achieved by cART in plasma, HIV-1 persists in long-lived cellular reservoirs, and low-level ongoing viral replication, undetectable by standard test, may be detected in plasma by ultrasensitive assays. Whether this low-level viremia is related to viral replication or release of virus from stable reservoirs without evidence

of replication remains controversial. However, even without ongoing cycles of replication, HIV-1 virions are persistently produced and released. The association between reservoir size and immune activation during cART (Hatano *et al.* 2013a) suggests that cellular reservoirs may contribute to chronic inflammation and potentially to HIV morbidities in HIV individuals with durable cART-induced suppression of HIV-1 replication (Deeks *et al.* 2013). The central nervous system (CNS) is considered an anatomic reservoir where HIV-1 can persist despite suppressive therapy and replicate at low level, due to low penetration of several antiretroviral drugs across the blood-brain barrier (Dahl *et al.* 2014). The development of the INSTIs antiretroviral drug class opens new interesting perspectives in treatment control of HIV-1 reservoirs, relating to both their mechanism of action and the possibility of penetration and acting in "sanctuary sites".

In this perspective, we will review pharmacokinetics studies addressing INSTIs penetration in the CNS, their *in vitro* properties to inhibit HIV-1 in cells from the monocyte-macrophage lineage, and the effect of cART intensification using INSTIs on cerebrospinal fluid (CSF) markers of replication and immune activation.

Cerebrospinal fluid pharmacokinetics studies

Most of the CSF pharmacokinetics studies have involved RAL and investigated its CSF concentrations in relation to *in vitro* inhibitory concentrations, plasma levels, permeability status of the blood-brain barrier and presence of polymorphisms in RAL transporter genes.

Overall, RAL penetration in CSF varies largely among subjects, with a variable proportion of patients achieving drug concentrations above the reported inhibitory concentrations for wild-type HIV-1 (Yilmaz *et al.* 2009; Croteau *et al.* 2010; Calcagno *et al.* 2014). Indeed, the proportion of patients with RAL CSF levels above the inhibitory concentrations (IC) depended on the reference value used, e.g., IC50% vs IC90% or IC95%. For instance, CSF RAL concentrations exceeded the IC95% levels for wild-type HIV-1 strains in approximately 50% of 25 specimens and in 29% of 41 specimens in two different studies (Yilmaz *et al.* 2009; Croteau *et al.* 2010; Calcagno *et al.* 2014). On the contrary, CSF drug concentrations exceeded the IC50% for wild-type HIV-1 in all of 21 patients, by a median of 4.5 fold and in all of 41 patients in two different series (Croteau *et al.* 2010; Calcagno *et al.* 2014).

Thus, if IC95% values are used as reference, only a proportion of patients seem to achieve inhibitory CSF RAL concentrations.

CSF RAL concentrations were higher in patients with higher albumin CSF: plasma quotient and CSF albumin concentrations, reflecting higher barrier permeability, and in those with higher plasma RAL concentrations (Yilmaz *et al.* 2009; Croteau *et al.* 2010; Calcagno *et al.* 2014). In an attempt to better define CSF pharmacodynamic targets, inhibitory quotients (IQ, CSF concentrations divided by *in vitro* 50% and 95% inhibitory concentrations) were calculated for different drugs and an adequate exposure was considered if IQ95 was >1. Compared to other drugs, and despite high CSF: plasma ratios, of an average of 17%, RAL recipients showed a low IQ95, of 0.7, resulting from the high proportion of samples with concentrations below the IC95% (Calcagno *et al.* 2015). However, because of large inter-patient and intra-patient concentration variability, generalized definitions for adequate CSF exposure may not always apply to individual cases.

The CSF RAL concentrations were also compared between patients with or without certain polymorphisms of cell membrane efflux transporter genes, to investigate whether altered expression of transporters - used by RAL to cross the blood-brain barrier - could be associated with differences in CSF drug concentration. Several membrane transporters expressed in the blood-brain barrier were investigated, including the major transport protein P-glycoprotein (Pgp), coded by the ATP-binding cassette sub-family B member 1 (ABCB1) and the ATP-binding cassette sub-family G member 2 (ABCG2), expressed by the homonymous gene. No different CSF RAL concentrations were found between patients with different ABCB1 variants, including the 3435C→T variant, which is associated with different Pgp expression level (Johnson *et al.* 201s3; Calcagno *et al.* 2014; Tsuchiya *et al.* 2014). In contrast, RAL concentrations in CSF, but not plasma,

were lower in CA and AA genotype holders at position 421 in the ABCG2 gene compared to CC genotype (Tsuchiya *et al.* 2014). Because these C to A nucleotide substitutions reduce transporter expression, they may impair RAL transport from blood to CSF, resulting in low CSF concentrations.

The fact that ABCG2 is diffusely expressed, whereas ABCB1 is only weakly expressed on the CSF side of choroid plexus epithelial cells, suggests that ABCG2 expression level is more likely to influence CSF RAL concentration than ABCB1. Finally, an association was also found between lower CSF RAL concentrations and the 613 CG genotype in the gene encoding the hepatocyte nuclear factor 4 α (HNF4α), an intranuclear factor that contributes to expression of OAT1, another RAL brain barrier transporter (Calcagno *et al.* 2014). Overall, it seems that polymorphisms in the transporter genes might influence the CSF RAL penetration. However, given the current evidence, they seem more to contribute to the large concentration variability observed among patients than to represent a potentially useful marker of CSF RAL penetration.

Beyond RAL, there is no published data on the CSF pharmokinetics of EVG, and only initial, although encouraging data on DTG. A recent study of a group of 11 naïve patients showed CSF DTG concentrations similar to unbound plasma concentrations and exceeding the *in vitro* IC50% for wild-type HIV-1 in all of the cases (Letendre *et al.* 2014). Of note, DTG CSF concentrations exceeded the IC50% value by 90-fold at week 2 and by 66-fold at week 16, i.e., figures that were much higher than those observed with RAL (Croteau *et al.* 2010). The clinical relevance of these findings is unknown, however, the high CSF DTG concentrations, together with its known activity against most integrase inhibitor single-mutant viruses suggest an effect of DTG also in case of resistance to integrase inhibitors, including in the CNS.

In vitro studies of integrase inhibitors susceptibility on macrophage cells

Recent *in vitro* studies showed that the anti-HIV-1 activity of RAL in macrophages was similar or slightly higher than that observed in PBMCs and T cell lines (Scopelliti *et al.* 2011). Despite significantly lower intracellular concentrations in macrophages than in lymphocytes, however, RAL was similarly effective in inhibiting HIV-1 replication, both in resting and activated macrophages (Gavegnano *et al.* 2013). The efficacy of RAL on macrophage cell infection was also confirmed by measurement of pro-inflammatory cytokines in tissue culture supernatants from primary brain derived macrophages infected with HIV-1 and treated with RAL.

Indeed, RAL significantly suppressed or decreased the rate of cytokine production in HIV-1 infected microglia, including IFN-γ, IL-10, IL-12-p70, IL-1, TNFα and IL-6 (Tatro *et al.* 2014). In general, these *in vitro* studies support the efficacy of RAL also in CNS macrophages and their potential in inhibiting CNS HIV-1 replication.

During macrophage infection, however, the presence of a single primary RAL resistance mutation (Q148H, Q148R, N155H, or N155S) was sufficient to confer virus resistance, similarly to what is observed in co-stimulated CD4 T cells (Canducci *et al.* 2011). On the other hand, single mutations were associated with a different level of susceptibility to RAL and EVG in human primary monocyte derived macrophages, and DTG was able to inhibit infection of RAL-resistant N155H and Y143C mutants in these cells (Marsden *et al.* 2011; Pollicita *et al.* 2014). These observations suggest that macrophages, which may play a role as a reservoir of HIV infection, may also enable the development of RAL resistance. In this view, the efficacy of DTG in macrophages, including against RAL-resistant mutants, may be clinically relevant.

INSTI intensification and effects on reservoirs

Several studies have been implemented using an INSTI for the intensification of cART regimens in HIV-1 infected individuals with stable virological suppression. An increase of episomal circular DNA forms (2-LTR circles) has been detected following RAL intensification in patients receiving a standard cART suppressive regimen (Buzon *et al.* 2010; Llibre *et al.* 2012; Hatano *et al.* 2013b), although this observation was not confirmed by others (Gandhi *et al.*

2012; Gutierrez *et al.* 2013). This observation supports the hypothesis that low-level viral replication persists in some individuals even after long-term cART, and that INSTIs may impact on this residual replication.
Nevertheless, no significant changes in ultrasensitive plasma HIV RNA levels were observed with RAL intensification (McMahon *et al.* 2010; Gandhi *et al.* 2012; Gutierrez *et al.* 2013; Hatano *et al.* 2013b), suggesting that residual viremia is prevalently produced by latent stable reservoirs, rather than reflecting ongoing active replication in plasma. In addition, INSTI intensification has been associated, although not in all studies, with a decrease of CD4 and CD8 T-cell activation (Gandhi *et al.* 2012; Llibre *et al.* 2012; Gutierrez *et al.* 2013; Hatano *et al.* 2013b) and of coagulation markers levels (D-dimer) (Hatano *et al.* 2013b).
A low-level persistent HIV-1 replication has also been detected in CSF in patients with more than 10 years of suppressive cART (Dahl-Peterson). Around 17% of CSF samples had detectable low-level HIV-1 RNA (compared with 57% in plasma), and detectable HIV-1 RNA in CSF was associated with increased CSF immune activation, suggesting that HIV-1 may possibly continue to drive neurological injury even during suppressive therapy.
These findings also indicate that the CNS could be a possible reservoir for HIV-1 during suppressive therapy.
However, no effect of INSTI intensification was observed on CSF markers of viral replication and immune activation.
Adding RAL to a current suppressive cART regimen did not affect HIV-1 RNA or neopterin levels, or intrathecal CD4 and CD8 T-cell activation (Dahl *et al.* 2011), similar to what observed in intensification studies with non-INSTI antiretroviral drugs (Yilmaz *et al.* 2010).
Therefore, although CNS is considered a reservoir where antiretroviral drug concentrations are generally lower than in blood, it seems not to be affected by cART intensification with differentially CSF-penetrating drugs (Yilmaz *et al.* 2010; Dahl *et al.* 2011).

Conclusions

Recent data are accumulating that support the neuroprotective potential for INSTIs. While CSF penetration of RAL seems to vary among subjects, due to the barrier status and, possibly, host genetic factors, DTG CSF concentrations are more consistently exceeding the desired inhibitory HIV-1 concentrations.
On the other hand, *in vitro* observations on the efficacy of INSTIs in monocytic-macrophage cells support their potential to inhibit HIV-1 replication in CNS macrophages, and confirm the efficacy of DTG against RAL-resistant mutants, which may be clinically relevant.
Although preliminary intensification studies with RAL have failed to show an effect on CSF markers of replication and immune activation, additional clinical and pharmacodynamics studies may help understand the full potential of INSTIs, including the newer drugs, in inhibiting CNS HIV-1 replication and purging the CNS reservoir.

BUDGET IMPACT MODELS

Davide Croce, Giuliano Rizzardini

Recently, Budget Impact Models (BIM), a tool allowing to evaluate the economic impact of drug regimens, have captured a lot of attention.
The economic crisis and its effects on health system funding have made this tool, which before was considered as an accessory tool in health economic evaluation, a fundamental instrument in the evaluation process that precedes the marketing authorization of pharmaceutical products.

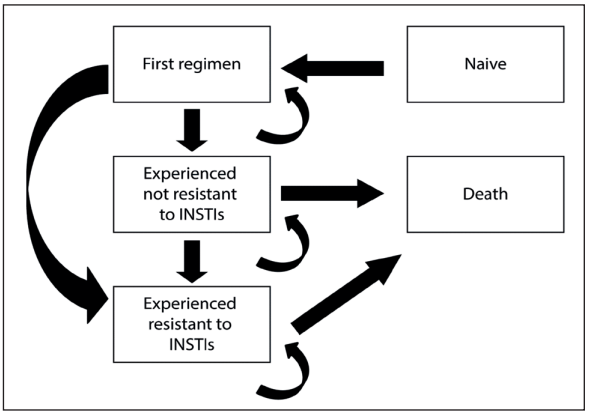

FIGURE 3 - *Schematic representation of the BIM for the introduction of INSTI in clinical practice*

The use of these models is particularly helpful for the evaluation of the overall convenience of a product and the allocation of the budget resources.

It is generally accepted that a full economic evaluation for a new health system intervention requires a cost-efficacy analysis together with a budget impact analysis (BIA).

The aim of BIA is to estimate the economic consequences deriving from the introduction of a new health intervention in a specific health system or setting (Mauskopf *et al.* 2007; Sullivan *et al.* 2014). Results of BIM might be of particular interest for hospital managers and, in general, decision makers of health system balance since they provide a useful simulation for balance planning. This is one of the main reasons why companies selling diagnostic and treatment tools provide BIAs as sale support information. Moreover, nowadays the main national regulatory agencies such as the National Institute for Health and Care Excellence (NICE, UK), the Pharmaceutical Benefits Advisory Committee, as well as the managed care organizations in the US require that companies submit the estimates of both the cost-efficacy analysis and the expected impact of the new interventions on the national and local and health budgets in order to support the authorization process of a new product.

TABLE 3 - *Description of the model.*

Entry	*Specification*	*Notes*
Population (HIV treated patients)	85500	Population size was estimated as the sum of the total number of patients living with HIV in Italy in the year 2012 plus the number of newly diagnosed patients in the year 2013 (3853) according to the Italian Health Institute (Istituto Superiore di Sanitá, ISS). Number of deaths was estimated to be 1% of total number of treated patients
Analysis	Total HIV-1 positive population, stratified in three groups	Stratification was performed as follows: subjects undergoing cART for the first time (first regimen), experienced patients (i.e. patients who experienced virological failure or changed cART regimen due to tolerability issues, simplification or other reasons - experienced patients harboring virus not resistant to INSTIs), and subjects currently in treatment with INSTIs (RAL) who experienced virological failure due to INSTIs resistance (experienced patients harboring virus resistant to INSTIs).
Annual cycle	Each year 3853 new infections occur, 1% of the treated population is considered to have resulted in death, and 7.82% of patients switches to a different therapy*.	Among the patients who change their regimen, 24,4% belong to the first regimen group, whereas 65,6% are experienced patients (based on the observations in a hospital with more than 6000 patients).
Scheme	Patients belonging to the first regimen group who switch to a different therapy are moved to the group of experienced patients not resistant to INSTIs unless they received INSTIs in the previous year. In the latter case they are moved to the group of experience patients resistant to INSTIs. It is estimated that 3.3% of the INSTIs treated population will develop resistance.	Patients belonging to the experienced patients group not resistant to INSTIs who switch to a different therapy can either remain in the same group (with a different regimen) if the failed regimen does not include INSTIs, or can be moved to INSTIs experienced patients group. Patients belonging to the INSTIs experienced group cannot switch to other groups. In case of decease, patients are removed from the group

*Based on observations performed in six infectious diseases operative units in the Lombardia Region (Italy) and a total number of 10.600 patients across a 12 months time window (years 2011-2012).

TABLE 4 - *Cost impact analysis for the reference scenario.*

	Year 1	*Year 2*	*Year 3*	*Total*
Cost for first regimen patients	€ 300.603.714	€ 323.683.526	€ 346.620.493	€ 970.907.733
Cost for experienced patients not resistant to INSTIs	€ 691.463.058	€ 701.285.752	€ 712.586.587	€ 2.105.335.396
Cost for experienced patients resistant to INSTIs	€ 30.621.224	€ 37.944.464	€ 45.419.131	€ 113.984.819
Total cost	**€ 1.022.687.995**	**€ 1.062.913.743**	**€ 1.104.626.210**	**€ 3.190.227.948**

TABLE 5 - *Cost impact analysis for the DTG scenario.*

	Year 1	*Year 2*	*Year 3*	*Total*
Cost for first regimen patients	€ 300.974.523	€ 323.734.563	€ 345.692.341	€ 970.401.427
Cost for experienced patients not resistant to INSTIs	€ 692.796.927	€ 702.576.612	€ 712.876.491	€ 2.108.250.029
Cost for experienced patients resistant to INSTIs	€ 29.836.487	€ 36.172.603	€ 42.447.282	€ 108.456.373
Total cost	**€ 1.023.607.937**	**€ 1.062.483.779**	**€ 1.101.016.113**	**€ 3.187.107.829**

TABLE 6 - *Percent differences between the DTG scenario and the reference scenario.*

	Year 1	*Year 2*	*Year 3*	*Total*
Cost for first regimen patients	0.1%	0.0%	- 0.3%	- 0.1%
Cost for experienced patients not resistant to INSTIs	0.2%	0.2%	0%	0.1%
Cost for experienced patients resistant to INSTIs	- 2.6%	- 4.7%	- 6.5%	- 4.9%
Total cost	**0.1%**	**0%**	**- 0.3%**	**- 0.1%**

TABLE 7 - *Absolute differences between the DTG scenario and the reference scenario.*

	Year 1	*Year 2*	*Year 3*	*Total*
Cost for first regimen patients	€ 370.810	€ 51.037	- € 928.152	- € 506.306
Cost for experienced patients not resistant to INSTIs	€ 1.333.869	€ 30.621.224	€ 30.621.224	€ 30.621.224
Cost for experienced patients resistant to INSTIs	- € 784.737	- € 1.771.861	- € 2.971.849	- € 5.528.446
Total cost	**€ 919.942**	**- € 429.964**	**- € 3.610.097**	**- € 3.120.118**

BIM analysis for the INSTIs

The following model investigates the overall costs for Italian national health system (Sistema Sanitario Nazionale, SSN) in a scenario in which DTG is introduced in clinical practice. The model estimates the effects of DTG introduction in terms of health system costs (including ARVs, hospitalizations, visits, examinations and other concomitant non HAART drugs cost) by comparing a standard scenario corresponding to the present situation in Italy with the estimated scenario after the introduction of DTG in clinical practice.

The model includes a common scenario (year 0) which is represented by the year 2013, starting from which some hypothesis based on public available literature and on the studies for the establishment of the diagnostic workup and therapeutic options document (PDTA, Lombardia Region) have been formulated. The assumptions considered in the model are described in Figure 3 and Table 3.

Scenario: The pharmacological regimens included in the model were selected to mirror the regimens most frequently used in Italy, based on market surveys. The model simulates the evolution of the market within a three year time frame, changing the market share in presence or absence of DTG. The model considers the expected economic impact of:

1) the introduction of DTG*;
2) the shift to other existing therapies.

Dosage of DTG in the subgroup of patients who are resistant to INSTIs (patients who where treated with RAL and developed resistance) is estimated to be 50 mg twice a day combined with an OBT based on the data available from the VIKING-3 study (Castagna *et al.* 2014) and will be compared with a salvage therapy including FTC/TDF, etravirine (ETR), DRV, and maraviroc (MVC).

The costs included in the model refer to the year 2013. To establish the annual pharmacy costs for each treatment, the average annual costs were calculated multiplying monthly costs by the number of months corresponding to the annual basic need for a patient. Monthly drug costs were based on the data reported in the Lombardia Region administrative order 1725 (01/03/2013) (14).

Previous analysis have shown that the basic need per patients corresponds to the number of therapeutic units distributed in a time window of ten months, due to the incomplete adherence to cART and other factors.

The remaining cost data were calculated based on the clinical records taken from (Rizzardini *et al.* 2012), and considering the results of the Markov model used for cost-efficiency analysis of DTG. The present model compares two scenarios differing by the presence or absence of DTG with the aim to calculate the differential impact and the overall impact of DTG on the SSN.

In order to assess the robustness of the study, a sensitivity analysis was carried out on the variables that determine the cost of treatment and the number of patients in therapy: drug cost were decrease (-20%), and number of new patients, percentage of therapy switches and percentage of INSTIs resistant patients were allow to vary by ±20%.

Table 4 to 7 show the results of the three year model projection for the two alternative scenarios.

As reported in the tables, it is estimated that the introduction of DTG in clinical practice will lead to a three million euro saving for the SSN over a three year time frame.

Estimated total costs calculated in the sensitivity analysis differed by shortcoming or excess; these differences demonstrate the complexity and issues currently faced in the pharmacological management of HIV-1 positive patients deriving both from the introduction of generic drugs and new molecules and the consequent price adjustments and from the financial difficulties of public health institutions.

The results of economic analysis as well as the needs of health care operators show significant variations due the high dynamism of the current situation.

*It is estimated that naïve patients will be given DTG together with a backbone constitute either by FTC/TDF (60% of patients) or ABC/3TC (40% of patients) whereas in experienced patients, DTG will be used in association with an OBT estimated from the data reported in the SAILING study (Cahn *et al.*, 2013). In the latter group it is estimated that DTG will be used in association with a backbone in 40% of cases or with an OBT in the remaining 60% of cases.

The estimates for the introduction of DTG predicted by the model show how a drug with a well-known efficacy entails an improvement of the economic performances for the health system despite the elevated cost. However, it must be considered that the model is strongly dependent from the assumptions on which is based and that the BIM is intended for providing a general framework to the health care decision makers.
Future assessment of economic impact for the introduction of new drugs will likely need some degree of adjustment due to the increasing variability in the market conditions, which are becoming more and more specific between different hospitals.
Indeed, from the BIM perspective, co-existence of different drug costs prevents the possibility to a priori and completely define the choice with highest economic gain for each health care institution.
Moreover, an additional factor contributing to the dynamism of the field of pharmacological treatments for HIV-1 infected patients is the introduction of generic drugs, which will become available in increasing numbers as patents expire. Despite the expressions of concern from the patient's associations, generic drugs could find large application in HIV-1 treatment depending on drug market regulation and obligatoriness of use.
This will eventually lead to a decrease in the branded drugs price, which will need to be corrected to be competitive with the generic drug.
This situation will result in a significant increase of public health savings which in turn will increase the available funds for more expensive regimens for the patients who require them.
This virtuous circle needs to be carefully monitored in order to maximize the benefits and minimize the "escape from generic drug treatment" which has been previously observed in other pharmacological branches.

CONCLUSIONS

Massimo Andreoni

The development of drugs targeting critical steps in the life cycle of HIV-1 has been central to treatment success. While RAL is the first approved HIV-1 integrase inhibitor, two other new INSTIs, DTG and EVG, have been recently approved The integrase enzyme is essential for retroviral replication, and the absence of a host-cell equivalent of integrase means that INSTI do not interfere with normal cellular processes, and therefore have a high therapeutic index. INSTIs target the strand transfer step of HIV-1 integration; strand transfer integrase inhibitors bind in the catalytic core domain of the enzyme and compete for binding with host DNA. It's important to note that the stable integration of the reverse transcribed viral genome into host chromatin forms an important point-of-no- return during HIV infection.
In this review different aspects of the use of INSTIs in the prophylaxis and treatment of HIV infection are presented.
For both PEP and PrEP, the ideal drug should prevent the infection of cells when exposed to HIV, or at least abort the virus replication cycle acting before viral integration with cellular DNA, providing a theoretical advantage in preventing establishment of HIV infection rather than limiting infection. For both PEP and PrEP, other drug characteristics critical when selecting the appropriate ARVs are safety, tolerability, adequate penetration into target tissues, convenient dosing, and few potential drug-drug interactions. Currently available INSTIs have many of the characteristics of an ideal agent and are currently recommended in first line regimens for PEP, while for PrEP, new agents in this class are being developed which show promising results and might represent a new option in HIV-1 prevention.
Another topic addressed in this review is the use of INSTIs in the primary infection treatment. There are not controlled study of this scenario, but in several clinical trials a triple therapy with INSTIs showed high efficacy in a subset of patients with high viral load (above 100,000 copies/mL) as can be found in primary infection. Furthermore, the need to quickly start the treatment, in absence of data relating

to resistance test could be justify the use of INSTIs drugs for which the presence of resistance mutations are completely exceptional.

The demonstration that INSTIs may extinguish the kinetics of viral replication faster and to a greater extent justifies the assumption that they are more effective than the other antiretrovirals of acting on the pathogenetic mechanisms at the basis of the processes of immune reconstitution. This is particularly interesting in regards to the mechanism of T lymphocyte homeostasis, which allows a more complete recovery of the immune function. Integrase inhibitors may be able to interact significantly on two essential mechanisms: immune-activation and immune-proliferation, as they are closely related to the mechanisms underlying the phenomena of immune exhaustion and the depletion of CD4 T lymphocytes related to HIV-1 replication. These effects are mirrored by the CD4 T lymphocytes gain and the improvement in the quality of immune system as seen in both naïve and drug-experienced patients.

The three INSTIs recently introduced in clinical practice (RAL, EVG, DTG) proved to be efficient in suppressing viral load in naive patients, supporting their use also in patients with high levels of HIV-1 replication. Moreover, they were well tolerated and showed no relevant pharmacological interactions supporting their use in patients under treatment for HIV-1 associated comorbidities. Taken together, these observations, promote the use of INSTIs as a component of first line cART.

In recent years, thanks to the improved efficacy of current antiretroviral treatment regimens, virological failures occur less frequently in clinical practice. However, virological failures may sill occur in patients treated with different lines of antiretroviral therapy and different combinations of drug classes. Several studies, that have analyzed the role of INSTIs in this particular setting, have demonstrated their effectiveness after virological failure in patients naive or experienced to INSTIs.

However, in patients failing INSTIs, substantial differences are found in regard to HIV-1 drug resistance. Indeed, because of the low/moderate genetic barrier of RAL and EVG, to avoid the resistance development, particular attention should be deserved on adherence levels and on background drugs used in both cART - naïve and cART - experienced patients treated with these INSTIs. The broad cross-resistance profile between RAL and EVG precludes their sequential use in individuals failing either of them. Differently, in patients who have previously failed to RAL or EVG, even though the presence of Q148 mutation with at least two additional INSTI mutations can decrease their response rate, DTG bis die administration may warrant a residual efficacy. Moreover, the emergence of novel resistance under DTG treatment is a rare event that select viruses less prone to accumulate further resistance because of their low replication capacity, putting DTG as a good third drug option in first line treatment.

INSTIs show a good tolerability and safety profile combined with absence of significant drug to drug interactions. However, some precaution should be adopted regarding renal function before prescribing EVG in the fixed formulation with COBI. It is recommended to check eGFR at baseline when using EVG/COBI/TDF/FTC and avoid use of this compound when eGFR is <70 mL/min. A strictly monitoring of kidney function with a novel set point of eGFR after one month from starting EVG/COBI/TDF/FTC is recommended. It is also mandatory monitoring tubular function by using urine glucose, urine proteins and serum phosphate at baseline and then routinely. These procedures are addressed to allow the discrimination of an increment of eGFR due to impaired creatinine secretion or a more concerning onset of proximal renal tubular dysfunction due to other drug used in the regimen. Nevertheless, a serum creatinine change from baseline ≥0.4 mg/dL on consecutive visits is considered an acceptable threshold for targeting subjects with potentially serious renal adverse events who need an enhanced renal monitoring. The same procedure is suggested for DTG only when it is administered in association with TDF or other potentially nephrotoxic drugs.

Moreover, in recent years, the number of elderly persons living with HIV-1 has increased as the result of both the availability of effective antiretroviral therapy, which has reduced AIDS-related mortality, and newly diagnosed infections occurring in older adults. It is projected that by 2015, more than half of all HIV-

infected people in the United States will be over the age of 50. The overlapping epidemics between HIV-1 and aging, changes profoundly the clinical picture of HIV-1 disease. In this scenario, the availability of the new HIV drug class of INSTIs represent a valuable option for the treatment of HIV infection, for the unique tolerability and potential impact in the capacity to contrast mechanisms of residual viral burden and secondary reduce the burden of chronic inflammation.

New antiretroviral agents like the INSTIs offer the opportunity to maintain or promote CD4 T cell increase and a prompt and durable HIV-1 suppression, to treat HIV-1 with a minimal impact on renal function, metabolic disturbances and bone metabolism with minimal hepatotoxicity and a potential for administration in End Stage Liver Disease.

In addition they offer the opportunity of modify cART for the duration of anti-HCV therapy to circumvent interactions with ribavirin, or the new anti HCV drugs and especially with regimen including anti HCV protease inhibitors. Thus RAL and DTG, may constitute a relevant treatment options in the setting of HIV-1 and HCV co-infection.

In conclusion, patients with HIV-1 infection have several comorbidities requiring multiple pharmacotherapies that can increase their risk of polypharmacy and related adverse events. However, little is known about the impact of aging on medication use in HIV-1 infected older individuals, the potential for interactions with cART and administered medications, and the impact of this on therapy tolerability and virological response with aging.

Reducing pill burden, careful titration of medications, and increasing awareness of common drug to drug interactions can prevent co-administration of potentially harmful combinations and reduce unnecessary polypharmacy-related adverse events in this population.

Finally, recent data are accumulating that support the neuroprotective potential for INSTIs. While CSF penetration of RAL seems to vary among subjects, due to the barrier status and, possibly, host genetic factors, DTG CSF concentrations are more consistently exceeding the desired inhibitory HIV-1 concentrations. On the other hand, in vitro observations on the efficacy of INSTIs in monocytic-macrophage cells support their potential to inhibit HIV-1 replication in CNS macrophages, and confirm the efficacy of DTG against RAL-resistant mutants, which may be clinically relevant. Although preliminary intensification studies with RAL have failed to show an effect on CSF markers of replication and immune activation, additional clinical and pharmacodynamics studies may help under- stand the full potential of INSTIs, including the newer drugs, in inhibiting CNS HIV-1 replication and purging the CNS reservoir.

Future assessment of economic impact for the introduction of new drugs will likely need some degree of adjustment due to the increasing variability in the market conditions, which are becoming more and more specific between different hospitals. Indeed, from the BIM perspective, co-existence of different drug costs prevents the possibility to a priori and completely define the choice with highest economic gains for each health care institution.

In conclusion, given the potency and the relative safety of the INSTIs in initial trials and in clinical practice, today they are considered the reference drugs for the treatment of HIV-1 infection.

List of abbreviations

3TC: lamivudine; ABC: abacavir; ARV: antiretroviral; ATZ: atazanavir; ATZ/r: ritonavir boosted atazanavir; BIA: budget impact analysis, BIM: budget impact models; cART: combination antiretroviral therapy; COBI: cobicistat; DRV: darunavir; DRV/r: ritonavir boosted darunavir; DTG: dolutegravir; EFV: efavirenze; EVG: elvitegravir; ETR: etravirine; FTC: emtricitabine; LPV: lopinavir; INSTI: integrase strand transfer inhibitors; MVC: maraviroc; NNRTI: non nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhbitors; OBT: optimal backbone therapy; PEP: post-exposure prophylaxis; PrEP: pre-exposure prophylaxis; PI: protease inhibitors; SSN: Sistema Sanitario Nazionale (National Health System); TDF: tenofovir disoproxil fumarate.

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