New Microbiologica, 42, 2, 69-80, 2019, ISN 1121-7138

REVIEW

Italian expert panel consensus statements on two-drug antiretroviral regimens to treat naïve and virologically suppressed HIV-1 infected patients

Andrea Antinori[1], Maria Mercedes Santoro[2], Roberta Gagliardini[1], Giulia Marchetti[3], Annalisa Mondi[1], Valeria Cento[4], Carlo Federico Perno[5*], Massimo Andreoni[6], for the Two-Drug Antiretroviral Regimens expert panel[#]

[1]*National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy;*
[2]*Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy;*
[3]*Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, Presidio San Paolo, University of Milan, Italy;*
[4]*Residency in Microbiology and Virology, University of Milan, Italy;*
[5]*Dept of Oncology and Oncohematology, University of Milan, Italy;*
[6]*Infectious Diseases, University of "Rome "Tor Vergata". Rome, Italy*

[#]*Members of the Two-Drug Antiretroviral Regimens expert panel are listed in the acknowledgment section*

SUMMARY

New strategies for HIV treatment able to reduce drug-exposure, medium-long term toxicities and costs are a recognized clinical need. The availability of newer drugs with improved potency and tolerability, as well as high genetic barrier to resistance, makes antiretroviral-sparing strategies with two-drug regimens (2DRs) particularly attractive. Substantial evidence has been generated over the last few years supporting 2DR in virologically suppressed HIV infected patients for whom a therapy switch is planned. More recently, very promising data on 2DR in naïve patients have also been reported.
The main purpose of this consensus is to provide an overview of guideline indications and recommendations, and the most recent data from clinical studies of 2DR in both naïve and virologically suppressed patients. As an expert consensus, suggestions and indications on the use and management of 2DR are also provided.

Received January 31, 2019 *Accepted* February 20, 2019

INTRODUCTION

The use of combination antiretroviral therapy (cART) containing three active drugs from at least two different classes began in the mid-1990s. Since then, the use of a three-drug regimen (3DR), usually composed of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) plus one drug from a different class, has been the standard of care for HIV treatment worldwide.
The availability of newer drugs with improved potency and tolerability, as well as high barrier to resistance, makes antiretroviral-sparing strategies with two-drug regimens (2DRs) more feasible and attractive. Potential advantages of 2DR include reduced medium- and long-term toxicity, complexity, and costs of antiretroviral therapy. This approach may be particularly suited for maintenance therapy in patients with steadily controlled viral replication, who wish or need to simplify cART. In these settings, substantial evidence favoring 2DR has been accumulated. More recently, very promising data on the use of 2DR in ART-naïve patients have also been reported.

Key words:
HIV, Two-drug regimens, Dolutegravir, Antiretroviral therapy, HIV drug-resistance, Virological efficacy.

Corresponding author:
Carlo Federico Perno
E-mail: carlo.perno@unimi.it

The main purpose of this consensus is to provide an overview of guideline indications and recommendations (*Table 1*) (BHIVA 2016; SIMIT 2017; Antinori *et al.,* 2018; DHHS 2018; EACS 2018; GeSida 2018; Saag *et al.,* 2018), and the most recent results from clinical studies of 2DR in both ART-naïve and virologically suppressed patients. The use of 2DR in experienced patients failing therapy (thus with detectable viremia) is still matter of debate and is not reviewed in this work. In fact, very few data from both observational studies and pilot studies are currently available for the consideration of this strategy in clinical practice (Capetti *et al.,* 2017; Spagnuolo *et al.,* 2019). As an expert consensus, suggestions and indications on the use and management of 2DR are also provided (*Table 2*).

EFFICACY OF TWO-DRUG REGIMENS: EVIDENCES FROM RECENT CLINICAL TRIALS

Efficacy of two-drug regimens in ART-naïve patients

In the last few years, some randomized clinical trials have provided the most relevant information on 2DR in naïve patients.
The NEAT001/ANRS143 study was a randomized clinical trial that compared a 2DR with darunavir/ritonavir (DRV/r) + raltegravir (RAL) *vs* DRV/r + tenofovir disoproxil fumarate/emtricitabine (TDF/FTC), with approximately 800 patients enrolled (Raffi *et al.,* 2014). Though overall efficacy was similar, suboptimal response was observed in

Table 1 - *Two-drug regimens in recent national and international guidelines.*

Regimen	*DHHS 2018 (DHHS 2018)*	*IAS-USA 2018 (Saag et al., 2018)*	*EACS 2018 (EACS 2018)*	*BHIVA 2016 (BHIVA 2016)*	*SIMIT 2017 (SIMIT 2017)*	*GeSida 2018 (GeSida 2018)*
			Treatment naive			
DRV/r + RAL	Alternative [CI]* VL<100,000, CD4>200	Alternative [BI]* VL<100,000, CD4>200	Alternative^ VL<100,000, CD4>200	Alternative [AII]*** VL<100,000, CD4>200	Alternative* VL<100,000, CD4>200	
LPV/r + 3TC				Not recommended [BI]		
DRV/r + 3TC	Alternative [CI]*	Alternative [BI]*		Not recommended [BI]		
DTG + 3TC	Alternative [BI]*	Not yet recommended	Alternative^ VL<500,000			
			Switch with virological suppression			
DRV/r + 3TC	Alternative [BI]*	Recommended [AII]§	Recommended^^		Recommended [AI]^^^	Recommended [AI]ç
ATV/r + 3TC	Alternative [CI]*	Recommended [AII]§	Recommended^^	Alternative [AII]	Recommended [AI]^^^	Recommended [AI]ç
LPV/r + 3TC	Alternative [CI]*	Recommended [AII]§		Alternative [AII]		
DRV/r + RAL	Not yet recommended				Optional [CI]	
DRV/r + RPV					Optional [CI]	
DTG + RPV	Recommended [AI]**	Recommended [AI]§	Recommended		Recommended [AI]	Recommended [AI]
DTG + 3TC	Not yet recommended	Recommended [AII]§			Alternative [BII]	

**When the use of tenofovir disoproxil, tenofovir alafenamide, or abacavir is contraindicated or not desirable;*
***When the use of nucleoside reverse transcriptase inhibitors is not desirable and when resistance to either dolutegravir or rilpivirine is not expected;*
§*In patients with no prior virological failure or transmitted drug resistance;*
^*When none of the preferred regimens are feasible or available, whatever the reason;*
^^*Only to persons with a) no resistance, b) suppression of HIV viral load to < 50 copies/mL for at least the past 6 months and c) absence of chronic Hepatitis B Virus co-infection;*
***Where there is need to avoid abacavir, tenofovir disoproxil or tenofovir alafenamide;*
^^^***AI** only in patients with switch from boosted protease inhibitors regimens; **BI** for switch from other regimens;*
ç*Only to person treated by boosted protease inhibitors regimens, and with a) no resistance to protease inhibitors or lamivudine, b) suppression of HIV viral load to < 50 copies/mL for at least the past 6 months and c) absence of chronic Hepatitis B Virus co-infection.*

The strength of recommendations is reported as in each guideline. In DHHS, IAS-USA, Italian and GESIDA guidelines, each recommended statement includes a letter (**A**, **B**, or **C**) that represents the strength of the recommendation ("Strongly recommended," "Moderately recommended," "Optional," respectively) and a numeral (**I**, **II**, or **III**) that represents the quality of the evidence that supports the recommendation ("Data are obtained from at least one randomized controlled trial with sufficient potency, or metanalysis of controlled studies", "Data are obtained from non-randomized research, or observational cohort studies," "Recommendation based on case studies, or expert consent," respectively). In BHIVA guidelines, the modified Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for the assessment, evaluation and grading of evidence and the development of recommendations is used.

3TC, lamivudine; ATV/r, atazanavir/ritonavir; BHIVA, British HIV Association; DRV/r, darunavir/ritonavir; DTG, dolutegravir; EACS, European AIDS Clinical Society; DHHS, U.S. Department of Health and Human Services; GeSida/PNS, Grupo de Estudio de Sida/Plan Nacional sobre el Sida; IAS, International AIDS Society; LPV/r, lopinavir/ritonavir; RAL, raltegravir; RPV, rilpivirine; SIMIT, Società Italiana di Malattie Infettive e Tropicali; VL, viral load.

patients with high HIV-1 RNA and/or low CD4+ cell counts receiving 2DR (Raffi *et al.,* 2014).

The GARDEL study (N=426) compared a 2DR with lopinavir/ritonavir (LPV/r) + lamivudine (3TC) *vs*. LPV/r+2NRTI, and demonstrated similar virological efficacy, but this strategy now has some limitations related to twice-daily dosing, a relatively high pill burden, and the adverse effect profile of LPV/r (Cahn *et al.,* 2014). More recently, non-inferiority of DRV/r+3TC *vs* DRV/r+ TDF/3TC at 48-weeks (n=145) was demonstrated in the randomized ANDES clinical trial (93% *vs* 94% virological response, respectively) (Figueroa *et al.,* 2018).

The availability of dolutegravir (DTG), a second-generation integrase inhibitor (INI) with a high genetic barrier, has allowed 2DR to be designed with no need for a booster. Two single-arm pilot studies investigated the virological efficacy of DTG+3TC in naïve patients. The PADDLE pilot study enrolled 20 patients and demonstrated 100% virological efficacy at week 24, and 90% at week 48 (Cahn *et al.,* 2017).

The ACTG A5353 was a single-arm study conducted in 120 naïve patients (of whom 31% had an HIV-1 RNA level >100,000 copies/mL at baseline) that showed 90% virological success after 24 weeks, independently from baseline (89% virological success in the subset with HIV-1 RNA >100,000 copies/mL) (Taiwo *et al.,* 2018b).

The most robust data regarding dual therapy in the naïve setting come from GEMINI 1 and 2, two parallel, international, randomized, double-blind phase III non-inferiority studies, where DTG+3TC was compared with DTG+FTC/TDF. A total of 1441 patients with the following features were enrolled: ART-naïve adults with HIV-1 RNA 1,000-500,000 copies/mL, no pre-existing major viral resistance mutations, no HBV infection or HCV requiring therapy (Cahn *et al.,* 2018a). The primary endpoint of HIV-1 RNA <50 copies/mL at week-48 was achieved in 91% of patients on DTG+3TC, and in 93% of patients on DTG+FTC/TDF. A difference in virological response was observed in patients with $CD4^+$ counts ≤200/mm^3 at baseline (79% in DTG+3TC arm *vs* 93% in DTG+FTC/TDF arm); however, most failures were unrelated to virological efficacy. Given the small number of patients enrolled in this subgroup, a longer fol-

Table 2 - *Statements and recommendations by the expert panel.*

Efficacy of two-drug regimens: evidences from the recent clinical trials[a]

Efficacy of two-drug regimens in ART-naïve patients

1. In ART-naïve patients with HIV-RNA <500,000 copies/mL, without evidence of pre-existing major resistance-associated mutations and of active HBV infection, 2DR with DTG+3TC was not inferior to a regimen with 3 drugs (DTG+FTC/TDF) in term of virological efficacy at 48 weeks; thus it could be recommended for ART initiation [**AI**].
2. Unless further data are provided, in naïve patients with a baseline CD4 count <200 cells/mm^3, caution is necessary [**BII**].
3. Other 2DR options (as DRV/r+RAL, DRV/r+3TC, LPV/r+3TC) could not be considered as effective when compared to current preferred 3DR and cannot be recommended for starting ART in naïve patient [**AIII**].

Efficacy of two-drug regimens in ART-experienced virologically suppressed patients

1. Switching to a new regimen in the setting of virological suppression, when either triple or dual regimens are used, should consider the patient treatment history, the occurrence of virological failure, the presence of resistance, and chronic HBV infections [**AII**].
2. Due to inclusion criteria and patients' characteristics in the randomized trials on switching strategies, the duration of virological suppression before switch decision should be considered [**BII**].
3. In a proactive switch strategy in virologically suppressed patients, the following 2DRs could be used with a different level of recommendation:
   a. DTG + RPV [**AI**]
   b. ATV/r+3TC; DRV/r+3TC [**AI** for switch from a PI-based cART]
   c. DTG+3TC [**AIII**]
   d. DRV/r+RPV [**BI** for switch from a PI-based cART]
   e. DRV/r+RAL [**CI**]
4. Switching to 2DRs different from those in statement 3 are less effective than preferred therapies and should not be used outside research settings [**AI**].
5. Although no specific clinical data have been generated in this setting, considering the equivalence demonstration between boosters, ATV/r may be replaced with ATV/cobicistat and DRV/r with DRV/cobicistat [**AIII**].

Virological and immunological evaluations in the setting of two-drug regimens[a]

Viral parameters in the evaluation two-drug regimen efficacy

1. Virological efficacy, both as first-line and switch treatment, is primarily defined by the achievement and long-term maintenance of undetectable plasma HIV RNA with standard assays (e.g., <50 copies/ml) [**AI**].
2. Given the association of virological failure with low baseline (nadir) CD4 cell count (<200 cells/mm^3), high baseline viral load and high baseline HIV-DNA, when available, the above parameters should all be taken into account for identification of patients starting or switching a 2DR as well as for any antiretroviral combinations [**BII**].
3. Given data on the association between viral blips, low level viremia and residual viremia with viral failure and consequent development of resistance under 3DR, these markers should be considered in the assessment of patients who are candidates for a 2DR switch and in the monitoring of virologically suppressed patients [**BIII**].

Immunological parameters in the evaluation of two-drug regimen efficacy

1. Literature data show a role of CD4/CD8 ratio as predictor of morbidity/mortality on 3DR; CD4/CD8 ratio is therefore used in the clinical monitoring of patients in this setting [**AII**].
2. Although initial evidence on modification of the CD4/CD8 ratio upon switch to two-drug regimens are still contradictory, this parameter should be monitored during 2DR [**CII**].

Drug-resistance issue in two-drug regimens

1. Performance of a genotypic resistance test before starting a 2DR in drug-naïve patients is strongly recommended [**AII**].
2. Before switching to a 2DR in patients with previous virological failure or unknown previous treatment history, results of previous genotypic resistance test (and/or current resistance test on HIV-DNA) is required [**BII**].

Safety and toxicity[a]

1. The comparison between 2DR and 3DR did not generally show a significant difference in the occurrence of adverse events leading to treatment interruption. Accordingly, the decision to start/switch to a 2DR or a 3DR should not be based on concerns for treatment-limiting adverse events [**AII**].
2. When starting on or switching to a 2DR or 3DR from a TDF-based triple regimen, the advantages in terms of renal and bone toxicity-sparing should be balanced with the potential negative impact (if any) on the lipid profile, at least for patients on boosted PI-based dual regimens [**AII**].
3. The safety of 2DR (boosted PI- or DTG-based therapy) has largely been documented in studies comparing dual options with triple arms including TDF. Consequently, the above recommendations should not be applied in the setting of dual versus TAF-based regimen comparisons until results of ongoing clinical trials are available [**BIII**].

Health Technology Assessment[a]

1. With health budgets shrinking, this is the right time to formally introduce HTA to evaluate not only a single antiretroviral drug but also new strategies for the use of antiretroviral combinations (i.e., 2DR vs 3DR) [**BIII**].
2. Cost savings achieved by avoiding toxicities depend on the specific drug withdrawn or not introduced [**BIII**].
3. Since HTA would make it easier for stakeholders to demonstrate the impact of new antiretroviral combinations, the clinical expert should have a role in the HTA process [**BIII**].

[a]The strength of recommendations is reported as in each guideline. In DHHS, IAS-USA, SIMIT and GESIDA guidelines, each recommended statement includes a letter (**A**, **B**, or **C**) that represents the strength of the recommendation ("Strongly recommended", "Moderately recommended", "Optional", respectively) and a numeral (**I**, **II**, or **III**) that represents the quality of the evidence that supports the recommendation ("Data are obtained from at least one randomized controlled trial with sufficient potency, or metanalysis of controlled studies," "Data are obtained from non-randomized research, or observational cohort studies," "Recommendation based on case studies, or expert consent," respectively). In BHIVA guidelines, the modified Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for the assessment, evaluation and grading of evidence and the development of recommendations is used.

2DR, two-drug regimen; 3DR, three-drug regimen; 3TC, lamivudine; ART, antiretroviral therapy; ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; DTG, dolutegravir; FTC, emtricitabine; HBV, Hepatitis B virus; HTA, Health Technology Assessment; LPV/r, lopinavir/ritonavir; PI, protease inhibitor; RAL, raltegravir; RPV, rilpivirine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil.

low-up and further studies are warranted to confirm these results and possibly provide a biological explanation for such apparently lower response in this setting.

Efficacy of two-drug regimens in virologically suppressed patients

In treatment switch studies, 2DR strategies have been mainly studied in patients on long-lasting active cART, with steadily suppressed viremia and no previous history of virological failure (Nishijima *et al.,* 2013; Perez-Molina *et al.,* 2015; Maggiolo *et al.,* 2016; van Lunzen *et al.,* 2016; Di Giambenedetto *et al.,* 2017; Perez-Molina *et al.,* 2017; Pulido *et al.,* 2017; Fabbiani *et al.,* 2018; Llibre *et al.,* 2018; Taiwo *et al.,* 2018a).

Boosted PI-regimens combined with 3TC, and DTG combined with rilpivirine (RPV) are the most widely explored successful strategies in randomized clinical trials.

The combination of DTG+3TC has also been investigated, although only in small pilot studies, two small randomized trials and cohort analyses. Nevertheless, results were very promising, supported by those obtained in two large trials with naïve subjects (Hidalgo-Tenorio *et al.,* 2017; Maggiolo *et al.,* 2017; Reynes *et al.,* 2017; Blanco *et al.,* 2018; Borghetti *et al.,* 2018; Cahn *et al.,* 2018b; Joly *et al.,* 2018; Taiwo *et al.,* 2018a). In switch studies, DTG+RPV has demonstrated non-inferiority when compared to a standard regimen, with 95% virological success after 48 weeks, irrespective of the previous cART (Llibre *et al.,* 2018).

On the other hand, 2DR studies with PI/r+3TC usually included experienced patients, mostly already under a PI/r-based regimen, with few exceptions (i.e., in the SALT study, 32.5% of patients switched from an NNRTI-based regimen) (Perez-Molina *et al.,* 2015).

The PI/r+3TC combination only partially responds to the need to reduce potential toxicity, as issues due to the PI itself and booster still remain, mostly regarding the selection bias of patients on a long-term PI-based regimen. Concerning DRV/r+RAL strategy, a single small randomized clinical trial (N=58) with a main end-point on renal toxicity failed to demonstrate a benefit on eGFR; however, the large trial NEAT001/ANRS143 (N=805) in naïve patients versus a standard DRV/r+TDF/FTC confirmed the efficacy of the combination, except for patients with viral load higher than 100,000 copies/ml and CD4 counts lower than 200 cells/mm^3 (Nishijima *et al.,* 2013; Raffi *et al.,* 2014).

The combination of PI/r+ maraviroc (MVC) (Pett *et al.,* 2016; Rossetti *et al.,* 2017) or ATV/r+RAL (van Lunzen *et al.,* 2016) has demonstrated less efficacy compared to 3DR (perhaps driven in part by the cumbersome design of the studies), while there are no controlled data for DRV/r+ DTG (a potentially very promising combination, particularly in patients with previous failures and resistance to RT inhibitors, and/or in need of a therapeutic strategy containing drugs with high genetic barrier).

To date, no specific prognostic index able to predict the virologic outcome of a 2DR in the switch setting has been clearly identified. Therefore, any patient candidate for a switch to a 2DR should be carefully assessed in terms of therapeutic history, with particular attention to previous failures, evidence of pre-existing resistance, history of suboptimal adherence, treatment interruptions, and presence of chronic HBV infection. Along with these, other possible risk factors for virological failure (i.e., pre-ART HIV-RNA values, quantitative HIV-DNA, CD4 count at nadir) need to be further investigated in specifically designed studies, including current recommended regimens (*Table 3*).

The optimal time of switch during viral suppression (early or delayed) and, consequently, the optimal time under viral suppression before switch, is still a matter of debate. In randomized clinical trials, at least 6 months of viral suppression have usually been required among inclusion criteria, but the median time of viral suppression has varied significantly throughout the studies. In particular, when specific mutations such as M184V/I are involved, the time of viral suppression before switch may play a protective role; some recent data obtained in this specific setting do not seem to encourage 3TC-including 2DR in the presence of previously identified M184V virus, particularly with a

Table 3 - *Key open issues.*

Viro-immunological evaluations	
Risk of virological failure	According to other co-factors (quantitative HIV-DNA, CD4$^+$ T cells count at nadir, duration of virological suppression in individuals under virological control, specific previous resistance mutations, as M184V, and previous failures), a possible different risk of virological failure should be further investigated in specifically designed studies on the use of 2DRs, including current recommended regimens.
HIV-DNA	Standardized tests for HIV DNA quantification are still lacking. Thus, monitoring of intra-patient HIV DNA quantification should be performed using the same assay. Moreover, when possible, HIV DNA measurement on sorted lymphocyte populations should be considered. Finally, a relevant clinical cut-off of total HIV DNA should be defined in both in ART-naïve and ART-experienced virologically suppressed patients.
Critical drug-naïve patients	In ART-naïve patients, evidence from patients with very high pre-cART viral load (>500,000 copies/mL) is needed to eventually extend the use of DTG+3TC as an initial treatment to this high-viremia population.
Immunological profile	A broader immune characterization of patients on 2DR should include the study of the main CD4$^+$ and CD8$^+$T cell subpopulations (naive, memory, activated, exhausted, apoptotic) and their function, as well as of pro-inflammatory markers.
Safety and toxicity evaluations	
Tenofovir disoproxil vs. tenofovir alafenamide	It is still uncertain whether the modification of renal function, bone parameters and serum lipid levels observed in patients starting or switching to a 2DR is related to 2DR *per se*, or rather to the withdrawal/avoidance of TDF. Comparison with TAF-based triple therapies will probably better clarify this aspect.

2DR, two-drug regimen; cART, combination antiretroviral therapy; TDF, tenofovir disoproxil; TAF, tenofovir alafenamide.

short duration of viral suppression before switching to dual therapy (Gagliardini *et al.,* 2018). Further analyses are required to ascertain this important point.

VIROLOGICAL AND IMMUNOLOGICAL EVALUATIONS IN THE SETTING OF TWO-DRUG REGIMENS

Viral parameters in the evaluation of two-drug regimen efficacy

Similar to classical cART, the goal of 2DR is long-term control of HIV replication to undetectable levels, as assessed by plasma HIV RNA quantification with standard assays.

While, by definition, undetectable viremia denotes virological success, limited experience with 2DR at this time may advise assessment of additional parameters, to confirm that decreased treatment pressure compared to triple therapy does not result in an inapparent suboptimal response, eventually leading to overt treatment failure.

Such parameters may include surrogate markers of viral reservoir (e.g., total blood HIV DNA), residual viremia measured through non-routine ultrasensitive plasma HIV RNA assays, as well as HIV RNA blips, and the distinction between negative vs detected but not quantifiable viremia in standard assays (often referred to as very low-level viremia or VLLV) (Sarmati *et al.,* 2007; Ryscavage *et al.,* 2014; Amendola *et al.,* 2017; Bachmann *et al.,* 2018; Ceccherini-Silberstein *et al.,* 2018).

Notably, VLLV has been shown to predict virological failure, even with triple therapy, in the majority, but not all, of the analyses (Ryscavage *et al.,* 2014). Viral blips could negatively affect decay of the HIV reservoir in the long run (Bachmann *et al.,* 2018). A recent study has shown a positive correlation between pre-cART total HIV-1 DNA burden and the risk of virological rebound with standard first-line triple therapy (Ceccherini-Silberstein *et al.,* 2018). In addition, higher HIV-DNA burden has been shown to be predictive of failure with boosted PI- or DTG-based monotherapy (Lambert-Niclot *et al.,* 2012; Rutsaert *et al.,* 2017; Wijting *et al.,* 2018). Therefore, this parameter merits investigation as a novel or additional staging marker to better identify people at risk of viral rebound following start of 2DR strategies.

So far, few 2DR studies have investigated virological parameters other than standard viremia. Compared to triple therapy, 2DRs were not associated with higher frequency of viral blips in several switch studies evaluating LPV/r+3TC (Arribas *et al.,* 2015), atazanavir/ritonavir (ATV/r) + 3TC (Di Giambenedetto *et al.,* 2017; Fabbiani *et al.,* 2018), DRV/r+3TC (Pulido *et al.,* 2017), DTG+3TC (Blanco *et al.,* 2018; Taiwo *et al.,* 2018a). Other studies reported few or no blips following switch to 2DR, but no comparison with triple therapy was presented (Maggiolo *et al.,* 2017; Joly *et al.,* 2018). The SWORD1&2 studies excluded any association between viral blips and the extremely rare occurrence of virological failure, though a longer time of observation is required (Blanco *et al.,* 2018; Taiwo *et al.,* 2018a).

Recent evidences on residual viremia and VLLV have been also reported. In particular, in patients who switched to DTG+3TC, the levels of residual viremia were stable from baseline over week 48, and similar to those found in patients on 3DR, including after stratifying for therapy duration or CD4 T+ cell count (Li *et al.,* 2018). Similar proportions of individuals with target not detected (TND) viral load were found at each visit through week 48 for patients treated with DTG+RPV and those on 3DR, including after stratifying for baseline viral load category (40 copies/mL≤VL<50 copies, Target detected or TND) (Underwood *et al.,* 2018).

Although still requiring analysis, available data suggest similar rates of decline of viral DNA in 2DR and patients treated with 3DR (Lombardi *et al.,* 2017). Potentially reassuring data in this context include the similar evolution of HIV DNA in both MONI (Lambert-Niclot *et al.,* 2012) and MONET trials for up to 144 weeks (Geretti *et al.,* 2013) comparing switch to DRV/r monotherapy with triple therapy, as well as the maintenance of HIV DNA levels in the pilot DTG monotherapy MONODO study (Sculier *et al.,* 2018).

Quantification of total HIV DNA is an appealing marker of viral reservoir in 2DR as well as in 3DR (Sarmati *et al.,* 2007; Ceccherini-Silberstein *et al.,* 2018; Lanzafame *et al.,* 2018). Nevertheless, its use in clinical routine is hampered by several technical and conceptual issues that still need to be resolved (Table 3). A clinical cut-off of total HIV DNA should be defined in both drug-naïve and virologically suppressed patients. Concerning this last point, in drug-naïve patients starting a first-line regimen, the risk of virological rebound was significantly higher in patients with a pre-cART total HIV-1 DNA >10,000 copies/10^6 CD4+ T cells than in those with a total HIV-1 DNA ranging from 1,000 to 10,000 copies/10^6 CD4+ T cells and <1,000 copies/10^6 CD4+ T cells (Ceccherini-Silberstein *et al.,* 2018). In virologically suppressed patients who switched to a PI-sparing regimen, HIV DNA levels below the median 226 copies/10^6 PBMCs at baseline were independently associated to a reduced risk of virological failure or viral blip (Sarmati *et al.,* 2007). More data are required to set up specific cut offs relevant for the virological outcome and significant progress in this area is awaited depending on availability and widened use of standardized HIV DNA assays.

In conclusion, while 2DR trials exclusively used standard viremia to define virological outcome (Cahn *et al.,* 2017; Cahn *et al.,* 2018b; Taiwo *et al.,* 2018b), the data available indicate that 2DR is not associated with increased risk of blips. More data are required to define the impact of 2DR on either VLLV/residual viremia or HIV DNA, particularly when 2DR is used as a switch strategy in patients under successful triple therapy, where intra-patient changes over time can be assessed.

The question whether 2DR is efficient in controlling viral reservoirs can be approached not only directly (by measuring the above-mentioned viral parameters), but also empirically, by evaluating viral rebound after analytical treatment interruption, once viral replication has been suppressed for an adequate length of time, subject to specific ethical assessment in a research context. In fact, the kinetics of viral rebound following therapy interruption is expected to be a rough indicator of the ability of therapy to reduce the viral reservoir (Chun *et al.,* 2010; Martin *et al.,* 2017; Salantes *et al.,* 2018). No data on HIV rebound following 2DR interruption have been documented so far.

Immunological parameters in the evaluation of two-drug regimen efficacy

Little or no data exist on the fine immunological changes that occur during the switch from triple to dual therapy. Indeed, several virological aspects have been deeply investigated; however, in several papers, only the number

of peripheral blood CD4+ T cells, and not that of CD8+ T lymphocytes, has been reported. This must be underlined because in recent years the CD4/CD8 ratio has proven to be not only an important predictive biomarker of clinical progression (being strictly associated with CD4+ T-cell nadir), but also an indicator of T cell activation (Guiguet *et al.,* 2009; Serrano-Villar *et al.,* 2013; Serrano-Villar *et al.,* 2014). Such parameter is thus of utmost importance and should be an integral part of patient monitoring following switch to 2DR.

Indeed, switching to a 2DR has been shown to increase CD8+ T cells (Mussini *et al.,* 2018). A recent study showed that activated CD8+ T lymphocytes, which express both CD38 and HLA-DR molecules, do not increase significantly in patients who switch from triple to dual therapy. Another study on 429 patients reported that CD8+ cells increased in the group of patients who stop TDF (n=364), but not in the 65 who stop abacavir (ABC) (Quiros-Roldan *et al.,* 2018). It would be important to investigate in greater detail the changes that occur among the CD8+ T cell population, a complex area responding to different stimuli and intercurrent pathologies, including other viral infections. In other words, more data are needed to assess whether dual regimens can affect the quality of this population, considering the phenotype of naïve and memory cells along with markers of exhaustion (CD57 and PD-1/CD279), as well as its functional activities, propensity to undergo programmed cell death, and the capacity of these cells to respond to viral antigens and eventually to simultaneously produce multiple cytokines.

Another important aspect is related to changes that could occur among CD4+ T cells, not only in terms of phenotype and function, but also considering, for example, the intracellular DNA viral load, which can be differently distributed between naïve vs memory/activated CD4+ T lymphocytes (Gibellini *et al.,* 2017). It should be noted that the above-mentioned parameters have never been investigated by comparing different 3DRs and, so far, there is no biological support suggesting any role of single antiviral on them. In this case as well, it would be extremely important to perform in-depth analyses to compare the immunological effects of 2DRs versus 3DRs on CD4+ T lymphocytes.

Finally, given that immune system activation is closely linked to inflammation, and that HIV infection can be considered a chronic inflammatory disease, data on the level of soluble molecules (proinflammatory cytokines like IL-1, IL-6, TNF and many others) and on the quality of the innate cellular immune response (quality and activity of monocytes, number of neutrophils and activation of platelets) should be investigated in research studies to understand whether any parameter of this kind can be conveniently integrated in patient monitoring. However, so far data from SWORD trials (Orkin *et al.,* 2017) and ATLAS trial (Belmonti *et al.,* 2018) do not suggest any impact on such markers when switching from triple to dual regimens at week 48.

Along this line, data from pilot studies are being produced that investigate the possible effects of dual versus triple regimens on pro-inflammatory biomarkers (Lombardi *et al.,* 2018; Maggiolo *et al.,* 2018; Molano *et al.,* 2018).

Albeit still at an early stage, research on the role of 2DR on inflammation and immune activation appear to be worth pursuing to better define the optimal context of 2DR strategies, considering the role played by inflammation/immune activation in the pathogenesis of non-AIDS comorbidities (*Table 3*) (Deeks 2011).

In conclusion, the clinical implications of the first and simple immunological findings have still to be clarified, and prospective studies that use sophisticated techniques and technologies are needed to better understand the possible effects of a therapy switch on both the innate and adaptive immune system (*Table 3*).

DRUG-RESISTANCE ISSUE IN TWO-DRUG REGIMENS

Successful 2DRs were made possible by the availability of drugs combining potency and tolerability with high genetic barrier to resistance. How these approaches would deal with resistance development/re-emergence, compared with 3DR, is thus of paramount importance.

Drug-resistance in ART-naïve patients

In the ACTG 5353 pilot study (Taiwo *et al.*, 2018b), DTG plus 3TC demonstrated efficacy in individuals with pre-treatment HIV-1 RNA up to 500,000 copies/mL, but 1 out of 3 virological failing patients showed the emergence of resistance mutations (M184V in reverse transcriptase and R263K in integrase). In GEMINI-1&2 studies, the same strategy was not associated with treatment-emergent INSTI or NRTI at failure (Cahn *et al.,* 2018b).

Among trials exploring PI/r-based 2DR, NEAT001/ANRS143 showed the emergence of treatment resistant strains in 6/29 virological failures tested (5 to INSTI and 1 to NRTI) in the DRV/r plus RAL comparted to 0/13 virological failures tested in the comparator arm (Raffi *et al.,* 2014). On the contrary, in the ANDES study (Figueroa *et al.,* 2018), investigating DRV/r+3TC strategy, no virological failures were observed in the 2DR arm. However, general conclusions could not be drawn due to the small population size and the relatively short follow-up period of the study. Therefore, larger studies are warranted to evaluate emergent resistance in the PI-based 2DR option. Finally, in the GARDEL study, LPV/r plus 3TC was confirmed to be as effective as LPV/r plus 2NRTI with a very low level of resistance at failure in both arms: PI-resistance was never described, and the M184V mutation was detected only in two patients of the 2DR group (Cahn *et al.,* 2014).

In conclusion, findings on 2DR usage as first-line strategy showed a rare emergence of drug-resistance, in agreement with the high genetic barrier to resistance of DTG, DRV/r and LPV/r, which prevents resistance selection in standard initial therapy (Llibre *et al.,* 2015; Clutter *et al.,* 2016). However, it is important to underline that, based upon current knowledge, 2DR strategies as first-line treatment should be considered only in selected patients with no evidence of pre-existing drug-resistance, pre-ART plasma HIV-1 RNA ≤100,000 copies/mL (for use of DRV/r plus RAL) or ≤500,000 copies/mL (for use of DTG plus 3TC), and HBsAg negative, as shown in *Table 2*, according to the evidence so far available. Thus, dual-therapy as initial regimen is currently not recommended when rapid initiation of ART is warranted, before HIV drug resistance (and viral load) results are available.

Drug-resistance in ART-experienced virologically suppressed patients

Drug-resistance is a critical factor in switching strategies (SIMIT 2017; Antinori *et al.,* 2018; DHHS 2018; EACS 2018; Gunthard *et al.,* 2018), and the selection of any new regi-

men should consider all available results (either historical or current) from standard genotype. Proviral DNA genotype may be a valid alternative tool when there is inadequate information about prior plasma drug-resistance (Zaccarelli *et al.,* 2016; DHHS 2018). As a best-case scenario, the availability of both cumulative resistance by previous genotypic resistance tests and proviral DNA resistance would allow the best prediction of virological outcome after switch in virologically suppressed patients (Armenia *et al.,* 2018).

A 2DR may not be an appropriate strategy to be considered for switch in the case of previously selected mutations that could alter the virologic efficacy of the drugs to be used. Based on current knowledge, when specific mutations such as M184V/I are involved, the switch to 2DR with 3TC cannot be recommended, even though long-time viral suppression before switch may constitute a "protective" factor against failure (Gagliardini *et al.,* 2018).

To reduce the risk associated with previously selected resistance mutations on the maintenance of virological control during 2DR, switch-therapy studies (with boosted PI or DTG) enrolled only patients with either: a) long-standing virological suppression (at least six months); b) no previous treatment failure, or c) no documented previous resistance (Arribas *et al.,* 2015; Maggiolo *et al.,* 2016; Joly *et al.,* 2017; Maggiolo *et al.,* 2017; Perez-Molina *et al.,* 2017; Pulido *et al.,* 2017; Aboud *et al.,* 2018; Fabbiani *et al.,* 2018; Llibre *et al.,* 2018; Maggiolo *et al.,* 2018). In line with this, the SWORD1&2 studies excluded patients with any major PI, INSTI, NRTI, or NNRTI resistance-associated mutation (including also the INSTI R263K) before therapy switch (Aboud *et al.,* 2018; Llibre *et al.,* 2018). Therefore, evidence on the use of 2DR based on the time under virological suppression before switching, previous resistance, and previous failures is still needed (*Table 3*).

To date, the few patients that failed simplified 2DR with boosted PI or DTG, generally showed a very low (or null) rate of resistance emergence at weeks 24-100 after switch. After 100 weeks of treatment with DTG plus RPV in the SWORD 1&2 studies, only 10/990 (1%) of patients had confirmed virologic failure and consequent withdrawal from the study; three of them harbored drug resistance mutations (one at week 36, one at week 88 and one at week 100) (Aboud *et al.,* 2018). All three patients presented RPV-associated mutations, while no DTG specific mutations were found in the two patients with integrase genotypic test at failure. These results reflect the high genetic barrier to resistance of DTG, suggesting that DTG plus RPV is able to maintain HIV-1 suppression (at least up to 100 weeks) with no increased risk of resistance development.

Similarly, DTG plus 3TC had very good results in both clinical trials and observational studies, with very low failure rate and with no plasma resistance emergence in any drug-target (Joly *et al.,* 2017; Maggiolo *et al.,* 2017; Taiwo *et al.,* 2018a), with few exceptions involving 3TC resistance in two observational studies (Calvez *et al.,* 2017; Calvez *et al.,* 2018).

Further studies, especially in a real-life setting, are required to confirm the protective role of DTG toward its companion drug.

SAFETY AND TOXICITY ISSUES IN TWO-DRUG REGIMENS

One of the key aims of 2DR, and the main reason that clinicians investigated this option, is to reduce the potential risk of short- and long-term toxicities, mainly related to the NRTI backbone. The gradual ageing of the HIV population brings generates a greater burden of age-related comorbidities, which results in a higher risk of osteoporosis and cardiovascular, renal, hepatic, and metabolic diseases of the HIV-positive compared to the HIV-negative population. This makes a) the prevention of drug-related toxicity and b) the preservation of long-term health of HIV-infected individuals, two unmet clinical needs of paramount importance (Bertoldi *et al.,* 2017; Gallant *et al.,* 2017; Andreoni *et al.,* 2018).

In recent years, the safety of two *vs.* three drug regimens has been extensively explored in clinical trials, with particular focus on bone mineral density (BMD), renal function, and metabolic profile.

Renal function and bone mineral density

In the HIV-positive population, renal impairment and osteoporosis are important health concerns, probably consequent to a complex interaction between HIV, traditional risk factors/comorbidities, and drug-related factors (McComsey *et al.,* 2010; Salter *et al.,* 2011).

Several cohort studies have demonstrated an association between cumulative exposure to TDF, and progressive reduction of the estimated glomerular filtration rate (eGFR), along with a more rapid decline in BMD (Dazo *et al.,* 2011; Ryom *et al.,* 2013; Achhra *et al.,* 2016a). TDF-sparing 2DRs are thus particularly promising and positively supported by recent results in randomized clinical trials conducted with drug naïve patients, which showed significantly reduced impact on renal function and bone markers (evaluated either as BMD or through bone turn-over markers), compared to 3DRs (Reynes *et al.,* 2013; Raffi *et al.,* 2014; Bernardino *et al.,* 2015; Stellbrink *et al.,* 2016; Cahn *et al.,* 2018a).

Results are more controversial in virologically suppressed patients receiving PI-based 2DR. Switching to ATV/r+3TC or DRV/r+MVC was associated with an improvement in eGFR and bone safety, compared to those who maintained 3DR, despite virological inferiority with DRV/r+MVC in the GUSTA study (Di Giambenedetto *et al.,* 2017; Rossetti *et al.,* 2017; Fabbiani *et al.,* 2018). However, no improvement was observed in SALT and DUAL-GESIDA studies, which compared PI/b+3TC *vs* 2NRTI+PI/b (Perez-Molina *et al.,* 2015), nor in the SPARE study, specifically designed to evaluate renal toxicity, which failed to demonstrate a statistically significant improvement of baseline eGFR between DRV/r+RAL *vs* LPV/r+TDF/FTC (Nishijima *et al.,* 2013).

On the other hand, a switch to PI-sparing 2DR with DTG+RPV improved markers of bone resorption (type-1 collagen C-telopeptide), and bone formation (osteocalcin, bone-specific alkaline phosphatase), as well as markers of renal tubular function (urine retinol-binding protein and urine beta-2 microglobulin). These positive results were observed both at 48-weeks, in comparison with TDF-based 3DR (McComsey *et al.,* 2017; Llibre *et al.,* 2018), and at 100-weeks of treatment, in comparison with baseline (Orkin *et al.,* 2017).

Despite these findings, it is still uncertain whether the improvement in renal and bone parameters observed in patients starting or switching to a 2DR is related to 2DR *per se*, or rather to the withdrawal/avoidance of TDF, as almost all studies used a TDF-based 3DR as comparator. The results of ongoing clinical trials comparing 2DR with tenofovir alafenamide (TAF)-based 3DR will probably better clarify this aspect.

Serum lipid levels

TDF has shown an intrinsic lipid-lowering effect (Santos *et al.,* 2015; Postorino *et al.,* 2016), that can be lost in a switch to TDF-sparing 2DRs. In the SALT study, the patients who were switched from ATV/r+2NRTIs (80% of whom on TDF) to ATV/r+3TC had an increase in total cholesterol and total/HDL cholesterol ratio at week 48, compared to the patients who remained under triple-therapy (+4.1 *vs* –6.5, and +1.3 *vs* –4.7, respectively) (Perez-Molina *et al.,* 2015). Similar results were obtained in ATLAS-M and DUAL-GESIDA studies, where a significant increase of total, HDL and LDL cholesterol was observed in the 2DR arm (ATV/r or DRV/r+3TC), compared with the 3DR arm (ATV/r or DRV/r+2NRTIs, mostly TDF/FTC), yet without significant differences in triglycerides and total/HDL and HDL/LDL cholesterol ratios (Di Giambenedetto *et al.,* 2017; Pulido *et al.,* 2017).

Promising results were obtained in 2 randomized clinical trials with the switch to DTG+RPV, which did not modify lipid profile at week-48, compared to previous cART [PI- (26%), NNRTI- (54%) or INI-based (20%) therapy; 70% on TDF backbone] (Llibre *et al.,* 2018). Nevertheless, in the 1400 naïve subjects enrolled in the GEMINI trials, the 2DR arm with DTG/3TC had a higher increase in total, HDL and LDL cholesterol (for all $p<0.001$) and a greater decrease of total cholesterol/HDL ratio and TG levels ($p<0.05$ for both), compared to the 3DR arm with DTG+3TC/TDF (Cahn *et al.,* 2018a).

Taken together, these results indicate that the clinical relevance of serum lipid level alterations in 2DR compared to 3DR still remains to be defined, as in the majority of TDF-sparing 2DR the total/HDL cholesterol ratio (one of the strongest predictors of cardiovascular risk (Millan *et al.,* 2009) remained unchanged.

Neurocognitive performance

Although neurocognitive outcomes were reported in only a few studies, no differences were observed between the 2DR and 3DR arms (Winston *et al.,* 2017; Fabbiani *et al.,* 2018; Perez-Valero *et al.,* 2018). These findings are in line with previous de-intensification studies, showing no detrimental effects on short- and long-term neurocognitive performance in mono or 2DR arms (Perez-Valero *et al.,* 2014).

Adverse events

Although NRTI-sparing is supposed to improve the tolerability of the regimen, only a slight tendency toward a lower rate of adverse events (AEs) leading to treatment discontinuations for 2DRs over 3DRs without significant differences in the incidence of treatment-limiting and severe AEs was found in a recent meta-analysis (Achhra *et al.,* 2016b). Although the similar adverse events profile could potentially be explained by selection bias due to the long duration of the comparator therapy in trials exploring switching strategies, the same data in trials conducted in ART-naïve population seems to confirm a similar tolerability profile of dual and triple ART.

DRUG-DRUG INTERACTIONS ISSUE IN TWO-DRUG REGIMENS

In general, 2DRs by definition imply a lower threat of drug-drug interactions (DDIs) as compared to conventional 3DRs.

Although PI-based 2DRs (plus 3TC or RAL) generally showed optimal virological efficacy and safety, they are penalized by the non-negligible risk of DDIs, as boosted PIs are substrates and inhibitors of CYP3A4.

INI-based 2DRs with DTG or the newly-developed oral and injectable Cabotegravir (CAB), either in combination with 3TC or RPV (Margolis *et al.,* 2017; Rusconi *et al.,* 2017; Cahn *et al.,* 2018a; Llibre *et al.,* 2018), are particularly promising because they are associated with a reduced potential for being either perpetrators or victims of significant metabolic interplays leading to clinically significant DDIs.

DTG is a substrate of UDP-glucuronosyltransferase (UGT), breast cancer resistance protein (BCRP) and P-glycoprotein (Pgp) and, to a lesser extent, of CYP3A4 (Di Perri *et al.,* 2018). DTG inhibits organic cationic transporter (OCT) 2, which translates to a higher exposure of dofetilide and metformin (Di Perri *et al.,* 2018). RPV is a CYP3A4 substrate and a Pgp inhibitor (Weiss and Haefeli 2013), although only slight increases in both DTG and RPV are seen when co-administered. According to *in vitro* studies, CAB has a low potential of interaction with either CYP inhibitors/inducers or CYP3A substrates and, despite being a Pgp and BCRP substrate, high intrinsic membrane permeability limits transporter effects on absorption (Reese *et al.,* 2016). This latter property is of some relevance because RPV, with which the drug is combined in the LATTE strategy (Margolis *et al.,* 2017), is an inhibitor of both Pgp and BCRP *in vitro*. Caution is advised in case of co-administration of organic anionic transporter (OAT) 1/3 substrates (i.e., methotrexate), in that CAB inhibits their renal transporters, potentially leading to higher systemic exposure (Reese *et al.,* 2016). No clinical data are available on the DDIs profile of CAB as injectable formulation: the drug is released directly into the systemic circulation, and it is unknown to what extent the by-pass of intestinal absorption and primary hepatic processing will modify the chance of interacting with drugs, exerting or undergoing metabolic effects at that level (Reese *et al.,* 2016).

With the exception of injectable CAB, for which no clinical experience is yet available, these regimens cannot be co-administered with strong CYP3A inhibitors, such as rifampicin, or antiepileptics, such as carbamazepine and phenobarbital.

HEALTH TECHNOLOGY ASSESSMENT

Health Technology Assessment (HTA) refers to the systematic evaluation of properties, effects, and/or impacts of health technology. It is a multidisciplinary process that evaluates the social, economic, organizational and ethical issues of a health intervention or health technology. Despite the success of 3DR, the idea of treating HIV infection with fewer drugs is captivating, due to issues of convenience, long-term toxicities and costs. Several studies investigated the impact on a local health budget of the use of antiretrovirals in mono or dual therapy (most of them with PI-based regimens), but very few performed an HTA analysis (Restelli *et al.,* 2014a; Restelli *et al.,* 2014b; Girouard *et al.,* 2016; Papot *et al.,* 2017); to our knowledge, no one has done this with INI. Due to the worldwide shrinking of health budgets, this is the right time to adopt HTA as a key tool to evaluate not only a single antiretroviral drug but also new strategies for the use of antiretroviral combinations (i.e., dual *vs*. triple regimens).

CONCLUSIONS

Consistent and favorable evidence on 2DRs has been accumulated in recent years, paving the way to a new paradigm shift toward this strategy.
2DRs now represent a safe and effective therapeutic option in terms of virological suppression, prevention of resistance to failure, and favorable renal, bone, and metabolic parameters in treatment-experienced patients. In addition, substantial evidence is accumulating that demonstrates the potential advantages of this approach in naïve patients as well. The possibility of prescribing such first-line and maintenance regimens is a valuable tool for reducing toxicities and costs as well as for achieving the goal of increasing global access to cART. Nevertheless, additional tools are worth exploring in a research setting to help define patients who could benefit most from 2DR strategies.

Acknowledgements
This article is based on a consensus conference held in Florence, Italy on October 19-20, 2018 and made possible by an unrestricted educational grant from ViiV.

The Two-Drug Antiretroviral Regimens expert panel.
Massimo Andreoni, *Infectious Diseases, University of Rome "Tor Vergata". Rome, Italy*; Andrea Antinori, *National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy;* Francesca Ceccherini-Silberstein, *Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy;* Valeria Cento, *Residency in Microbiology and Virology, University of Milan, Milan, Italy;* Andrea Cossarizza, *Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena, Italy;* Mario Clerici, *Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy, and IRCCS Don Carlo Gnocchi Foundation, Milan, Italy;* Antonella D'Arminio Monforte, *Department of Health Sciences, ASST Santi Paolo e Carlo, Institute of Infectious Diseases, University of Milan, Milan, Italy;* Andrea De Luca, *Infectious Diseases Unit, Siena University Hospital, Siena, Italy;* Antonio Di Biagio, *Infectious Diseases Clinic, Policlinico Hospital San Martino, Genoa, Italy;* Giovanni Di Perri, *Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy;* Massimo Galli, *Infectious Diseases Unit, DIBIC Luigi Sacco - University of Milan, Italy;* Roberta Gagliardini, *National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy;* E. Girardi, *National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy;* Andrea Gori, *Infectious Diseases Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, University of Milan, Italy;* Adriano Lazzarin, *IRCCS San Raffaele Scientific Institute, Clinic of Infectious Diseases, Milan, Italy;* Miriam Lichtner, *Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy;* Sergio Lo Caputo, *Clinic of Infectious Diseases, University of Bari Aldo Moro, Bari, Italy;* Giordano Madeddu, *Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy*; Franco Maggiolo, *Division of Infectious Diseases, AO Giovanni XXIII, Bergamo, Italy;* Giulia Marchetti, *Clinic of Infectious Diseases, Department of Health Sciences, ASST Santi Paolo e Carlo, Presidio San Paolo, University of Milan, Italy;* Annalisa Mondi, *National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy;* Cristina Mussini, *Infectious Diseases Clinics, Azienda Ospedaliero-Universitaria Policlinico di Modena, Italy;* Carlo Federico Perno, *Oncology and Oncohematology, University of Milan, Italy*; Maria Carla Re, *Microbiology Section of the Department of Experimental, Diagnostic and Specialty Medicine, School of Medicine University of Bologna Italy;* Diego Ripamonti, *Infectious Diseases Unit, ASST Papa Giovanni XXIII, Bergamo;* Giuliano Rizzardini, *1st Division of Infectious Diseases, ASST Fatebenefratelli-Sacco, Milano, Italy;* Maria Mercedes Santoro, *Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy;* Maurizio Zazzi, *Department of Medical Biotechnology, University of Siena, Italy*.

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