

Management of HIV infection after triple class failure

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SUMMARY

Resistance of Human Immunodeficiency Virus (HIV) to antiretrovirals is a clinically important issue despite the availability of five antiretroviral drug classes. Although the incidence of HIV resistance might have stabilized or even decreased in patients starting their first Highly Active Antiretroviral Therapy (HAART) regimen in recent years, the prevalence of failure to the three original antiretroviral classes is estimated to range from 2.1% to 16% after HAART initiation. International guidelines recommend the use of at least two active drugs in constructing a new antiretroviral regimen to obtain virologic success, and adding a compound with a different mechanism of action often increases the chances of virologic response. With the introduction of new drug classes and new-generation compounds of older classes in the antiretroviral armamentarium, the chances of achieving virologic success in patients with resistance to all three original antiretroviral classes are certainly higher than in the past. Patients who experience virologic failure and show resistance to new antiretrovirals are, however, described both in randomized trials and clinical settings. Although HAART regimens using various associations of the newest antiretrovirals led to very high rates of virologic success in patients with previous failure to all three original drug classes, there are circumstances in which patients cannot benefit from two fully active drugs, especially after prior exposure to several suboptimal therapies or functional monotherapies. These patients often need a holding regimen while awaiting new and effective antiretrovirals. This article reviews strategies that might be effective options to obtain virologic success in patients with triple class failure, and treatment strategies for patients who do not have two active drugs to construct a new effective antiretroviral regimen after virologic failure.

KEY WORDS: HIV, AIDS, Virologic failure, Resistance, Triple class failure.

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INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) has made the most substantial change in the natural history of HIV-infected individuals thus far (Palella *et al.* 1998; Mocroft *et al.* 1998, Palella *et al.* 2006).

The aim of HAART is to suppress HIV replication and allow for immune restoration, which secures patients from acquiring opportunistic infections

(Mellors *et al.*, 1997; Egger *et al.*, 2002; Phillips *et al.*, 2007). Thanks to the large number of available antiretroviral compounds, virologic success is now generally attainable also in patients with previous failures and drug resistance (Hicks *et al.* 2006; Clotet *et al.*, 2007; Lazzarin *et al.*, 2007; Steigbigel *et al.*, 2008; Pichenot *et al.* 2012). The issue of drug resistance is, however, of increasing concern especially in individuals with long-standing HIV infection who have often been exposed to suboptimal treatment and sequential monotherapies in the past. These previous treatments often resulted in the accumulation of resistance mutations that might have jeopardized the effectiveness of subsequent HAART regimens (Yamashita *et al.*, 2001). Perhaps even more importantly extensive resistance to the three origi-

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nal antiretroviral classes (triple class resistance) has been associated with a higher risk of disease progression and death (Zaccarelli *et al.*, 2005; Grover *et al.*, 2008; Di Giambenedetto *et al.*, 2008; Cozzi-Lepri *et al.*, 2008).

Thanks to the recent availability of several new drug classes and new generation compounds within the three original classes, the issue of triple class failure and resistance might seem less urgent than in the past. However, failure with these new drugs has been reported both in randomized trials and in clinical settings (de Meyer *et al.*, 2008; Cooper *et al.*, 2008; Canducci *et al.* 2009; Hatano *et al.*, 2010). The management of such patients is extremely challenging even for the most experienced HIV physicians.

This article will review the possible management strategies for these patients. Published articles (either original data or review articles) were searched for in PubMed using the terms of interest: "HIV", "resistance", "virologic failure", "class failure", "triple class failure", "mortality", "survival", "progression". Single antiretroviral names were also searched for as terms of interest to present updates on the newest treatment options. Abstracts presented at recent international conferences were also included for the latest update. Data from randomized clinical trials and observational studies were used for this article.

Epidemiology and risk factors for triple class failure

Data on the prevalence of triple class failure (TCF) might change depending on the definition of triple class failure itself. Older studies tended to use a looser definition, which defined TCF as having failed at least one compound of each of the three original antiretroviral classes. This definition also allowed for inclusion in this category patients who had failed a single unboosted protease inhibitor (PI) or only one nucleoside reverse transcriptase inhibitor (NRTI). According to this definition, an overall prevalence of TCF was reported as high as 16% in a large collaborative cohort study in the early 2000s (Ledergerber *et al.* 2004). Similar results were obtained by the EuroSIDA Study Group: TCF prevalence was measured in patients starting a HAART regimen from 1997 to 2003 and an overall prevalence of 13% was observed (Mocroft *et al.*, 2004). A study from North Carolina University enrolling patients

from 2000 onwards reported an overall prevalence of triple class resistance - TCR - (defined as the presence of at least one resistance mutation to the three original antiretroviral drug classes) of 7.6% (Napravnik *et al.*, 2007). When TCR prevalence was estimated only considering patients with available genotype, results ranged from 6.6% prevalence in the UK Collaborative HIV Cohort (Jones *et al.*, 2008), to 13% in an American cohort in the late 1990s (Richman *et al.*, 2004), to 20% in the North Carolina University Study, and was reported as high as 27% in a recent CDC report (Buchacz *et al.*, 2012).

Using a more stringent definition of TCF (having failed at least one ritonavir boosted PI, one NRTI and one compound of each subclasses of NRTIs: zidovudine and stavidine; lamivudine and emtricitabine; didanosine, tenofovir and abacavir) Phillips and colleagues found an overall prevalence of TCF as high as 2.1% (Phillips *et al.*, 2007). In this and the previous studies, the rate of TCF was higher in patients with previous evidence of virologic failure than in those who had not experienced failure previously.

Recently, the Pursuing Later Treatment Options II Project (PLATO II) from the CHOERE Group examined the risk of TCF in previously naïve patients who started HAART starting from 1998 and found an overall TCF prevalence of 3.0% over a follow-up period of 4 years (Pursuing Later Treatment Options II (PLATO II) project team *et al.*, 2012). A British Columbia Study evaluated TCR in previously naïve patients starting their first HAART regimen from January 2000 on and found a 1.9% prevalence in patients with available genotypes (Lima *et al.*, 2010).

As for the incidence of TCF over time, The EuroSIDA Study Group estimated an incidence of TCF of 1.6 cases/100 person years of follow-up (PYFU) and of 3.9 cases/100 PYFU in treatment-naïve and treatment-experienced patients respectively (Mocroft *et al.*, 2004). The authors found a constant increased risk of TCF over time for treatment-naïve patients and a more dynamic evolution for treatment-experienced patients. In this group of patients there was an increase in the incidence of TCF in the first three years after HAART initiation with a peak in the 2 to 3 year period. After that time the incidence remained substantially stable.

Conversely, a Danish cohort study found a peak

in the incidence rate of TCF during the fourth year of HAART, and declined thereafter during the study period (Lohse *et al.*, 2005). They also observed a decline in the prevalence of TCF after 2001, which is in contrast to the EuroSIDA Study Group finding of a constant increase in the prevalence of TCF up to 2003. More recently, the PLATO II group observed an increase in the incidence rate of TCF in the first 5 years of HAART treatment, rising from 3.9 cases/1000 PYFU in 2000 to 8.8 cases/1000 PYFU in 2005, declining thereafter to an incidence of 5.8 cases/1000 PYFU in 2009 (Pursuing Later Treatment Options II (PLATO II) project team *et al.*, 2012). These results are consistent with those reported in the North Carolina University Study considering patients who developed genotypic resistance to all three original antiretroviral drug classes. In previously naïve patients starting an HAART regimen the incidence rate of TCR was 8 cases per 1000 PYFU (Napravnik *et al.*, 2007). The UK Collaborative HIV Cohort reported a somewhat higher incidence of TCR at 17% (Jones *et al.*, 2008). In the smaller British Columbia Cohort the incidence rate for developing TCR was estimated as 0.9 per 1000 person-months during a median time of follow-up of 23 months (Lima *et al.*, 2010).

Although not always consistent, mainly due to differences in measurement methods, these data collectively suggest that the incidence of TCF might have plateaued, if not decreased in recent years, at least in patients starting their first line treatment as an HAART regimen. This trend is certainly reassuring for the future evolution of TCF and TCR, considering that virtually every HIV-infected patient starts treatment with a HAART regimen.

Some of these cohort studies also investigated the factors associated with a higher risk of developing TCF: in the EuroSIDA study, higher baseline HIV-RNA levels and IVDU route of transmission were associated with a higher risk of TCF in previously treatment-experienced patients while higher baseline CD4+ cell count and the number of new NRTIs in the new HAART regimen were protective against TCF (Mocroft *et al.*, 2004). In the same study only higher baseline HIV-RNA was a risk factor for TCF in previously naïve patients.

In the PLATO II Study, which considered only previously naïve patients, higher baseline HIV-

RNA and lower baseline CD4+ cell count were associated with a higher risk of TCF. Conversely, MSM route of transmission and older age at the time of HAART initiation were associated with a protective effect. In this study no difference in the risk of TCF was found according to the drug class used in the initial HAART regimen (Pursuing Later Treatment Options II (PLATO II) Project Team for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) *et al.* 2010). In the North Carolina University Study, factors associated with an increased risk for TCR in previously naïve patients starting a HAART regimen were a higher peak HIV-RNA level, a greater number of antiretrovirals received and the use of an unboosted single-PI regimen. In the same study factors associated with a greater risk of TCR in previously experienced patients were again a greater number of antiretrovirals received and having started treatment with a non-HAART regimen (Napravnik *et al.*, 2007). The most consistent trend across all these studies seems to be the higher risk for TCF/TCR in patients who have high levels of viral replication prior to starting treatment. Close monitoring to avoid the rapid selection for resistance mutations should be considered in patients starting antiretroviral treatment with high HIV-RNA levels.

Consequences of triple class failure on HIV disease progression

Since the main mechanism by which HAART had such great effect on the survival of HIV-infected patients is suppression of plasma HIV replication, it is easily predictable that loss of viral suppression in plasma (virologic failure) might have clinical consequences. Taken to the extreme, the lack of a chance to obtain virologic suppression (triple class failure) might have a deep clinical impact. The clinical consequences of triple class failure with respect to disease progression and survival were investigated in a number of studies and results have not always been consistent. The Johns Hopkins cohort did not find any increased risk of death or disease progression in patients showing extensive antiretroviral drug resistance (identified by the number of mutations) (Lucas *et al.*, 2004).

Using a slightly different approach, Recsky and colleagues examined the prevalence of drug resistance in HIV-infected patients followed at the

British Columbia HIV/AIDS Drug Treatment Program. They compared the prevalence of drug resistance in patients with plasma HIV-RNA >500 copies/ml who died between 1997 and 2001 and living patients who had a genotype done over the same period of time. A much higher prevalence of drug resistance was found in the virologic failure group (76%) compared to patients who died (44%). Similarly, the prevalence of triple class resistance was higher in the virologic failure group compared to patients who died (11% vs 6%) (Recsky *et al.*, 2004).

In a study by Zaccarelli *et al.* the proportions of patients progressing to death, AIDS-related death and new AIDS event or death were 27.1%, 21.5% and 35.9% for patients with three class-wide resistance compared to 8.9%, 6.1% and 16% of patients with no class-wide resistance. At multi-variable analysis three class-wide resistance, as well as HIV-RNA levels prior to resistance testing, were associated with a higher risk of death by any cause, AIDS-related death and new AIDS event or death (Zaccarelli *et al.*, 2005).

Consistent with these data, the Danish HIV Cohort Study reported a higher risk of mortality for patients found to have a greater number of genotypic mutations: the mortality rate ratio was 2.3 (95% CI: 2.1-4.8) for patients harboring ≥ 9 mutations compared to those who showed <8 mutations in their HIV genotype (Lohse *et al.*, 2007).

Grover *et al.* analysed treatment strategies after virologic failure possibly influencing the outcome. They studied 692 patients with evidence of multi-drug-resistance in the UK between 1997 and 2004 and they estimated a mortality rate of 3.7 death/100 PYFU in this population. Changing therapy with a higher GSS at treatment failure compared to staying on the same treatment had a protective effect on subsequent virologic success, but not on overall survival (Grover *et al.*, 2008).

Although not always consistent in terms of design, population studied, outcome measure and follow-up, these data indicate that virologic failure and drug resistance are associated with poor clinical outcome and need to be addressed carefully.

Recent data from the PLATO II group show a more encouraging perspective on the virologic and clinical outcome of patients experiencing

TCF. They studied 2476 patients who started antiretroviral therapy after 1998 and experienced TCF. They found a steady increase in the proportion of patients with virologic response after TCF, rising from 19.5% in 2000 to 57.9% in 2009 ($p < 0.0001$). They also found a significant decrease in the proportion of new AIDS diagnosis after TCF over time, and a marked decrease in mortality (Pursuing Later Treatment Option II (PLATO II) project team *et al.*, 2012).

While earlier data reported no impact of triple class failure on clinical outcomes, more recent studies showed how having fewer treatment options can impact the development of AIDS events and mortality. On the other hand, the recent availability of new antiretrovirals has also allowed the achievement of viral suppression in multi-drug experienced patients, leading to a decrease in the incidence of AIDS events and mortality. These data should reassure clinicians on the good outcomes such patients can aim for when virologic suppression is attained.

Goals and treatment options for triple class-experienced patients

Achieving viral suppression

Until a few years ago sustained virologic suppression was not considered an attainable goal for treatment-experienced patients. Causes of the low success rate in experienced patients were the presence of resistant viral strains, often due to previous suboptimal therapies; the complexity of HAART regimens for experienced patients, which often consisted of a high pill burden at least twice daily; the lack of new drug classes with different mechanisms of action or new compounds of older classes with activity against resistant viral strains. These factors certainly compromised the efficacy of salvage regimens in the early years of HAART when PIs were often administered without the ritonavir booster, thereby reducing their efficacy.

Nevertheless in past years several new compounds targeting different steps in the life cycle of HIV besides reverse transcription and proteins excision became available. In addition, new generation PIs and NNRTIs retaining activity against PI and NNRTI-resistant viral strains were recently approved.

Phase-III trials of these new compounds showed a high proportion of virologic success in experi-

TABLE 1 - Summary of randomized clinical trials evaluating the efficacy and safety of most recent antiretrovirals in treatment-experienced HIV infected patients.

	Study	Number of patients and study design	Inclusion criteria	Primary endpoint	Primary outcome
New compounds of older drug classes	Resist 1 and 2	N = 1529 Open-Label, 1:1 randomization Study Arm: TPV/r + OBR, n= 775 Control Arm: PI/r + OBR, n=754	HIV-RNA>1000 cp/ml on failing PI-based ART Previous treatment with ≥ 1 NRTIs, NNRTIs and ≥ 2 PIs	HIV-RNA reduction ≥ 1 Log cp/ml at week 48	Study Arm: 34% Control Arm: 15% p<0.0001
	Power 1 and 2	N = 255 (pooled analysis for patients receiving DRV/r 600/100 mg BID vs Placebo) Open-Label, Randomized Study Arm: DRV/r + OBR, n= 131 Control Arm: PI/r + OBR, n= 124	HIV-RNA>1000 cp/ml on failing PI-based ART Previous failure to ≥ 1 NRTIs, NNRTIs and PIs ≥ 1 PI resistance mutations	Original: HIV-RNA reduction ≥ 1 Log cp/ml at week 24 Pooled analysis: HIV-RNA reduction ≥ 1 Log cp/ml at week 48	At week 48 Study Arm: 61% Control Arm: 15% p<0.0001
	Duet 1 and 2	N= 1203 Double-blinded, 1:1 randomization Study Arm: ETV + DRV/r + OBR, n=599 Control Arm: Placebo + OBR, n=604	HIV-RNA>5000 cp/ml on failing ART ≥ 1 NNRTIs and ≥ 3 major PI resistance mutations	HIV-RNA<50 copies/ml at week 24	Overall Study Arm: 58% Overall Control Arm: 41% p<0.05 in both Duet 1 and 2
New antiretroviral classes	Toro 1 and 2	N= 1013 Open-Label, 2:1 randomization Study Arm: ENF + OBR, n= 661 Control Arm: OBR alone, n= 334	HIV-RNA>1000 cp/ml on failing PI-based ART Previous treatment with ≥ 1 NRTIs, NNRTIs and ≥ 2 PIs	Mean plasma HIV-RNA change from baseline at week 24	Toro 1 Study Arm: -1.7 Log cp/ml Control Arm: -0.8 Log cp/ml Toro 2 Study Arm: -1.4 Log cps/ml Control Arm: -0.8 Logcp/ml p<0.001 for both studies
	Benchmark 1 and 2	N = 699 Double-blinded, 2:1 randomization Study Arm: RAL + OBR, n= 462 Control Arm: Placebo + OBR, n = 237	HIV-RNA>1000 cp/ml on failing ART Documented genotypic or phenotypic resistance to ≥ 1 NRTIs, NNRTIs, PIs	HIV-RNA <400 copies/ml at week 16	Overall Study Arm: 72% Overall Control Arm: 37% p<0.001 for both studies individually and for the combined analysis
	Motivate 1 and 2	N = 1049 Double-blinded, 1:1:1 randomization Study Arm A: MVC BID + OBR, n= 426 Study Arm B: MVC QD + OBR, n= 414 Control Arm: Placebo + OBR, n= 209	HIV-RNA>5000 cp/ml on failing ART Treatment experience and documented genotypic or phenotypic resistance to at least 3 ARV classes (NRTIs, NNRTIs, PIs, Fusion Inhibitors)	Mean plasma HIV-RNA change from baseline at week 48	Study Arm A: -1.84 Log cp/ml Study Arm B: -1.68 Log cp/ml Control Arm: -0.79 Log cp/ml p<0.001 for each comparison of a MVC Group compared to Control Group, both in individual studies and in the combined analysis.
	Elvitegravir Molina JM, 2012	N = 724 Double-blinded 1:1 randomization Study Arm: ELV + OBR, n= 361 Control Arm: RAL + OBR, n= 363 In both arms, OBR consisted of a PI/r + 1 other agent	HIV-RNA>1000 cp/ml on failing ART ≥ 6 months experience or documented resistance to ≥ 2 ARV classes	HIV-RNA<50 copies/ml at week 48	Study Arm: 59% Control Arm: 58% p=0.001 for non inferiority

Abbreviations: TPV/r: Tiplranavir/ritonavir; DRV/r: Darunavir/ritonavir; ETV: Etravirine; ENF: Enfuvirtide; RAL: Raltegravir; MVC: Maraviroc; ELV: Elvitegravir; OBR: Optimized Background Regimen; cp/ml: copies/milliliter; NRTI: Nucleoside Reverse Transcriptase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; PI/r: ritonavir-boosted PI.

enced patients. Table 1 summarizes the results of the Phase-III trials for the newest antiretrovirals approved for treatment-experienced patients. Consistently in all these studies, higher proportions of virologic success were observed when two or more active antiretrovirals were part of the new regimen and when at least one of them belonged to a new drug class (Hicks *et al.*, 2006; Clotet *et al.*, 2007; Lazzarin *et al.*, 2007; Steigbigel *et al.*, 2008; Pichenot *et al.*, 2012).

The availability of such new drugs increased the possibility of sustained virologic suppression in experienced patients to a great extent and HIV-RNA <50 copies/ml is now the recommended goal for treatment-experienced patients as well as for naïve patients (Panel on Antiretroviral Guidelines for Adults and Adolescents. Department of Health and Human Services. 2012).

As an example of such sustained virologic success, Eron *et al.* recently presented the 5-year efficacy results of raltegravir for treatment-experienced patients. Overall, in the ITT analysis, 42% of the 462 patients randomized to raltegravir had HIV-RNA <50 copies/ml, compared to 16% of the 237 patients in the control arm and 80% of the 199 patients in the raltegravir arm, who achieved HIV-RNA <50 copies/ml at week 16, continued to show virological suppression after 5 years of treatment (Eron *et al.*, 2012).

Data on the feasibility of achieving sustainable viral suppression in drug-experienced patients do not only come from randomized clinical trials, but also from observational studies of clinical practice. A study at the University of Alabama analyzed the effectiveness of the introduction of a novel antiretroviral treatment for three-class experienced patients. Patients treated with darunavir/ritonavir were 4.24 times more likely to achieve virologic suppression compared to other non-PIs strategies (McKinnell *et al.*, 2009).

Efficacy of raltegravir plus OBT in multidrug-experienced patients has been assessed in clinical practice as well. A study from the French ANRS CO3 Aquitaine Cohort showed a 78% virologic response frequency and a median 57 cells/mm³ CD4+ Tcells increase after 24 weeks of treatment (Wittkop *et al.*, 2009).

Similar promising results were also shown recently from an HIV Swiss Cohort Study, where the authors studied 123 multi-experienced patients starting raltegravir treatment as either part

of a new suppressive regimen or for treatment simplification reasons. 72% and 96% of patients in whom raltegravir was included in a new suppressive regimen or for simplification strategy had virologic success after 24 weeks of treatment respectively (Scherrer *et al.*, 2009).

A study from Spain recently evaluated the efficacy of etravirine in drug-experienced patients reporting a 73% treatment response (HIV-RNA <50 copies/ml) at week 48 (Santos *et al.*, 2011). Data from the Etravirine Early Access Program in the United States also showed the efficacy of etravirine in drug-experienced patients. The response rate in patients who received etravirine without either raltegravir or darunavir/ritonavir was 62.3 % at week 48 (Towner *et al.*, 2010).

High rates of virologic success were also reported in clinical practice for maraviroc. In a Study from the University of Düsseldorf, 80% of drug-experienced patients failing their current regimen and harboring an R5-tropic virus, achieved HIV-RNA <50 copies/ml after 6 months of a new maraviroc-including HAART (Reuter *et al.* 2010). A Spanish study evaluating 46 patients failing their current regimen and harboring and R5-tropic virus reported a proportion of virologic success of 96% after 48 weeks of treatment (Genebat *et al.*, 2010).

Despite the wide availability of new drugs in the recent past, only few additional new antiretrovirals are currently in clinical trials for multi-experienced HIV-infected patients.

Dolutegravir is a second generation integrase inhibitor which showed a potentially higher genetic barrier to resistance *in vitro* compared to raltegravir or elvitegravir (Kobayashi *et al.*, 2011; Canducci *et al.*, 2011). Dolutegravir is currently being studied in clinical trials for efficacy and safety. In a phase 2 international study which enrolled 205 treatment-naïve patients, all dolutegravir once-daily dose groups showed a similar efficacy in the primary analysis at week 16 (van Lunzen *et al.*, 2012). The VIKING trial is an ongoing study evaluating the efficacy and safety of dolutegravir in integrase inhibitor-resistant, multidrug-experienced patients. Preliminary results suggest that dolutegravir maintains its efficacy against viral strains with major integrase inhibitors resistance mutations. 183 subjects with current (n=124) or historical (n=59) resistance to raltegravir and documented resistance to ≥2 oth-

er ART classes were enrolled in the study. The primary endpoint was antiviral efficacy measured as mean HIV-RNA decline at day 8 (patients optimized their OBR at that time point) and as the proportion of patients with HIV-RNA <50 copies/ml at week 24. Mean HIV-RNA declined by 1.4 log₁₀ c/mL (95% CI: 1.3, 1.5; p<0.001) at day 8, with 82% of patients showing >1 log HIV-RNA decline or <50c/mL at the same time point. Of the 114 subjects who completed 24 weeks on study or discontinued before data cutoff, 72 (63%) had <50 c/mL RNA at week 24. Although results varied according to the number and type of resistance mutations in the integrase gene at baseline and the overall susceptibility score, dolutegravir represents a promising second line integrase inhibitor (Nichols *et al.*, 2012).

Combining new antiretrovirals

All the phase III trials testing the efficacy and safety of new antiretrovirals usually add the investigational compound to an OBT based on the resistance testing. Having a single active agent in a new regimen is not generally recommended because it leads to rapid selection of resistance mutations. However, in recent phase III trials 15% to 28% of patients had the investigational agent as the only active compound in the regimen as measured by either genotypic or phenotypic sensitivity score. These patients had a consistently lower rate of response across all trials (Hicks *et al.* 2006; Clotet *et al.*, 2007; Lazzarin *et al.*, 2007; Steigbigel *et al.*, 2008; Pichenot *et al.*, 2012).

Patients who still have active agents among PIs and NNRTIs may benefit from adding a recently approved compound to an OBT containing older PIs, NRTIs or NNRTIs (Panel on Antiretroviral Guidelines for Adults and Adolescents. Department of Health and Human Services. 2012).

For example, in the UTILIZE study Baxter and colleagues showed that 59% of treatment-experienced patients failing a PI-based regimen had viral strains harboring PI resistance and the majority of these patients' viruses remained sensitive to the newer PIs, either tipranavir or darunavir (Baxter *et al.*, 2010).

Furthermore, due to the potential overlap in mutations conferring resistance to tipranavir and darunavir, the question as to whether a darunavir/ritonavir-based regimen is effective af-

ter tipranavir/ritonavir failure is of clinical significance. Our group studied patients failing a tipranavir/ritonavir-based regimen and found that only 19.2% of patients showed an increase in the darunavir resistance score. A 54% response rate was observed at week 24 in patients subsequently starting a darunavir/ritonavir based-HAART (Spagnuolo *et al.*, 2009).

For patients who do not have active compounds in the three original antiretroviral classes, recent data from our and other groups showed the efficacy and safety of combining new compounds only in a fully active regimen.

In the TRIO trial (Yazdanpanah *et al.*, 2009), Yazdanpanah and colleagues studied 103 treatment-experienced patients who started a new HAART regimen based on darunavir/ritonavir, etravirine and raltegravir. Patients were heavily pretreated and 43% were classified as CDC stage C. The efficacy of the new regimen was shown both at weeks 24, 48 and recently 96 of treatment (Fagard *et al.*, 2012): 90%, 86% and 88% of patients had HIV-RNA <50 copies/ml at each time point. Although they did not have a specific control group for this study, the authors argued that such efficacy results are higher than the results obtained in other studies of treatment-experienced patients and approach results obtained with naïve patients.

Similar results were obtained by Imaz and colleagues (Imaz *et al.*, 2009). The authors followed 32 three class-experienced patients with a median of nine previous HAART regimens and across thirteen years of therapy starting darunavir/ritonavir, raltegravir and etravirine. At week 24 HIV-RNA was less than 50 copies/ml in 94% of patients. The same group also reported data on an extended cohort of multi-experienced patients starting a new HAART regimen with a combination including at least three drugs among the most recently approved (darunavir/r, etravirine, raltegravir, maraviroc) reporting an overall virological suppression rate of 78% at week 48 (Imaz *et al.*, 2011). The association of these three new compounds was also tested in a group of twelve adolescents with perinatal HIV infection and a multidrug-resistant viral strain (Thuret *et al.*, 2009). At week 48 a less robust percentage of virologic success was observed: 50% of patients had HIV-RNA <50 copies/ml. Nonetheless, 92% had HIV-RNA <400 copies/ml.

Florence *et al.* reported data on the efficacy of new antiretroviral combination strategies from the Etravirine Early Access Program in Europe. 70% of patients who were prescribed etravirine in association with darunavir/ritonavir and raltegravir, with or without background NRTIs, achieved virological success at week 24 (Florence *et al.* 2010). Similarly, Towner and colleagues reported data from the Etravirine Early Access Program in the United States. In the subgroup of patients who started a new HAART with etravirine, darunavir/ritonavir and raltegravir the proportion of virologic success at week 48 was 64% (Towner *et al.*, 2010).

Our group recently reported the results on efficacy and safety of the combination of raltegravir, etravirine and maraviroc in heavily pretreated patients harboring R5 tropic virus (Nozza *et al.* 2010). Patients had been HIV-infected for a median of 17 years and had been on antiretrovirals

for a median of 14. At week 48, 93% of patients had HIV-RNA <50 copies/ml and all of them had HIV-RNA <400 copies/ml. These results were confirmed in the recently published extended 96-week follow-up of this cohort of patients (Nozza *et al.* 2011). Figure 1 summarizes the efficacy results of these new antiretroviral combination studies. Concerns regarding the potential pharmacokinetic (PK) interactions when combining new antiretrovirals might arise, given the common metabolic pathway of most of them. Both darunavir/ritonavir, etravirine and maraviroc are substrates of the cytochrome P-450 isoenzyme, with etravirine being an inducer and darunavir/ritonavir being an inhibitor. Raltegravir is metabolized via hepatic glucuronidation, nonetheless interactions with cytochrome P-450 substrates have been reported (Fabbiani *et al.*, 2011; Goldwirt *et al.*, 2011; Anderson *et al.*, 2008). In addition, the coadministration of raltegravir and maraviroc led to a

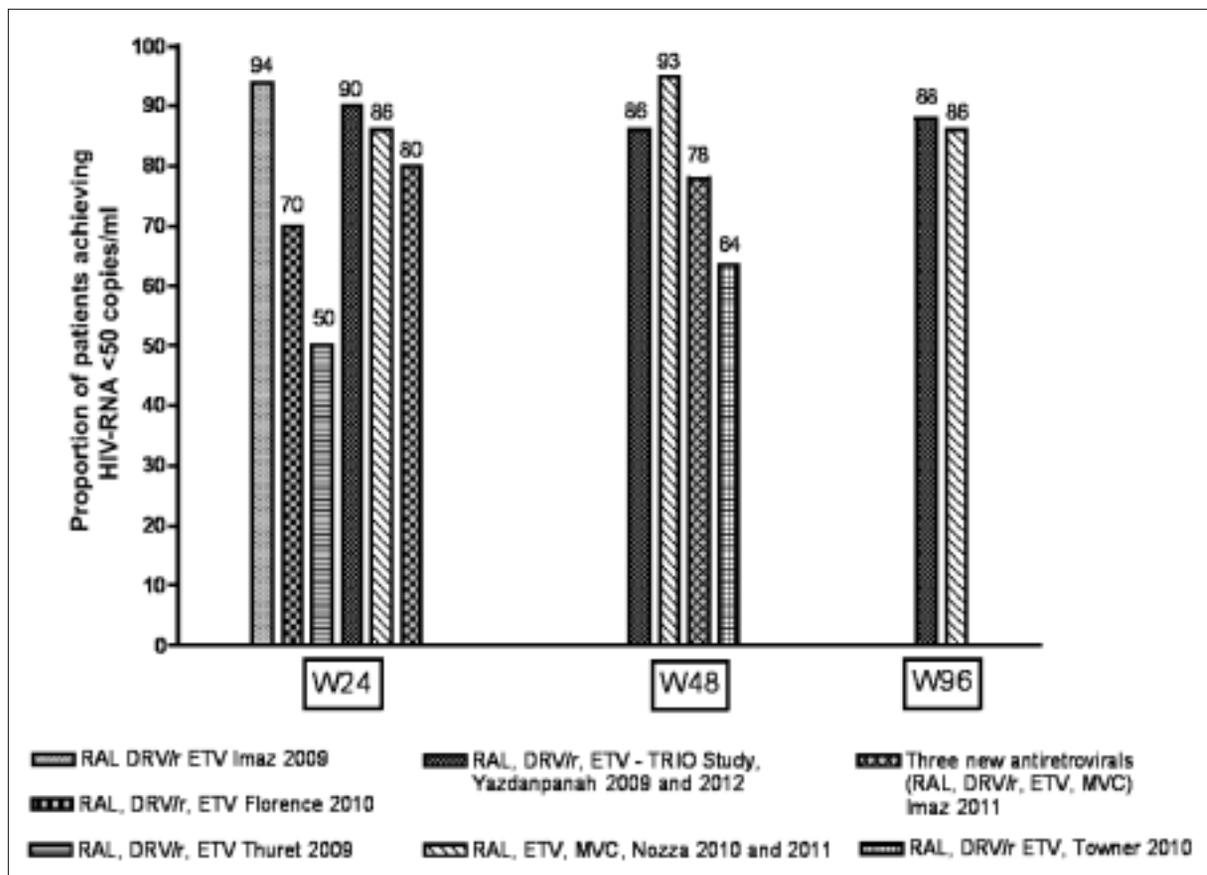


FIGURE 1 - Independent studies assessing the virologic efficacy of combinations of new antiretrovirals to obtain virologic suppression in heavily pretreated patients.

33% decrease in the C_{max} and 37% of AUC for raltegravir and a 20% decrease in the C_{max} and 14% of AUC for maraviroc when compared to the separate administration of these two compounds (Andrews *et al.* 2010). Nonetheless a PK study of plasma samples from ten HIV-infected, drug-experienced patients treated with darunavir/ritonavir, raltegravir and etravirine failed to reveal detrimental drug-drug interactions (Barrail-Tran *et al.*, 2010). A collaboration between our group and the University of Turin evaluated the PK profiles of 32 patients treated with the NRTIs- and PIs-sparing regimen consisting of raltegravir, etravirine and maraviroc. Results showed no significant change in the PK parameters for either drug was observed over 48 weeks of treatment, nor any correlation between antiretroviral C_{trough} concentration and any efficacy or safety parameter (Calcagno *et al.*, 2011). These PK data and the promising results in terms of virological efficacy, immunological recovery and safety profiles, should reassure practising physicians on the feasibility of new antiretroviral combination strategies.

Holding regimens for patients without effective drug options

Patients harboring viral strains resistant to all or almost all antiretrovirals are an existing, although small, subset of patients in the majority of clinical settings. However, failure and resistance to newer compounds is shown in all the most recent phase-III trials as well as in clinical settings and there is a subset of patients who do not currently have valid treatment options to be re-suppressed. Management of such patients is not easy. Whenever investigational compounds are in development, these patients might benefit from being enrolled in clinical trials. However, this option is not available in all clinical settings and very few new antiretrovirals are reasonably expected on the market in the very near future (Pozniak 2009). In addition, such heavily pre-treated patients might already have a virus that is not susceptible to these new compounds. Maturation inhibitors, for instance, showed reduced activity against PI-resistant viruses (Lalezari *et al.* 2008; McCallister *et al.*, 2008) and elvitegravir is unlikely to be effective in patients harboring raltegravir-resistant viruses (Shimura *et al.*, 2008).

In patients with no effective treatment options, guidelines consider continuing treatment to prevent CD4+ T cell loss and disease progression. Cohort studies even suggest that continuing treatment in the absence of an CD4+ cell increase might decrease the risk of disease progression (Ledergerber *et al.*, 2004; Raffanti *et al.*, 2004). However, maintaining treatment in the presence of viral replication is the main factor responsible for the emergence of resistance mutations potentially compromising future treatment options (Kantor *et al.*, 2004; Goetz *et al.*, 2006; Delaugerre *et al.*, 2012).

This group of patients needs a bridge from their current failing HAART to the next effective treatment. The following strategies have been investigated as holding regimens which these patients may be candidates for:

- using a greater number of antiretrovirals to achieve a tighter HIV replication control;
- recycling previously used drugs to which the virus might have reverted resistance mutations
- partial treatment interruption aiming at preventing too great a CD4+ T cells drop.

Using a greater number of antiretrovirals (Mega-HAART, double PI strategies) was fairly common in the late 1990s and early 2000s for patients failing first-generation HAART and showing resistance mutations (Miller *et al.*, 2000; Montaner *et al.*, 2001; Hammer *et al.*, 2002; Ruiz *et al.*, 2003; Katlama *et al.*, 2004; Staszewski *et al.*, 2006). Such strategies, however, became less common over time because of their numerous side-effects and thanks to the great efficacy of boosted-PI regimens and second generation compounds.

The concept of including a high number of antiretrovirals in patients with limited therapeutic options is currently limited usually only to certain NRTIs which have proved tolerability enhancing practicing physicians' confidence towards their use. In multiclass-experienced patients, however, it is very rare to have fully active NRTIs available to build a new HAART regimen, and the use of additional NRTIs in the salvage regimen usually revolves into recycling older compounds. This practice is fairly widespread among clinicians and the rationale relies on data suggesting the possibility of a residual antiviral activity despite the presence of resistance mutations, reduced viral fitness when maintaining certain mutations, or the hypersensitization ef-

fect towards other compounds (Miller *et al.*, 2002; Campbell *et al.*, 2005; Stebbing *et al.*, 2004; Llibre *et al.*, 2008). With the availability of new extremely effective antiretrovirals, however, recycling of NRTIs might not be necessary. This was shown in a recent work by Imaz *et al.* who reported very similar proportions of virologic suppression in patients who received a new regimen with two to three fully active drugs regardless of whether it included partially active NRTIs or not (Imaz *et al.*, 2011).

Other antiretroviral classes have been investigated in terms of their potential recycling role. Our group studied the reintroduction of enfuvirtide with an OBT in patients with a previous failure to this compound, and clinical outcomes in multidrug-resistant patients receiving fewer than three active drugs who reintroduced ENF compared to those who did not. We observed a higher survival probability associated with the recycling of enfuvirtide. We also showed a significant virologic and immunologic benefit during each cycle, even in the absence of complete virologic suppression (Cossarini *et al.*, 2009).

Very recently, Zhao and colleagues reported on the outcome of reintroducing raltegravir despite previous failure of this drug. The proportion of patients achieving HIV-RNA < 50 copies/ml at week 48 after raltegravir reintroduction was 23% in patients harboring the H155N mutation only, similar to what observed in patients showing no raltegravir signature mutations (22%), and much higher than patients harboring the Q148H/K/R only: 4%. The authors concluded suggesting the possibility for certain subsets of patients to reintroduce raltegravir in a new optimized regimen after a previous failure (Zhao *et al.* 2010). This option might be considered a last resort in the light of the availability of a second generation integrase inhibitor (dolutegravir) whose efficacy can be hampered by accumulation mutations due to raltegravir failure.

Our group and others have explored the possibility of a partial treatment interruption with the goal of resuming wild-type virus preventing CD4+ cells drop. Taking advantage of the reduced replication capacity of HIV viral strains carrying the M184V mutation (Wei *et al.* 2002; Eron *et al.* 2004), we compared the clinical and immunological outcome of lamivudine monotherapy and treatment interruption in patients experiencing

virologic failure (Castagna *et al.*, 2006). Our pilot study showed a longer time to clinical or immunological failure for the lamivudine monotherapy group compared to the treatment interruption group. In addition, over 48 weeks of study, patients receiving lamivudine monotherapy had a lower reduction in the percentage of CD4+ cells and lower HIV-RNA levels. Further, patients in the lamivudine monotherapy group had similar responses to 24 weeks of HAART as patients in the treatment interruption group suggesting that lamivudine monotherapy is safer than complete treatment interruption for patients harboring the M184V.

We also wanted to investigate whether the smaller loss in the percentage of CD4+ cells was due to lamivudine residual activity or to the reduced viral fitness thanks to the maintenance of the M184V. We randomized patients to receive either lamivudine once daily, emtricitabine once daily or emtricitabine once a week. Emtricitabine once daily was chosen to test for any beneficial effect from a cytidine analogue with a longer half life and the once a week arm was designed to test the least amount of drug that would be necessary to maintain the M184V without having any substantial antiviral activity. We showed that patients treated with emtricitabine once weekly had a similar reduction in the percentage of CD4+ cells to patients receiving 3TC (Soria *et al.* 2010).

The role of lamivudine monotherapy was also recently studied in the Swiss HIV Cohort. Results from this study suggested some caution in applying this strategy to all patients harboring the M184V mutation. The authors reported a high rate of immunological failure in 26 patients undergoing lamivudine monotherapy. A greater proportion of patients who had failed a PI-based regimen before monotherapy was introduced experienced immunologic failure compared to patients who had failed an NRTIs-based regimen. The authors therefore recommend such strategy only as a transient possibility for limited periods of time and their conclusions stress how it should not be offered to patients failing a PI-based regimen. (Opravil *et al.* 2009).

Preventing immunological deterioration with a holding regimen was also the purpose of Llibre and colleagues who studied multiexperienced patients failing an antiretroviral regimen and starting a nucleoside-only holding regimen. 24 weeks

after starting an zidovudine/lamivudine/abacavir/tenofovir-containing regimen the median CD4+ T Cell drop was -53 cells/mm³. The authors also observed an overall 0.71 Log₁₀ decrease in HIV-RNA, proving the residual antiviral activity of these compounds. No change in the number of resistance mutations was observed at week 24 compared to baseline, showing the safety of such strategy in preventing the accumulation of additional resistance mutations (Libre *et al.* 2008).

CONCLUSION

Patients with failure of all three original classes of antiretrovirals are a sizable - although small - subset of patients in most clinical settings. There is

currently no consensus on the best treatment strategies for such patients. The past few years represented a unique occasion to achieve sustained viral suppression for multidrug-resistant patients thanks to the concomitant availability of several new antiretroviral agents. Different combinations of such compounds with - or in some cases even without - older drugs represent an effective treatment option for triple class-experienced patients. Results from independent studies are extremely encouraging with proportions of virologic success comparable to those observed in trials on naïve patients. Since the recommended goal of salvage therapy is to achieve viral suppression, this approach should be the first used whenever possible. For patients with no options even considering the new drug classes, no

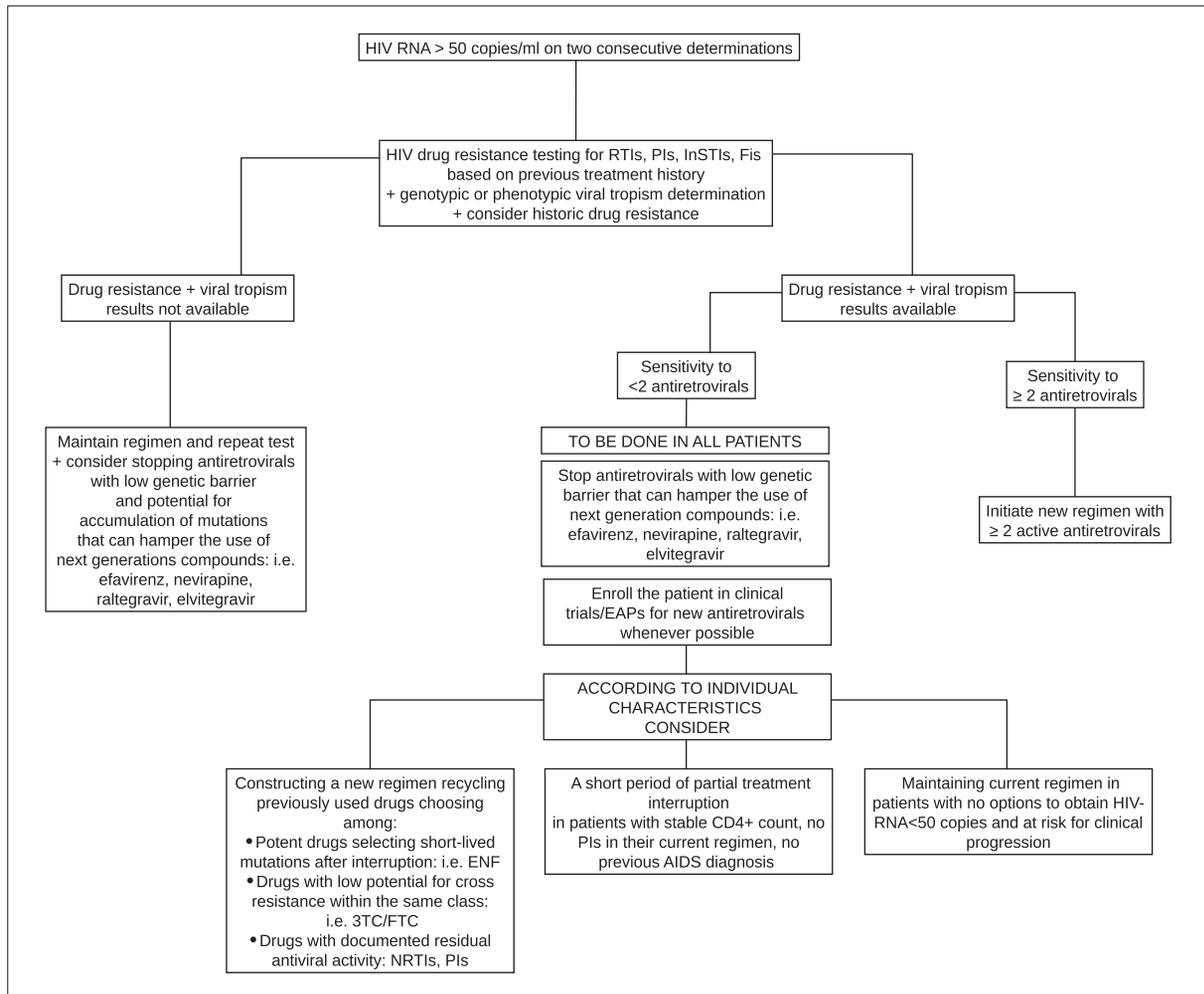


FIGURE 2 - Suggested algorithm for the management of patients presenting with triple class failure.

consensus treatment is available and only few management strategies have been studied. Figure 2 represents a possible algorithm for the management of virological failure in patients with multi-class drug experience.

Stopping current antiretrovirals with low genetic barrier and potential cross-resistance with next generation compounds in the same class seems a mandatory recommendation for all patients. In addition, enrolling patients in investigational trials with new compounds should be considered the first possibility in order to achieve virologic suppression. There are, however, settings in which investigational compounds are not immediately available and few holding regimen strategies have been investigated for such patients waiting for a new effective regimen to be initiated. These holding regimens include recycling previously used drugs, a short course of partial treatment interruption and maintaining the patients on their current treatment. Clinicians should consider each patient's history and clinical characteristics and the antiretroviral toxicity profile and interactions to tailor the most appropriate strategy on an individual basis. Results from studies evaluating these strategies should reassure clinicians in terms of their feasibility for a limited time frame without risk for patient safety, but they should always be considered a temporary bridge between virologically successful regimes. Given the complexity of the management of such patients and the chronic nature of HIV disease, there is a great need for new strategies aiming at finding a better outcome for multidrug-experienced patients.

REFERENCES

- ANDERSON M.S., KAKUDA T.N., HANLEY W., J.T. MILLER KOST, STOLT R., WENNING L.A., ET AL. (2008). Minimal pharmacokinetic interaction between the human immunodeficiency virus nonnucleoside reverse transcriptase inhibitor etravirine and the integrase inhibitor raltegravir in healthy subjects. *Antimicrobial Agents and Chemotherapy*. **52** (12), 4228-4232.
- ANDREWS E., GLUE P., FANG J., CROWNOVER P., TRESSLER R., DAMLE B. (2010). Assessment of the pharmacokinetics of co-administered maraviroc and raltegravir. *British Journal of Clinical Pharmacology*. **69** (1), 51-57.
- BARRAIL-TRAN A., YAZDANPANA H., GOLDWIRT L., CHENE G., COLIN C., PIKETTY C., BOLLENS D., ET AL. (2010). Pharmacokinetics of etravirine, raltegravir and darunavir/ritonavir in treatment experienced patients. *AIDS (London, England)*. **24** (16), 2581-2583.
- BAXTER J.D., BHATTI L., COAKLEY E., BARTCZAK J., McDONOUGH M., VINISKO R., PILIERO P.J. (2010). Susceptibility of HIV-1 to tipranavir and other antiretroviral agents in treatment-experienced patients: The UTILIZE study. *Current HIV Research*. **8** (4), 347-354.
- BUCHACZ K., BAKER R., WARD D.J., PALELLA F.J., CHMIEL J.S., YOUNG B., YANGCO B.G., NOVAK R.M., BROOKS J.T. (2012). Trends in decline of antiretroviral resistance among ARV-experienced patients in the HIV outpatient study: 1999-2008. *AIDS Research and Treatment*. **2012**, 230290.
- CALCAGNO A., NOZZA S., BONORA S., CASTAGNA A., GONZALEZ DE REQUENA D., D'AVOLIO A., LAZZARIN A., DI PERRI G. (2011). Pharmacokinetics of the raltegravir/maraviroc/etravirine combination. *The Journal of Antimicrobial Chemotherapy*. **66** (8), 1932-1934.
- CAMPBELL T.B., SHULMAN N.S.V, JOHNSON S.C., ZOLOPA A.R., YOUNG R.K., BUSHMAN L., FLETCHER C.V., LANIER E.R., MERIGAN T.C., KURITZKES D.R. (2005). Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*. **41** (2), 236-242.
- CANDUCCI F., CERESOLA E.R., BOERI E., SPAGNUOLO V., COSSARINI F., A. CASTAGNA, LAZZARIN A., CLEMENTI M. (2011). Cross-resistance profile of the novel integrase inhibitor dolutegravir (S/GSK1349572) using clonal viral variants selected in patients failing raltegravir. *The Journal of Infectious Diseases*. **204** (11), 1811-1815.
- CANDUCCI F., SAMPALO M., MARINOZZI M.C., BOERI E., SPAGNUOLO V., GALLI A., CASTAGNA A., LAZZARIN A., CLEMENTI M., GIANOTTI N. (2009). Dynamic patterns of human immunodeficiency virus type 1 integrase gene evolution in patients failing raltegravir-based salvage therapies. *AIDS (London, England)*. **23** (4), 455-460.
- CASTAGNA A., DANISE A., MENZO S., GALLI L., GIANOTTI N., CARINI E., BOERI E., ET AL. (2006). Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: A randomized pilot study (E-184V study). *AIDS (London, England)*. **20** (6), 795-803.
- CLOTET B., BELLOS N., MOLINA J.M., COOPER D., GOFFARD J.C., LAZZARIN A., WOHRMANN A., ET AL. (2007). Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: A pooled subgroup analysis of data from two randomised trials. *Lancet*. **369** (9568), 1169-1178.
- COOPER D.A., STEIGBIGEL R.T., JGATELL M., ROCKSTROH

- J.K., KATLAMA C., YENI P., LAZZARIN A., ET AL. (2008). Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *The New England Journal of Medicine*. **359** (4), 355-365.
- COSSARINI F., GALLI L., SAGNELLI C., GIANOTTI N., HASSON H., CLEMENTI M., SORIA A., SALPIETRO S., LAZZARIN A., CASTAGNA A. (2009). Survival of HIV-1 infected multidrug-resistant patients recycling enfuvirtide after a previous failure. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **51** (2), 179-184.
- COZZI-LEPRI A., PHILLIPS A.N., CLOTET B., MOCROFT A., RUIZ L., KIRK O., LAZZARIN A., ET AL. (2008). Detection of HIV drug resistance during antiretroviral treatment and clinical progression in a large European cohort study. *AIDS (London, England)*. **22** (16), 2187-2198.
- DE MEYER S., VANGENEUGDEN T., VAN BAELEN B., DE PAEPE E., VAN MARCK H., PICCHIO G., LEFEBVRE E., DE BETHUNE M.P. (2008). Resistance profile of darunavir: Combined 24-week results from the POWER trials. *AIDS Research and Human Retroviruses*. **24** (3), 379-388.
- DELAUGERRE C., GALLIEN S., FLANDRE P., MATHEZ D., AMARSY R., FERRET S., TIMSIT J., MOLINA J.M., AND DE TRUCHIS P. (2012). Impact of low-level-viremia on HIV-1 drug-resistance evolution among antiretroviral treated-patients. *PLoS One*. **7** (5), e36673.
- DI GIAMBENEDETTO S., COLAFIGLI M., PINNETTI C., BACARELLI A., CINGOLANI A., TAMBURRINI E., CAUDA R., DE LUCA A. (2008). Genotypic resistance profile and clinical progression of treatment-experienced HIV type 1-infected patients with virological failure. *AIDS Research and Human Retroviruses*. **24** (2), 149-154.
- EGGER M., MAY M., CHENE G., PHILLIPS A.N., LEDERGERBER B., DABIS F., COSTAGLIOLA D., ET AL. (2002). Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: A collaborative analysis of prospective studies. *Lancet*. **360** (9327), 119-129.
- ERON J.J., JR, BARTLETT J.A., SANTANA J.L., BELLOS N.C., JOHNSON J., KELLER A., KURITZKES D.R., ST CLAIR M.H., JOHNSON V.A. (2004). Persistent antiretroviral activity of nucleoside analogues after prolonged zidovudine and lamivudine therapy as demonstrated by rapid loss of activity after discontinuation. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **37** (5), 1581-1583.
- ERON J., COOPER D., STEIGBIGEL R.T., CLOTET B., WAN H., ZHAO, LY T., HEPLER D., SKLAR P., ET AL. (2012). Final five-year results of the BENCHMRK studies: sustained antiretroviral effect of raltegravir and exploratory analysis of the late outcomes based on early virologic response. 19th International AIDS Conference, July 22-27, 2012, Washington, DC, USA.
- FABBIANI M., DI GIAMBENEDETTO S., RAGAZZONI E., D'ETTORRE G., PARRUTI G., PROSPERI M., BRACCIALE L., CAUDA R., NAVARRA P., DE LUCA A. (2011). Darunavir/ritonavir and raltegravir coadministered in routine clinical practice: Potential role for an unexpected drug interaction. *Pharmacological Research: The Official Journal of the Italian Pharmacological Society*. **63** (3), 249-253.
- FAGARD C., COLIN C., CHARPENTIER C., RAMI A., JACOMET C., YENI P., VITTECOQ D., ET AL. (2012). Long-term efficacy and safety of raltegravir, etravirine, and darunavir/ritonavir in treatment-experienced patients: Week 96 results from the ANRS 139 TRIO trial. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **59** (5), 489-493.
- FLORENCE E., DE WIT S., CASTAGNA A., RIBERA E., HILL A., VANAKEN H., VAN DELFT Y., MARKS S. (2010). HIV RNA suppression rates after 24 weeks of treatment with etravirine, darunavir/ritonavir and raltegravir in the etravirine early access programme. *International Journal of STD & AIDS*. **21** (3), 224-225.
- GENEBAT M., RUIZ-MATEOS E., PULIDO I., GONZALEZ-SERNA A., GARCIA-PERGANEDA A., MENDEZ G., ROMERO-SANCHEZ M.C., FERRANDO-MARTINEZ S., LEAL M. (2010). Long-term immunovirological effect and tolerability of a maraviroc-containing regimen in routine clinical practice. *Current HIV Research*. **8** (6), 482-486.
- GOETZ M.B., FERGUSON M.R., HAN X., McMILLAN G., ST CLAIR M., PAPPAS K.A., MCCLENNON D.R., O'BRIEN W.A. (2006). Evolution of HIV resistance mutations in patients maintained on a stable treatment regimen after virologic failure. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **43** (5), 541-549.
- GOLDWIRT L., BRAUN J., DE CASTRO N., CHARREAU I., BARRAIL-TRAN A., DELAUGERRE C., RAFFI F., ET AL. (2011). Switch from enfuvirtide to raltegravir lowers plasma concentrations of darunavir and tipranavir: A pharmacokinetic substudy of the EASIER-ANRS 138 trial. *Antimicrobial Agents and Chemotherapy*. **55** (7), 3613-3615.
- GROVER D., COPAS A., GREEN H., EDWARDS S.G., DUNN D.T., SABIN C., PHILLIPS A., ALLEN E., PILLAY D., AND UK COLLABORATIVE GROUP ON HIV DRUG RESISTANCE AND UK COLLABORATIVE HIV COHORT STUDY (UK CHIC). (2008). What is the risk of mortality following diagnosis of multidrug-resistant HIV-1? *The Journal of Antimicrobial Chemotherapy*. **61** (3), 705-713.
- HAMMER S.M., VAIDA F., BENNETT K.K., HOLOHAN M.K., SHEINER L., ERON J.J., WHEAT L.J., ET AL. (2002). Dual vs single protease inhibitor therapy following antiretroviral treatment failure: A randomized trial. *JAMA : The Journal of the American Medical Association*. **288** (2), 2: 169-180.
- HATANO H., LAMPIRIS H., FRANSEN S., GUPTA S., HUANG W., HOH R., MARTIN J.N., ET AL. (2010). Evolution

- of integrase resistance during failure of integrase inhibitor-based antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **54** (4), 389-393.
- HICKS C.B., CAHN P., COOPER D.A., WALMSLEY S.L., KATLAMA C., CLOTET B., LAZZARIN A., ET AL. (2006). Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the randomized evaluation of strategic intervention in multi-drug resistant patients with tipranavir (RESIST) studies: An analysis of combined data from two randomised open-label trials. *Lancet*. **368** (9534), 466-475.
- IMAZ A., DEL SAZ S.V., RIBAS M.A., CURRAN A., CABALLERO E., FALCO V., CRESPO M., ET AL. (2009). Raltegravir, etravirine, and ritonavir-boosted darunavir: a safe and successful rescue regimen for multidrug-resistant HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **52** (3), 382-386.
- IMAZ A., LLIBRE J.M., MORA M., MATEO G., CAMACHO A., BLANCO J.R., CURRAN A., ET AL. (2011). Efficacy and safety of nucleoside reverse transcriptase inhibitor-sparing salvage therapy for multidrug-resistant HIV-1 infection based on new-class and new-generation antiretrovirals. *The Journal of Antimicrobial Chemotherapy*. **66** (2), 358-362.
- JONES R., NELSON M., BOWER M., POWLES T., MANDALIA S., GAZZARD B., STEBBING J. (2008). Triple-class antiretroviral agent resistance in a large cohort: Prevalence and clinical outcomes. *Archives of Internal Medicine*. **168** (17), 1926-1927.
- KANTOR R., SHAFER R.W., FOLLANSBEE S., TAYLOR J., SHILANE D., HURLEY L., NGUYEN D.P., KATZENSTEIN D., FESSEL W.J. (2004). Evolution of resistance to drugs in HIV-1-infected patients failing antiretroviral therapy. *AIDS (London, England)*. **18** (11), 1503-1511.
- KATLAMA C., DOMINGUEZ S., GOURLAIN K., DUVIVIER C., DELAUGERRE C., LEGRAND M., TUBIANA R., ET AL. (2004). Benefit of treatment interruption in HIV-infected patients with multiple therapeutic failures: A randomized controlled trial (ANRS 097). *AIDS (London, England)*. **18** (2), 217-226.
- KOBAYASHI M., YOSHINAGA T., SEKI T., WAKASA-MORIMOTO C., BROWN K.W., FERRIS R., FOSTER S.A., ET AL. (2011). In vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrobial Agents and Chemotherapy*. **55** (2), 813-821.
- LALEZARI J., MCCALLISTER S., GIGLIOTTI M., ET AL. (2008). A phase 2 safety and efficacy study on bevirimat (BVM) in heavily treatment experienced HIV+ patients identifies the target phase 3 study profile. 48th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). October 25-28, 2008, Washington, D.C., USA.
- LAZZARIN A., CAMPBELL T., CLOTET B., JOHNSON M., KATLAMA C., MOLL A., TOWNER W., ET AL. (2007). Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in Duet-2: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet*. **370** (9581), 39-48.
- LEDERGERBER B., LUNDGREN J.D., WALKER A.S., SABIN C., JUSTICE A., REISS P., MUSSINI C., ET AL. (2004). Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. *Lancet*. **364** (9428), 51-62.
- LIMA V.D., HARRIGAN P.R., SENEAL M., YIP B., DRUYTS E., HOGG R.S., MONTANER J.S. (2010). Epidemiology of antiretroviral multiclass resistance. *American Journal of Epidemiology*. **172** (4), 460-468.
- LLIBRE J.M., BONJOCH A., IRIBARREN J., GALINDO M.J., NEGREDO E., DOMINGO P., PEREZ-ALVAREZ N., ET AL. (2008). Targeting only reverse transcriptase with zidovudine/lamivudine/abacavir plus tenofovir in HIV-1-infected patients with multidrug-resistant virus: a multicentre pilot study. *HIV Medicine*. **9** (7), 508-513.
- LOHSE N., JORGENSEN L.B., KRONBORG G., MOLLER A., KVINESDAL B., SORENSEN H.T., OBEL N., GERSTOFT J., AND DANISH HIV COHORT STUDY. (2007). Genotypic drug resistance and long-term mortality in patients with triple-class antiretroviral drug failure. *Antiviral Therapy*. **12** (6), 909-917.
- LOHSE N., OBEL N., KRONBORG G., LAURSEN A., PEDERSEN C., LARSEN C.S., KVINESDAL B., SORENSEN H.T., GERSTOFT J. (2005). Declining risk of triple-class antiretroviral drug failure in danish HIV-infected individuals. *AIDS (London, England)*. **19** (8), 815-822.
- LUCAS G.M., GALLANT J.E., MOORE R.D. (2004). Relationship between drug resistance and HIV-1 disease progression or death in patients undergoing resistance testing. *AIDS (London, England)*. **18** (11), 1539-1548.
- MCCALLISTER S., LALEZARI J., RICHMOND G., ET AL. (2008). HIV-1 gag polymorphisms determine treatment response to bevirimat (PA-457).
- MCKINNELL J.A., LIN H.Y., NEVIN C.N., WILLIG J.H., MCFARLAND G., GENZ M., RAPER J.L., ET AL. (2009). Early virologic suppression with three-class experienced patients: 24-week effectiveness in the darunavir outcomes study. *AIDS (London, England)*. **23** (12), 1539-1546.
- MELLORS J.W., MUNOZ A., GIORGI, J.V. MARGOLICK J.B., TASSONI C.J., GUPTA P., KINGSLEY L.A., ET AL. (1997). Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Annals of Internal Medicine*. **126** (12), 946-954.
- MILLER V., COZZI-LEPRI A., HERTOOGS K., GUTE P., LARDER B., BLOOR S., KLAUKE S., RABENAU H., PHILLIPS A., STASZEWSKI S. (2000). HIV drug susceptibility and treatment response to mega-HAART regimen in pa-

- tients from the frankfurt HIV cohort. *Antiviral Therapy*. **5** (1), 49-55.
- MILLER V., STARK T., LOELIGER A.E., LANGE J.M. (2002). The impact of the M184V substitution in HIV-1 reverse transcriptase on treatment response. *HIV Medicine*. **3** (2), 135-145.
- MOCROFT A., LEDERGERBER B., VIARD J.P., STASZEWSKI S., MURPHY M., CHIESI A., HORBAN A., ET AL. (2004). Time to virological failure of 3 classes of antiretrovirals after initiation of highly active antiretroviral therapy: Results from the EuroSIDA study group. *The Journal of Infectious Diseases*. **190** (11), 1947-1956.
- MOCROFT A., VELLA S., BENFIELD T.L., CHIESI A., MILLER V., GARGALIANOS P., D'ARMINIO MONFORTE A., ET AL. (1998). Changing patterns of mortality across europe in patients infected with HIV-1. EuroSIDA study group. *Lancet*. **352** (9142), 1725-1730.
- MONTANER J.S., HARRIGAN P.R., JAHNKE N., RABOUD J., CASTILLO E., HOGG R.S., YIP B., HARRIS M., MONTESSORI V., O'SHAUGHNESSY M.V. (2001). Multiple drug rescue therapy for HIV-infected individuals with prior virologic failure to multiple regimens. *AIDS (London, England)*. **15** (1), 61-69.
- NAPRAVNIK S., KEYS J.R., QUINLIVAN E.B., WOHL D.A., MIKEAL O.V., ERON J.J. JR. (2007). Triple-class antiretroviral drug resistance: risk and predictors among HIV-1-infected patients. *AIDS (London, England)* **21** (7), 825-834.
- NICHOLS G., MILLS A., GROSSBERG R., LAZZARIN A., MAGGIOLO F., MOLINA J.-M., PIALOUX G., ET AL. (2012). Antiviral activity of dolutegravir in subjects with failure on an integrase inhibitor-based regimen: week 24 phase 3 results from VIKING-3. Glasgow, UK.
- NOZZA S., GALLI L., BIGOLONI A., NICOLA G., POGLIAGHI M., COSSARINI F., SALPIETRO S., ET AL. (2011). Durability and safety of a novel salvage therapy in R5-tropic HIV-infected patients: Maraviroc, raltegravir, etravirine. *Journal of Acquired Immune Deficiency Syndromes* (1999). **56** (4), e113-5.
- NOZZA S., GALLI L., VISCO F., SORIA A., CANDUCCI F., SALPIETRO S., GIANOTTI N., ET AL. (2010). Raltegravir, maraviroc, etravirine: an effective protease inhibitor and nucleoside reverse transcriptase inhibitor-sparing regimen for salvage therapy in HIV-infected patients with triple-class experience. *AIDS (London, England)*. **24** (6), 924-928.
- OPRAVIL M., KLIMKAIT T., LOUVEL S., WOLF E., BATTEGAY M., FUX C.A., BERNASCONI E., ET AL. (2009). Prior therapy influences the efficacy of lamivudine monotherapy in patients with lamivudine-resistant HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes* (1999) (Oct 15).
- PALELLA F.J. JR, DELANEY K.M., MOORMAN A.C., LOVELESS M.O., FUHRER J., SATTEN G.A., ASCHMAN D.J., HOLMBERG S.D. (1998). Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *The New England Journal of Medicine*. **338** (13), 853-860.
- PALELLA F.J. JR, BAKER R.K., MOORMAN A.C., CHMIEL J.S., WOOD K.C., BROOKS J.T., HOLMBERG S.D., AND HIV OUTPATIENT STUDY INVESTIGATORS. (2006). Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *Journal of Acquired Immune Deficiency Syndromes*. **43** (1) (Sep): 27-34.
- Panel on Antiretroviral Guidelines for Adults and Adolescents. Department of Health and Human Services. *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents*. 2012.
- PHILLIPS A.N., GAZZARD B., GILSON R., EASTERBROOK P., JOHNSON M., WALSH J., LEEN C., ET AL. (2007). Rate of AIDS diseases or death in HIV-infected antiretroviral therapy-naive individuals with high CD4 cell count. *AIDS (London, England)*. **21** (13), 1717-1721.
- PHILLIPS A.N., LEEN C., WILSON A., ANDERSON J., DUNN D., SCHWENK A., ORKIN C., ET AL. (2007). Risk of extensive virological failure to the three original antiretroviral drug classes over long-term follow-up from the start of therapy in patients with HIV infection: an observational cohort study. *Lancet*. **370** (9603), 1923-1928.
- PICHENOT M., DEUFFIC-BURBAN S., CUZIN L., YAZDANPANAH Y. (2012). Efficacy of new antiretroviral drugs in treatment-experienced HIV-infected patients: a systematic review and meta-analysis of recent randomized controlled trials. *HIV Medicine*. **13** (3), 148-155.
- POZNIAK A.L. (2009). Investigational agents for salvage. *Current Opinion in HIV and AIDS*. **4** (6), 524-530.
- PURSING LATER TREATMENT OPTION II (PLATO II) PROJECT TEAM, OBSERVATIONAL HIV EPIDEMIOLOGICAL RESEARCH EUROPE (COHERE) GROUP, COSTAGLIOLA D., LODWICK R., LEDERGERBER B., TORTI C., VAN SIGHEM A., ET AL. (2012). Trends in virological and clinical outcomes in individuals with HIV-1 infection and virological failure of drugs from three antiretroviral drug classes: A cohort study. *The Lancet Infectious Diseases*. **12** (2), 119-127.
- PURSING LATER TREATMENT OPTIONS II (PLATO II) PROJECT TEAM FOR THE COLLABORATION OF OBSERVATIONAL HIV EPIDEMIOLOGICAL RESEARCH EUROPE (COHERE), LODWICK R., COSTAGLIOLA D., REISS P., TORTI C., TEIRA R., DORRUCCI M., ET AL. (2010). Triple-class virologic failure in HIV-infected patients undergoing antiretroviral therapy for up to 10 years. *Archives of Internal Medicine*. **170** (5), 410-419.
- PURSING LATER TREATMENT OPTIONS II (PLATO II) PROJECT TEAM, COLLABORATION OF OBSERVATIONAL HIV EPIDEMIOLOGICAL RESEARCH EUROPE (COHERE) GROUP, NAKAGAWA F., LODWICK R., COSTAGLIOLA D.,

- VAN SIGHEM A., TORTI C., ET AL. (2012). Calendar time trends in the incidence and prevalence of triple-class virologic failure in antiretroviral drug-experienced people with HIV in Europe. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **59** (3), 294-299.
- RAFFANTI S.P., FUSCO J.S., SHERRILL B.H., HANSEN N.I., JUSTICE A.C., D'AQUILA R., MANGIARDI W.J., FUSCO G.P., AND COLLABORATIONS IN HIV OUTCOMES RESEARCH/UNITED STATES PROJECT. (2004). Effect of persistent moderate viremia on disease progression during HIV therapy. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **37** (1), 1147-1154.
- RECKY M.A., BRUMME Z.L., CHAN K.J., WYNHOVEN B., YIP B., DONG W.W., HEATH K.V., ET AL. (2004). Antiretroviral resistance among HIV-infected persons who have died in British Columbia, in the era of modern antiretroviral therapy. *The Journal of Infectious Diseases*. **190** (2), 285-292.
- REUTER S., BRAKEN P., JENSEN B., SIERRA-ARAGON S., OETTE M., BALDUIN M., KAISER R., HAUSSINGER D. (2010). Maraviroc in treatment-experienced patients with HIV-1 infection - experience from routine clinical practice. *European Journal of Medical Research*. **15** (6), 231-237.
- RICHMAN D.D., MORTON S.C., WRIN T., HELLMANN N., BERRY S., SHAPIRO M.F., BOZZETTE S.A. (2004). The prevalence of antiretroviral drug resistance in the United States. *AIDS (London, England)*. **18** (10), 1393-1401.
- RUIZ L., RIBERA E., BONJOCH A., ROMEU J., MARTINEZ-PICADO J., PAREDES R., DIAZ M., ET AL. (2003). Role of structured treatment interruption before a 5-drug salvage antiretroviral regimen: The retrogene study. *The Journal of Infectious Diseases*. **188** (7), 977-985.
- SANTOS J.R., LLIBRE J.M., DOMINGO P., IMAZ A., FERRER E., PODZAMCZER D., BRAVO I., RIBERA E., VIDELA S., CLOTET B. (2011). Short communication: High effectiveness of etravirine in routine clinical practice in treatment-experienced HIV type 1-infected patients. *AIDS Research and Human Retroviruses*. **27** (7), 713-717.
- SCHERRER A.U., VON WYL V., FUX C.A., OPRAVIL M., BUCHER H.C., FAYET A., DECOSTERD L.A., ET AL. (2009). Implementation of raltegravir in routine clinical practice: Selection criteria for choosing this drug, virologic response rates, and characteristics of failures. *Journal of Acquired Immune Deficiency Syndromes (1999)*. (Oct 16).
- SHIMURA K., KODAMA E., SAKAGAMI Y., MATSUZAKI Y., WATANABE W., YAMATAKA K., WATANABE Y., ET AL. (2008). Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). *Journal of Virology*. **82** (2), 764-774.
- SORIA A., DANISE A., GALLI L., TIBERI S., SEMINARI E., COSSARINI F., BIGOLONI A., MARCOTULLIO S., LAZZARIN A., CASTAGNA A. (2010). Viro-immunological dynamics in HIV-1-infected subjects receiving once-a-week emtricitabine to delay treatment change after failure: a pilot randomised trial. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology* (Jan 5).
- SPAGNUOLO V., GIANOTTI N., SEMINARI E., GALLI L., FUSETTI G., SALPIETRO S., LAZZARIN A., CASTAGNA A. (2009). Changes in darunavir/r resistance score after previous failure to tipranavir/r in HIV-1-infected multidrug-resistant patients. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **50** (2) 192-195.
- STASZEWSKI S., BABACAN E., STEPHAN C., HABERL A., CARLEBACH A., GUTE P., KLAUKE S., ET AL. (2006). The LOPSAQ study: 48 week analysis of a boosted double protease inhibitor regimen containing lopinavir/ritonavir plus saquinavir without additional antiretroviral therapy. *The Journal of Antimicrobial Chemotherapy*. **58** (5), 1024-1030.
- STEBBING J., NELSON M., ORKIN C., MANDALIA S., BOWER M., POZNIAK A., GAZZARD B. (2004). A randomized trial to investigate the recycling of stavudine and didanosine with and without hydroxyurea in salvage therapy (RESTART). *The Journal of Antimicrobial Chemotherapy*. **53** (3), 501-505.
- STEIGBIGEL R.T., COOPER D.A., KUMAR P.N., ERON J.E., SCHECHTER M., MARKOWITZ M., LOUTFY M.R., ET AL. (2008). Raltegravir with optimized background therapy for resistant HIV-1 infection. *The New England Journal of Medicine*. **359** (4), 339-354.
- THURET I., CHAIX M.L., TAMALET C., RELIQUET V., FIRTION G., TRICOIRE J., RABAUD C., FRANGE P., AUMAITRE H., BLANCHE S. (2009). Raltegravir, etravirine and r-darunavir combination in adolescents with multidrug-resistant virus. *AIDS (London, England)*. **23** (17), 2364-2366.
- TOWNER W., LALEZARI J., SENSION M.G., WOHLFEILER M., GATHE J., APPELBAUM J.S., BELLMAN P., ET AL. (2010). Efficacy, safety, and tolerability of etravirine with and without darunavir/ritonavir or raltegravir in treatment-experienced patients: Analysis of the etravirine early access program in the United States. *Journal of Acquired Immune Deficiency Syndromes (1999)*. **53** (5), 614-618.
- VAN LUNZEN J., MAGGIOLO F., ARRIBAS J.R., RAKHMANOVA A., YENI P., YOUNG B., ROCKSTROH J.K., ET AL. (2012). Once daily dolutegravir (S/GSK1349572) in combination therapy in antiretroviral-naïve adults with HIV: Planned interim 48 week results from SPRING-1, a dose-ranging, randomised, phase 2b trial. *The Lancet Infectious Diseases*. **12** (2), 111-118.
- WEI X., LIANG C., GOTTE M., WAINBERG M.A. (2002). The M184V mutation in HIV-1 reverse transcriptase reduces the restoration of wild-type replication by attenuated viruses. *AIDS (London, England)*. **16** (18), 2391-2398.
- WITTKOP L., BREILH D., DA SILVA D., DUFFAU P., MERCIÉ

- P., RAYMOND I., ANIES G., ET AL. (2009). Virological and immunological response in HIV-1-infected patients with multiple treatment failures receiving raltegravir and optimized background therapy, ANRS CO3 aquitaine cohort. *The Journal of Antimicrobial Chemotherapy*. **63** (6), 1251-1255.
- YAMASHITA T.E., PHAIR J.P., MUNOZ A., MARGOLICK J.B., DETELS R., O'BRIEN S.J., MELLORS J.W., WOLINSKY S.M., JACOBSON L.P. (2001). Immunologic and virologic response to highly active antiretroviral therapy in the multicenter AIDS cohort study. *AIDS (London, England)*. **15** (6), 735-746.
- YAZDANPANA Y., FAGARD C., DESCAMPS D., TABURET A.M., COLIN C., ROQUEBERT B., KATLAMA C., ET AL. (2009). High rate of virologic suppression with raltegravir plus etravirine and darunavir/ritonavir among treatment-experienced patients infected with multidrug-resistant HIV: Results of the ANRS 139 TRIO trial. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*. **49** (9), 1441-1449.
- ZACCARELLI M., TOZZI V., LORENZINI P., TROTTA M.P., FORBICI F., VISCO-COMANDINI U., GORI C., ET AL. (2005). Multiple drug class-wide resistance associated with poorer survival after treatment failure in a cohort of HIV-infected patients. *AIDS (London, England)*. **19** (10), 1081-1089.
- ZHAO J., TEPPLER H., NGUYEN B., HAZUDA D., MILLER M. (2010). Raltegravir (RAL) resistance mutations observed after virologic failure differentially affect subsequent response to a RAL-containing regimen. 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), September 12-15, 2010, Boston, MA, USA.

