REVIEW ARTICLE

Drugs 2008; 68 (1): 43-72
0012-6667/08/0001-0043/$53.45/0

Antiretroviral Therapy
Optimal Sequencing of Therapy to Avoid Resistance

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Abstract

In the second decade of highly active antiretroviral therapy, drug regimens offer more potent, less toxic and more durable choices. However, strategies addressing convenient sequential use of active antiretroviral combinations are rarely presented in the literature. Studies have seldom directly addressed this issue, despite it being a matter of daily use in clinical practice. This is, in part, because of the complexity of HIV-1 resistance information as well as the complexity of designing these types of studies. Nevertheless, several principles can effectively assist the planning of antiretroviral drug sequencing. The introduction of tenofovir disoproxil fumarate, abacavir and emtricitabine into current nucleoside backbone options, with each of them selecting for an individual pattern of resistance mutations, now permits sequencing in the context of previously popular thymidine analogues (zidovudine and stavudine). Similarly, newer ritonavir-boosted protease inhibitors could potentially be sequenced in a manner that uses the least cross-resistance prone protease inhibitor at the start of therapy, while leaving the most cross-resistance prone drugs for later, as long as there is rationale to employ such a compound because of its utility against commonly observed drug-resistant forms of HIV-1.

Combination antiretroviral therapy (ART) prevents immune deterioration, reduces morbidity and mortality,[1-5] and prolongs the life expectancy of people infected with HIV.[6] However, current therapies are only capable of partially and temporarily halting the replication of HIV. Multiple studies suggest that residual low level replication occurs in patients who otherwise show good response to ART.[7-11] It is also clear that 'archived' proviruses can persist for many years in reservoirs of latently infected CD4+ cells and in the cells of tissue compartments into which drug penetration is limited. Consequently, viral replication can resume quickly upon withdrawal of ART[11-14] and the opportunity for development of HIV drug resistance always exists. Resistance mutations have been described for all antiretroviral drugs currently in use. This has led to the conclusion that current therapeutic options will be required lifelong in order to prevent HIV disease progression.

Under real-life circumstances, factors that are responsible for drug resistance cannot be completely eliminated. In fact, only about one-half of patients who commence treatment with combination ART in a non-clinical trial setting achieve the goal of viral suppression[15,16] compared with the 70–90% success rates commonly observed in clinical trials.[17-19] This substantial difference is likely to be due to suboptimal adherence in the non-trial setting, among other factors, and because participants in clinical trials are usually highly motivated. Similarly, nonadherence to therapy is perhaps the best recognized risk factor for development of virological failure and drug resistance.[20] A minimal adherence level of 95% has been traditionally recommended in order to minimize emergence of drug resistance. However, in the long-term, this goal may be difficult, if not impossible, to maintain.

Antiretroviral drug sequencing refers to the preferred use of a particular antiretroviral drug (or drug class) in initial therapy on the assumption that virological failure and drug resistance might later develop, which could then be overcome by the use of a second drug (of the same or different class). The concept is that sequencing will provide a strategy to deal with virological failure. In this sense, ART sequencing anticipates that therapy will fail in a proportion of patients in the presence of resistance mutations. Clinicians may then switch to use of an active agent with the lowest likelihood of cross-resistance as aided by resistance testing. The primary objectives are the avoidance of accumulation of mutations and selection of multidrug-resistant viruses. In addition, sequencing can be used when mutations responsible for resistance to one drug are hypersusceptibility mutations for other drugs or are associated with hypersusceptibility mutations to such drugs. Alternating antiretroviral drugs has also been used to maintain viral suppression in the absence of treatment failure, although this strategy is not recommended.[21]

A previous review on ART sequencing[22] made the following two assumptions: (i) different mutations are selected after exposure to certain antiretroviral agents within the same class; and (ii) not all mutations confer the same degree of cross-resistance to other agents of the same class. These assumptions have been further confirmed in recent years. As an example, it is clear that protease inhibitor (PI) resistance mutations do not all affect all drugs equally and that known cross-resistance mutations can affect different drugs to different extents.[23] At a molecular level, it has been shown that each PI interacts with the protease enzyme in a unique way and that PI mutations can mediate against such interaction.[24]

Thus, there is valid justification to find optimal long-lasting drug combinations. Although the first objectives of ART are to suppress viral replication and facilitate immune recovery for as long as possible, the attainment of this goal may oblige us to select therapeutic options that might avoid or delay the appearance of drug resistance or to overcome it, when it emerges. Appropriate sequencing should also minimize the likelihood of drug toxicities.

1. The Concept of Virological Failure and its Clinical Relevance

In general, the stronger the suppression of viral replication the less likely it will be that clinically

significant resistance will emerge.[25,26] It is also accepted that immunological restoration may have the best chance of success if virological suppression is maintained.[27] The level of virological suppression, as measured by nucleic acid amplification techniques, correlates with the clinical benefit of ART, durability of suppression,[27] reduced development of drug resistance[28] and lack of disease progression.[29] In this regard, viral RNA undetectability is the current accepted virological goal, which usually means a viral burden of <400 or <50 copies/mL, depending on the detection system used. From a clinical viewpoint, virological suppression translates into absence of virological resistance. This assumption, although somewhat virologically inaccurate, may be practical for clinical decision making. There is evidence that resistance mutations can develop at very low viral replication levels[11,30] (lower than the detectability cut-offs of commercial kits for plasma viral RNA quantification), but the frequency with which these mutations lead to higher viral replication is often clinically insignificant. Nevertheless, a persistent detection (usually twice or more) of viral RNA over the usual cut-off is considered to represent a risk for further viral evolution and a time at which a switch in therapy might be considered.[31,32]

On the other hand, ongoing viral replication in patients receiving therapy does not always lead to continued immunological deterioration.[33] It is now clear that immunological impairment responds to several factors, including the patient's immunological reserve, age and probably the pathogenic potential of infecting HIV strains. It has been reported that older patients have a slower and less pronounced CD4+ cell count recovery at the same levels of virological suppression than younger patients,[32,34-36] although others have not found this association.[37] It is also known that defective HIV-1 strains are the cause of HIV infection among a subset of long-term non-progressive patients.[38-41] In fact, immunological recovery can be seen in patients with partial virological suppression, including patients who initially responded but later experience therapeutic failure with highly resistant HIV.[33] In general, two different scenarios are seen when HIV strains overcome the pressure of drugs with either lower (typically non-nucleoside reverse transcriptase inhibitors [NNRTIs]) or with higher genetic barriers for resistance (typically boosted PI). It is common to see immunological recovery in ART-adherent patients no longer responding to PIs, but this is rare when therapy fails while patients are receiving NNRTIs. This has been attributed to the higher fitness cost that PI resistance and some nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations represent for the virus compared with the NNRTI resistance mutations.[42-46] However, it is also true that immunological recovery in the face of virological failure may not be sustained in the long-term for some patients.[32] This later deterioration may be related to further evolution of resistant virus towards variants with higher fitness and additional mutations that could also affect the activity of other antiretroviral drugs.[47-49] Whether patients can tolerate a low viral replication rate and experience continued immunological reconstitution is an individual phenomenon and is very difficult to predict. Therefore, as long as active therapeutic options are available, complete viral suppression must be the goal of therapy and virological failure must be treated as soon as it is detected.

Leading international agencies have published recommendations for the treatment of chronic HIV infection, e.g. the US International AIDS Society, the US Department of Health and Human Services (US-DHHS), the British HIV Association and others.[50-52] All advocate that the combination of two or more classes of antiretroviral drugs offers the best opportunity to avoid virological failure and resistance. However, it is also true that short- and long-term adverse effects may increase as more drugs are used simultaneously. Therefore, the current norm is to use the fewest number of drugs that achieve the highest level and duration of viral suppression, while trying to avoid significant toxicity. The US-DHHS guidelines document offers a detailed section on the advantages and drawbacks of each antiretroviral drug.[52]

There is general agreement among international panels that newer and less toxic drugs are preferred as initial therapy in most settings. These drugs also select for resistance mutations in HIV-1 that are more or less specific for each of them. In addition, when given as part of a three- or four-drug combination, the resistance mutations that could emerge in HIV-1 tend not to overlap with those selected for by older standard regimens. Hence, there are opportunities for rational ART sequencing.

2. Drug Resistance and its Clinical Relevance

Drug resistance is closely related to virological failure and poorer prognosis in drug-experienced patients.[53-55] Also, several cohort studies of patients taking antiretrovirals who were stratified by CD4+ cell count, have demonstrated that detectable viral load in plasma is associated with a higher risk of opportunistic infection and death.[55] For instance, in a cohort of 623 prospectively followed patients who had experienced virological failure and for whom a first genotypic resistance test was performed, the presence of class-wide resistance was associated with lower survival and a higher frequency of the endpoints relating to death for any cause, AIDS-related death and AIDS-defining event/death.[56] In addition, the more that drug classes were compromised by resistance the higher was the risk of such endpoint events. Interestingly, class-wide resistance for NRTI drugs was associated with a higher risk of death, while the use of a new boosted PI had a protective effect. In a different study, drug resistance to NNRTIs was found to be associated with a higher risk of death in a cohort of drug-naive patients followed for a median of 56.4 months.[53] This study carefully controlled for patient adherence to therapy and was carried out in a setting of free access to HIV care, including ART. Similar observations have been made in another drug-naive cohort in which NNRTI resistance was the strongest predictor of virological failure and disease progression.[54] Consequently, it seems clear that patients infected with drug-resistant viruses have a lower chance of attaining viral suppression or may have viral suppression for a shorter time than those infected with completely susceptible viruses. This also suggests that ongoing virological failure in the presence of resistance to NNRTIs may be more dangerous than virological failure in the presence of resistance to NRTIs or PIs.

As global availability of antiretrovirals increases, the prevalence of drug-resistant HIV-1 strains will also increase. Transmission of drug-resistant HIV has increased in countries in which ART is widely available and threatens the benefits of ART for many patients. This poses a challenge for clinicians whose role it is to maintain optimal drug efficacy for the longest period possible and for health research systems that must maintain availability of active drugs.

3. Important Considerations in Antiretroviral Therapy

There are several important issues concerning antiretroviral therapy.

1. Life-long therapy is expected to be required for HIV-infected people who have indications for ART.
2. In order to achieve long-term efficacy, combinations of antiretroviral drugs must meet the following requirements: (i) be potent (leading to virological suppression in the vast majority of patients); (ii) be forgiving (such that efficacy is minimally affected by suboptimal patient adherence); (iii) be minimally toxic (few and mild adverse effects associated with its use); and (iv) have a high genetic barrier to resistance (i.e. clinically significant resistance to the drug should require an accumulation of at least several mutations).
3. Drug sequencing strategies should be used to maximize the probability of maintaining long-term efficacy of ART. For patients, it also becomes fundamental that a drug combination, in the event of failure, leaves as many other potent therapeutic options as possible available for the future.

There are currently 21 antiretroviral drugs in clinical use for treating HIV infection. International guidelines have assessed the literature and recommended first-choice combinations. The large majority of patients are treated with drugs belonging to the

NRTIs plus a NNRTI or a PI. Fusion, entry and integrase inhibitors are currently restricted to highly drug-experienced patients facing drug exhaustion and who require expanded access to new classes of drugs. Although many ART drug combinations are highly efficacious, not all can be sequenced in a manner that might translate into durable viral suppression. For the purpose of planning a successful sequencing strategy, specific questions need to be answered.

1. What is the likelihood of virological failure over time with the different first- and second-line combinations now available?
2. How frequent are certain resistance mutations when patients fail particular regimens?
3. What mutations are selected by a particular drug or drug regimen and what is the impact of such mutations on cross-resistance?
4. What are the prevalence and patterns of transmitted drug resistance and which drug classes are affected by such mutations?

ART sequencing is principally applicable for therapy of drug-naive individuals, as this is the group that may benefit the most from avoiding or delaying the emergence of drug resistance. Drug-experienced patients are a heterogeneous group who require a more personalized approach, since complex resistance mutations may be present. Many such patients may harbour multidrug-resistant viruses, which were selected by older drug regimens that are now obsolete. The ART sequencing strategies discussed herein may not, unfortunately, be as useful in such patients.

4. What is the Likelihood of Virological Failure Over Time?

Drugs that fail less often are preferable to those that fail more frequently. For practical purposes, a drug sequencing strategy should be uppermost in the mind of the treating physician well before a patient is initiated on ART. First-line drugs should therefore be those with the best chance of attaining sustained virological suppression and, in the event of failure, the least likelihood of broad cross-resistance. Thus, use of drugs and/or regimens with a high potential for high cross-resistance could be delayed. This strategy necessitates addressing other factors that can influence therapeutic outcomes: regimen potency, short- and long-term toxicity, drug interactions with other antiretrovirals and other drugs, and suitability of the regimen for the patient's ability and motivation to be adherent.

It has been emphasized that the accumulation of drug resistance will commonly begin with the failure of the first antiretroviral regimen, and that resistance mutations that emerge at each subsequent step of ART will remain archived and affect the efficacy of subsequent regimens.[57] Therefore, it becomes paramount that a rational sequencing approach must carefully weigh the pros and cons of the first ART combination with respect to the likelihood of causing cross-resistance and to the prospective number of future therapeutic options that will remain active upon drug failure. Some have argued for avoidance of NRTI combinations that might result in broad class cross-resistance, e.g. stavudine plus didanosine, which lead to thymidine-associated mutations (TAMs).[57] Today, newer NRTI drugs represent a switch away from thymidine analogues and offer improved long-term therapeutic options.

A summary of the most recent clinical trials and their virological outcomes is presented in table I, which contains information that may assist the clinician in the selection of regimens and the formulation of an ART sequencing strategy.

4.1 Current First-Line Combinations

Modern ART combinations typically consist of an NRTI backbone (two NRTI drugs together) plus either an NNRTI or a ritonavir-boosted PI, or sometimes a non-boosted PI.

Studies in treatment-naive patients who begin ART, evaluate the efficacy of drug regimens by measuring and comparing morbidity/mortality rates, T-cell count recovery and the proportion of patients attaining viral suppression (alternatively, proportion of virological failure) between treatment arms. NNRTI- and PI-based regimens have been intensively compared in recent years, but an answer to the question of which regimen is best remains obscure.

Table I. Principal antiretroviral therapy (ART) clinical trials: efficacy and resistance patterns

Study	Regimens tested	No. of pts	Follow-up (wk)	<50 copies/mL (% pts)	<400 copies/mL (% pts)	VF (%)	Genotyped /VF (n)	Pts with resistance (n)	Major resistance mutations seen in isolates from pts with therapy failure (type and number when reported)			Antiviral effect comparison
									NRTI	NNRTI	PI	
ACTG 384[58a]	d4T + ddI + EFV	155				32			≈17%	≈24%	≈5%	
	d4T + ddI + NFV	155				37			≈7%	≈5%	≈10%	
	AZT + 3TC + EFV	155				13			≈8%	≈7%	0	SUP
	AZT + 3TC + NFV	155				40			≈22%	≈5%	≈7%	
	d4T + ddI + EFV + NFV	178				19			≈1%	≈10%	≈2%	
	AZT + 3TC + EFV + NFV	182				16			≈4%	≈7%	≈1%	
Gilead 934[59]	TDF + FTC + EFV	244	96	75	67	12	14/14	10	184V = 2; TAM = 0; 65R = 0	10		SUP
	AZT + 3TC + EFV	243	96	62	61	6	27/29	20	184V = 9; TAM = 1; 65R = 0	18		
Gilead 903[19]	TDF + 3TC + EFV	299	48	76		16	47/47	29	184V = 18; other = 1; 65R = 8	26		EQU
	d4T + 3TC + EFV	301	48	80		16	49/49	26	184V = 17; other = 4; 65R = 2	24		EQU
SEAL (ESS30008)[60]	ABC + 3TC (od tab) + EFV or PI	130	48	81	NA	1.5	2/2	} 4	184V = 3; 65R = 2	NR	NR	EQU
	ABC + 3TC (bid tab) + EFV or PI	130	48	82	NA	3	4/4			NR	NR	EQU
ACTG A5095[61]	ABC + AZT + 3TC	382	48	61		21	57/82	39	184V = 37; TAM = 11	103N = 1		EQU
	ABC + AZT + 3TC + EFV or AZT + 3TC + EFV	765	48	83		11	NR	NR	NR	NR		EQU
ACTG A5095[62]	AZT + 3TC + EFV	382	192	85		26	71/99[b]	34	184V = 19; other = 2	29		EQU
	ABC + AZT + 3TC + EFV	383	192	88		25	72/94[b]	37	184V = 15; other = 6	34	Any = 3	EQU
ZODIAC (CNA30021)[63]	ABC + 3TC (od tab) + EFV	384	48	66	NA	7	16[c]	13	184V = 10; 65R = 1; 74V = 5; 115F = 1	103N = 6		EQU
	ABC + 3TC (bid) + EFV	386	48	68	NA	5	15[c]	10	184V = 5; 74V = 3; 115F = 1	103N = 8		EQU
CNA30024[64]	ABC + 3TC + EFV	324	48	70		28.6	10/20	4	184V = 2; 65R = 0	103N = 4		EQU

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Table I. Contd

Study	Regimens tested	No. of pts	Follow-up (wk)	<50 copies/mL (% pts)	<400 copies/mL (% pts)	VF (%)	Genotyped /VF (n)	Pts with resistance (n)	Major resistance mutations seen in isolates from pts with therapy failure (type and number when reported)			Antiviral effect comparison
									NRTI	NNRTI	PI	
	AZT + 3TC + EFV	325	48	69		19	6/13	4	184V = 4; other = 0	103N = 4		EQU
CLASS	ABC + 3TC (bid) + EFV	97	96	72		15	6/15	5	184V = 2; 65R = 0	103N = 3		SUP
(ESS40001)[65]	ABC + 3TC + APV/r (or FPV/r)	96	96	59		25	18/24	6	184V = 4; 115F = 1; 74V = 1; 77L = 1; 118I = 1; 65R = 0		54V	
	ABC + 3TC + d4T	98	96	60		25	17/24	10	184V = 9; 215Y = 2; 65R = 2; 74V = 1; 215Y = 2; 115F = 2; 77L = 1			
GESIDA 3903[66]	ddI + 3TC + EFV	186	24	71	77.8	NA	NA	NA	NA	NA	NA	SUP[d]
	AZT + 3TC + EFV	183	24	65.9	71	NA	NA	NA	NA	NA	NA	
FTC-301[67]	FTC + ddI + EFV	286	60	76		4	13/13	12	184V = 6; 65R = 1	11		SUP
	d4T + ddI + EFV	285	60	54		12	35/37	31	TAM = 7	31		
START I[68]	d4T + 3TC + IDV	101	48	49	53	27	NR	NR	NR	NR	NR	EQU
	AZT + 3TC + IDV	103	48	47	52	31	NR	NR	NR	NR	NR	EQU
Dupont 006[69]	AZT + 3TC + EFV	154		64		NR	NR	NR	NR	NR	NR	SUP
	AZT + 3TC + IDV	148		43		NR	NR	NR	NR	NR	NR	
	EFV + IDV	148		47		NR	NR	NR	NR	NR	NR	
ACTG 5142[70]	LPV/r + 2 NRTIs	253	96	77		37	52/94	10	184V = 7; 65R = 0	Any = 2; 103N = 0		
	EFV + 2 NRTIs	250	96	89		24	33/60	27	184V = 8; 65R = 3	Any = 16; 103N = 9		SUP
	LPV/r + EFV	250	96	83		29	39/73	34	184V = 1; 65R = 0; other = 3	Any = 27; 103N = 21	Any = 2	
M98-863[17,71]	LPV/r + d4T + 3TC	326	48	67	75	23[e]	51/74	0	184V = 19; TAM = 0		None	SUP
	NFV + d4T + 3TC	327	48	52	63	38[e]	96/123	43	184V = 79; TAM = 9		30N = 29 90M = 15	
KLEAN study[72]	FPV/r + (ABC/3TC od tab)	434	48	66	73	4[e]	14/16	4	184V = 3; TAM = 0	Any = 0	Any = 2	EQU
	LPV/r + (ABC/3TC od tab)	444	48	65	71	5[e]	21/24	6	184V = 4; TAM = 1	Any = 2	Any = 2	EQU

Continued next page

Table I. Contd

Study	Regimens tested	No. of pts	Follow-up (wk)	<50 copies/mL (% pts)	<400 copies/mL (% pts)	VF (%)	Genotyped /VF (n)	Pts with resistance (n)	Major resistance mutations seen in isolates from pts with therapy failure (type and number when reported)			Antiviral effect comparison
									NRTI	NNRTI	PI	
COL103952 ALERT[73]	FPV/r + (TDF/FTC od tab)	53	24	79	89	NA	NA	NA	NA	NA	NA	EQU
	ATV/r + (TDF/FTC od tab)	53	24	83	89	NA	NA	NA	NA	NA	NA	EQU
AI 424-034 (BMS)[18]	AZT + 3TC + ATV	404	48	32	70	NR	NR	NR	NR	NR	NR	EQU
	AZT + 3TC + EFV	401	48	37	64	NR	NR	NR	NR	NR	NR	EQU
M03-603	Induction LPV/r bid + AZT + 3TC											
LPV/r monotherapy[74]	→ LPV/r (400/100mg) bid	104	96	50	65	25	15/25	5	184V = 2		3	
	→ EFV + AZT + 3TC	51	96	61	72	NR	5/NR	2	184V = 1	1		SUP
once-daily LPV/r[75]	LPV/r (400/100mg) bid + TDF + FTC	75	48	64		14.6	8/11		184V = 2		None	EQU
	LPV/r (800/200mg) od + TDF + FTC	115	48	70		9.6	7/11		184V = 1		None	EQU
MONARK[76]	LPV/r	83	48	71	79	25	21/21	2	None		Any = 2[f]	EQU
	LPV/r + AZT + 3TC	53	48	75	77	6	3/3	1	184V = 1			EQU

a Type and number of major resistance mutations for: NRTI, 184 = 80%, 74 = 5%, 65 = 3%, 215 = 3%, 75 = 1%, >1 mut = 5%; NNRTI, 103 = 72%, 190 = 6%, 188 = 3%, >1 mut = 5%; PI, 30 = 61%, 90 = 17%, 46 = 7%, 84 = 3%, >1 mut = 12%.

b From 175 patients with pre-existing resistance and who experienced VF, genotyping could be done in 143.

c From 70 patients in both arms who experienced VF successful genotyping could be performed in 31.

d Preliminary report.

e VF = >400 copies/mL. Total VF events between weeks 24 and 108.

f No phenotypic resistance.

3TC = lamivudine; **ABC** = abacavir; **APV** = amprenavir; **AZT** = zidovudine; **bid** = twice daily; **d4T** = stavudine; **ddI** = didanosine; **EFV** = efavirenz; **EQU** = equivalent regimens; **FPV** = fosamprenavir; **FTC** = emtricitabine; **IDV** = indinavir; **LPV** = lopinavir; **mut** = mutation; **NA** = not available; **NFV** = nelfinavir; **NNRTI** = non-nucleoside reverse transcriptase inhibitor; **NR** = not reported; **NRTI** = nucleoside reverse transcriptase inhibitor; **od** = once daily; **PI** = protease inhibitor; **pts** = patients; **r** = ritonavir; **SUP** = superior regimen; **tab** = tablet; **TAM** = thymidine-associated mutation; **TDF** = tenofovir disoproxil fumarate; **VF** = virological failure; → indicates followed by.

Although efavirenz has so far had better overall performance than PIs with respect to antiviral potency and duration of viral suppression, patient nonadherence to therapy may affect the NNRTI class to a higher extent than occurs for PIs or NRTIs, principally as a result of its low genetic barrier for resistance and a higher risk of significant resistance at low levels of patient adherence. A second and similarly potent NNRTI,[77] nevirapine, is more widely used in many resource-poor settings because of cost and the risk of teratogenicity associated with efavirenz.

4.1.1 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Efavirenz is potent and can be given once daily. To date, efavirenz has outperformed its competitors in almost all clinical trials of drug-naive patients with virus that did not carry NNRTI-resistance mutations. Efavirenz has been favourably compared against non-boosted indinavir, non-boosted atazanavir, nelfinavir, ritonavir-boosted fosamprenavir and lopinavir/ritonavir. Consequently, efavirenz has become the NNRTI of choice for first-line therapy (except in pregnancy and when drug interactions prohibit its use). Currently, the most popular combinations including efavirenz are tenofovir disoproxil fumarate (DF)/emtricitabine plus efavirenz, abacavir/lamivudine plus efavirenz, and zidovudine/lamivudine plus efavirenz.

The AIDS Clinical Trials Group (ACTG) 384 study,[58] a large trial in treatment-naive patients, demonstrated the superiority of both efavirenz-based regimens when compared with a non-boosted PI (nelfinavir, which does not require ritonavir boosting) and of the zidovudine/lamivudine backbone when compared with a stavudine plus didanosine backbone. In this trial, four-drug regimens were associated with a stronger and more durable viral suppression than when three drugs were used, except for the triple combination zidovudine/lamivudine with efavirenz. It became evident that efficacy greatly depended on how individual drugs were combined. The regimen of stavudine plus didanosine led to higher toxicity (peripheral neuropathy and pancreatitis) and the use of nelfinavir without efavirenz had lower virological efficacy. Importantly, zidovudine/lamivudine plus efavirenz was the most durable three-drug regimen in naive patients. Since this trial, subsequent studies have used zidovudine/lamivudine plus efavirenz as a regimen against which efficacy and toxicity have been compared. Modern combinations have had to show higher potency than this regimen as well as more balanced profiles between antiviral potency and toxicity.

Recent comparisons of efavirenz versus newer boosted PI-based regimens were performed by the Clinically Significant Long-Term Antiretroviral Sequential Sequencing Study Team (CLASS or ESS40001) trial and the ACTG 5142 trial. The former, comparing three arms (abacavir/lamivudine plus efavirenz vs abacavir/lamivudine plus ritonavir-boosted fosamprenavir [initially ritonavir-boosted amprenavir] vs abacavir/lamivudine plus stavudine) found a trend towards better performance of the NNRTI arm after 96 weeks of follow-up and at the <50 copies/mL endpoint but not at <400 copies/mL.[65] It is important to note that this study had limited statistical power to detect the smaller differences that can exist among newer drug regimens. ACTG 5142, which compared NRTI/lamivudine plus efavirenz versus NRTI/lamivudine plus lopinavir/ritonavir versus efavirenz plus lopinavir/ritonavir (NRTI = zidovudine, stavudine extended release or tenofovir DF selected by the investigator before randomization), reported higher efficacy in the efavirenz group than in the zidovudine/lamivudine plus lopinavir/ritonavir arm. These data indicate that efavirenz is a very potent antiretroviral when used in appropriate combinations. Interestingly, resistance mutations in two drug classes (NRTI-associated [M184V] and NNRTI-associated [K103N]) were more common in the efavirenz plus NRTI arm than in the lopinavir/ritonavir arm, and major PI mutations were detected in only 2 of 91 patients in whom a lopinavir/ritonavir-containing regimen failed, both patients in the group receiving efavirenz plus lopinavir/ritonavir.[70] Thus, efavirenz is widely considered as a first option for therapy of treatment-naive HIV-1 infected patients. However, there are circum-

stances in which efavirenz may easily be lost as an active drug, as discussed in section 4.2.

4.1.2 Protease Inhibitors (PIs)

A recent systematic review elucidated the consequences of virological failure after various initial combinations of therapy.[78] The study included 15 clinical trials selected from 176 citations initially obtained by literature search. On the basis of genotypic testing of viruses from patients failing therapy, the analyses performed in this study calculated the number of drugs and drug classes that would remain active upon virological failure with different ART combinations. This is now known as future drug options (FDOs) scoring systems. The authors found that NNRTI-based and boosted PI-based combinations were similar in potency and duration of response, but the use of boosted PI regimens offered the benefit of delayed resistance, which may in turn preserve active therapeutic options in the event of failure to the first regimen. These findings gain relevance as boosted PIs have become preferable over non-boosted PIs. The results on NRTIs regarding sequencing could not be assessed in that study.

Using data from an open cohort (from January 2000 to December 2005), another study compared FDOs after virological failure for different drug classes when given as initial therapy.[79] FDOs were reduced in patients failing a thymidine analogue-including regimen (median 3.65, interquartile range [IQR] 1.29) compared with patients who did not receive thymidine analogues (median 3.82, IQR 1.12; $p = 0.011$). FDOs were higher in those who received NNRTIs compared with those who received PIs (median 3.64, IQR 1.15; $p = 0.027$). The effect of some class sequencing options was also tested. This revealed that switching from NNRTI-based ART to a boosted PI-based regimen had a higher chance of successful virological suppression (48.1%) than switching from NNRTI to PI-based ART (28.2%) or from PI to NNRTI-based ART (21.4%). It is important to highlight that the strategy of switching from a boosted PI regimen to a NNRTI-based regimen was not specifically reported in this study, and neither were study subjects stratified according to adherence to therapy. Therefore, these data can at most support the concept that boosted PIs do offer a higher degree of efficacy than non-boosted PIs when NNRTI-based ART fails. Whether boosted PIs or NNRTIs are more advantageous as initial therapy was not specifically compared in this study.

PIs have been found to have lower potency than efavirenz in drug-naive patients in clinical trials and are second to efavirenz plus NRTI regimens in regard to proportion of patients who remain virologically suppressed at the efficacy measurement timepoints. Nevertheless, PIs possess a very high genetic barrier for resistance, which means that a greater number of mutations must be selected to render PIs inactive. In general, HIV-1 protease must acquire three or more mutations in order for it to become significantly resistant to newer PIs.[80-82] Several newer PIs, e.g. lopinavir and tipranavir, may maintain activity despite the presence of four or five mutations in protease.[81,83] In multiple recent clinical trials, it has become evident that the development of HIV resistance is very rare with boosted PI drugs. The older PI indinavir, and the newer PIs, lopinavir, amprenavir (and fosamprenavir), atazanavir, darunavir and tipranavir, are now almost always given with ritonavir boosting.

Regarding the low propensity for boosted PIs to select for resistance in patients, it is important to note that the pharmacokinetics of boosted PIs may add one more protecting factor against emergence of HIV drug resistance. In contrast to the very prolonged plasma half-life of NNRTIs, which results in a long decreasing plasma concentration slope, most PIs have a short half-life and sharp plasma concentration decreasing slopes.[84] Ritonavir boosting substantially increases the peak and trough plasma concentrations of PIs but only marginally lengthens their half-life. In this manner, exposure of virus to suboptimal drug levels is short for PIs (hours) and boosted PIs, while it is very long for NNRTIs (days).

For instance, to date, only two cases of stavudine/lamivudine plus lopinavir/ritonavir failure in initially treatment-naive patients have been reported to harbour significant resistance mutations after 360 weeks of follow-up.[85,86] For ritonavir-boosted

fosamprenavir (given with abacavir/lamivudine), only two cases of virological failure have been associated with drug resistance after 160 weeks of follow-up.[87,88] Only one case has been reported of virological failure associated with ritonavir-boosted atazanavir therapy (given with abacavir/lamivudine plus tenofovir DF). As yet, there is no report of resistance developing in treatment-naive patients taking either ritonavir-boosted darunavir or ritonavir-boosted tipranavir.

Virological failure in boosted PI-treated patients has typically been related to poor adherence. However, virological failure not associated with resistance, despite excellent adherence, has been described in some patients receiving PIs and boosted PIs.[11,89] Several explanations for this phenomenon are possible: (i) non-reported (or non-detected) non-adherence; (ii) abnormally low drug absorption or rapid drug elimination leading to low drug exposure; (iii) active replication in sanctuary sites to which drug penetration is low; and (iv) low-level adherence.[11] Also, non-recognized resistance mutations can partially explain this observation, although this is less likely.[11] In order to determine the impact of these possible mechanisms of virological failure, better tools for measurement of adherence and determination of plasma drug concentrations may be required. As the role of cell membrane transporters that pump drugs out from the intracellular environment may also have an impact on antiviral efficacy of these drugs, the use of inhibitors of cell transporters could potentially improve drug penetration to sanctuary sites.

4.2 PIs versus NNRTIs

Adherence is undoubtedly the most important factor that affects the risk of virological failure and drug resistance. International guidelines acknowledge the important role of adherence in therapeutic outcome and prognosis, but no clear discussion on the preferable role of PIs versus NNRTIs in non-adherent patients is available. Instead, these decisions are left to clinical judgement but the decisions are not simple. Therefore, a discussion of this issue is required.

It has been traditionally recommended that >95% adherence is necessary to minimize the risk of treatment failure and resistance. However, such high-level adherence may not be attainable in many patients over the long-term.[90,91] Adherence levels vary widely, from <50% to close to 100%, and multiple factors are involved.

In general, NNRTIs, are more forgiving in regard to adherence than the other drug classes. Newer boosted PIs also appear to perform well when adherence is <95%.[92,93] However, it is worrisome that poor adherence conditions (facilitated either by patient or health system-related factors) can compromise the efficacy of NNRTIs. It has been reported that adherence <60% resulted in more resistance to NNRTIs than to the other drug classes, including PIs.[94] Although very low adherence might seem unlikely to result in enough antiviral pressure to select resistance, it is important to recognize that the chance of losing NNRTI efficacy is high in these circumstances. PIs might be more appropriate for initiation of therapy, if adherence is likely to be problematic. In this sense, adherence factors could play a role in decisions about sequencing. At acceptably high adherence (ideally ≥95%), NNRTIs followed by boosted PI is probably the best sequencing strategy, as it can guarantee higher response rates and durability. In contrast, at very low adherence (e.g. <80%), the risk of drug resistance can be minimized if the order chosen is boosted PI followed by NNRTIs, in which case timing the switch when adherence issues have been resolved may be wise. These strategies appear logical, as they are based on appropriately tested and approved regimens. Also, PI use has been associated with immunological preservation in patients experiencing virological failure, and lower morbidity and mortality when resistance, which is closely linked to non-adherence, is present.[53,54,95,96] However, clinical advantages regarding future treatment options and durability of viral suppression will need further testing.

The ACTG 5142 trial (comparing lopinavir/ritonavir plus two NRTIs vs lopinavir/ritonavir plus efavirenz vs efavirenz plus two NRTIs) provides

data that can be used to decide which drug classes may be most convenient for therapy initiation.[70] The combination of efavirenz plus two NRTIs showed a lower frequency of virological failure at week 24 than the regimen lopinavir/ritonavir plus two NRTIs (24% vs 37.1%, respectively); however, at the time of virological failure, no patient in the lopinavir/ritonavir group had NRTI-resistance mutations with the exception of M184V (7 of 53 genotyped samples [15%]). On the other hand, 11 of 33 genotyped samples showed NRTI-resistance mutations, including eight with M184V/I and three with K65R. Although there is always a concern that NRTI resistance may still develop in a boosted PI plus two NRTIs starting regimen, it seems clear that there is a lower likelihood of drug loss with such a combination than with efavirenz plus two NRTIs. A low frequency of NRTI resistance mutations (with the exception of M184V/I) has been reported in all clinical trials that used ritonavir-boosted PIs.[17,70-72,76] Therefore, a sequencing strategy needs to anticipate a failure of both drug classes. In circumstances of poor adherence, NNRTI plus NRTI regimens may be more prone to affect both drug classes, and therefore it may be wiser to maintain boosted PI therapy until acceptably high adherence becomes consistent.

There is obviously no direct way to assess adherence unless the patient has had the chance to receive therapy for a certain period of time. However, factors known to negatively affect adherence should be addressed before therapy is negatively affected by them. Delaying this assessment until patients are already receiving therapy may lead to missed opportunities to prevent the negative impact of non-adherence on efficacy of therapy and emergence of drug resistance. Such factors vary with the clinical, geographical and sociodemographic characteristics of patients. In Western countries, conditions found to disfavour patient adherence to ART include, but are not limited to: (i) active mental illness (depression); (ii) alcohol or drug abuse; (iii) lack of patient education; and (iv) lack of reliable access to medication. Other factors can predict optimal adherence, such as availability of emotional and practical life supports, or the patient's ability to fit therapy into his or her daily routine.[52] For instance, patient readiness for starting HIV therapy could be estimated before initiating ART by using a scale that evaluates the potential impact of several factors that influence adherence to therapy, and that could help distinguish between patients who will likely have optimal adherence from those who will not.[97] More recent research suggests that interventions aimed at correcting patient-related risk factors for non-adherence can improve adherence in patients who will soon start ART.[98] These clinical tools can be used to discern when a drug with high genetic barrier to resistance (viz. boosted PIs) might be more convenient for some patients. When facing 'grey-zone' patients, many clinicians may decide to wait until these factors are corrected before starting ART. However, it is not infrequent to have patients in whom therapy needs to be initiated urgently or very soon. In these situations, PIs are more suitable from the standpoint of resistance.

Using at least two new drugs in a regimen has been traditionally recommended in cases of virological failure. In the case of virological failure of an NRTI plus NNRTI regimen, and because of the high likelihood of resistance to the NNRTI, a switch to an active NRTI plus boosted PI regimen should probably be considered as the best subsequent option. Genotypic resistance testing usually helps guide this process. The best PI drug candidates to replace NNRTIs currently include atazanavir plus ritonavir, lopinavir/ritonavir and fosamprenavir plus ritonavir. In addition, the NRTI backbone may also need to be sequenced. The utilisation of NRTI-sparing combinations is as yet too immature to support a plausible sequencing strategy.

4.3 Benefits and Potency of Nucleoside Reverse Transcriptase Inhibitor (NRTI) Backbones

The nucleoside analogues in clinical use include zidovudine, stavudine, didanosine, zalcitabine, abacavir, lamivudine and emtricitabine; the only approved nucleotide analogue is tenofovir DF.

Several benefits have been associated with the use of NRTI backbones. The Swiss Cohort Study showed that the rate of virological failure on PI-based ART (either indinavir or nelfinavir) was almost twice as high in NRTI-experienced as in NRTI-naive patients (24% vs 13% for nelfinavir and 27% vs 16% for indinavir, respectively).[99] In addition, it has been clearly demonstrated that some combinations of NRTIs contribute to durability and potency[58] of an antiretroviral regimen, while others result in rapid virological failure.[100,101] Furthermore, NRTI backbones continue to be an almost invariable component of ART regimens, since NRTI-sparing regimens have not yet been studied sufficiently. Appropriate sequencing of NRTIs can be fundamental for durability of therapeutic success. Data have been gathered on the efficacy of newer drugs compared with previously accepted first-line combinations (table I).

An added reduction in the rate of emergence of resistance mutations has been noticed when lamivudine was included in NRTI backbones in several clinical trials. This appears to be related not only to synergistic suppression of viral replication but also to the inherent effect of the lamivudine-selected resistance mutation, i.e. M184V, on the emergence of NRTI or PI resistance mutations.[102,103] The M184V mutation increases fidelity and decreases the processivity of the HIV-1 reverse transcriptase. It has been argued that M184V can reduce viral fitness and, consequently, the rate of selection of other resistance mutations.[104] Reduced emergence of resistance has been noticed in regimens with lamivudine (or emtricitabine) in combination with zidovudine, stavudine, tenofovir DF or abacavir. In addition, it has been demonstrated that M184V causes hypersusceptibility to zidovudine, stavudine and tenofovir.[63,65,105] Clinical trials have consistently shown higher efficacy for NRTI backbones that include lamivudine or emtricitabine, and these are associated with a lower rate of emergence of resistance; e.g. nelfinavir, indinavir, lopinavir/ritonavir and ritonavir-boosted fosamprenavir.[17,106,107]

The ACTG 5095 study compared abacavir/zidovudine/lamivudine versus zidovudine/lamivudine plus efavirenz versus abacavir/zidovudine/lamivudine plus efavirenz in treatment-naive patients and led to two very important findings:

1. The triple NRTI regimen (abacavir/zidovudine/lamivudine) alone was inferior to the NRTI plus efavirenz combination, which led to the disposal of the former regimen as a first-line choice for initiation of ART.[61]

2. The efficacy and toxicity profile of abacavir/zidovudine/lamivudine plus efavirenz and the combination zidovudine/lamivudine plus efavirenz were equivalent.[62]

These conclusions supported the use of two NRTIs over the triple NRTI combinations as a preferred backbone, and the continued preference of efavirenz over triple-nucleoside regimens alone.

The CNA30024 trial demonstrated the antiviral equivalence of twice daily abacavir/lamivudine plus efavirenz versus the standard comparator zidovudine/lamivudine plus efavirenz, along with better CD4+ cell count recovery and reasonably low toxicity, the exception being the hypersensitivity reactions associated with abacavir.[64] Furthermore, the same regimen of abacavir/lamivudine plus efavirenz had equal antiviral performance to a once-daily regimen and a fixed dose once-daily tablet form as shown by CNA30021 (ZODIAC [Ziagen® Once Daily in Antiretroviral Combination] study) and ESS30008 (SEAL study), respectively.[60,63] The major concern continues to be the 6–9% incidence of abacavir hypersensitivity reactions in patients receiving this regimen.

The Gilead 934 and Gilead 903 studies evaluated the performance of tenofovir DF/emtricitabine plus efavirenz versus zidovudine/lamivudine plus efavirenz and tenofovir DF, lamivudine plus efavirenz versus stavudine, lamivudine plus efavirenz, respectively.[19,59] The combination of tenofovir DF/emtricitabine plus efavirenz in the 934 study has the highest reported efficacy at 96 weeks among the NRTI plus NNRTI regimens studied to date, and was superior to zidovudine/lamivudine plus efavirenz regarding the proportion of patients with <50 viral RNA copies/mL at endpoint. When compared with other regimens, it offers several advan-

tages: it does not cause anaemia, has lower mitochondrial toxicity and has a low rate of emergence of resistance. Tenofovir DF, lamivudine plus efavirenz had similar antiviral potency as stavudine, lamivudine plus efavirenz.[19] The earlier 903 trial (which evaluated the combination tenofovir DF plus lamivudine backbone) reported somewhat higher levels of virological failure and resistance than that seen in the 934 study (which studied the tenofovir DF/emtricitabine backbone). The tenofovir DF-selected mutation K65R was not seen in the 934 trial in the arm treated with tenofovir DF/emtricitabine plus efavirenz, whereas it was present in 8 of 47 patients with virological failure in the 903 trial who received tenofovir DF, lamivudine plus efavirenz. TAMs were not seen in the 934 trial (tenofovir DF/emtricitabine plus efavirenz) but were present in 1 of 47 patients in the 903 trial (tenofovir DF/lamivudine plus efavirenz) among those who received tenofovir DF. The 934 comparator arm zidovudine/lamivudine plus efavirenz showed nine cases of 184V, one with TAMs, and none with K65R. The 903 comparator arm stavudine + lamivudine plus efavirenz resulted in 17 patients developing M184V, two cases with K65R and four cases with TAMs. Apparently, the use of emtricitabine instead of lamivudine in the 934 trial may have resulted in reduced virological failure and a lower proportion of resistance mutations in the patients in whom therapy failed. The lower incidence of resistance in the 934 trial has been attributed to the advantageous pharmacokinetic properties of emtricitabine (mainly, longer half-life that matches that of tenofovir DF). Whether this benefit will be sustained when using emtricitabine in other regimens, which typically had included lamivudine, awaits further research.

An early trial, the START (Selection of Thymidine Analog Regimen Therapy)-I study, reported a higher potency of a stavudine plus lamivudine backbone compared with zidovudine/lamivudine when both were given in addition to indinavir.[68] Despite the antiviral advantage of stavudine over zidovudine, its higher mitochondrial toxicity has made it less preferable among clinicians and patients. Didanosine plus emtricitabine has proven superiority compared with stavudine plus didanosine in both antiviral potency and toxicity profiles,[67] but has not been tested against a superior comparator (zidovudine/lamivudine or tenofovir DF/emtricitabine). A similar backbone, didanosine plus lamivudine, which is being compared with zidovudine/lamivudine both plus efavirenz in the GESIDA (Grupo de Estudio de SIDA) 3903 trial, appears promising, with good antiviral potency and less toxicity than that previously reported for stavudine plus didanosine.[66] A final analysis will provide definite conclusions on its role in ART and sequencing. The NRTI backbone stavudine plus didanosine has fallen out of favour as a result of its higher incidence of mitochondrial toxicity and its high risk of selection of TAMs and of the Q151M mutation.[58,108-110]

Emtricitabine and lamivudine have been compared in the Protocol 303 trial. Efficacy was almost identical with both and these drugs are thought to be interchangeable.[111] Whether emtricitabine offers advantages over lamivudine regarding selection of resistance is not known, as the Protocol 303 trial design could not answer this question. A pooled analysis of trials retrospectively compared stavudine plus emtricitabine plus abacavir versus zidovudine/lamivudine/abacavir in treatment-naive patients and reported similar efficacy but a lower rate of virological failure (9% vs 15%) and incidence of M184I/V (52% vs 74%) in the stavudine plus emtricitabine plus abacavir arm.[112] There are no data comparing the efficacy or resistance rates of emtricitabine and lamivudine as part of current NRTI regimens.

Combinations resulting in unacceptably high rates of virological failure include: two NRTIs alone, abacavir plus tenofovir DF and lamivudine, tenofovir DF plus didanosine and lamivudine, and stavudine plus didanosine and abacavir, because of either rapid selection of resistant virus or high toxicity rates. Zidovudine and stavudine have antagonistic effects and are not used together in any NRTI backbone.

5. Patterns of Antiretroviral Drug Resistance

The resistance mutations of the different drug regimens studied in recent important clinical trials are shown in table I. Additionally, the patterns of resistance mutations for each class of drug are summarized in table II, table III and table IV. Integrating these data may be helpful for clinicians to decide appropriate sequencing strategies.

However, it should be kept in mind that the resistance patterns reported in some clinical trials may sometimes be different from those seen in routine clinical practice. Patients in clinical trials are strictly monitored virologically and resistance testing is performed promptly when indicated. In contrast, in the non-clinical trial setting, the time between the actual occurrence of therapeutic failure in a patient and the detection of virological failure and resistance to an antiretroviral may generally be longer. Therefore, there is a greater chance that suboptimal therapy and resistance evolution will take place in such settings.[114] Published data argue for aggressive modification of therapy when virological failure occurs.[115] The result may be a tendency for higher detection of resistance mutations or more complex mutational patterns in patients treated in regular clinical practice. Clearly, aggressiveness in detection of virological failure and resistance as well as timely drug substitutions are of the utmost importance.

5.1 NNRTI Resistance and Intraclass Sequencing of NNRTIs

Efavirenz, nevirapine and delavirdine are the NNRTIs available for clinical use.

Table II shows the main NNRTI resistance mutations. In clinical trials involving NNRTI-based regimens, NNRTI-related mutations are present in the vast majority of patients undergoing virological failure (table I). Sequencing strategies for NNRTIs had been based on the fact that in patients receiving nevirapine in the absence of zidovudine, therapy failed with the Y181C mutation and without K103N in 50–80% of cases and that in patients receiving nevirapine with zidovudine plus didanosine, virological failure occurred with Y181C alone 50% of the time.[22,116] Efavirenz retains important activity for viruses that only have the Y181C mutation. Some have suggested that avoiding the concomitant use of zidovudine and nevirapine in the same regimen could favour the emergence of Y181C alone (instead of K103N ± Y181C) and the expected continuing activity of efavirenz.[22] However, this strategy has been refuted by a longitudinal study in which nevirapine-experienced patients harbouring only Y181C had an 82% reduced rate of response to efavirenz compared with nevirapine-naive patients.[117] Therefore, sequencing cannot be attempted with nevirapine and efavirenz.

Interest has been raised in regard to NNRTI hypersusceptibility mutations. These mutations are mainly selected by NRTI drugs, can be found in 10–20% of nucleoside-experienced patients, and include M41L, M184V, V118I, H208Y and T215Y.[118,119] Depending on whether these mutations are alone or in combination, a variable degree of susceptibility (or hypersusceptibility) can result. The M41L and T215Y mutations in combination with the M184V can cause hypersusceptibility to all three NNRTIs. NNRTI hypersusceptibility was also seen in viruses with the T69SSA insertion plus M41L, A62V and T215Y.[118] The V118I/H208Y/

Table II. Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations associated with HIV drug resistance

NNRTI	Resistance mutations							
Nevirapine	K103N[a,b]	V106AM[a,b]	V108I[a,b]	Y181C/I[a,b,c]	Y188C/L/H[a,b]	G190A[a,b]		
Delavirdine	K103N[a]	V106AM		Y181C/I[a]	Y188L	G190SA		P236[a,b]
Efavirenz	K103N[a,b]	V106AM	V108I[b]		Y188L		P225H[b]	

a Major resistance mutation.

b Mutation preferentially selected by drug.

c In absence of zidovudine, nevirapine therapy selects for Y181C about 80% of the time.

Table III. HIV protease inhibitor (PI) resistance mutations based on the US International AIDS Society Panel for Antiretroviral Resistance[113]a

PI	Cross-resistance mutations		Unique mutations	
	major	minor	major	minor
Saquinavir/r	*L90M, G48V*	L10IRV, L24I, I54VL, I62V, A71VT, G73S, V77I, V82AFTS, I84V		
Indinavir/r	*M46IL, 82AFT, I84V*	*L10I*RV, K20MR, L*24I*, M*32I*, M36I, I*54V*, A*71VT*, G73SA, V77I, L*90M*		
Nelfinavir	*L90M*	10FIRV, L24I, M36I, *M46I*L, A71VT, G73S, V77I, V82AFTS, I84V, *N88DS*	*30N*	
Fosamprenavir/r	*I50V*	*L10F*IRV, V32I, *M46IL*, *I47V*, *I54L*VM, G73S, V82AFST, L90M		
Lopinavir/r	*V32I*, *I47VA*, *V82AFT*S	*L10FIRV*, K20MR, L24I, L33F, *M46I*L, I50V, F53L, *I54V*LAMTS, A71VT, G73S, *I84V*,I90M		*L63P*
Atazanavir/r	*I84V*, *N88S*,	L10IFVC, K20RMITV, L24I, *V32I*, *L33I*FV, M36ILV, *M46IL*, G48V, F53LY, I54, LVMTA, I62V, *A71VITL*, G73CSTA, V82ATFI, L90M	*I50L*	G16E, E34Q, D60E, I64LMV, I93LM
Tipranavir/r	*L33F*, V82T, I84V	*L10VF*, K20MR, E35G, *M36I*, K43T, M46L, I47V, I54AM*V*, L90M	*V82L*	I13V, Q58E, H69K,T74P, N83D
Darunavir/r	I50V, I54ML, I84V	V11I, V32I, L33F, I47V, G73S,	L76V	V11I, L89V

a Italic font represents mutations preferentially selected by drug.

/r = boosted with ritonavir.

T215Y mutant has been found to confer the highest level of hypersusceptibility as well as the lowest replication capacity.[120] Conversely, the single T215Y mutation does not appear to confer increased susceptibility, and the Q151M mutation has not been found to be associated with NNRTI hypersusceptibility but was only able to cause a small reduction in susceptibility to the three NNRTIs.[118,120] With respect to the clinical importance of these mutations, a cohort study showed a better virological prognosis in patients with hypersusceptible viruses treated with NNRTIs than in patients with viruses that had wild type susceptibility, but another only found a short-lived virological benefit.[119,121] These findings may not offer practical applicability for ART sequencing strategies at this time.

5.2 PI Resistance and Intraclass Sequencing of PIs

PIs are associated with a complex array of multiple resistance mutations (see table III).

PIs are now almost always administered with ritonavir boosting. Longitudinal studies of the sequential use of newer PIs in drug-naive patients and their impact on clinical outcomes have not yet been published. The best evidence to guide sequencing to date has been generated from characterisation of drug cross-resistance among virus isolates from drug-experienced patients and virological outcomes of salvage therapies.

Several popular ART sequencing strategies have been used in clinical practice. For instance, nelfinavir selects for D30N (87% of the cases with PI resistance) in the majority of patients who have received either monotherapy or combination therapy.[122] Other PIs are not affected by this mutation. Early switching can therefore be made to a newer boosted PI in the event of virological failure associated with D30N. The mutation N88S alone or with other mutations is also selected by nelfinavir, which causes amprenavir hypersusceptibility.[123] Hence, ritonavir-boosted fosamprenavir can offer advantages in such cases. Lopinavir/ritonavir has been advocated in place of nelfinavir when the latter fails and no genotypic or phenotypic resistance information is available, as is the case in some resource-poor settings. Of note, delaying a switch to an active regimen after nelfinavir drug resistance-related virological failure may lead to further acquisitions of mutations, which can confer cross-resistance to other PIs. Interestingly, a pathway in which N88D

facilitates the coexistence of the generally mutually exclusive D30N and L90M has been described. The presence of D30N + N88D + L90M constitutes a stable genetic backbone for the accumulation of additional PI resistance mutations and can explain the acquisition of multi-PI resistance in patients receiving nelfinavir. The evolution of resistance appears to progress through the following steps: D30N → D30N + N88D → D30N + N88D + L90M → D30N + N88D + L90M + (L33F ± I84V or M46I/L ± I54V).[124]

In treatment-naive patients in whom newer boosted PI drugs have failed, scarce direct data are available because only a few cases of documented and characterized resistant viruses have been reported. Non-boosted atazanavir usually selects for the I50L mutation (along with A71V), which also causes hypersusceptibility to amprenavir, indinavir, nelfinavir, saquinavir and especially to lopinavir.[125-127] Virological failure with non-boosted atazanavir can usually be overcome by using ritonavir-boosted atazanavir. The mutation pattern seen in viral isolates from patients receiving boosted atazanavir differs from that described in patients receiving non-boosted atazanavir and from *in vitro* data. For instance, I50L has not been seen in patients receiving boosted atazanavir, but N88S (along with K20T, M36I/V, L63P, A71T) and G73S (along with L10I, L63P, V77I, I93L) have been reported in two separate patients exposed to this regimen.[128-130] N88S confers hypersusceptibility to fosamprenavir (and amprenavir) and this can represent a strategy for sequencing. In a large sample of PI-resistant isolates, the mutations associated with atazanavir resistance were found to differ from those associated with other PIs, and 86% of viruses resistant to one or two PIs (nelfinavir, indinavir, lopinavir, amprenavir or saquinavir) retained susceptibility to atazanavir.[131] The least chance of cross-resistance was found for amprenavir, lopinavir and saquinavir. Additionally, ritonavir-boosted atazanavir showed similar efficacy to lopinavir/ritonavir in salvage therapy in drug-experienced patients. This provides a certain degree of confidence that boosted atazanavir-related resistance provides numerous options for second-line therapeutic success. Therefore, failure with atazanavir could be followed by the use of ritonavir-boosted atazanavir, and failure of boosted atazanavir by the use of boosted fosamprenavir, boosted saquinavir or lopinavir/ritonavir.

Two cases of ritonavir-boosted fosamprenavir failure have been reported. The virus strain from one patient this regimen failed in had a genotype associated with amprenavir failure, i.e. M46I + I50V, and a cleavage site mutation at position L449F[87] along with other polymorphisms. This virus was susceptible to lopinavir and hypersusceptible to atazanavir. A second report of another patient who underwent viral rebound after a prolonged period with low viral load (<1000 copies/mL) showed the mutations V32I, I47V, M46I and A71V.[88] Clonal analysis of this virus before therapy had been started demonstrated the presence of small proportions of the resistance mutations V32I, I47V and V82A. Unfortunately, no phenotypic susceptibility data were reported for the latter case.

A recent meta-analysis pooled data from 922 ART-naive patients (NEAT, SOLO and KLEAN trials) who received either ritonavir-boosted fosamprenavir or non-boosted fosamprenavir plus abacavir/lamivudine.[132] Paired genotypes (baseline and follow-up) were obtained for 74 of 85 participants with virological failure. Fosamprenavir-associated resistance mutations were detected in 5 of 74 patients with virological failure, i.e. four of five receiving fosamprenavir and one boosted fosamprenavir. In four patients, viruses developed I54L or I54 M and one acquired the V32I + I47V combination. No virus from patients receiving boosted fosamprenavir had reduced fosamprenavir susceptibility. Three of four viral isolates from patients who received fosamprenavir and who acquired fosamprenavir mutations had fosamprenavir resistance. PI cross-resistance was observed for lopinavir, saquinavir, nelfinavir, atazanavir and indinavir in 0, 0, 2, 0 and 1 of 5 subjects, respectively.

Two cases of resistance developing with lopinavir/ritonavir plus a NRTI-backbone as initial therapy have been reported. The first involved the V32I, M46M/I and I47A mutations,[85] and other substitu-

tions at M36I, I54V.L63P, V82A, I93l and L33F.[86] The mutation I47A causes hypersusceptibility to saquinavir. Patients in whom this therapy fails and who had virus with the I47A mutation had commonly been treated with ritonavir-boosted saquinavir. In the absence of this mutation, a genotype (and/or phenotype)-informed selection appears warranted for second-line drug use. The OK04 trial compared maintenance therapy with lopinavir/ritonavir plus two NRTIs versus lopinavir/ritonavir monotherapy; in patients whose HIV-1 RNA had been suppressed to <50 copies/mL for at least 6 months and had no previous virological failure while receiving PI therapy, lopinavir resistance was found in 2 of 11 patients in the monotherapy arm (2% of all patients on monotherapy) and one of four in the combination therapy arm (1% of all patients in this arm). Protease genotypes of viral isolates from these three patients had the 10F and 46I mutations in the first arm, 54V, 77I and 82A in the second arm, and 54V, 63P, 71V and 82A in the third. Two isolates in the combination arm had TAMs. Phenotypic resistance to lopinavir/ritonavir was low (change was 1.5-fold, 7.1-fold and 7.2-fold, respectively) and phenotypes of these isolates revealed sensitivity to saquinavir, amprenavir, atazanavir, tipranavir and darunavir. Two patients had virological failure with boosted saquinavir. One patient was lost to follow-up.

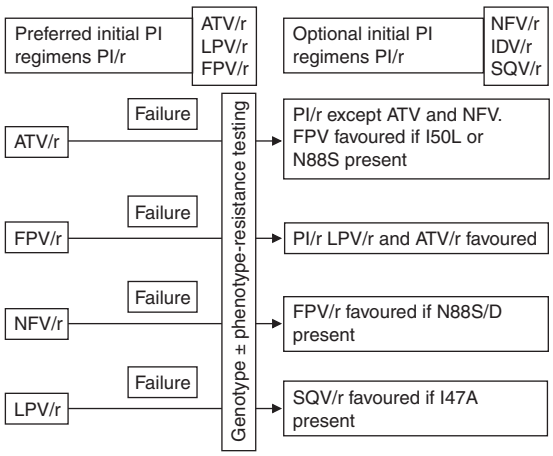

Fig. 1. Possible protease inhibitor (PI) intraclass sequencing options. Most PIs are used with ritonavir (r) boosting. Genotyping and sometimes phenotyping resistance testing are necessary to determine which mutations are present and which PIs are less affected by them. Switching of the accompanying non-nucleoside reverse transcriptase inhibitors is usually performed simultaneously. Arrows indicate direction of potentially advantageous sequencing, given good tolerance to regimens. **ATV** = atazanavir; **FPV** = fosamprenavir; **IDV** = indinavir; **LPV** = lopinavir; **NFV** = nelfinavir; **/r** = boosted with ritonavir; **SQV** = saquinavir.

To date, limited data are available to establish the best sequencing strategy for the current standard of boosted PIs in PI-naive patients. Each of the findings of minimal cross-resistance on the basis of case reports, the prolonged activity of boosted PI regimens, patient convenience, and drug adverse-effect profile can help in selection of a first- and second-line regimen. Figure 1 outlines possible boosted PI sequencing options. A lower potential for wide cross-resistance appears to favour atazanavir over fosamprenavir and lopinavir, but new information is required from patients who have recently started to take newer PIs.

5.3 NRTI Resistance and Intraclass Sequencing of NRTIs

NRTI resistance mutational pattern is summarized in table IV. TAMs are mainly selected by zidovudine, stavudine and sometimes didanosine.[133-136] These mutations include M41L, D67N, K70R, L210W, T215Y/F and K219Q. In general, clinical isolates with TAMs only harbour certain of these mutations.[137] T215Y is the mutation most frequently observed *in vivo* and is the most important mutation of this group, as it confers 10- to 15-fold resistance to zidovudine.[137,138] K70R and T215Y are seen together only when other TAMs are also present. This is probably because they are mutually antagonistic in their effect on zidovudine resistance.[139] The presence of four or more TAMs (mainly M41L, L210W and T215Y/F) results in >100-fold increase in the concentration that produces 50% inhibition for zidovudine, a 5- to 7-fold increase for abacavir, and 2- to 3-fold resistance to stavudine, didanosine and tenofovir DF.[140-145] The clinical efficacy of each of these drugs is affected by these TAMs.

The M184V mutation is principally selected by lamivudine and emtricitabine, and occasionally by abacavir.[140,146,147] It confers high-level resistance to

Table IV. Nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations

NRTI	Mutated residues in HIV-reverse transcriptase																									
	M41L		K65R		D67N		T69D		K70R		L74V		V75T		Q151M		M184V		L210W		T215Y/F		K219E/Q	
	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL	sel	RL
AZT	Y[a]	2↑	N	↓	Y[a]	3↑	Y[b]	1↑	Y[a]	3↑	N	↓	Y[b]	1↑	Y[b]	2↑	N	↓	Y[a]	2↑	Y[a]	4↑	Y[a]	3↑
d4T	Y[b]	4↑	Y[b]	1↑	Y[b]	3↑	Y[b]	1↑	Y[b]	1↑	N	O	Y	3↑	Y[b]	2↑	N	↓	Y[b]	2↑	Y[b]	2↑	Y[b]	3↑
TDF	Y[b]	3↑	Y	3↑	Y[b]	1↑	Y[b]	1↑	N	O	N	O	Y[b]	1↑	Y[b]	3↑	N	↓	Y[b]	3↑	Y[b]	3↑	Y[b]	1↑
ABC	Y[b]	3↑	Y	3↑	Y[b]	1↑	Y[b]	1↑	N	O	Y	3↑	Y[b]	1↑	Y[b]	2↑	Y[a]	3↑	Y[b]	3↑	Y[b]	3↑	Y[b]	1↑
ddI	Y[b]	3↑	Y	3↑	Y[b]	1↑	Y[b]	3↑	N	O	Y	3↑	Y[b]	3↑	Y[b]	2↑	Y[b]	2↑	Y[b]	3↑	Y[b]	3↑	Y[b]	1↑
ddC	Y[b]	1↑	Y	3↑	Y[b]	1↑	Y[b]	3↑	Y[b]	1↑	Y[b]	2↑	Y[b]	3↑	Y[b]	2↑	Y[b]	2↑	Y[b]	1↑	Y[b]	1↑	Y[b]	1↑
3TC	Y[b]	1↑	Y	2	N	O	Y	1↑	Y[b]	1↑	N	O	N	O	Y[b]	3↑	Y[a]	4↑	Y[b]	1↑	Y[b]	1↑	N	O
FTC	Y[b]	1↑	Y[b]	2↑	N	O	Y	1↑	N	O	N	O	Y	3↑	Y[b]	3↑	Y[a]	4↑	Y[b]	1↑	Y[b]	1↑	N	O

a Drug mainly selects for this mutation.

b Drug less frequently selects for this mutation

3TC = lamivudine; **ABC** = abacavir; **AZT** = zidovudine; **d4T** = stavudine; **ddC** = zalcitabine; **ddI** = didanosine; **FTC** = emtricitabine; **O** = no resistance; **RL** = resistance level conferred by a mutation; **sel** = selects for this mutation (Y = yes; N = no); **TDF** = tenofovir disoproxil fumarate. ↓ indicates hypersusceptibility; **1**↑ indicates contributes to resistance; **2**↑ indicates low level resistance; **3**↑ indicates moderate level resistance; **4**↑ indicates high level resistance.

lamivudine and emtricitabine, but low-level resistance to abacavir, didanosine and zalcitabine.[147] Abacavir and didanosine remain active against viruses containing the M184V mutation alone, but the presence of TAMs enhances the resistance level of M184V to abacavir and didanosine.[148]

The K65R mutation is selected in cell culture and can be selected, albeit rarely, during therapy with didanosine, zalcitabine, abacavir and tenofovir DF.[149,150] This mutation is the only one selected by tenofovir in tissue culture[145] and the only one found to be clearly associated with tenofovir DF in intensification therapy.[151] The use of concomitant tenofovir DF with abacavir or didanosine facilitates the emergence of K65R.[101] Stavudine can also select for K65R in cell culture.[152] K65R confers intermediate resistance to didanosine, zalcitabine, lamivudine, emtricitabine, stavudine, tenofovir DF and abacavir when associated with the mutations Y115F ± M184V.[152-156] It is important to highlight that K65R confers hypersusceptibility to zidovudine and its prevalence has been increasing as a result of the widespread use of NRTI backbones that exclude zidovudine. The effect of K65R on resistance to tenofovir DF is almost completely reversed by the mutation M184V.

The frequency of K65R is very low in patients in whom therapy that included both zidovudine and abacavir has failed. K65R is rarely found in patients in whom abacavir therapy has failed who had viruses already harbouring TAMs. One study reported that the frequency of K65R increased from 0.4% to 3.6% between 1998 and 2003. In contrast, other NRTI mutations including TAMs and M184I/V decreased in frequency: T215Y decreased by 19%, M41L by 17% and L210W by 13%. This study also performed virological assays that demonstrated that K65R antagonized the resistance of TAMs to zidovudine and that TAMs antagonized K65R resistance to abacavir, tenofovir DF and zalcitabine. Interestingly, TAMs do not antagonize K65R resistance to lamivudine or emtricitabine, but do enhance resistance by M184V to abacavir, didanosine and tenofovir DF.[157]

The L74V mutation (as well as other substitutions at the same position) has been typically seen in patients in whom didanosine monotherapy has failed, but has also emerged in patients receiving abacavir monotherapy.[140,147,158] It causes 5- to 10-fold loss of susceptibility to didanosine, but only 3- to 4-fold reduction in abacavir susceptibility and 2-fold reduction in tenofovir DF susceptibility.[142,154,159] L74V partially antagonizes the effect of T215Y on a zidovudine- or stavudine-resistant phenotype.[159] It is usually not seen when zidovudine is part of the ART regimen.

The T69D mutation has been seen most commonly after long-term zalcitabine exposure and it reduces HIV susceptibility to zalcitabine by five to ten times. The T69A/S mutations have emerged mainly after didanosine therapy and cause intermediate resistance to this drug. T69D also confers certain levels of resistance to other NRTIs.[160,161]

Insertions between positions 69 and 70 (SA, SG or SS) are associated with prolonged exposure to multiple NRTIs, and when accompanied by other mutations (e.g. T215Y, other TAMs and T69S) result in high level resistance to zidovudine and moderate resistance to stavudine, zalcitabine, didanosine and tenofovir.[162-164]

The V75T mutation is primarily selected by stavudine *in vitro* but it is rare *in vivo*,[165] and confers intermediate resistance to stavudine, didanosine and zalcitabine.[165,166] The '151 complex', which includes substitutions Q151M, A62V, V75I, F77L and F116Y, confers partial resistance to all NRTIs except tenofovir (only 1.8-fold decreased susceptibility) and has been primarily associated with didanosine-containing combinations such as didanosine plus zidovudine and didanosine plus stavudine.[108,142,167,168]

The duration and degree of viral suppression achieved by the tenofovir DF/emtricitabine backbone in clinical trials favours its use as the combination of choice for ART initiation. Consequently, most current clinical guidelines suggest NRTI backbones that include tenofovir DF/emtricitabine as first-line therapy. Additionally, zidovudine/lamivudine and abacavir/lamivudine are also among first-line therapies due to ample experience with such regimens. Stavudine plus lamivudine (or emtricitabine) is a less favoured option since it causes more mitochondrial toxicity. TAMs appears to emerge more frequently after use of thymidine analogues than does K65R after tenofovir DF use (69% after a mean of 5.4 ± 2.9 years vs 2–4% after 1 year of therapy).[151] In addition, data from the 907 trial demonstrated that the antiviral response to intensification therapy with tenofovir DF was reduced when three or more TAMs were present in the viral baseline genotype.[169] Therefore, future therapies seem more likely to be compromised if thymidine analogues are used first.

With a tenofovir DF/emtricitabine (or lamivudine) backbone, the selected mutations should not affect zidovudine[145] and only minimally affect stavudine.[170] A subsequent regimen might potentially include zidovudine/lamivudine or stavudine plus lamivudine. In most cases, tenofovir DF resistance will not be found, as reported in two major clinical trials.[19,59] But when detected, it usually relates to K65R ± M184V, and both confer hypersusceptibility to zidovudine. Therefore, zidovudine/lamivudine (or emtricitabine) becomes the most logical subsequent regimen. Three cases have been reported of NRTI-resistant HIV harbouring the K65R mutations with no TAMs, which rapidly suppressed after addition of zidovudine to the baseline regimen.[171] Stavudine plus lamivudine (or emtricitabine) could also be substituted for tenofovir DF/emtricitabine in a subsequent regimen, since the level of resistance that K65R confers to stavudine is low, and at least one study has described a more favourable virological outcome with stavudine than with zidovudine for rescue therapy in the presence of the K65R mutation.[172] If virological failure occurs without tenofovir DF resistance mutations in a regimen of tenofovir DF/emtricitabine plus efavirenz, it could be argued that substituting a boosted PI for efavirenz in the following regimen and keeping tenofovir DF in the backbone might be an acceptable choice. No data on clinical repercussions yet exist to inform this decision, but hasty switching to a different backbone cannot be justified when resistance is absent (unless

toxicity factors play a role). Tenofovir DF is likely to be active in virus with few TAMs, and thymidine analogues are likely to be active against viruses containing only K65R, but there is a tendency to start therapy with the tenofovir DF/emtricitabine backbone instead of a zidovudine/lamivudine backbone. A careful assessment of the toxicity profile generally guides the selection of the initial regimen. Keeping lamivudine or emtricitabine in the presence of the M184V mutation appears valid when zidovudine, stavudine or tenofovir DF are the accompanying NRTIs, because of the hypersusceptibility that this mutation confers to these drugs. However, this may be less applicable when didanosine or abacavir are used, although there may still be an opportunity to benefit from the reduced replicative fitness conferred by M184V. Further discussion on this issue can be found elsewhere.[45,173,174]

Abacavir/lamivudine plus efavirenz is now considered an excellent option for initial therapy in several international guidelines. The risk of the hypersensitivity reaction to abacavir can now be significantly reduced by pharmacogenetic testing of patients prior to starting antiretrovirals (about 70–80% reduction), but not completely eliminated.[175,176] In *in vitro* and monotherapy studies, abacavir selected for the resistance mutations L74V, K65R, M184V and 115F.[140,154] However, when given together with lamivudine plus efavirenz or a PI, very few mutations were observed in patients in whom therapy failed (table I).[60-62] Analysis of the risk factors in those patients in whom therapy failed identified poor adherence as a probable major cause. Importantly, virological failure with abacavir/lamivudine backbones infrequently revealed K65R. The L74V and M184V mutations were more frequent; they are known to hypersensitize HIV-1 to a thymidine analogue (zidovudine or stavudine). Hence, either zidovudine or stavudine along with lamivudine or emtricitabine can be used in second-line regimens after failure of abacavir/lamivudine. Further research is needed to validate this approach. Sequencing of abacavir/lamivudine or tenofovir DF/emtricitabine with thymidine analogue-based backbones is outlined in figure 2.

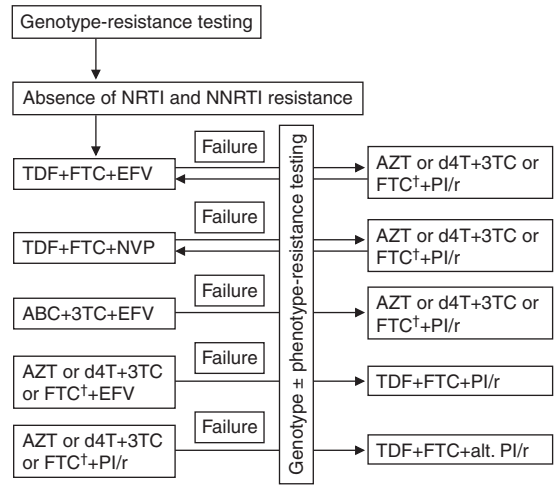

Fig. 2. Possible intraclass non-nucleoside reverse transcriptase inhibitor (NNRTI) sequencing. Generally, subsequent regimens include a new nucleoside reverse transcriptase inhibitor (NRTI) backbone plus a drug that replaces the initial NNRTI or protease inhibitor (PI) drug. Sequencing of NNRTI does not seem to be an option with current drugs. Emtricitabine (FTC) and lamivudine (3TC) appear to be interchangeable. Arrows indicate direction of potentially advantageous sequencing, given good tolerance to regimens. **ABC** = abacavir; **alt.** = alternate; **AZT** = zidovudine; **d4T** = stavudine; **EFV** = efavirenz; **NVP** = nevirapine; **/r** = boosted with ritonavir; **TDF** = tenofovir disoproxil fumarate; **†** indicates FTC is considered equivalent to 3TC, but rarely used in this NRTI backbone.

Establishing whether abacavir/lamivudine or tenofovir DF/emtricitabine backbones offer any resistance-related advantage in formulation of a sequencing strategy is difficult for several reasons. Firstly, very few cases of proven resistance are available for comparison. Secondly, second-line regimens usually lead to long-term suppression, such that studies on clinical impact would require long follow-ups in order to monitor durability of sequencing strategies. Thirdly, genotypic resistance testing is not generally possible when viral load is <1000 copies/mL. Many patients in whom modern ART combinations fail have low viral loads and resistance mutations cannot be characterized. Improving the sensitivity of current genotype-based testing for RNA levels <1000 copies/mL may necessitate analysis of a larger proportion of samples from patients in whom therapies fail. Furthermore, the clinical relevance of therapy failures in patients with low viral loads will

need to be assessed in the context of newer therapeutic options.

The other question is whether tenofovir DF/emtricitabine and abacavir/lamivudine can be sequenced. Viruses recovered from patients in whom tenofovir DF/lamivudine or emtricitabine and abacavir/lamivudine therapy has failed have been genotypically characterized; the predominant resistance mutations are presented in table I. *In vitro* resistance selection with tenofovir DF/emtricitabine and abacavir/lamivudine was recently reported.[177] Resistant viruses selected with tenofovir DF/emtricitabine displayed intermediate to high resistance to abacavir. Therefore, sequencing tenofovir DF/lamivudine or emtricitabine followed by abacavir/lamivudine cannot be recommended. On the other hand, resistant viruses selected with abacavir/lamivudine were, in general, partially susceptible to tenofovir (0.8- to 2.6-fold change in the concentration that produces a 50% effective response [EC_{50}]), but a higher level of resistance was found when the K65R substitution was present. In viruses with K65R, the increase in EC_{50} was 2.6- to 5.9-fold. Additionally, in the 907 trial (tenofovir DF intensification therapy), some observations deserve attention. Four of eight patients with baseline K65R had been receiving didanosine or abacavir; eight cases without K65R at baseline developed this mutation after addition of tenofovir DF, and three of them were also taking abacavir. The latter three cases experienced virological failure within 48 weeks. Although many viruses from patients in whom abacavir/lamivudine had failed may have a phenotypic pattern that suggests susceptibility to tenofovir DF/emtricitabine, several reports have warned that previous therapy with abacavir and didanosine has been associated with tenofovir DF failure.[178] Minority quasispecies of K65R can be detected by allele-specific polymerase chain reaction or clonal analysis in viruses from abacavir- and didanosine-treated patients containing L74V, and K65R has been associated with subsequent virological failure of a tenofovir DF-based regimen.[178,179] Even though K65R represents the hallmark tenofovir DF mutation, it has also been shown that tenofovir DF can maintain activity against viruses carrying this mutation. The clinical implications of this residual effect are still unknown. A study reported that in treatment-experienced patients carrying viruses with the K65R mutation along with one or more of M184V, Q151M, L74V and TAMs, only one-half of the patients achieved virological suppression (<400 copies/mL). In this study, the use of thymidine analogues did not improve the response, while the presence of the M184V mutation and the use of tenofovir DF were associated with an improved response.[180] These results support a residual activity of tenofovir DF against K65R-carrying viruses. Nevertheless, several clinical trials have found rapid virological failure when tenofovir DF plus abacavir (and tenofovir DF plus didanosine) have been used as part of an antiretroviral combination, and a convincing explanation for this result is still lacking.[7] In conclusion, tenofovir DF/emtricitabine ↔ abacavir/lamivudine sequencing cannot be recommended in light of available evidence.

6. Frequency of Transmitted Drug Resistance

Transmitted HIV drug resistance varies worldwide. In countries in which ART is widely available, the incidence of transmitted drug resistance is 5–20%. In Europe and North America, primary resistance to NRTIs (especially TAMs) is generally higher (4–16%), followed by that against NNRTIs (1.4–13%) and then PIs (1.5–7.1%).[181-185] With few exceptions, NRTI resistance is the most prevalent form in South America (1–13%).[186-190] In contrast, NNRTI transmitted drug resistance is more frequent (1.4–55%) in sub-Saharan Africa.[191-195] Transmission of HIV drug resistance usually follows the therapeutic preferences of different geographical areas.

Transmitted drug resistance is measured by detection of antiretroviral resistance mutations in viruses from drug-naive, recently infected patients (6–12 months earlier) and because of obvious reasons, not at the time of infection. Under these circumstances, the rate of resistance transmission may be underestimated. Firstly, there is no international

consensus yet on what mutations are representative of transmitted drug resistance. A current effort by the WHO is attempting to solve this limitation.[196] And secondly, because of the tendency of resistance mutations to revert in the absence of ART (e.g. T215Y revertants [T215S/C/D/E/N/L]), the sensitivity of assays for detection of resistant viruses may be limited. Initially resistant transmitted viruses may be sequenced as minority quasispecies among more numerous non-resistant species.

Because the risk of treatment failure can be increased among patients infected with resistant virus (primarily for NNRTI-based therapy), pre-treatment resistance testing has become a standard of care in developed countries.[197-200] Also, viruses with revertant mutations evolve faster than wild-type viruses do into fully resistant variants, and this has been associated with reduced virological response to thymidine analogues.[201,202] Lack of recognition of these intermediate mutations may compromise future therapies. Primary NNRTI resistance mutations might render the entire class compromised. In developed countries, resistance testing can partially eliminate the problem of inappropriate treatment in treatment-naive patients, despite the sensitivity limitations in regard to minority species. However, in the event of non-availability of resistance testing, e.g. need of emergent ART, it is important for clinicians to be aware of the patterns and time trends of transmitted drug resistance in their geographical areas. In resource-poor settings, in which resistance testing cannot usually be performed, ART sequencing may have to be decided based on resistance surveillance data. Surveillance in such scenarios requires continuity of results, so as to adequately inform clinicians on the prevalence and patterns of local transmitted drug resistance.

7. Conclusion

ART sequencing strategies are used in HIV care. Sequencing newer NRTI backbones with older thymidine analogue-based backbones may offer an important opportunity for lengthening the benefit of ART. Boosted PIs are also amenable to sequencing, although resistance data on truly treatment-naive patients exposed to such regimens are limited. Currently approved NNRTIs do not offer any option for sequencing, although newer NNRTIs now under development may circumvent this problem.

The clinical impact of sequencing strategies has not yet been confirmed by clinical research. Durability of clinical benefit of sequencing will be important for coming generations of antiretroviral drugs. Therefore, longitudinal studies of large cohorts of HIV-infected patients who initiate ART sequencing strategies are needed in order to estimate the effect of such interventions regarding the long-term benefit of ART. Access to newer therapeutic options is warranted in resource-poor countries because of the potential to reduce the burden of virus, reduce rates of antiretroviral drug resistance and benefit from future sequencing options.

Acknowledgements

The work of Dr Martinez-Cajas has been supported by a Canadian HIV Trials Network Post-Doctoral Fellowship. Neither Dr Martinez-Cajas nor Dr Wainberg have any conflicts of interest to report in regard to this article.

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