Mutations Associated with Reduced Susceptibility to NRTIs

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This talk is about mutations responsible for resistance to nucleoside RT inhibitors most commonly referred to as NRTIs.

Disclosures

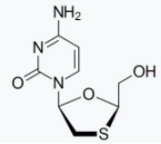
- Gilead Sciences (2022): Advisory board and speaking honorarium.
- ViiV Healthcare (2022): Speaking honorarium.

These are my disclosures.

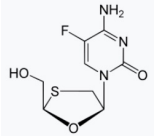
NRTIs and Resistance Mutations

Cytosine analogs

Lamivudine (3TC)

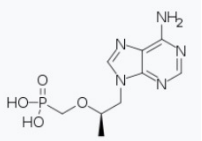


Emtricitabine (FTC)



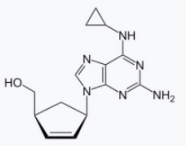
Adenosine analog

Tenofovir (TFV)



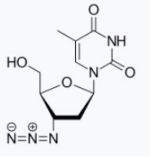
Guanosine analog

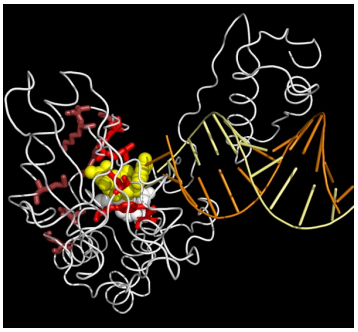
Abacavir (ABC)



Thymidine analog

Zidovudine (AZT)





Active site

Inhibitor

DRMs

Discriminatory mutations

- M184V/I
- K65R/N, K70E/Q, L74V, Y115F

Primer unblocking mutations (TAMs)

- M41L, D67N, K70R, L210W, T215Y/F, K219Q/E

1. These are the five NRTIs that are currently being used.
2. Tenofovir has one phosphate moiety and is therefore also referred to as a nucleotide. There are two tenofovir prodrugs - tenofovir disoproxil fumarate and tenofovir alafenamide.
3. In this presentation I use TFV as the abbreviation for tenofovir, TDF as the abbreviation for tenofovir disoproxil fumarate, and TAF as the abbreviation for tenofovir alafenamide.
4. This figure shows part of the 3-D structure of the HIV-1 RT enzyme in which an NRTI is approaching the active site.
5. The NRTI-resistance mutations are shown in red. Most are situated close to the active site where the incoming nucleoside or nucleoside analog is added to the growing primer strand.
6. The NRTI-resistance mutations act by two mechanisms. The discriminatory mutations reduce the rate at which NRTIs are added to the growing viral nucleic acid primer.
7. The thymidine analog mutations or TAMs make it more likely that a chain-terminating NRTI will be removed from the growing viral nucleic acid primer. As a result, these mutations are also called primer unblocking mutations.
8. The TAMS are rarely, if ever, selected by current NRTI combinations. They are

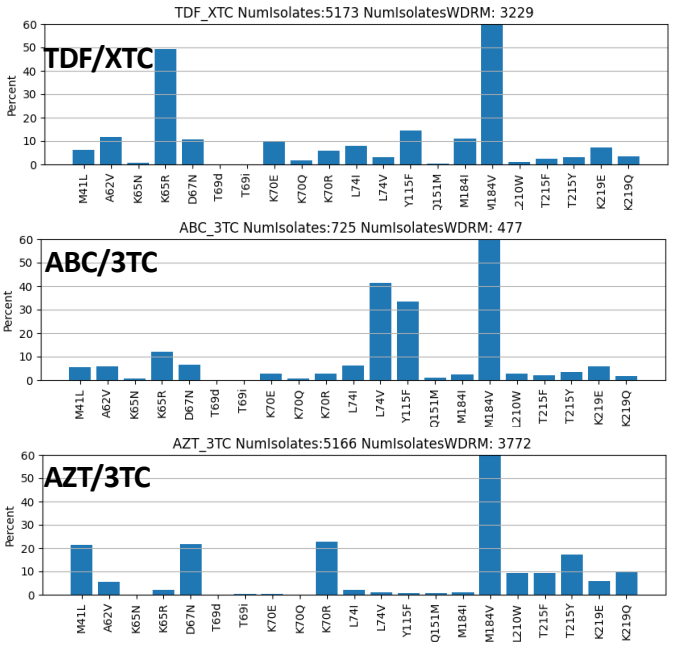
primarily observed in patients with histories of ART dating back to the years in which AZT and d4T were used.

9. Some of the TAMs are also among the most commonly transmitted DRMs because their fitness generally allows them to persist for longer periods of time even in the absence of drug exposure..

Outline

- DRMs selected by dual NRTI regimens
  - TFV/XTC
  - ABC/3TC
  - AZT/3TC
- Effects of DRMs on susceptibility
  - TFV
  - ABC
  - AZT
- Impact of DRMs on response to TFV, ABC, and 3TC

1. In this presentation, I will first review the DRMs selected by the three main dual NRTI combinations including TDF or TAF in combination with 3TC or FTC, ABC which is usually administered with 3TC, and AZT which is usually administered with 3TC.
2. Then, I'll review the effect of different DRMs and DRM combinations on susceptibility to TFV, ABC, and AZT.
3. Finally, I'll review the clinical significance of different DRMs, specifically how they influence the virological response to different NRTIs and NRTI combinations



DRMs Selected by Dual NRTIs

- M184VI in >80% of patients with DRMs.
- TDF and ABC select for the same DRMs but:
  - TDF/XTC - increased A62V, K65R, K70EQTN
  - ABC/3TC - increased L74V, Y115F
- AZT selects for TAMs
- MDR DRMs: T69i and Q151M exceedingly rare

1. These figures show the prevalence of 21 of the most common NRTI-resistance DRMs in patients receiving TDF/XTC, ABC/3TC, and AZT/3TC.
2. Sequenced viruses were available from about 5200 patients receiving TDF/XTC of whom about 3200 had at least one NRTI DRM, from 725 patients receiving ABC/3TC of whom about 500 had at least one DRM and from about 5200 patients receiving AZT/3TC of whom about 3800 had a DRM.
3. For each of the three dual NRTI combinations, more than 80% of samples with a DRM had M184V or M184I. As you can see from these figures M184V is much more common then M184I, which is believed to result from the increased replicative fitness of M184V relative to M184I.
4. TDF/XTC and ABC/3TC select for overlapping profiles but TDF selects more often for A62V, K65R, and mutations at position 70 while ABC/3TC selects more often for L74V and Y115F.
5. About 10% of patients receiving TDF or ABC also had TAMs. I suspect that this may represent patients with transmitted drug resistance and patients who received either AZT or d4T in the past that was not recorded in their treatment history.
6. AZT selects almost exclusively for TAMs at positions 41, 67, 70, 210, 215, and 219
7. The MDR DRMs - a double amino acid insertion at position 69 and Q151M were

exceedingly rare. Most of these DRMs data back to the time before 3TC and FTC became a standard part of dual NRTI therapy.

NRTI Phenotypic Susceptibility Data

- 3TC/FTC
  - Highest levels: >200-fold
  - 5-10-fold reductions occur with K65R and with multiple TAMs
- TFV and ABC
  - Highest levels: rarely >10 fold
  - TDF: low-level clinical resistance begins at about 1.5-fold
  - ABC: low-level clinical resistance begins at about 3-fold
- AZT
  - Highest levels: >100-fold
  - AZT: low-level clinical resistance likely begins at low levels

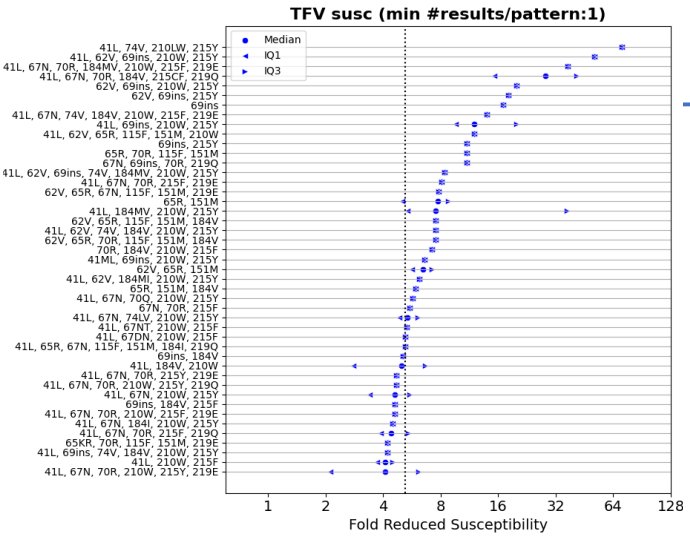
1. The range in possible reductions in susceptibility between the NRTIs. and the clinical significance of these reductions differs between NRTIs.
2. >200-fold reductions in 3TC/FTC susceptibility occur with M184V/I.
3. Much lower-level reductions in 3TC/FTC susceptibility occur with K65R and with multiple TAMs. However, we don't know the clinical significance of these smaller reductions in susceptibility.
4. For TFV and ABC, reductions in susceptibility rarely exceed 10-fold but low-level reductions in susceptibility are clinically significant.
5. Like 3TC/FTC, AZT has a high range in possible fold reductions in susceptibility.
6. Because AZT is weaker than other NRTIs, low-levels of reduced susceptibility as observed with certain individual TAMs are clinically significant.

NRTI Susceptibilities Associated with Common NRTI DRMs

DRM Pattern	AZT (Fold ↓)	TFV (Fold ↓)	ABC (Fold ↓)	3TC (Fold ↓)
Common discriminatory mutations				
184VI	0.4 (n=135)	0.5 (n=71)	3.2 (n=149)	**>200 (n=206)**
184VI + 65R	0.4 (n=19)	1.3 (n=19)	**8.7 (n=19)**	**>200 (n=29)**
184VI + 70E	0.2 (n=5)	0.6 (n=5)	3.4 (n=6)	**>200 (n=10)**
184VI + 74V	0.3 (n=12)	0.4 (n=10)	**5.0 (n=11)**	**>200 (n=12)**
184VI + 115F	0.7 (n=3)	0.9 (n=3)	**11 (n=3)**	**>200 (n=3)**
65R	0.6 (n=22)	**1.8 (n=19)**	2.5 (n=21)	8.8 (n=31)
TAMs (without and width 184VI)				
41L+215Y	11_{16}	**1.5_{11}**	2.4_{13}	2.0 (n=27)
41L+210W+215Y	144_{32}	**3.1_{28}**	3.6_{29}	3.2 (n=59)
67N+70R+215Y	36_{3}	2.5_{2}	2.7_{3}	3.9 (n=5)
41L+215Y+184V	4.8_{52}	1.0_{33}	**5.3_{51}**	**>200 (n=85)**
41L+210W+215Y+184V	11_{81}	1.3_{63}	**6.9_{75}**	**>200 (n=153)**

https://hivdb.stanford.edu/pages/phenoSummary/Pheno.NRTI.Simple.html

1. This table summarizes the effects of the most common DRMs on NRTI susceptibility. It is based on PhenoSense assay data in the Stanford HIVDR database.
2. M184VI are associated with >200-fold reductions in 3TC and FTC susceptibility. And about 3-fold reductions in ABC susceptibility.
3. M184VI increase susceptibility to AZT and TFV.
4. The main difference between TFV and ABC is the effect of M184VI which typically increases TFV susceptibility by about 2-fold and reduces ABC susceptibility by about 2-fold.
5. K65R alone reduces TFV susceptibility nearly two-fold, but in combination with M184V, the median reduction in susceptibility is just 1.3-fold
6. K70E, a TFV selected mutation, is associated with a minimal effect on susceptibility to any of the NRTIs
7. K65R, L74V, and Y115F reduce ABC susceptibility, particularly when they occur in combination with M184V.
8. The TAMs particularly those at positions 41, 210, and 215 reduce susceptibility to each of the NRTIs. Their effect on TFV and ABC are clinically significant.



TFV Resistance

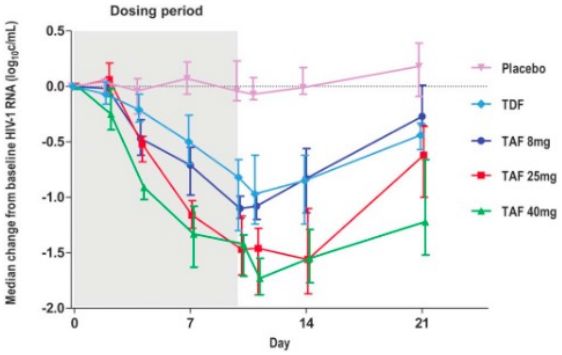
- Highest levels of reduced susceptibility
  - T69 insertions
  - K65R + Q151M
  - Large # of TAMS especially M41L+ L210W + T215YF
- Intermediate reductions in susceptibility
  - K65R +/- accessory DRMs including A62V, S68 mutations, T69 deletion, Y115F

https://hivdb.stanford.edu/cgi-bin/Phenotypes.cgi?Gene=RT

1. This figure shows the patterns of DRMs associated with a median-fold reduction in susceptibility of at least 4-fold.
2. The median fold reduction in TFV susceptibility is shown on the X-axis associated.
3. The Y axis shows those patterns of DRMs.
4. Median fold reductions in susceptibility are indicated by blue circles while the triangles indicate the IQRs.
5. A line is drawn at 4-fold which has become accepted as the clinically relevant cut-offs for high-level reduction in TFV susceptibility. I will soon describe how these cut-offs were derived.
6. There is variability in the fold reduction in susceptibility because there are other rare NRTI-associated DRMs that are not shown and because NNRTI-resistance mutations, which are present in a large proportion of these clinical isolates can affect susceptibility.
7. The patterns of mutations that are associated with the highest levels of reduced susceptibility are T69 insertions which usually occur in combination with one or more TAMs, K65R plus the MDR mutation Q151M, and large numbers of TAMs particularly the Type 1 TAMs M41L, L210W, and T215Y
8. As noted earlier, the MDR mutations have become exceedingly rare.
9. K65R rarely leads to high level resistance particularly in combination with

M184VI. Several accessory DRMs increase the fitness of viruses with K65R but they rarely reduce susceptibility to a level of 4-fold or more.

TAF Monotherapy

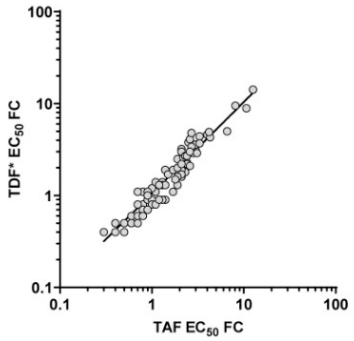


Ruane PJ. Antiviral activity, safety, and PK/PD of TAF as 10-day monotherapy. JAIDS 2013

TDF 300 mg: 0.97 log10 median VL decrease.

TAF 25 mg: 1.46 log10 median VL decrease.

In Vitro TAF Susceptibility



Margot N. Antiviral Activity of TAF against HIV-1 with TAMs and M184V. AAC 2020

TAF, TDF, and TFV select for K65R in vitro.

TAF and TDF have similar resistance profiles.

1. Phase 1 and 2 studies have shown that a 25-mg dose of TAF achieves higher intracellular TFV-DP concentrations than 300 mg TDF due to the greater plasma stability of TAF and intracellular conversion of TAF to TFV.

2. The figure shows that monotherapy with 25 mg of TAF achieved a median 1.46-log10-unit decrease in plasma HIV-1 RNA at day 10 compared to 0.97 log10 unit decrease for 300 mg of TDF.

3. However, TAF and TDF exhibit similar fold reductions in susceptibility to drug-resistant viruses in PhenoSense assay and in Gilead Sciences multi-cycle assays.

TAF Activity Against Drug-Resistant Variants

TABLE 4 Time to viral breakthrough at physiological concentrations of TAF or TFV

Isolate ID	TAF FC[a]	Mutant RT sequence	No. of TAMs[b]	Time to viral breakthrough (days)[d]	
				TFV	TAF
WT	1.0	No mutations	WT	>28	>28
101	1.9	M41L, L210W, T215Y	3 TAMs	13	>28
110	2.4	D67N, T69N, K70R, T215V, K219Q, M184V	3 TAMs + M184V	22	>28
114	2.7	D67N, T69TA, K70R, T215F, K219Q	4 TAMs	4	>28
67	2.7	M41L, D67N, L210W, T215Y	4 TAMs	14	>28
85	3.0	M41L, D67N, K70R, L210W, T215Y	5 TAMs	19	>28
87	3.0	M41L, D67N, K70R, T215Y, K219Q	5 TAMs	13	>28
108	3.3	M41L, D67N, K70R, T215F, K219Q, M184V	5 TAMs + M184V	20	>28
81	3.3	M41L, D67N, L210W, T215Y, K219Q	5 TAMs	18	>28
95	4.0	M41L, D67N, K70R, L210W, T215Y, K219Q	6 TAMs	19	>28
112	4.2	M41L, D67N, T69N, K70R, T215F, K219E, M184V	5 TAMs + M184V	10	>28
111	4.3	M41L, L210W, T215Y	3 TAMs	4	>28
120	6.6	M41L, D67N, L210W, T215Y, K219R	5 TAMs	10	20
113	8.1	M41L, D67N, L210W, T215Y	4 TAMs	10	>28
121	10.7	M41L, D67N, T69D, L74I, L210W, T215Y, K219R	5 TAMs	4	13
117	12.6	M41L, D67N, T69D, L210W, T215Y, K219R	5 TAMs	5	10

Margot N. Antiviral Activity of TAF against HIV-1 with TAMs and M184V. AAC 2020

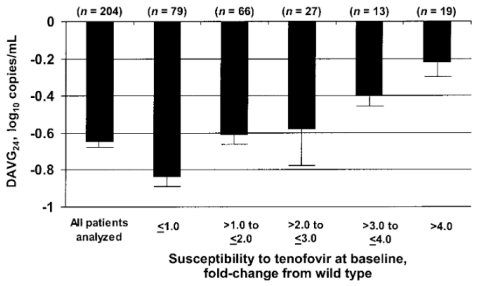
- In viral breakthrough assays TAF is better able to suppress resistant viruses compared with TFV.
- There are no clinical data showing that TAF is more effective than TDF in treating patients with resistant viruses.

1. Because standard in vitro phenotypic assays do not capture the 4-fold increase in intracellular TFV-DP concentration obtained with TAF dosing compared to TDF, Gilead has developed a viral breakthrough assay in which drug-resistant variants are cultured at physiologically relevant concentrations of TAF and TDF that mimick the 4-fold increase of TFV-DP that TAF provides.
2. This table shows that viral breakthrough in vitro of viruses containing multiple TAMs in vitro much earlier with TFV than with TAF. A follow-up paper published similar findings for K65R-containing viruses.
3. TFV is used for comparison rather than TDF because TDF is less stable than TFV in cell culture.
4. However, there are no clinical data showing that TAF is more effective that TDF at treating patients with drug-resistant viruses. But it is not unreasonable to infer this based on its increased activity against wildtype viruses.

Tenofovir Intensification Study

Table 1. HIV-1 RNA response to tenofovir disoproxil fumarate (tenofovir DF), by genotype at baseline.

	Mean HIV-1 RNA response						
	Subjects given tenofovir DF			Subjects given placebo			
Genotype at baseline[a]	*n*	$DAVG_{24}$[b]	$DAVG_{48}$[b]	*n*	$DAVG_{24}$[b]	*P*[c]	*P*[d]
All	222	−0.59	−0.57	110	−0.03	<.001	…
No M184V	73	−0.42	−0.43	40	0.08	<.001	…
M184V	149	−0.67	−0.64	70	−0.09	<.001	.003
M184V and no TAMs	51	−0.96	−0.88	20	−0.12	<.001	<.001
No TAMs	68	−0.80	−0.74	29	−0.11	<.001	…
TAMs	154	−0.50	−0.50	81	0.00	<.001	<.001
And no M184V	56	−0.45	−0.46	31	0.13	<.001	.001
And M184V	98	−0.52	−0.52	50	−0.08	<.001	.002
1 or 2	55	−0.66	−0.63	33	−0.04	<.001	.11
⩾3	99	−0.40	−0.43	48	0.03	<.001	<.001
With M41L or L210W	57	−0.21	−0.24	29	0.01	.013	<.001
Without M41L or L210W	42	−0.67	−0.67	19	0.07	<.001	.15
D67N	79	−0.53	−0.58	43	−0.03	<.001	.004
K70R	67	−0.71	−0.70	40	−0.03	<.001	.17
K219Q/E/N/R	57	−0.60	−0.59	27	0.11	<.001	.03
T215Y/F	106	−0.35	−0.37	53	0.03	<.001	<.001
M41L	81	−0.26	−0.29	40	0.06	<.001	<.001
L210W	46	−0.17	−0.21	22	0.06	.025	<.001
T215Y/F without M41L or L210W	25	−0.70	−0.66	13	−0.01	.012	.32



- **The 6 patients with K65R did not respond to TDF intensification.**

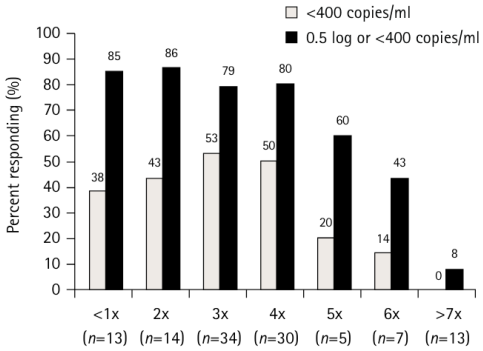
Miller MD Genotypic and phenotypic predictors of the magnitude of response to TDF treatment in ARV-experienced patients. AIDS 2004

1. Much of what we know about the clinical significance of NRTI DRMs on TDF activity comes from a 2004 publication.
2. The table summarizes the results from 2 placebo-controlled intensification trials in which TDF was added a single agent to the regimen of treatment-experienced patients who had experienced VF on one or more previous regimens.
3. The table shows that by **week 24**, the mean reduction in RNA levels was 0.59 logs.
4. It was particularly high in patients with M184V and no TAMs - 0.96 logs
5. It was low among those with 3 or more TAMs, particularly those containing M41L or L210W. These two mutations usually occur with T215Y and in fact T215YF was also associated with a small reduction in VL.
6. The figure on the right shows that at a reduction in susceptibility above 3-4 fold, there was a minimal response to TDF intensification.
7. Although the results were obtained using the Antivirogram assay which is no longer available, it does demonstrate that low level reductions in susceptibility too TDF can be clinically significant.
8. The 6 patients with K65R did not respond to TDF intensification.

Abacavir Intensification: Genotypic and Phenotypic Predictors

Baseline reverse transcriptase mutations	Median baseline vRNA	vRNA reduction (log_{10})
No mutations		
WT (*n*=15)	4.65	−0.96
1 mutation		
184V only (*n*=75)	3.60	−0.74
1 TAM (*n*=6)	3.63	−0.56
Other (*n*=1)	3.21	−0.60
Total (*n*=82)	**3.60**	**−0.72**
2 mutations		
184V + 1 TAM (*n*=14)	4.08	−0.95
2 TAMs (*n*=5)	4.51	−0.38
Other (*n*=3)	4.48	−0.72
Total (*n*=22)	**4.21**	**−0.82**
3 mutations		
184V + 2 TAMs (*n*=12)	4.16	−0.37
3 TAMs (*n*=6)	4.06	−0.32
Other (*n*=1)	2.71	+0.15
Total (*n*=19)	**4.14**	**−0.30**
≥4 mutations		
184V + 3 TAMs (*n*=6)	3.60	−0.18
184V + >3 TAMs (*n*=6)	4.71	−0.00
≥4 TAMs (*n*=5)	4.39	−0.36
Other (*n*=11)	4.50	−0.05
Total (*n*=28)	**4.31**	**−0.07**

- 68% had a virological response defined as ≥0.5 logs 4 weeks after ABC was added.
- No significant difference in response between those with WT virus and those with 1-2 DRMs.

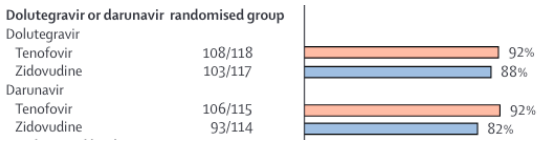


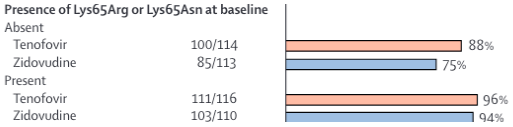
Lanier ER. Antiviral efficacy of ABC in ART-experienced adults harboring specific patterns of NRTI-resistance mutations. Antivir Ther 2004

1. Much of what we know about the clinical significance of NRTI DRMs on ABC activity also comes from a 2004 publication.
2. The table summarizes the results of a combined analysis of 5 multicenter trials in which ABC was added as a single agent to background ART in treated patients with ongoing virus replication
3. In the small group of patients with WT virus there was an approximately 1 log VL reduction - measured at W4
4. Among 75 patients with M184V, there was an approximately three-fourths of a log reduction while among 14 patients with M184V + 1 TAM there was also a log reduction.
5. There was a significantly reduced response only occurred in those with 3 or more DRMs.
6. The figure on the right shows how the cut-offs on the PhenoSense phenotypic assay were developed because a reduction in response was not observed until a reduction in susceptibility of more than 4-fold occurred and no activity was observed for those with a >6-fold reduction in susceptibility.
7. It is important to keep in mind that the TDF intensification study looked at W24 data, while this study looked at W4 data.

Implications of the NADIA Trial

- Patients with VF on a first-line NRTI/NNRT-regimen.
- About 85% had M184VI and 50% had K65R.
- By week 96, VF (RNA ≥400) was lower with TDF compared to AZT (15% to 8%; p=0.02).
- AZT was not superior to TDF even in those patients containing K65R.

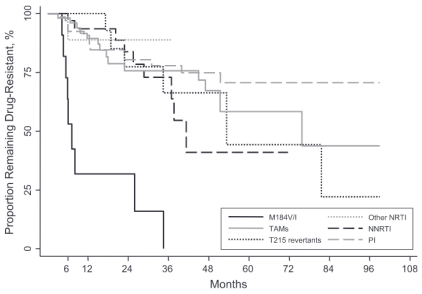




- Among 9 patients developing VF and emergent INSTI-DRMs on the DTG arms:
  - 6 had received AZT and 3 had received TDF
  - 5 of 6 receiving AZT and 0 of 3 receiving TDF developed high-level DTG resistance.

1. The NADIA trial is a more recent study that influenced our thinking about the clinical significance of NRTI DRMs, specifically the effect of K65R on a TDF-containing regimen.
2. In this trial, the participants had previously experienced VF on an NRTI/NNRTI regimen. In a factorial design, they were randomized to either DTG or DRV and to either TDV or AZT.
3. At baseline, 85% had an M184VI mutation and 50% had K65R.
4. By W96, trail participants were significantly more likely thad significantly better virological responses to TDF than to AZT regardless of treatment arm.
5. Moreover, AZT was not superior to TDF in those with a baseline K65R mutation which reduces TDF susceptibility by about 2-fold but increases AZT susceptibility by about 2-fold.
6. Finally, those receiving AZT were at greater risk of developing DTG-resistance mutations.

Replication Fitness of NRTI-Associated DRMs



Jain V. Differential Persistence of Transmitted HIV-1 Drug Resistance Mutation Classes. JID 2011

DRM	Median Rate of Loss In Years (95% CI)
Any NRTI	4.6 (3.3–6.4)
M184V	1.0 (0.5–2.0)
T215F	1.2 (0.3–4.6)
T215Y	1.7 (0.8–3.4)
D67N	6.0 (2.1–16.9)
M41L	8.6 (4.6–16.0)
T215 revertants	8.6 (4.6–16.0)

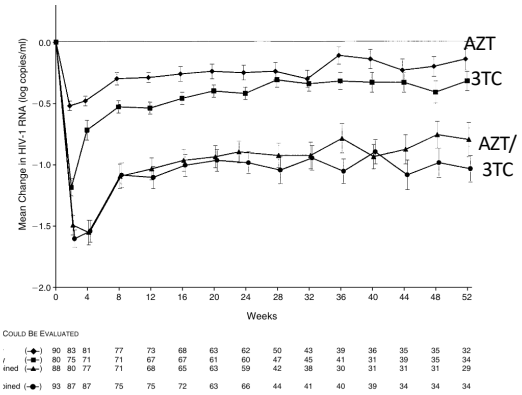
Castro H. Persistence of HIV-1 Transmitted Drug Resistance Mutations. JID 2013

DRM	Total	Clustering
41L	500	31%
67N	189	20%
219Q	151	18%
215C	128	31%
215FY	59	7%
184V	169	4%
65R	13	0%

Wertheim JO. Transmission fitness of drug-resistant HIV in a surveillance system transmission network. Virus Evol 2017

1. A discussion of NRTIs would not be complete without discussing the effect of DRMs on virological fitness and the clinical significance of the most common NRTI-resistance DRMs M184VI.
2. There have been two main papers that have looked at what happens to mutations in patients who are infected with a virus containing a DRM and do not go onto ARV therapy.
3. The figure on the left shows that M184VI mutations are no longer detectable in plasma in about 50% of patients some time between 6 and 12 months. This rate of decline is much quicker than the rate of decline of mutations associated with other drug classes and TAMS.
4. The table on the right is from a different study but shows very similar rates. M184VI is no longer detected by 1 year in 50% of the patients. Among the TAMs, the two with the greatest effects on susceptibility have a similar short half life, while several other TAMs usually persist for years.
5. Finally, a third study from the US CDC that created a transmission network from newly diagnosed patients found that viruses with the most impactful DRMs - T215FY, M184V, and K65R - were rarely found to cluster with one another suggesting that if they had been transmitted they faded rapidly over time.

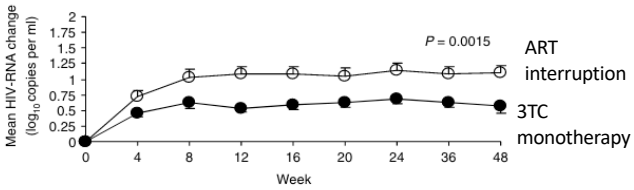
3TC Monotherapy Retained Residual Activity Against Viruses With M184V



COULD BE EVALUATED

(◆)	90	83	81	77	73	68	63	62	50	43	39	36	35	35	32
(■)	80	75	71	71	67	67	61	60	47	45	41	31	39	35	34
(▲)	88	80	77	71	68	65	63	59	42	38	30	31	31	31	29
(●)	93	87	87	75	75	72	63	66	44	41	40	39	34	34	34

Eron J. Treatment with Lamivudine, Zidovudine, or Both in HIV-Positive Patients with 200 to 500 CD4+ Cells per Cubic Millimeter. NEJM 1995



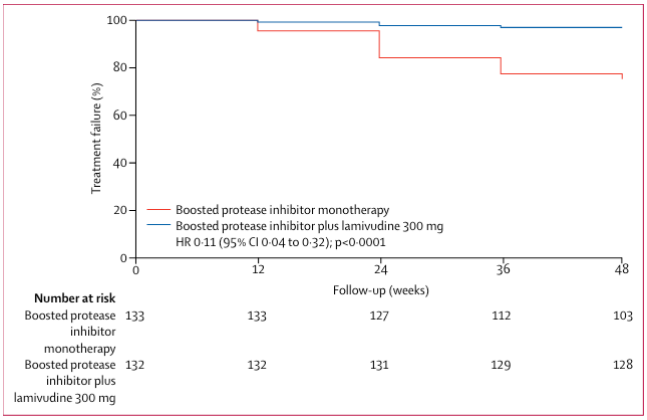
Castagna A. 3TC monotherapy in HIV-1-infected patients harbouring a 3TC-resistant virus: a randomized pilot study (E-184V study) . AIDS 2006

1. There have been more studies about M184V than any other HIV DRM.
2. On this and the next slide, I will present three studies indicating that viruses with M184V retain some degree of susceptibility to 3TC and FTC.
3. The figure on the left is from a clinical trial published in 1995 that shows the mean changes in HIV-1 RNA levels from patients receiving AZT monotherapy, 3TC monotherapy, and AZT/3TC combination therapy,
4. In this study 3TC led to a greater one log reduction in RNA levels by week 2. However, virus levels rapidly rebounded coincident with development of M184VI.
5. Nonetheless virus levels remained about one half log lower than baseline for one year.
6. Two other smaller clinical trials that I'm not showing demonstrated very similar results -- a sustained one-half log reduction in virus load associated with 3TC monotherapy treatment of viruses containing M184VI.
7. To this day, I'm not sure if the sustained activity results solely from the reduced replication capacity associated with M184VI.
8. The figure on the right is from the more recent E-184 open-label pilot trial that randomized patients receiving 3TC-containing ART and harboring the M184V mutation to monotherapy with 3TC 300 mg once daily or to the discontinuation of all ARV drugs.

9. This study and at least one other similarly designed study were consistent with the very early 3TC monotherapy trials showing that the use of 3TC in persons with viruses harboring M184V was associated with a sustained approximately 0.5 log reduction in virus load.

b-PI Monotherapy vs b-PI/3TC as 2nd-Line Maintenance ART (MOBIDIP trial)

- Sub-Saharan Africa
- PLWH on 2nd-line ART regimen
  - bPI + 2 NRTIs >= 48 weeks
  - 2 VL measurements < 200 copies / 6 months
- 97% had a history of M184V/I at the time of 1st-line VF
- Randomized to bPI (DRV/r or LPV/r) vs bPI/3TC



Ciaffi L. Boosted protease inhibitor monotherapy versus boosted protease inhibitor plus lamivudine dual therapy as second-line maintenance treatment for HIV-1-infected patients in sub-Saharan Africa (ANRS12 286/MOBIDIP): a multicentre, randomised, parallel, open-label, superiority trial. Lancet HIV 2017

1. A third clinical trial which demonstrated the benefit of 3TC despite the presence of M184V was the MOBIDIP study.
2. The study population included individuals in SSA who were virologically suppressed for 6 or more months on a 2nd-line regimen containing boosted LPV or DRV.
3. 97% of patients had a history of M184VI following VF on a 1st-line regimen.
4. By week 48, there were 30 patients with VF in the monotherapy group but only 4 in the dual therapy group, a finding that was highly statistically significant.

Summary

- DRMs selected by dual NRTI regimens
  - TFV/XTC
  - ABC/3TC
  - AZT/3TC
- Effects of DRMs on susceptibility
  - TFV
  - ABC
  - AZT
- Impact of DRMs on response to TFV, ABC, and 3TC

1. In this presentation, I summarized 3 types of evidence that inform what we know about the biological and clinical significance of NRTI DRMs.
2. We use these data to inform the Stanford GRT interpretation program.
3. On the following slides, I will summarize online resources that we provide for this program.

NRTI Notes and Handout

The table lists the most common clinically significant NRTI-resistance mutations. Mutations in bold red are associated with the highest levels of reduced susceptibility or virological response to the relevant NRTI. Mutations in bold reduce NRTI susceptibility or virological response. Mutations in plain text contribute to reduced susceptibility in combination with other NRTI-resistance mutations.

There are six widely used NRTIs: the cytidine analogs 3TC and FTC (jointly referred to as XTC), the TFV prodrugs TDF and TAF, ABC, and AZT. The NRTIs are most frequently used in the following two-drug combinations: AZT/3TC, ABC/3TC, TDF/XTC, and TAF/XTC. However, XTC is frequently used alone in combination with an anchor drug that has a high genetic barrier to resistance such as DTG or DRV/r.

Assessing the antiviral activity of NRTIs and the loss of that activity through NRTI-resistance mutations is complicated for several reasons. First, the NRTIs are prodrugs that must be tri-phosphorylated (in the case of XTC, AZT, and ABC) or di-phosphorylated (in the case

https://hivdb.stanford.edu/dr-summary/resistance-notes/NRTI/

Major Nucleoside RT Inhibitor (NRTI) Resistance Mutations

	Non-TAMs					TAMs						MDR	
	184	65	70	74	115	41	67	70	210	215	219	69	151
Cons	M	K	K	L	Y	M	D	K	L	T	K	T	Q
3TC	**VI**	R										**Ins**	**M**
FTC	**VI**	R										**Ins**	**M**
ABC	VI	**R**	E	**VI**	**F**	L			W	FY		**Ins**	**M**
TFV	***	**R**	E		F	L		R	W	FY		**Ins**	**M**
AZT	**	**		*		**L**	**N**	**R**	**W**	**FY**	QE	**Ins**	**M**

Bold underline: High-level reduced susceptibility or virological response. **Bold**: Reduced suceptibility or virological response. Plain text: Reduced susceptibility in combination with other NRTI-resistance mutations. Asterisk: Increased susceptibility.

M184VI: Although they cause high-level *in vitro* resistance to 3FTC, they are not contraindications to 3FTC because they increase TFV and AZT susceptibility and decrease viral replication fitness.

K65R: The most common DRM in patients with VF on a TFV-regimen. It causes a clinically relevant 2-fold reduction in TFV susceptibility. However, K65R+M184VI reduces TFV susceptibility <1.5-fold. INSTI-/PI-naive patients with K65R+M184VI who receive TFV/3FTC and a highly potent 3rd drug (e.g., DTG or DRV/r) respond as well or better than those receiving AZT/3TC even though K65R increases AZT susceptibility.

TFV, TDF, & TAF: Tenofovir (TFV) disoproxil fumarate (TDF) and TFV alafenamide (TAF) are TFV triphosphate prodrugs. Although TDF and TAF have similar resistance profiles, TAF attains higher intracellular levels. Additional TFV-selected mutations of uncertain phenotypic and clinical significance include A62V, K65N, K70GQNTdel, and L74I.

TAMs: Thymidine analog mutations. Selected by AZT and d4T; facilitate primer unblocking. Non-TAMs prevent NRTI incorporation. T215SCDEIVALN (T215 revertants) emerge from T215YF in the absence of NRTIs. **MDR**: Multidrug resistance mutations. T69 insertions occur with TAMs. Q151M occurs with non-TAMs and the accessory mutations A62V, V75I, F77L, and F116Y.

https://cms.hivdb.org/prod/downloads/resistance-mutation-handout/resistance-mutation-handout.pdf

1. The data that I reviewed in this presentation are summarized to a large extent in the Notes section of the HIV GRT interpretation program and in a very brief format in a PDF handout.
2. No major changes were made to the Notes and PDF handout since October 2022.

Individual DRM Scores

Rule	ABC	AZT	FTC	3TC	TDF
M41L	5	15	0	0	5
A62V	5	5	0	0	5
K65E	10	0	0	0	10
K65N	30	0	15	15	45
K65R	45	-10	30	30	50
D67E	5	15	0	0	5
D67G	5	15	0	0	5
D67H	5	15	0	0	5
D67N	5	15	0	0	5
D67S	5	15	0	0	5
D67T	5	15	0	0	5
D67del	30	30	15	15	30
S68del	15	0	15	15	15
T69G	10	5	0	0	5
T69ins	60	60	30	30	60
T69del	15	0	15	15	15
K70E	15	0	10	10	15
K70G	15	0	10	10	15
K70N	15	0	10	10	15
K70Q	15	0	10	10	15
K70R	5	30	0	0	5
K70S	15	0	10	10	15
K70T	15	0	10	10	15
K70del	15	0	10	10	15
L74I	15	0	0	0	5
L74V	30	0	0	0	0
V75I	5	5	5	5	5
F77L	5	10	5	5	5

Rule	ABC	AZT	FTC	3TC	TDF
Y115F	30	0	0	0	15
F116Y	5	10	5	5	5
Q151L	30	30	10	10	10
Q151M	60	60	15	15	15
M184I	15	-10	60	60	-10
M184V	15	-10	60	60	-10
L210W	5	15	0	0	5
T215F	10	60	0	0	10
T215I	5	20	0	0	5
T215V	5	20	0	0	5
T215Y	10	60	0	0	10
K219E	5	10	0	0	5
K219N	5	10	0	0	5
K219Q	5	10	0	0	5
K219R	5	10	0	0	5
K219W	5	10	0	0	5
V75M	0	10	0	0	0
V75A	0	10	0	0	0
V75S	0	10	0	0	0
V75T	0	10	0	0	0
T215A	0	10	0	0	0
T215C	0	10	0	0	0
T215D	0	10	0	0	0
T215E	0	10	0	0	0
T215L	0	10	0	0	0
T215N	0	10	0	0	0
T215S	0	10	0	0	0

Combination DRM Scores

Combination Rule	ABC	AZT	FTC	3TC	TDF
Q151M + M184IV	10	10	0	0	15
K65RN + Q151M	10	10	10	10	10
F77L + F116Y + Q151ML	15	10	15	15	15
L74V + M184IV	15	0	0	0	0
Y115F + M184IV	15	0	0	0	5
M41L + T215FY	10	10	5	5	10
L210W + T215FY	10	10	0	0	10
M41L + L210W	10	10	0	0	10
M41L + L210W + T215FY	10	0	15	15	10
M41L + E44AD + L210W + T215FY	5	5	0	0	5
E40F + M41L + L210W + T215FY	5	5	0	0	5
M41L + D67EGNHST + T215FY	5	5	0	0	5
D67EGNHST + T215FY + K219ENQRW	5	5	0	0	5
D67EGNHST + K70R + K219ENQRW	10	15	10	10	10
M41L + M184VI + T215FY	10	0	0	0	0
D67EGNHST + K70R + M184VI + K219ENQRW	10	0	0	0	0
M41L + T215ACDEILNSV	0	10	0	0	0
L210W + T215ACDEILNSV	0	10	0	0	0
K70EGNQST + M184IV	0	0	0	0	10
K65R + S68NGR	0	0	0	0	5
A62V + K65R	0	0	0	0	5

https://hivdb.stanford.edu/dr-summary/mut-scores/NRTI/

1. The HIVDB website also contains a list of all scores, which were last updated March 2024
2. There are individual mutation penalty scores for nearly all DRMs and several penalties that go into effect only when certain DRM combinations are present.
3. The total mutation penalty score for a drug is based on adding all of the individual and combination penalty scores.

NRTI Comments

Condition	Comment/ Mutation Type	Comment
115F	NRTI	Y115F causes intermediate resistance to ABC and low-level resistance to TDF.
116Y	NRTI	F116Y usually occurs in combination with the multi-NRTI resistance mutation Q151M. When it occurs alone, its clinical significance is unknown.
118I	Other	V118I is a polymorphic accessory NRTI-resistance mutation that often occurs in combination with multiple TAMs.
151L	NRTI	Q151M causes intermediate/high-level resistance to AZT and ABC, and low-level resistance to TDF, 3TC and FTC. In combination with two or more accessory mutations at positions 62, 75, 77, and 116, it confers high-level resistance to AZT and ABC and intermediate resistance to TDF, 3TC and FTC. Q151L is an extremely rare transitional mutation that may precede the emergence of the Q151M.
151M	NRTI	Q151M causes intermediate/high-level resistance to AZT and ABC, and low-level resistance to TDF, 3TC and FTC. In combination with two or more accessory mutations at positions 62, 75, 77, and 116, it confers high-level resistance to AZT and ABC and intermediate resistance to TDF, 3TC and FTC.
184VI	NRTI	M184V/I cause high-level in vitro resistance to 3TC and FTC and low/intermediate resistance to ABC (3-fold reduced susceptibility). M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT and TDF and are associated with clinically significant reductions in HIV-1 replication.
210W	NRTI	L210W is a TAM that usually occurs in combination with M41L and T215Y. The combination of M41, L210W and T215Y causes high-level resistance to AZT and intermediate resistance to ABC and TDF.
215SCDEIVALN	NRTI	T215Y/F are TAMs that causes intermediate/high-level resistance to AZT and potentially low-level resistance to ABC and TDF. T215S/C/D/E/I/V/N/A/L do not reduce NRTI susceptibility but arise from viruses that once contained T215Y/F. The presence of one of these revertant mutations suggests that the patient may have once been infected with a virus containing T215Y/F.
215YF	NRTI	T215Y/F are TAMs that causes intermediate/high-level resistance to AZT and potentially low-level resistance to ABC and TDF.

https://hivdb.stanford.edu/dr-summary/comments/NRTI/

1. All DRMs that receive a mutation penalty score and some that don't are accompanied by a comment.
2. The complete list of comments for each drug class can be viewed on the website
3. The comments have last been updated March 2024

Pre-Computed Scores for All DRM Patterns

Pattern	# Sequences	ABC	AZT	FTC	3TC	TDF
M184V	16214	15	-10	60	60	-10
M41L + M184V + T215Y	1944	50	75	65	65	15
A62V	1753	5	5	0	0	5
D67N + K70R + M184V + K219Q	1414	50	60	70	70	15
M41L + M184V + L210W + T215Y	1196	85	110	80	80	50
K65R + M184V	1110	60	-20	90	90	40
K70R + M184V	1105	20	20	60	60	-5
M184V + T215Y	1070	25	50	60	60	0
M184I	889	15	-10	60	60	-10
M41L	859	5	15	0	0	5
K65R	832	45	-10	30	30	50
M41L + T215Y	821	25	85	5	5	25
K70R	763	5	30	0	0	5
T215S	750	0	10	0	0	0
A62V + M184V	733	20	-5	60	60	-5
L74V + M184V	732	60	-10	60	60	-10
M41L + L210W + T215Y	720	60	120	20	20	60

https://hivdb.stanford.edu/dr-summary/pattern-scores/NRTI/

1. There is also a table that lists precomputed scores for all combinations of DRMs present in the database.
2. The table can be sorted by the # sequences so that the most common DRM patterns are shown at the top or by those DRMs associated with the highest scores for an NRTI.
3. It is very useful for us to check this table to make sure that updates to the mutation penalty scores lead to the results intended for actual virus isolates

Mutations Associated with Reduced Susceptibility to NRTIs

For questions and suggestions:
hivdbteam@lists.Stanford.edu

1. Thank you for your attention.
2. If you have any questions or suggestions don't hesitate to email us.