Antiviral Activity of Tenofovir Alafenamide (TAF) against HIV-1 Subtypes and Emergence of K65R

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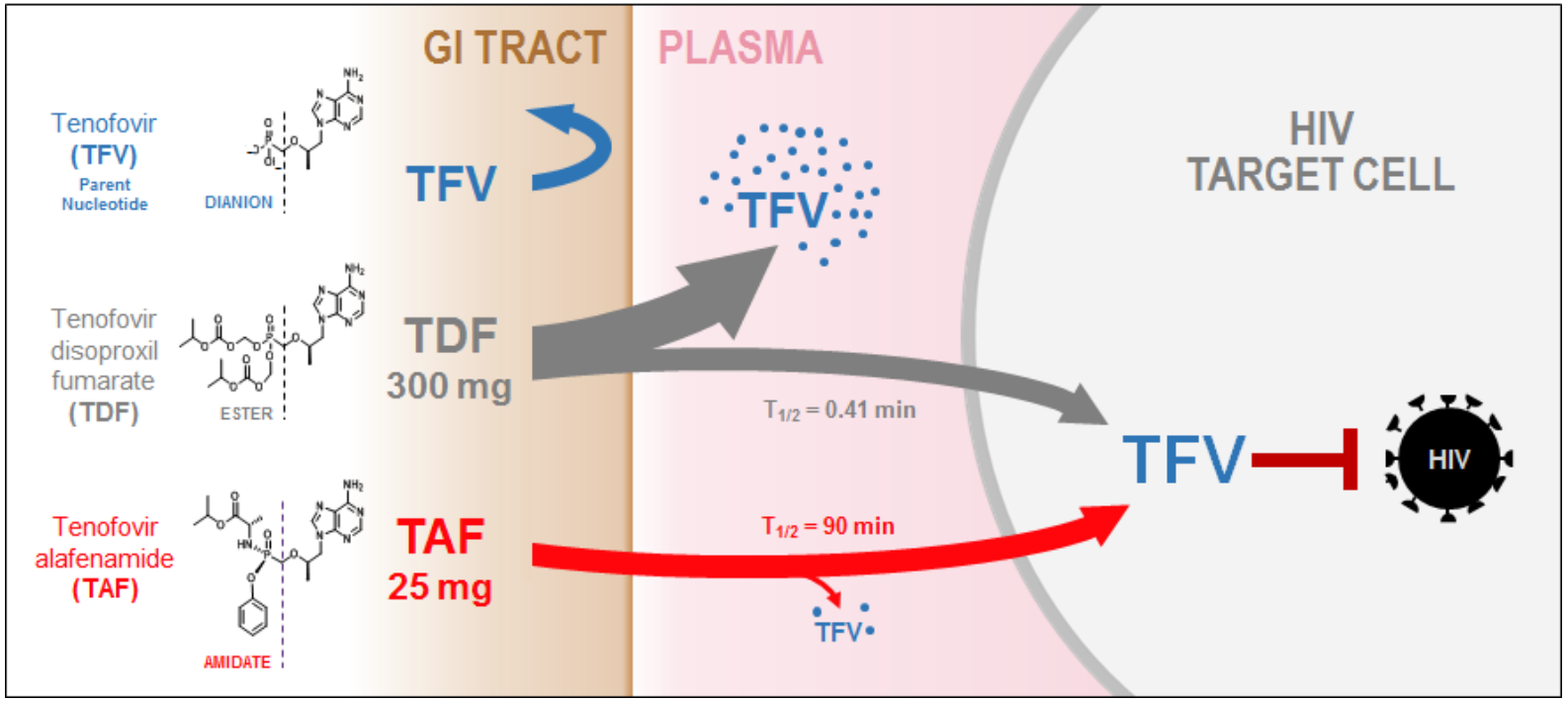
(See Cox *et al* - Poster O-07)

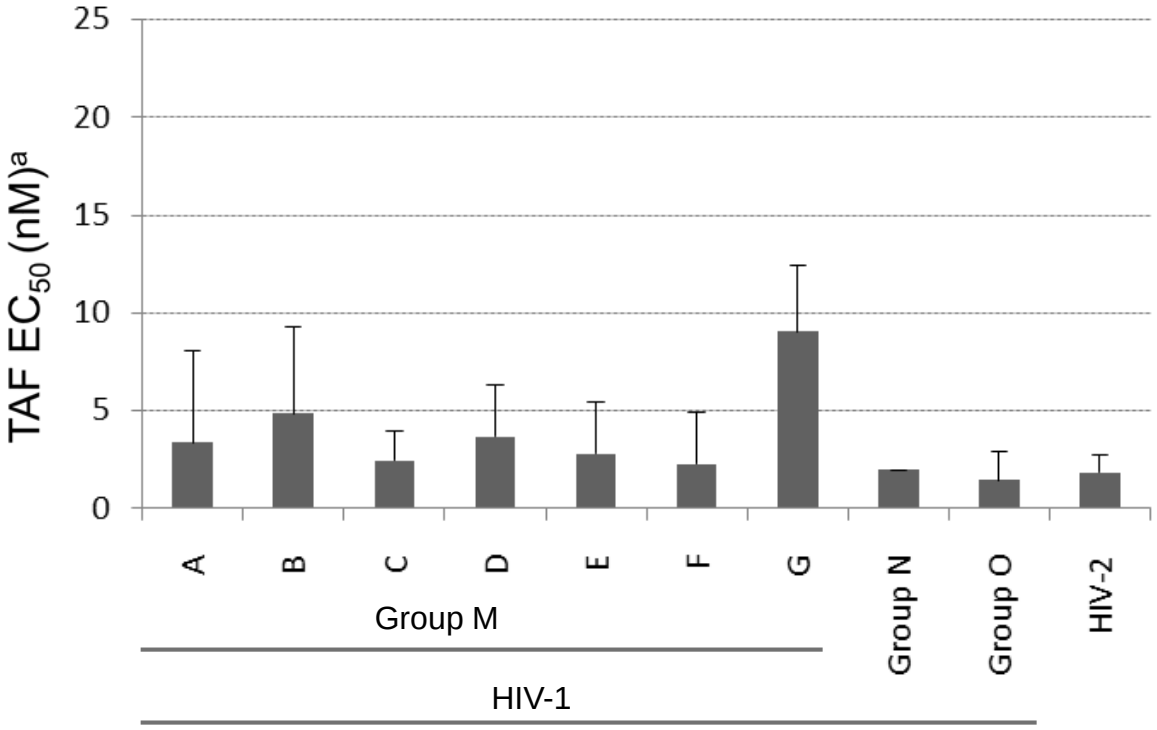
Tenofovir Alafenamide (TAF)



TAF:

- novel prodrug of the HIV-1 NRTI tenofovir (TFV)
- requires a lower dose (vs. TDF)
- has greater plasma stability (vs. TDF)
- reduces circulating TFV
- improves the delivery of intracellular TFV

TAF Antiviral Activity



[a] Mean EC_{50} and standard deviation

- Panel of HIV-1 and HIV-2 primary isolates tested in PBMCs (~ 3 each).
- TAF is active against all HIV-1 and HIV-2 isolates tested
- TAF mean EC_{50} = 3.5 nM (from 29 HIV-1 isolates tested in PBMCs)

From Callebaut et al AAC, 2015

Clinical Data & Resistance

- TAF has been co-formulated with other ARVs:
  - with FTC, EVG, COBI → E/C/F/TAF
  - with FTC, RPV → R/F/TAF
  - with FTC → F/TAF

- E/C/F/TAF: Clinical Studies conducted worldwide
  - More than 14 subtypes represented in treated population
  - Tx-Naïve: B (86.1%), AE (7.3%), C (1.5%), AG (1.5%), all others (3.6%)

- E/C/F/TAF: Highest efficacy ever seen in treatment naïve population (>90%)
  - Very low resistance observed :
    - 0.8% by Week 48
    - 1.2% by Week 96

K65R in HIV-1 Subtype C

- ***Observation:***
- Brenner *et a*l., AIDS, 2006
  HIV-1 subtype C rapidly develop K65R resistance to tenofovir in cell culture
- In vitro TFV dose escalation selections
- K65R:
  - Subtype B → ~ 55-75 weeks
  - Subtype C → ~ 12 weeks
- MOA associated with stretch of AAAAA in codons 64-65 in RT

- ***Response:***
- Miller *et al*., AIDS, 2006
  K65R development among subtype C HIV-1-infected patients in tenofovir DF clinical trials
- In vitro TFV dose escalation selections
- K65R:
  - Subtype B → ~ 8-16 weeks
- HIV-1-infected patients in tenofovir DF
  - Phase 3 Clinical Studies:
    (Study 903, Study 934)
    No K65R in patients w/ subtype C

"*Updated*" Response

- ***In vitro***
- Selection of virus panel: subtypes B and C (64-65-66 in RT)
- Panel Characterization
- Dose-escalation Resistance Selection
- Breakthrough Viral Growth (Resistance Barrier TAF vs. TFV)

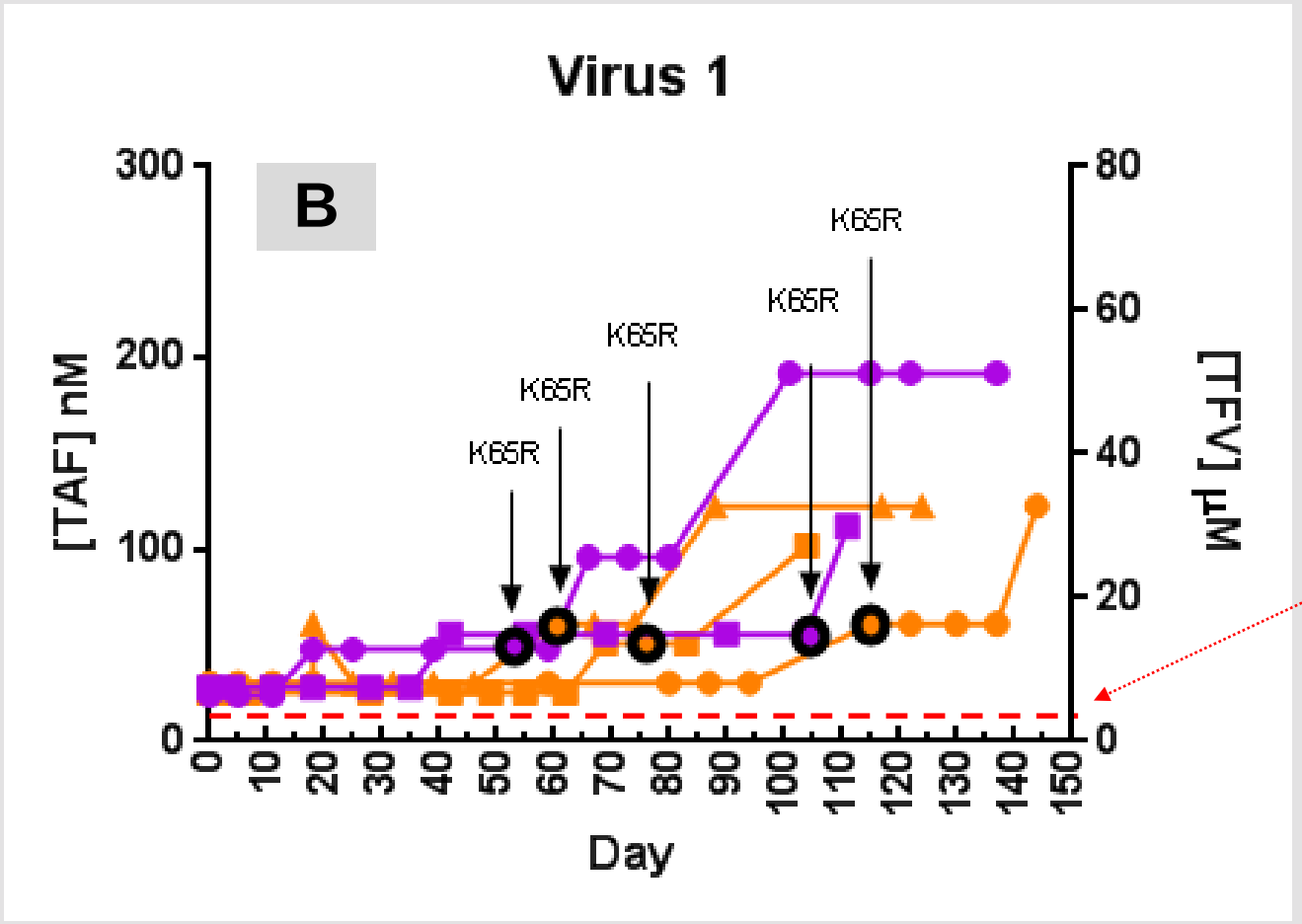
- ***Analysis of Clinical Studies***
- Multi-study Database Analysis

Genotypic & Phenotypic Characteristics of Viral Isolates

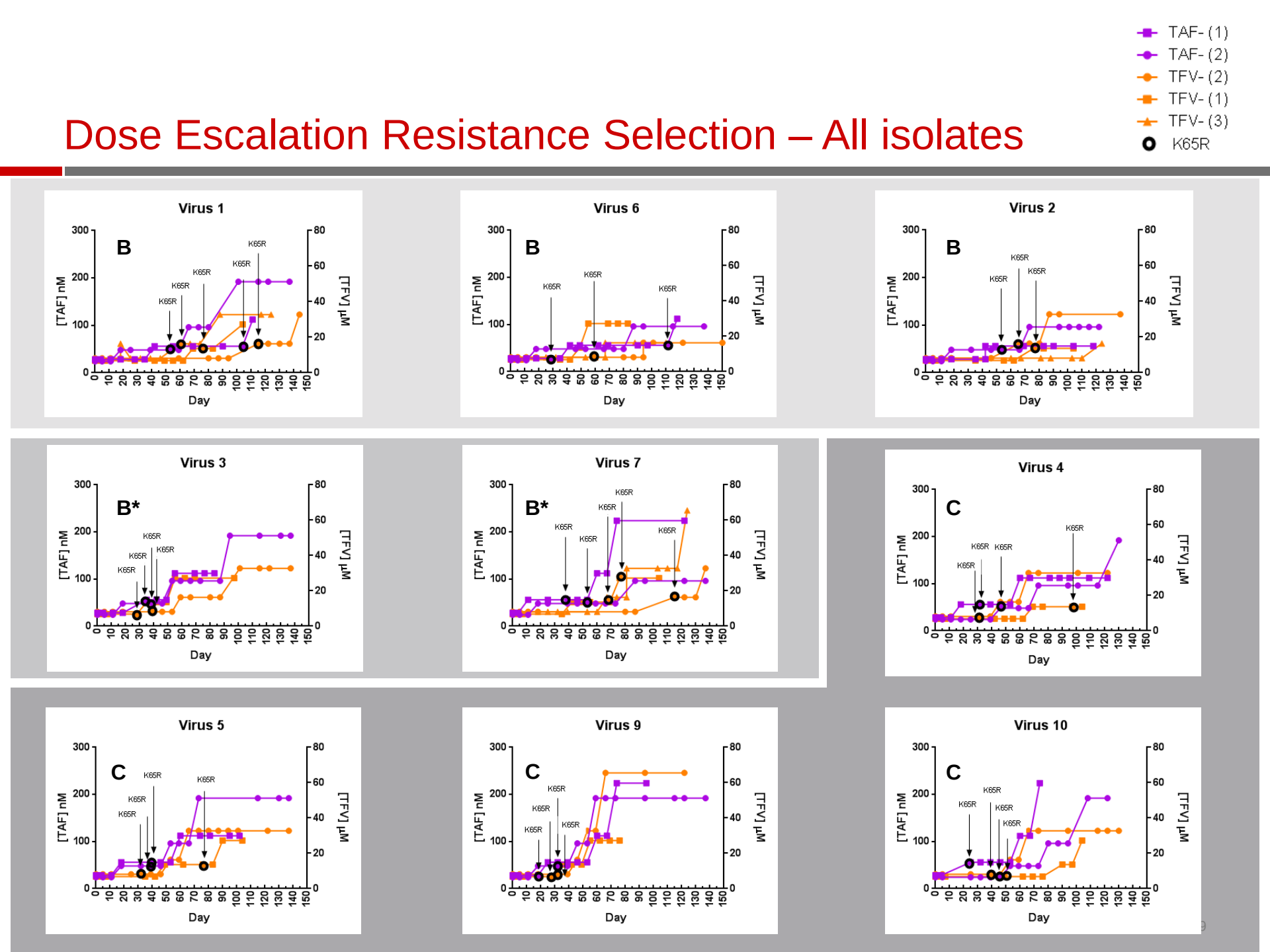
Isolate ID	Subtype	RT Codons 64 - 65 - 66	Fold Change (EC_{50} FC from Wild-Type Control)			
			MT-2 Assay			
			TAF	TFV	RAL	ATV
1	B	AAG AAA AAA	1.1	1.0	1.1	0.9
6	B	AAG AAA AAA	1.3	1.0	1.2	0.9
2	B	AAG AAG AAA	0.9	0.7	0.9	0.7
3	B*	AAA AAG AAA	0.9	0.8	1.0	0.9
7	B*	AAA AAG AAA	1.3	0.9	1.1	0.9
4	C	AAA AAG AAG	1.0	0.8	0.9	0.8
5	C	AAA AAG AAG	1.1	1.0	1.0	0.9
9	C	AAA AAG AAG	1.0	1.1	0.8	0.7
10	C	AAA AAG AAG	1.0	0.7	1.3	1.0

B*: subtype B with RT codons at positions 64 and 65 that are identical to most prevalent subtype C isolates.

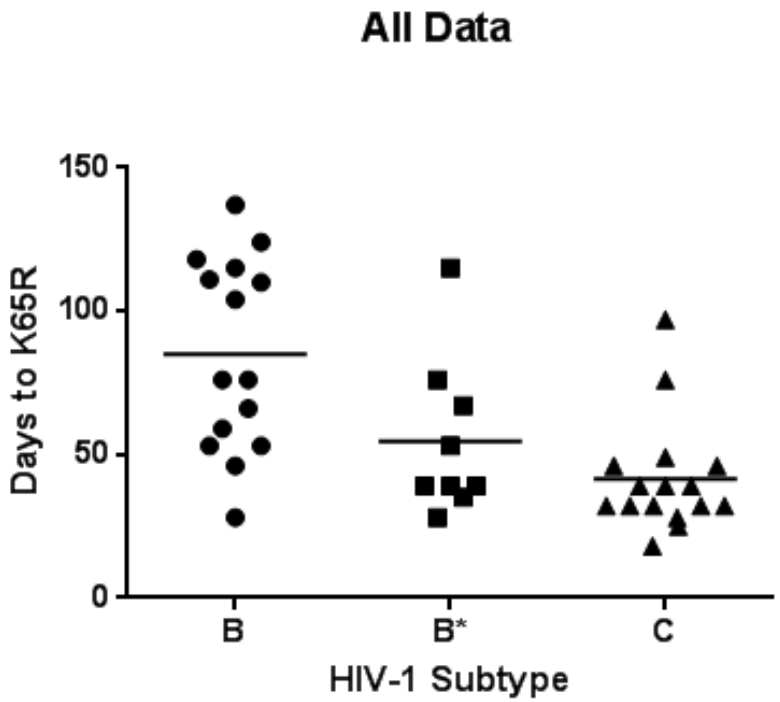
Dose Escalation Resistance Selection (Virus 1 – sub. B)



Dose Escalation Resistance Selection – All isolates

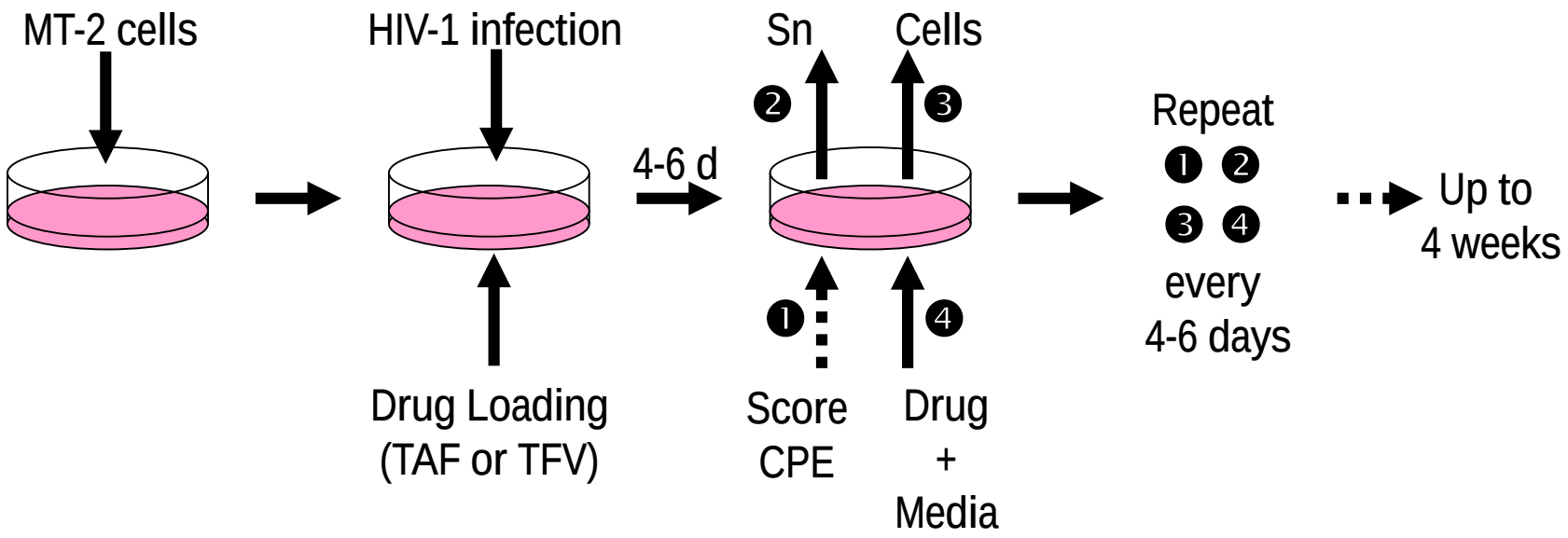


Summary of Resistance Selections



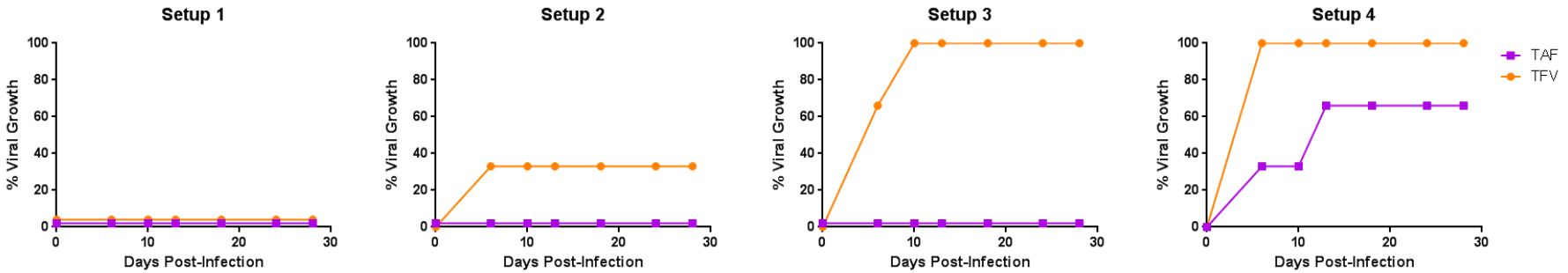
- Variability in the time to gain K65R upon repeat with the same isolates
- Trend for subtype C viruses to gain the K65R mutation more rapidly
- Similar trend for the B*viruses (same 64-65 motif as subtype C)
- No consistent difference in time of K65R development between TAF and TFV

Breakthrough Viral Growth



Breakthrough Viral Growth – Subtype B

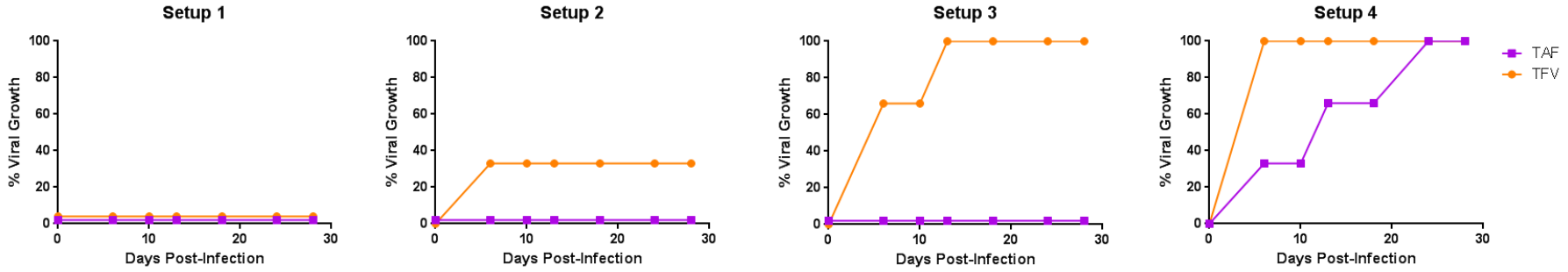
Viral Isolate 1: Subtype B



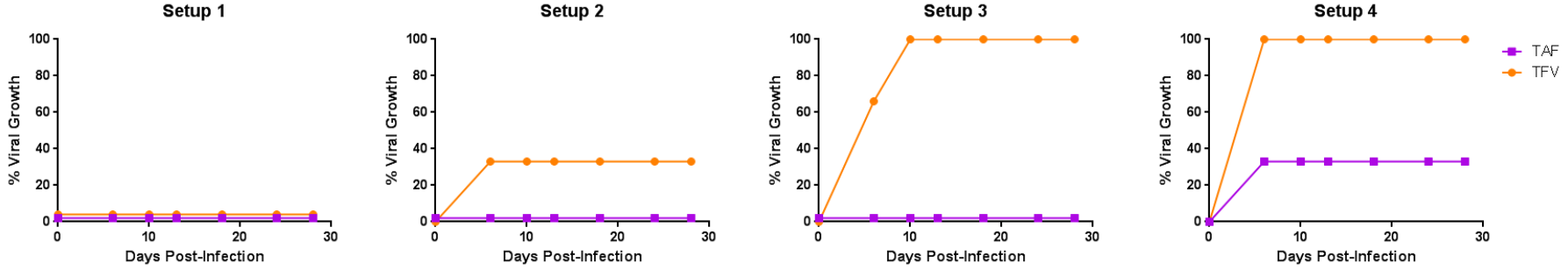
Drugs used at physiological concentration → 2X less drug → 2X less drug → 2X less drug

Breakthrough Viral Growth – Subtype B (continued)

Viral Isolate 6: Subtype B

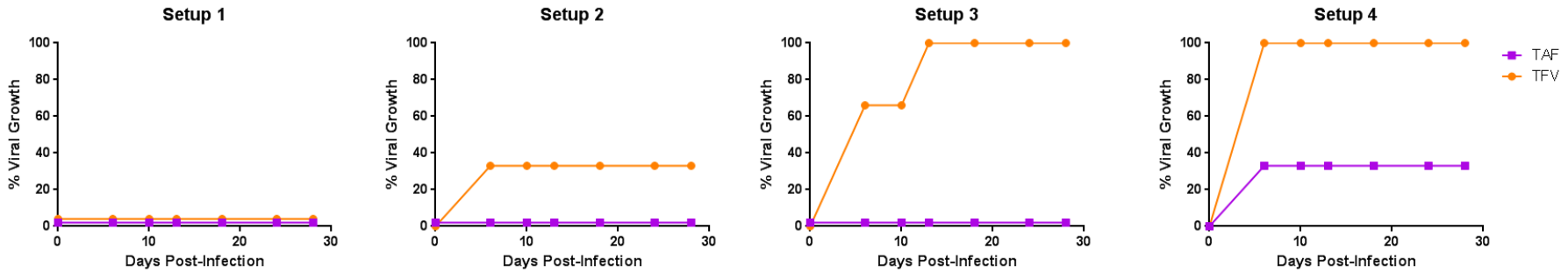


Viral Isolate 2: Subtype B

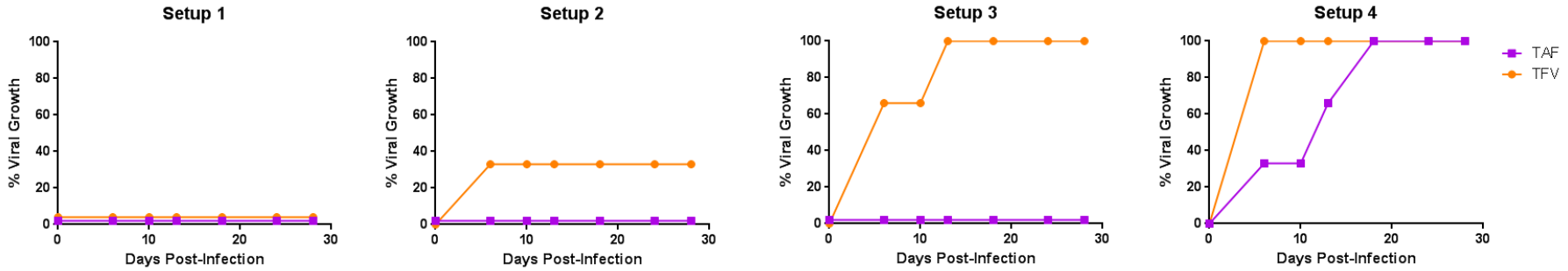


Breakthrough Viral Growth – Subtype B*

Viral Isolate 3: Subtype B*

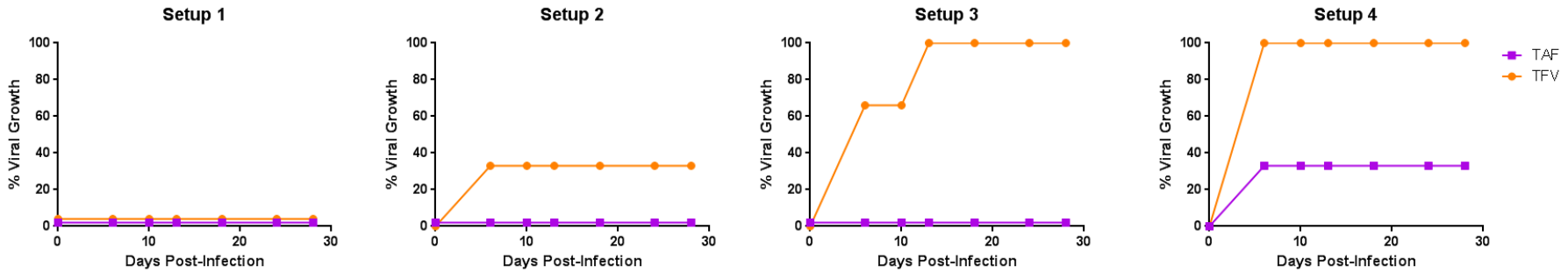


Viral Isolate 7: Subtype B*

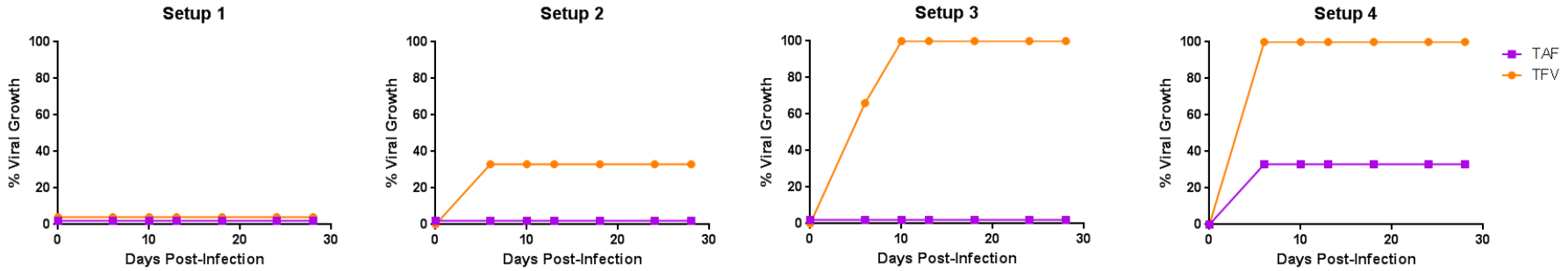


Breakthrough Viral Growth – Subtype C

Viral Isolate 4: Subtype C

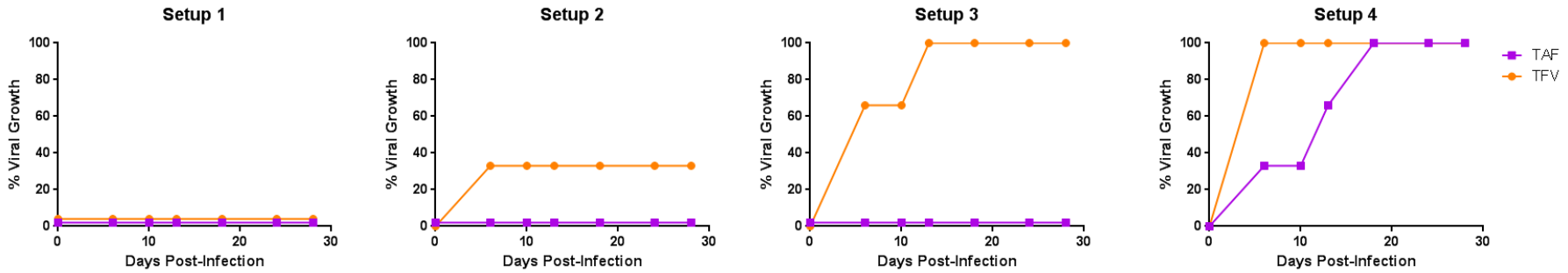


Viral Isolate 5: Subtype C

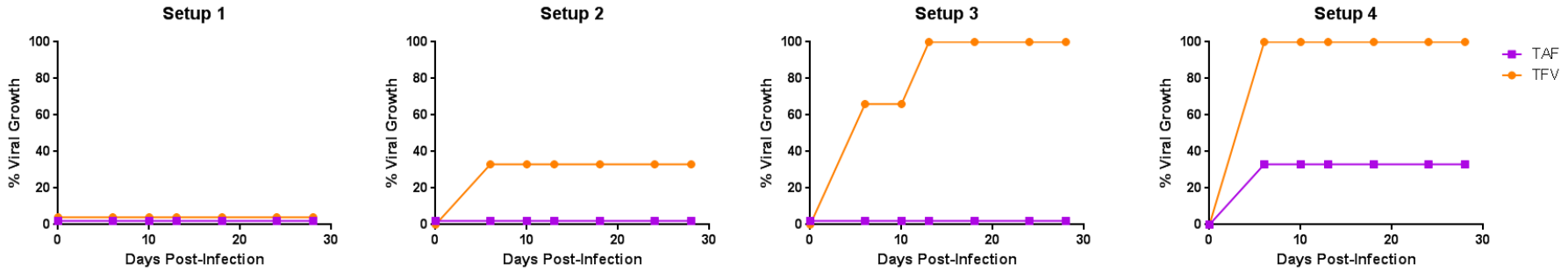


Breakthrough Viral Growth – Subtype C (continued)

Viral Isolate 9: Subtype C



Viral Isolate 10: Subtype C



Summary of Breakthrough Viral Growth

Isolate ID	Subtype	Codon RT 64-65-66	TAF Phenotypic Fold-Change	Time to Viral Breakthrough (days)							
				Setup#1		Setup#2		Setup#3		Setup#4	
				TAF 400 nM	TFV 25.0 µM	TAF 200 nM	TFV 12.5 µM	TAF 100 nM	TFV 6.3 µM	TAF 50 nM	TFV 3.1 µM
M	B	AAG AAA AAA	1.0	>28	>28	>28	6	>28	6	6	6
1	B	AAG AAA AAA	1.1	>28	>28	>28	6	>28	6	6	6
6	B	AAG AAA AAA	1.3	>28	>28	>28	6	>28	6	6	6
2	B	AAG AAG AAA	0.9	>28	>28	>28	6	>28	6	6	6
3	B*	AAA AAG AAA	0.9	>28	>28	>28	6	>28	6	6	6
7	B*	AAA AAG AAA	1.3	>28	>28	>28	6	>28	6	6	6
4	C	AAA AAG AAG	1.0	>28	>28	>28	6	>28	6	6	6
5	C	AAA AAG AAG	1.1	>28	>28	>28	6	>28	6	6	6
9	C	AAA AAG AAG	1.0	>28	>28	>28	6	>28	6	6	6
10	C	AAA AAG AAG	1.0	>28	>28	>28	6	>28	6	6	6

- No difference in TFV or TAF antiviral activity between subtype B vs. C viruses
- All WT viruses inhibited by TFV & TAF at pharmacologic concentrations (Setup #1)
- Breakthrough viral growth with TFV, but not TAF, at 2x and 4x lower drug levels (Setups #2-3)
- Breakthrough viral growth with TFV and TAF (8x) (Setup #4)

Analysis of Clinical Studies

Time of Analysis	Subtype	Patients with Data (N)	Virologic Failures (All) n	Virologic Failures (K65R) n (%)
2006	C	10	1	0
	Non-C	540	62	8 (1.5%)
2016	C	77	1	0
	Non-C	5479	245	17 (0.3%)

- 2006: Analysis of available clinical trial data evaluating 550 patients treated with tenofovir (TDF): K65R not observed in any subtype C patient
- 2016: Analysis of available clinical trial data evaluating 5556 patients from 9 clinical studies treated with TDF or TAF: K65R not observed in any subtype C patient and a low incidence (0.3%) was observed in non-subtype C patients

Conclusion

- As previously reported, subtype C HIV-1 isolates can select K65R more quickly than subtype B viruses *in vitro*
- This difference appears correlated with codon usage at positions 64-65-66 in RT, and is also found in some subtype B isolates (B*)
- At pharmacologic drug concentrations, both TAF and TFV were able to prevent viral breakthrough all viruses from both subtypes
- At sub-therapeutic drug concentrations, only TAF maintained antiviral activity against subtype B and C viruses, suggesting that higher intracellular drug concentrations achieved with TAF may result in reduced resistance development among individuals with lower drug adherence regardless of subtype
- Analyses of patients in clinical studies (n=5556) shows limited virologic failure for patients on TDF- and TAF-containing regimens, with K65R rarely seen overall and not observed in with any subtype C patient (n=77)

Acknowledgements

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- Clinical Virology
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- Clinical Pharmacology
- Clinical Operations
- Clinical Research
- Biometrics
- Regulatory Affairs
- Medical Affairs