

Diagnosing & Managing Treatment Failure

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Overview

- CD4 and VL as biomarkers
- Causes of treatment failure
- Mechanism of Virologic resistance
- Defining Virologic failure
- Limitations of genotypic testing
- Genetic barriers to resistance
- Mutations- rationale for stepwise regimens
- Case Discussion depending on time.

Goal of HAART

Durable Viral Suppression
Undetectable Levels

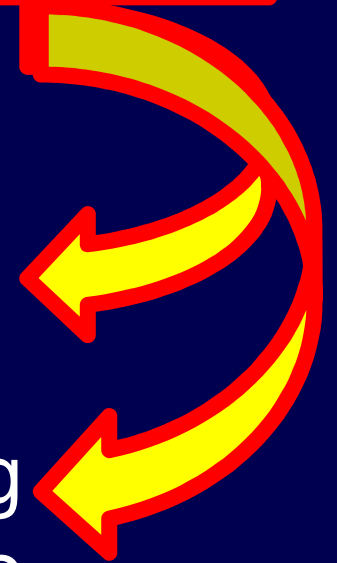
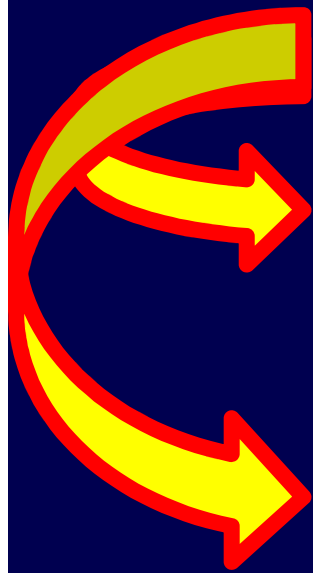
Halt disease
progression

Immunological
recovery

Reduce OIs

Prevent drug
resistance

Reduce viral
transmission



Factors that Contribute to Treatment Failure

- Sub-optimal potency of regimen
- Insufficient drug levels

Note!

– Non adherence

– Not all treatment

– Malabsorption

– failure is due to

– Drug interactions (herbal meds,

OTCs)

– resistance

- Resistant virus

Bio Marker

- You can only monitor if you can measure
 - Viral load
 - CD4 count
- Need to know what to expect to interpret

Value of CD4 count

- Therapeutic decisions -antiviral treatment, prophylaxis)
- Differential diagnosis of OIs
- Predicting prognosis

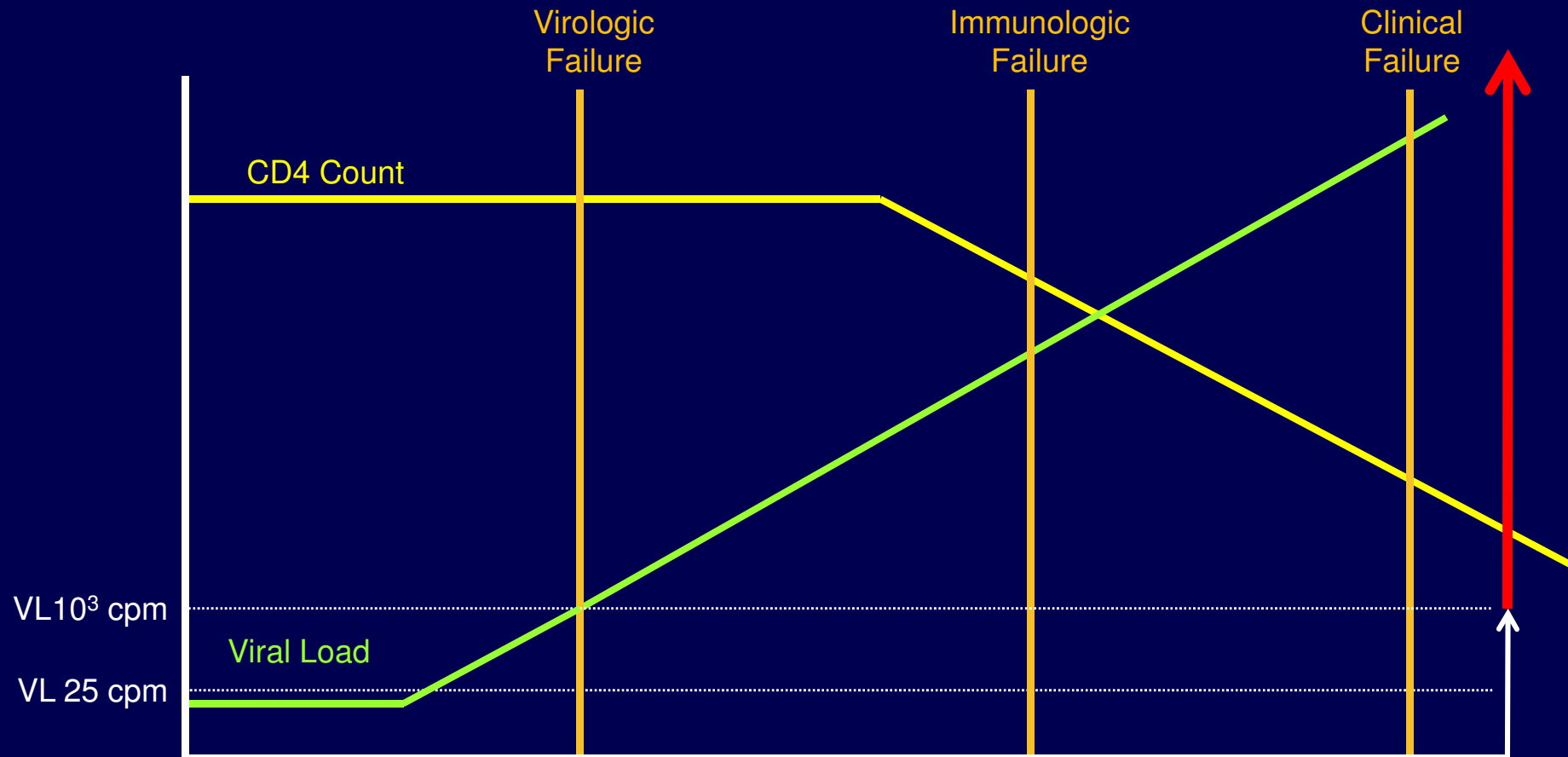
CD4 count - 500-1400/ μ L

- 3 analytic steps \Rightarrow total WCC, % LC, % CD4
 \Rightarrow wide analytic variation
- Seasonal variation, diurnal variation.
- Inter-current illness
- Corticosteroids.
- Splenectomy.
- Age in adults, gender, psychological stress, physical stress, pregnancy \Rightarrow no effect
- Trend needs to be monitored

Viral load

- Plasma HIV RNA load \Rightarrow most representative and sensitive test for monitoring:
 - Risk of progression.
 - Response to ART
 - Failure of ART.
- VL change >0.3 log (2 fold) is signif.

Measuring Viral Load → Earliest & most sensitive Marker of Rx Failure



Murri R, et al. *JAIDS*. 2006;41:23-30.

Losina E et al, *15th CROI* 2008, #823

Pillay D, et al. *14th CROI*, Los Angeles 2007, #642

Viral Load Response

- Expected decay in VL in ART naïve patients on potent ART:
 - 0.75 - 1 Log_{10} in one week
 - 1.5 - 2 log_{10} in 4 weeks (<5000cpm)
 - <500cpm in 8-16 weeks
 - <50 24-48 weeks

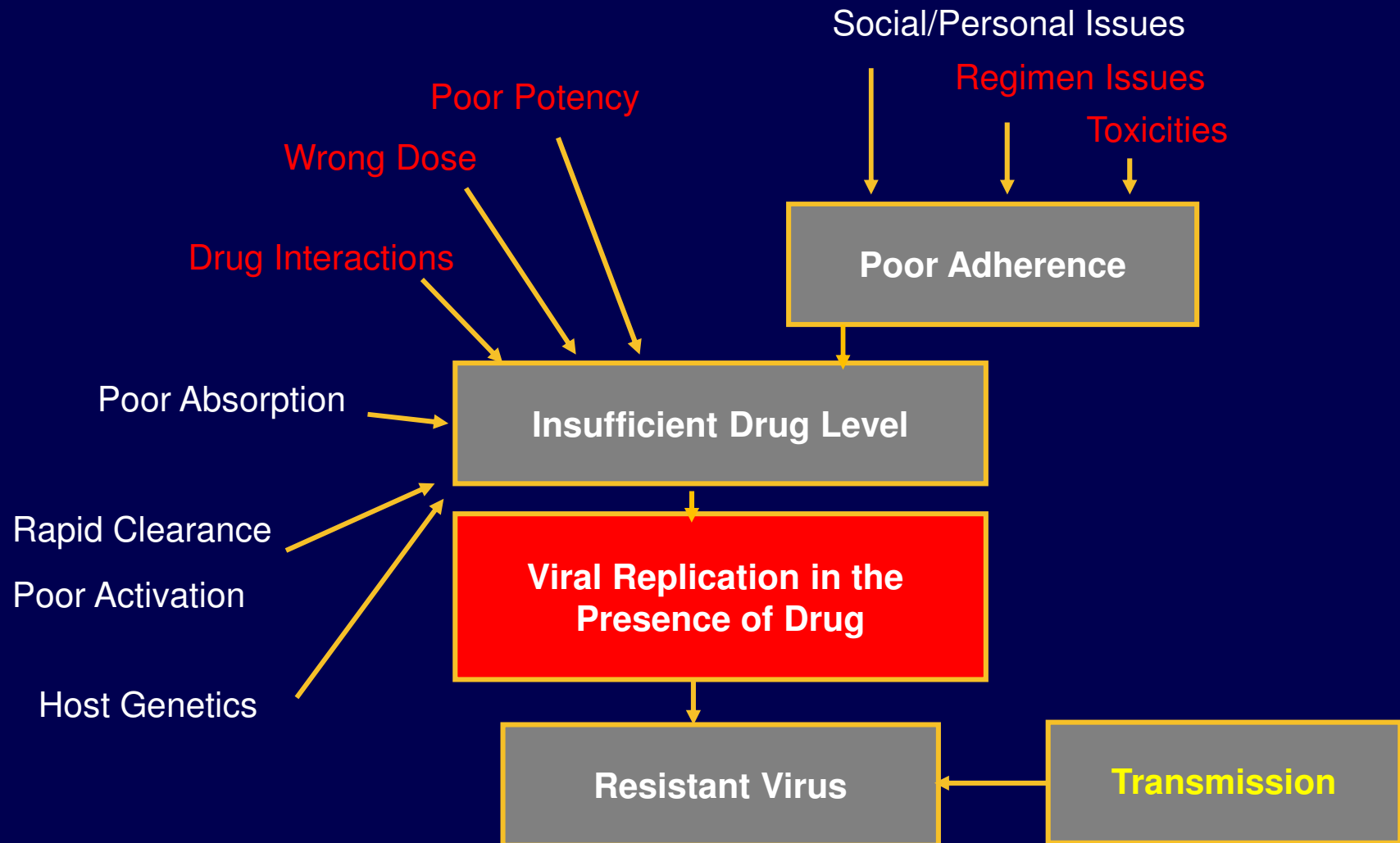
Virologic/Treatment failure

2 consecutive viral loads
>1000cpm

Treatment failure

- Check for:
 - Adherence
 - Tolerability
 - Dosing schedule
 - Drug interactions
- Repeat VL in 2 months $>1000 \Rightarrow$
change regimen

Factors that contribute to the Development of Resistance

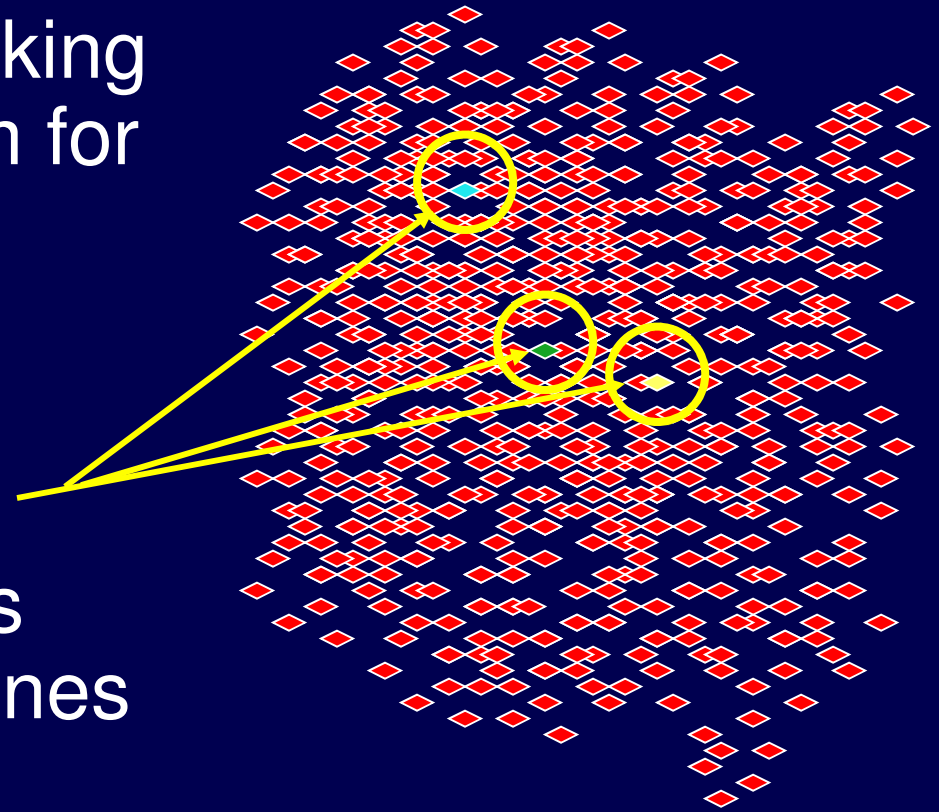


How Resistance Mutations Arise

- HIV replication \Rightarrow error prone:
 - DNA Replication 1:10⁹
 - HIV Replication 1:10⁴
 - RNA Synthesis 1:10⁴
 - Airline Baggage Loss 1:200
 - Good Typist 1:100
- 10⁹ viral particles produced/day
 - All possible mutations emerge daily
- Persistence of mutant depends on fitness

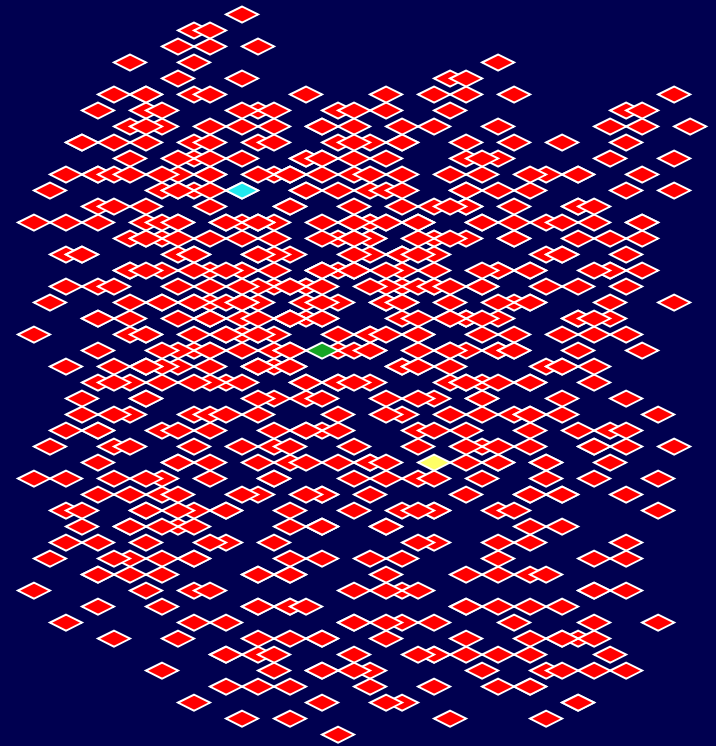
Growth in the absence of inhibitory pressure

- HIV multiplies freely taking the most optimum form for rapid growth → wt.
- As it proliferates, HIV undergoes spontaneous mutations in random genes due to error prone RT enzyme.



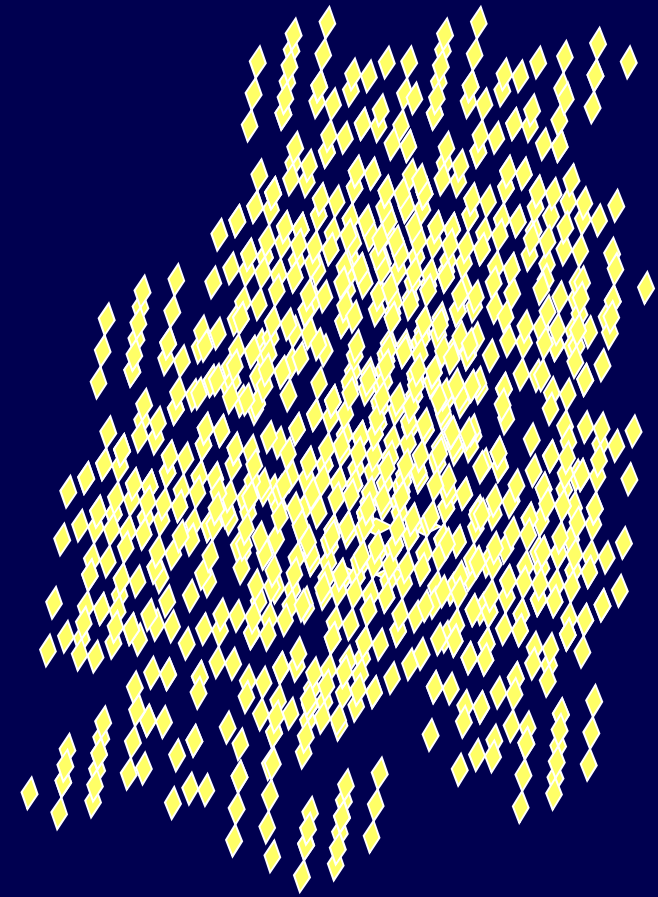
Growth in the presence of ARV pressure

- ARVs kill all of the original wild type organisms
- but**
- The mutated virus which is **RESISTANT** survives.



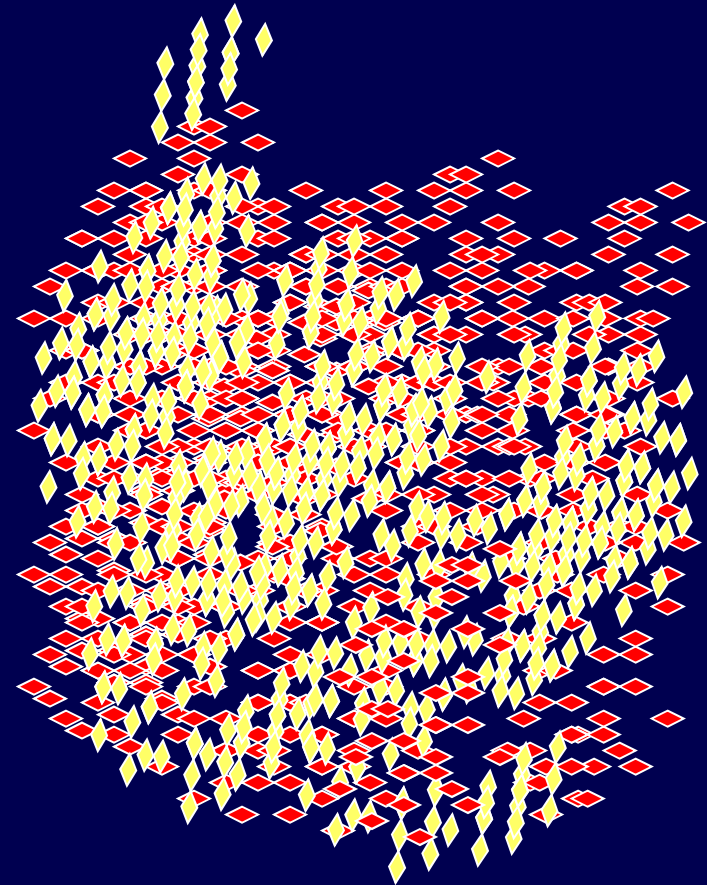
Growth in the presence of ARV pressure

- The mutated HIV grows and multiplies, even in the presence of ARVs.
- This virus is now **RESISTANT** and will continue to replicate albeit at a slower rate due to reduced fitness.



Growth in the absence of ARVs Treatment Interruption

- Wt. - replicative advantage
- Wt. - dominant species



Resistance is Irreversible

- Once selected resistance mutations remain archived in mononuclear cells
- When drug pressure is discontinued, mutations ↓ below 20% ⇒ not detected
- Recycling drugs ⇒ rapid reappearance (>20%)
– history of drug use is critical.

Facts on resistance testing

- Minimum VL required 1000 cpm
- Measures dominant HIV strains (>20%)
- Does not detect virus in sanctuary sites
- Does not detect mutant viruses selected by previous treatments that are “archived”
- Important to obtain comprehensive past drug history & outcome of past regimens

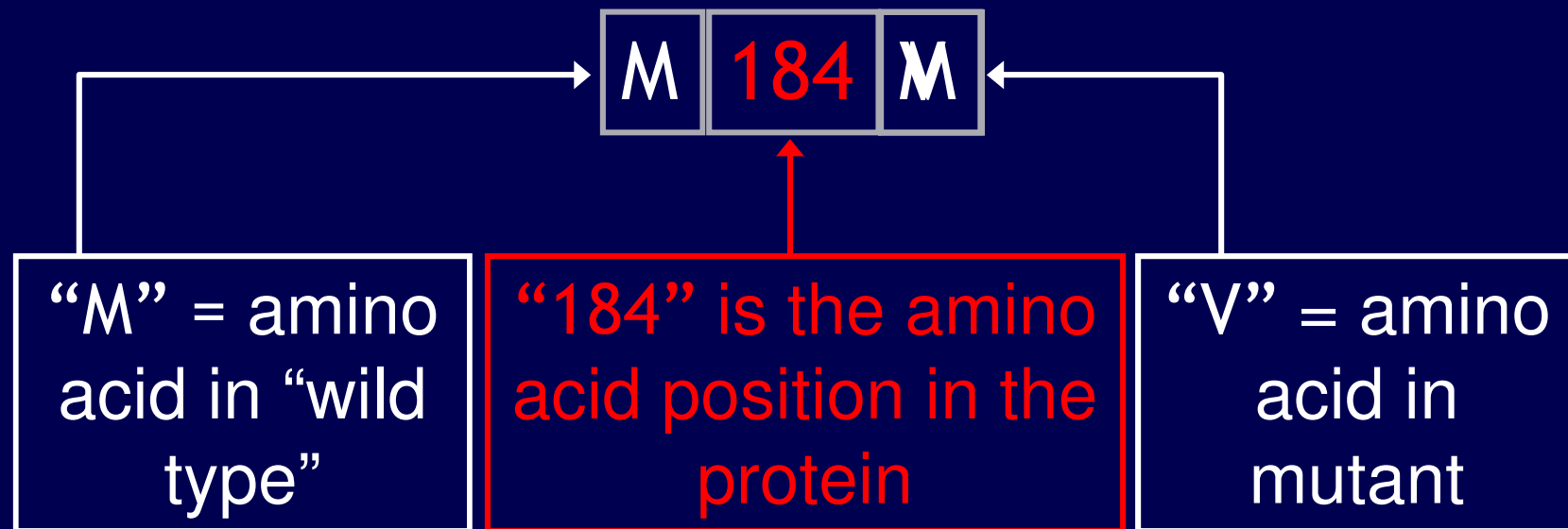
Facts on resistance testing

- Tells you what will not work, not what will work
- Most reliable for indicating Ω to drugs pt is currently on or recently discontinued

Resistance testing must be done
when the patient is on the failing
regimen

Designation of Mutations

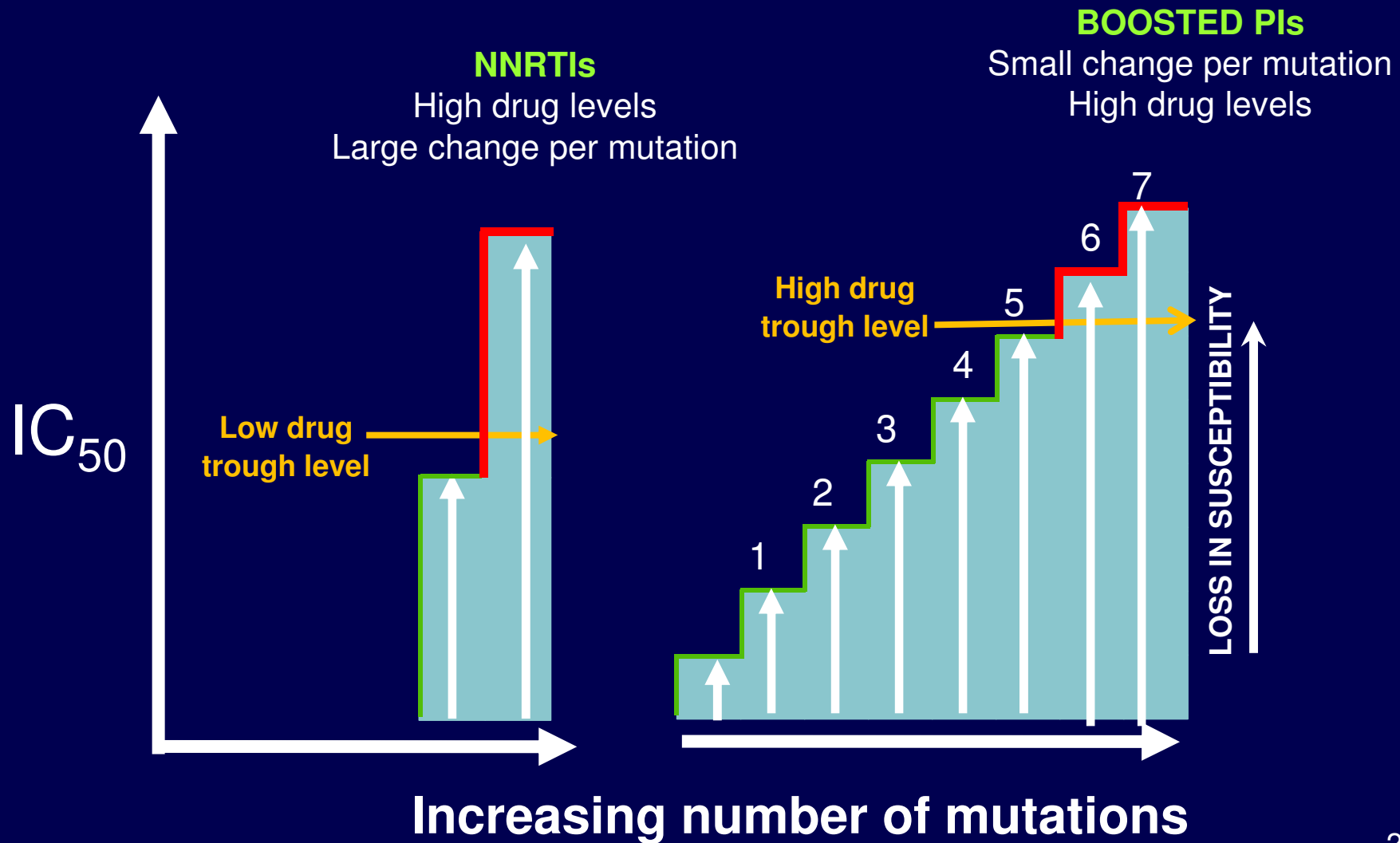
- How do we identify a resistance mutation?



All drugs are not equally susceptible to resistance: Genetic Barrier to Ω

- The genetic barrier to resistance describes:
 - The number of mutations the genome has to undergo to make the virus resistant to the drug.

Pharmacokinetic & Genetic Barriers to Resistance

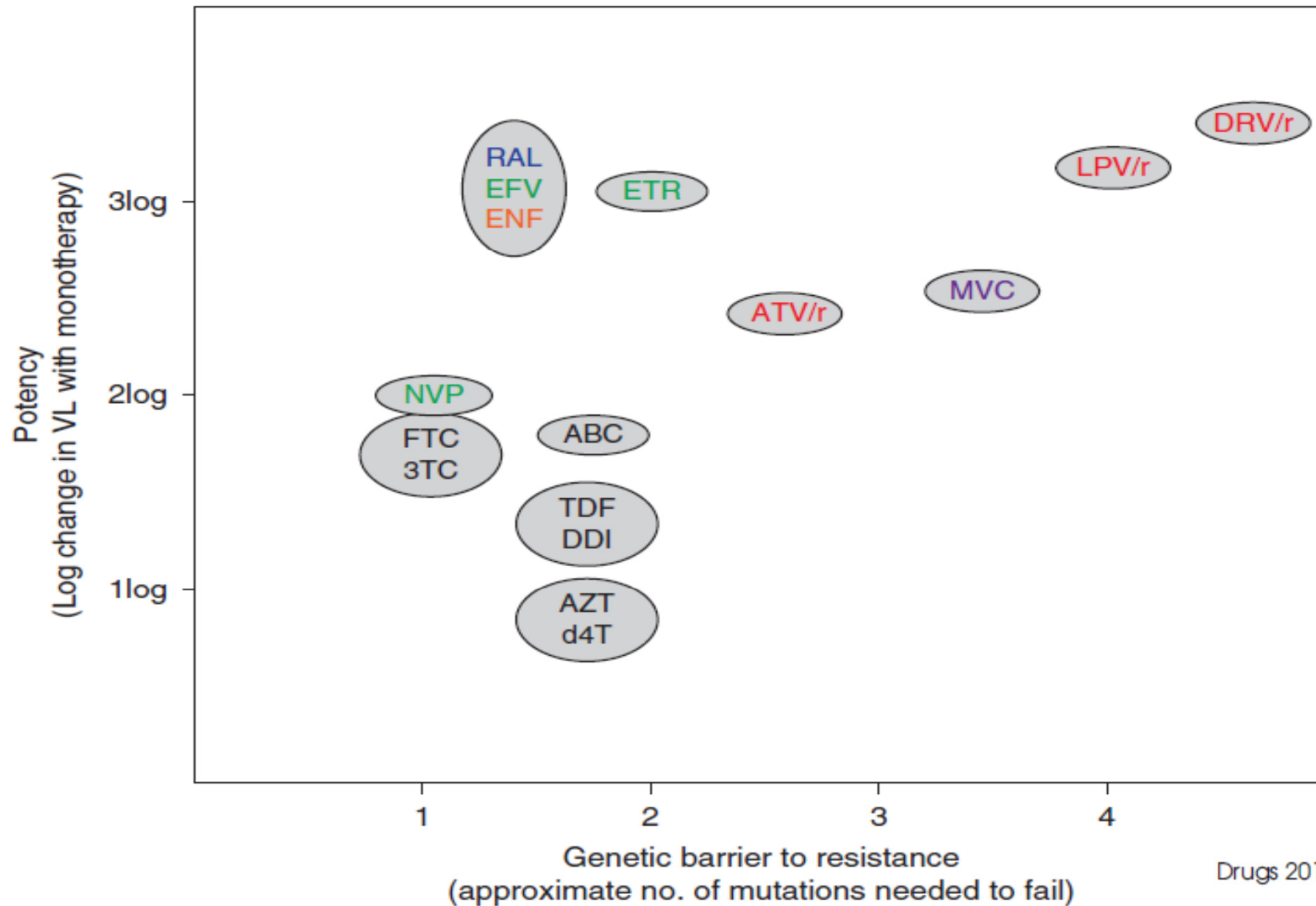


Genetic Barrier of Drug Classes

DRUG CLASS	GB
Unboosted PI	1
NNRTI	1
NRTI	1/2/3 *
Fusion Inhibitor	1
Boosted PI	3–8

***Up to 3 for thymidine analog mutations**

Potency vs. Genetic barrier



Summary: GB to Resistance

- GB of 1 = 1 specific mutation for the drug to lose all activity
- GB of 6 = 6 mutations required for the drug to lose all activity
- Ritonavir boosted PI have a high GB
- NNRTI have a low GB
- A high GB implies there is far less selection of resistance when on a boosted PI based regimen compared to NNRTI based regimen

ABC of HIV Mutations

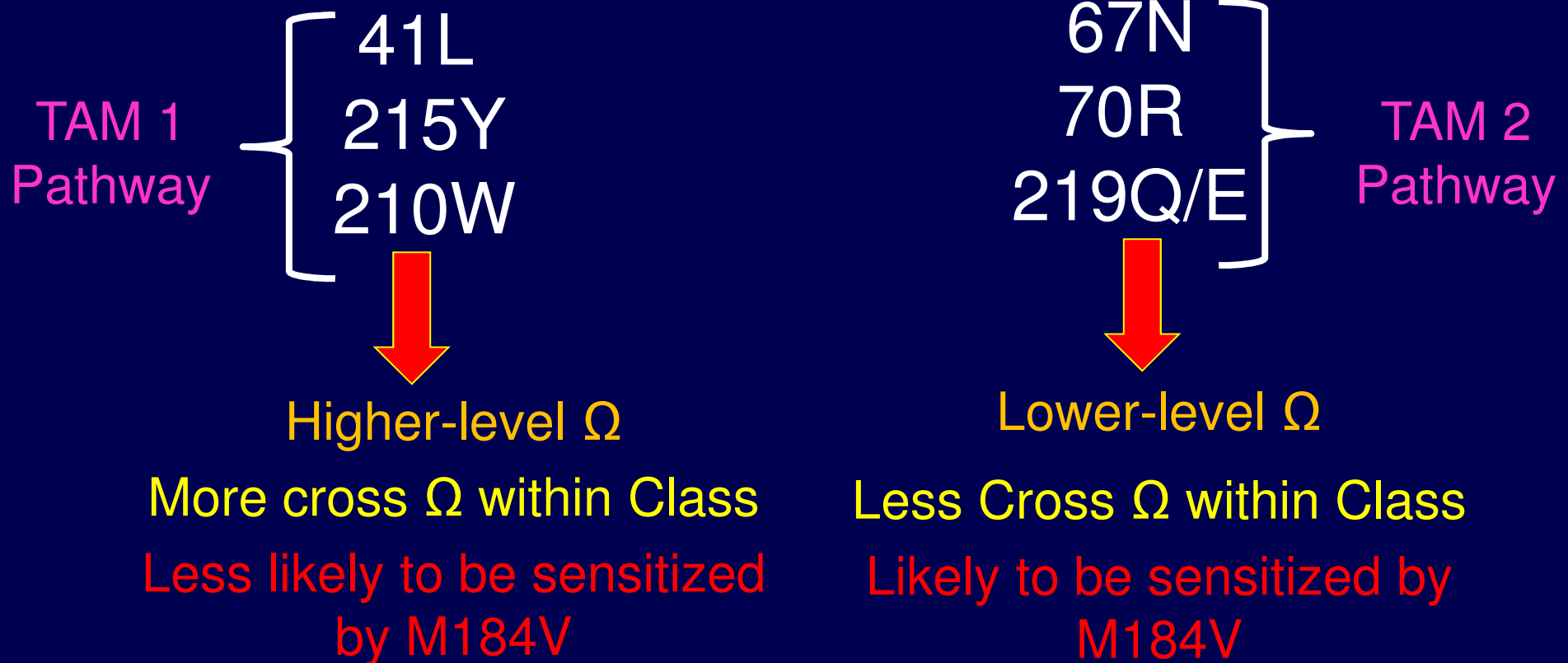
Effects of M184V

- High-level resistance to 3TC / FTC
- AZT, d4T activity enhanced
- TDF activity may be enhanced.
- Decreases 'viral fitness' – decrease VL by about 0.5Log_{10}

Thymidine Analog Mutations TAMs

- Selected for by AZT and d4T
- 3-6 such mutations \Rightarrow reduces AZT susceptibility by 100 fold
- Accumulation of several mutations causes cross-resistance to other NRTIs
- M41L, D67N, K70R, L210W, T215Y/F, K219Q/E

Two Pathways in the Evolution of Thymidine Analog Mutations (AZT/d4T)



K65R Mutation (Non-TAM)

- Selected by ABC, ddi, TDF, d4T.
- Decreases susceptibility to ABC, ddi, TDF & 3TC.
- Increases susceptibility to AZT in the presence of few TAMs
- Rarely occurs with TAMs & L74V
- Does not affect susceptibility to d4T.
- Reduces viral replication esp. with M184V

L74V Mutation (Non-TAM)

- Selected by ddI and ABC,
- Results in resistance to both drugs either alone (ddI) or together with other mutations (ABC)
- HIV quasi species expressing L74V are more sensitive to AZT and TDF

Summary - NRTI Mutations to Remember

- TAMs: M41L, D67N, K70R, L210W, T215Y/F, K219Q/E – AZT, d4T, TDF, ABC, ddI
- M184V - 3TC
- K65R – TDF, ddI, ABC, 3TC
- L74V – ddI and ABC

NNRTIs Resistance Mutations

- NNRTI mutations common at failure
- Often occurs as 1st Ω mutation.
- Most mutations \Rightarrow high level cross- Ω to other NNRTIs
- Mutations do NOT \downarrow replicative fitness
- Do not continue NNRTI if VL not suppressed \Rightarrow additional mutations will compromise 2nd generation NNRTIs

NNRTI Resistance Mutations

- K103N \Rightarrow high level resistance EFV & NVP
- Y181C - high level resistance to NVP & low level resistance to EFV, sensitizes to AZT
- New generation NNRTI- have higher genetic barrier to resistance – main mutation is Y181C, active against K103N mutants
- Etravirine is more robust active against most strains resistant to 1st generation NNRTIs

PI Mutations

- Resistance most complex.
- 2 groups of mutations major and minor
- Major mutations develop first.
- Minor are usually compensatory mutations

Major Protease Inhibitor (PI) Resistance Mutations

	30	32	46	47	48	50	54	76	82	84	88	90
<i>Cons</i>	D	V	M	I	G	I	I	L	V	I	N	L
ATV/r		I	IL	V	VM	L	VTALM		ATFS	V	S	M
DRV/r		I		VA		V	LM	V	F	V		
FPV/r		I	IL	VA		V	VTALM	V	ATSF	V		M
IDV/r		I	IL	V			VTALM	V	AFTS	V	S	M
LPV/r		I	IL	VA	VM	V	VTALM	V	AFTS	V		M
NFV	N		IL	V	VM		VTALM		AFTS	V	DS	M
SQV/r					VM		VTALM		AT	V	S	M
TPV/r		I	IL	VA			VAM		TL	V		

Don't have to know mutations

HIV Drug Resistance Database Stanford

<http://hivdb.stanford.edu/>

Early Warning Indicators

HIVDR Early Warning Indicators (EWI)

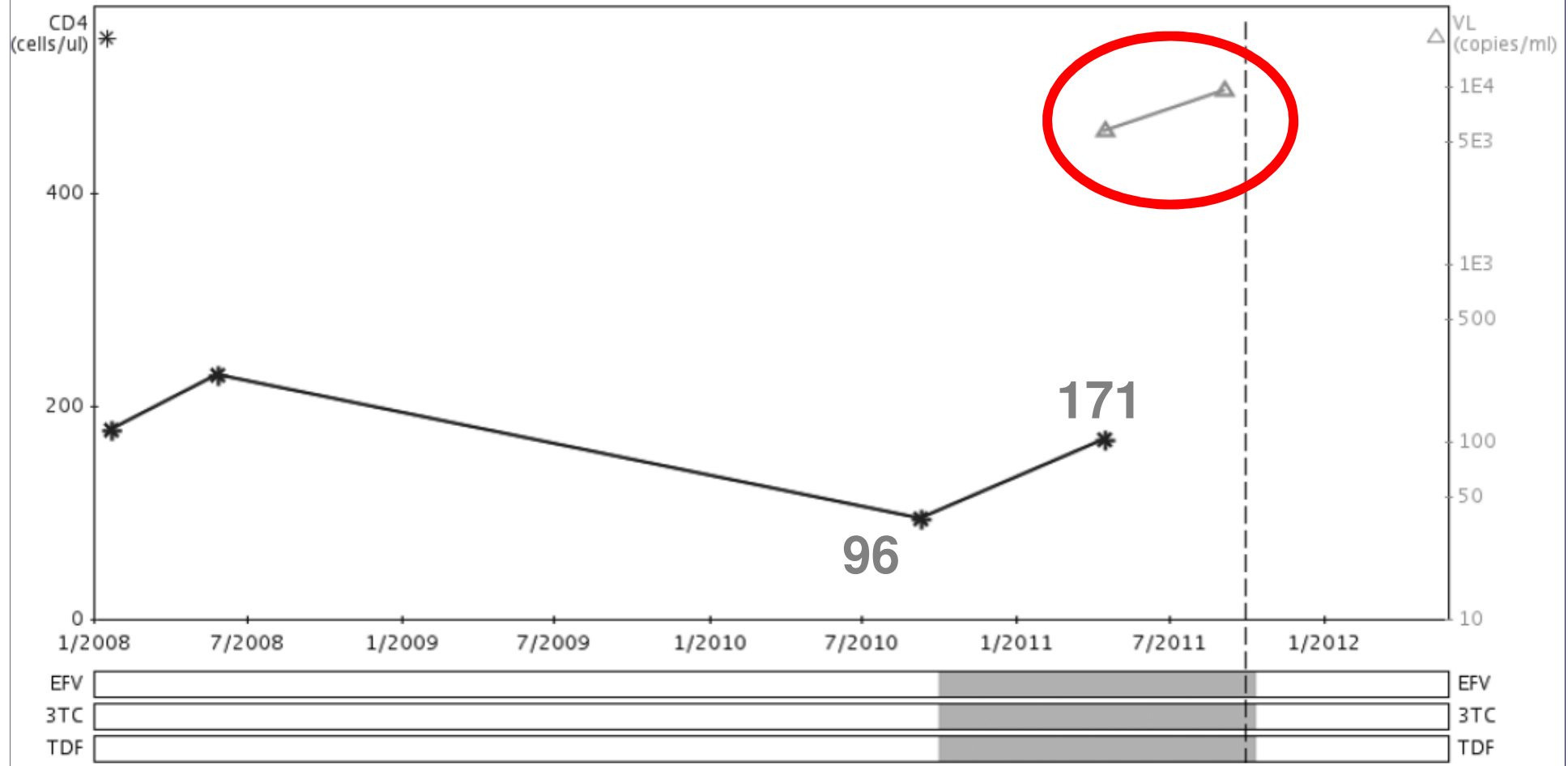
- Pharmacy refill
- Clinic visits
- Pill counts – self reported adherence
- Clinical risk factors
- Psychosocial risk factors

Case 1

39yr ♀ diagnosed HIV in pregnancy in 2007
CD4<200, sdNVP at delivery. 09/2010 initiated
TDF/3TC/EFV. She attended every each clinic
visit on time, knew names & dosages of her
ART, disclosed to family Counseling ⇒ no
specific barriers to adherence. History revealed
a diagnosis of epilepsy on phenobarbital 30mg
dly x ~20yrs & asthma on budesonide &
salbutamol inhalers.

Case 1: Clinical chart

- At 6/12 and 12/12 suboptimal viral



Case 1: Mutations

Drug	Mutations	Description	Level	GSS
zidovudine	184V	Susceptible	1	1.0
zalcitabine	N/A	N/A	N/A	N/A
didanosine	184V	Susceptible	1	1.0
lamivudine	184V	High-level resistance	5	0.0
stavudine	184V	Susceptible	1	1.0
abacavir	184V	Potential low-level resistance	2	1.0
emtricitabine	184V	High-level resistance	5	0.0
tenofovir	184V	Susceptible	1	1.0
nevirapine	103N 108I 225H	High-level resistance	5	0.0
delavirdine	103N 108I 225H	High-level resistance	5	0.0
efavirenz	103N 108I 225H	High-level resistance	5	0.0
etravirine	103N 225H	Low-level resistance	3	0.5

Case 1: Interpretation

- Pt. failing for a short time
- Only NNRTI resistance (K103N, P225H, V108I) and the M184V mutation
- AZT & TDF remain viable options.
- Both std. 2nd line regimens (AZT/3TC/LPVr and TDF/3TC/LPVr) are genotypically susceptible

Case 1: Recommendations

- Should do well on a standard second-line
- Can use AZT if Hb > 10 g/dl and does not have a high risk of metabolic complications.
- Test for HBV- if has active HBV use TDF.
- Intensive adherence support needed
- Use of alternative remedies & social deterrents to adherence must be explored.
- Monitor for IRIS
- Monitor renal function at baseline & 3 months – more frequently if risk factors for renal Dx

Case 1: Question

- Can you give 2 reasons why this patient might have developed ART resistance?
- Would you make any other changes to her medication?

Case 1

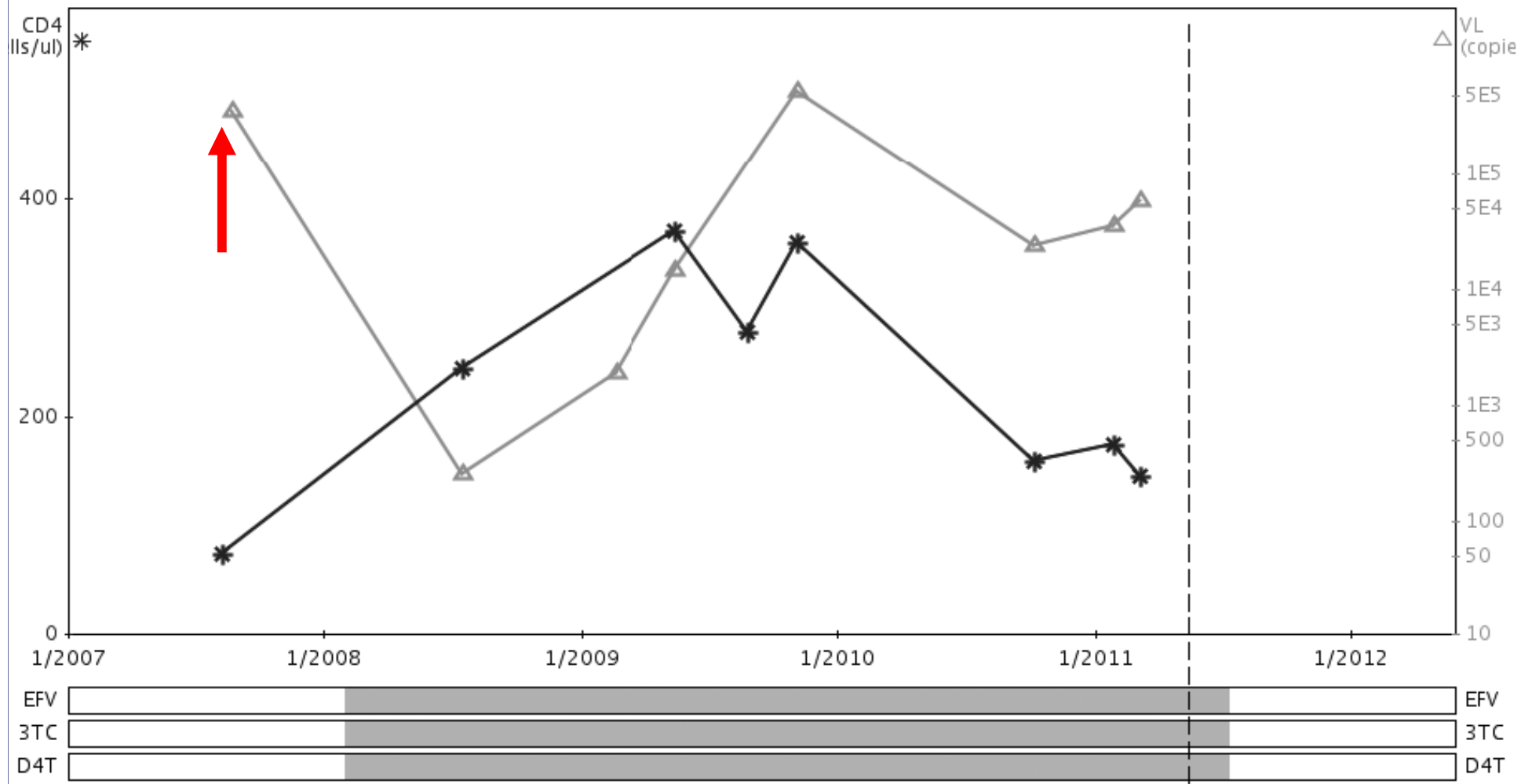
- sdNVP
- Phenobarbitone
- Switch antiepileptic Rx

Case 2

- 17yr ♀ on d4T/3TC/EFV since age 14 (2005).
- Baseline CD4 77cells/ μ l
- At initiation she severe wasting wt 23.4kg
- It was discovered there was poor disclosure to her by her family until 2010 with poor understanding of HIV and ART

Case 2: Clinical Chart

- 1st yr good response. The VL was never fully suppressed. Yr later VL ↑ & CD4 ↓



Drug	Mutations	Description	Level	GSS
zidovudine	41L 44D 69N 74V 118I 184V 210W 215Y	High-level resistance	5	0.0
zalcitabine	N/A	N/A	N/A	N/A
didanosine	41L 44D 69N 74V 118I 184V 210W 215Y	High-level resistance	5	0.0
lamivudine	41L 44D 69N 118I 184V 210W 215Y	High-level resistance	5	0.0
stavudine	41L 44D 69N 118I 184V 210W 215Y	High-level resistance	5	0.0
abacavir	41L 44D 69N 74V 118I 184V 210W 215Y	High-level resistance	5	0.0
emtricitabine	41L 44D 69N 118I 184V 210W 215Y	High-level resistance	5	0.0
tenofovir	41L 44D 69N 118I 184V 210W 215Y	Intermediate resistance	4	0.5
nevirapine	103N 106M 108I 227L 230L	High-level resistance	5	0.0
delavirdine	103N 106M 108I 227L 230L	High-level resistance	5	0.0
efavirenz	103N 106M 108I 227L 230L	High-level resistance	5	0.0
etravirine	103N 106M 227L 230L	Intermediate resistance	4	0.5

Case 2: Recommendation

- Pt. failing for long time \Rightarrow complex resistance pattern
- Durable suppression on std 2nd line regimen likely limited.
- Need new class of ARV \Rightarrow best combination integrase inhibitor, TDF/3TC & LPVr.

Case2: Questions

- Why so many resistance mutations?
- Outcomes in adolescents vs. older adults?
- What interventions would you put in place for this patient before switching her antiretroviral therapy?

Case2: Answers

- On failing regimen for a very long time, probably in the presence of suboptimal adherence- allowed virus to replicate in the presence of drug \Rightarrow multiple mutations.
- Adolescents well known for poorer treatment outcomes
- Intensive adherence support by a counselor, adolescent support group.

Case 4

- 45 yr ♀ with extensive ARV resistance.
- D4T/3TC/EFV- 03/03/2006 - 02/01/2009.
- AZT/ddI/LPV/r- 02/01/2009- 25/7/2011

Adherence has always been good except when admitted to hospital in 2008⇒ claims ARVs were not given to her.

Date	1/06	1/08	8/08	1/10	7/11
CD4	20	43	228	213	337
VL		1 500 000	12 000	24 712	139075
Rx	B/L	Reg1A	Reg 2	Reg 2	Reg 2

Case 4: Genotype: 06/2011

- Major PI: M46I, I54V, L76V, V82C, I84V,
- Minor PI: Q58E
- NRTI: M41L, D67N, K70R, V75M, T215F, K219Q
- NNRTI: V90I, K103S, V106M, E138A, F227L

27th July 2011 Started on TDF/3TC/DRV/r
600/100mg BD

Drug Resistance Interpretation: PR

PI Major Resistance Mutations: M46I, I54V, L76V, V82C, I84V

PI Minor Resistance Mutations: None

Other Mutations: None

Protease Inhibitors

atazanavir/r (ATV/r)	High-level resistance
darunavir/r (DRV/r)	Intermediate resistance
fosamprenavir/r (FPV/r)	High-level resistance
indinavir/r (IDV/r)	High-level resistance
lopinavir/r (LPV/r)	High-level resistance
nelfinavir (NFV)	High-level resistance
saquinavir/r (SQV/r)	High-level resistance
tipranavir/r (TPV/r)	High-level resistance

When maintain
regimen for a lon
develop mutatio
from the same cl
exposed to them

PR Comments

PIMajor

- M46I/L are nonpolymorphic PI-selected mutations that reduce susceptibility to IDV/ATV when present with other mutations. M46L also reduces susceptibility to TPV.

Case 4: progress

- 4th August 2011 (1/52 into 3rd line Rx) developed cough/night sweats/fever. Went to local clinic \Rightarrow diagnosed smear negative TB \Rightarrow Rifafour.
- 10th August 2011 returned for follow up:

What would you do at this point?

Case 4: Progress

- 24th August patient still on rifafour and 3rd line agents despite suggestion to stop rifampicin.
- Counseled and now on H/E/Z
- 21 Sept 2011- MDR TB diagnosed and referred to KGV for MDR treatment

Case 4: Progress

Jul 2012	Feb 2013	June 2013
310 11.2%	-	363 15%
15123 (log value)	62160	60584
Reg 3 and MDR TB tx	Reg3 MDR TB Tx	Reg3 and MDR TB tx

Case 4: Genotype: 03/2013

- Major PI: V32I, M46I, I54V, L76V, V82C, I84V
- Minor PI: L10F, L33F, Q58E
- NRTI: M41L, D67N, K70R, V75M, M184V, T215F, K219Q
- NNRTI: K103S, V106M, F227L

HIVdb: Genotypic Resistance Interpretation Algorithm

Date: 12-Jun-2015 21:50:52 UTC

Drug Resistance Interpretation: PR

PI Major Resistance Mutations: V32I, M46I, I54V, L76V, V82C, I84V

PI Minor Resistance Mutations: L10F, L33F, Q58E

Other Mutations: None

Protease Inhibitors

atazanavir/r (ATV/r) High-level resistance

darunavir/r (DRV/r) High-level resistance

fosamprenavir/r (FPV/r) High-level resistance

indinavir/r (IDV/r) High-level resistance

lopinavir/r (LPV/r) High-level resistance

nelfinavir (NFV) High-level resistance

saquinavir/r (SQV/r) High-level resistance

tipranavir/r (TPV/r) High-level resistance

PR Comments

PIMajor

Reasons for failure

- Drug drug interactions
- Poorly potent regimen
- Considering etraverrine, maraviroc, Raltegravir, DRV/r, tenofovir, 3TC

Conclusion

- Monitor treatment with VL
- All treatment failure is not due to viral resistance
- Must exclude other causes of treatment failure
- Genotypic resistance testing has limitations
- Early detection of resistance and early switch to suppressive regimen imp to prevent amplification of resistance