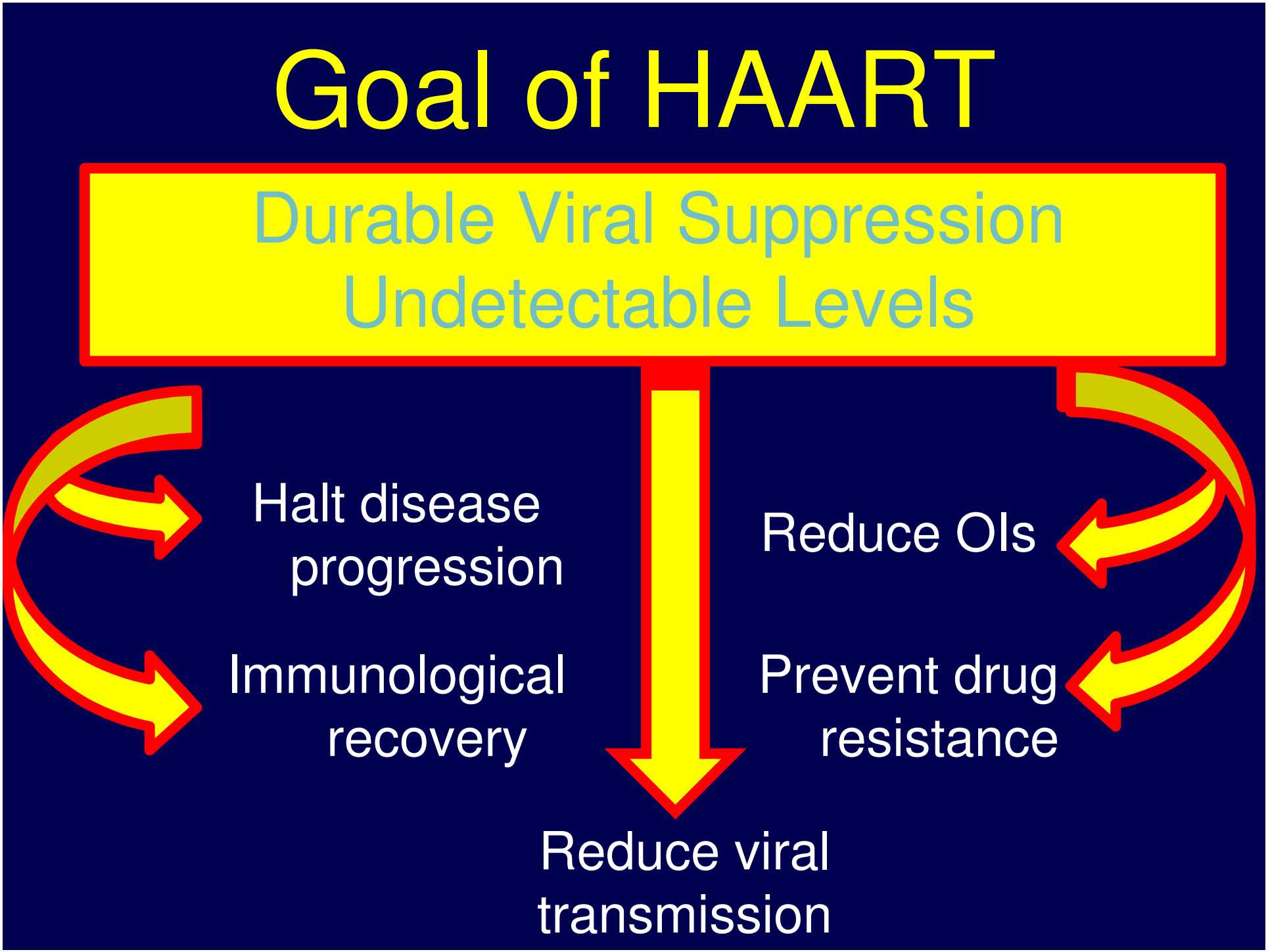
Diagnosing & Managing Treatment Failure

Yunus Moosa
UKZN

Overview

- CD4 and VL as biomarkers
- Causes of treatment failure
- Mechansim 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



Factors that Contribute to Treatment Failure

- Sub-optimal potency of regimen
- Insufficient drug levels
  - Non adherence
  - Malabsorption
  - Drug interactions (herbal meds, OTC's)
- Resistant virus

Note!

Not all Treatment failure is due to resistance

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/µL

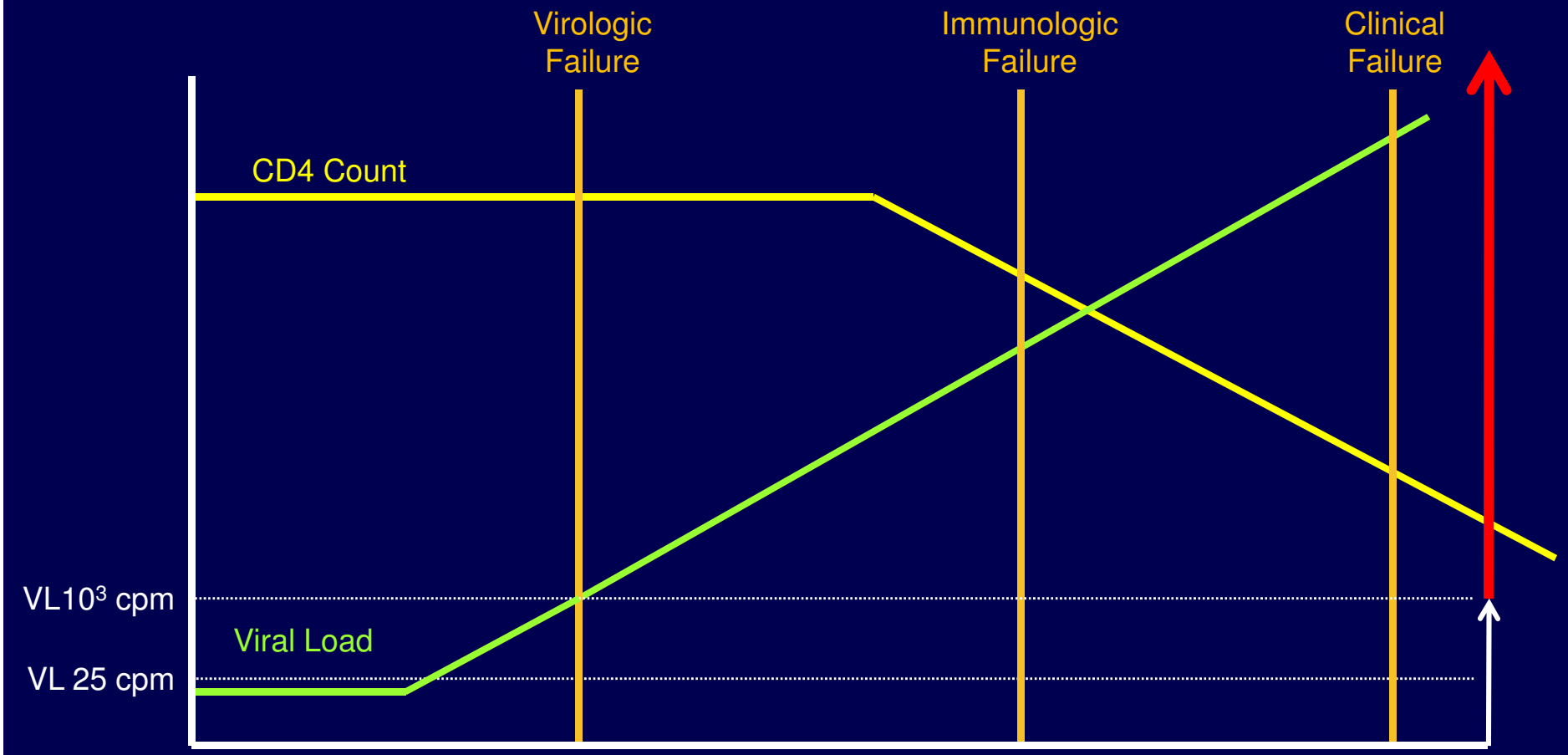
- 3 analytic steps ⇒ total WCC, % LC, % CD4 ⇒ wide analytic variation
- Seasonal variation, diurnal variation.
- Inter-current illness
- Corticosteroids.
- Splenectomy.
- Age in adults, gender, psychological stress, physical stress, pregnancy ⇒ no effect

- 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

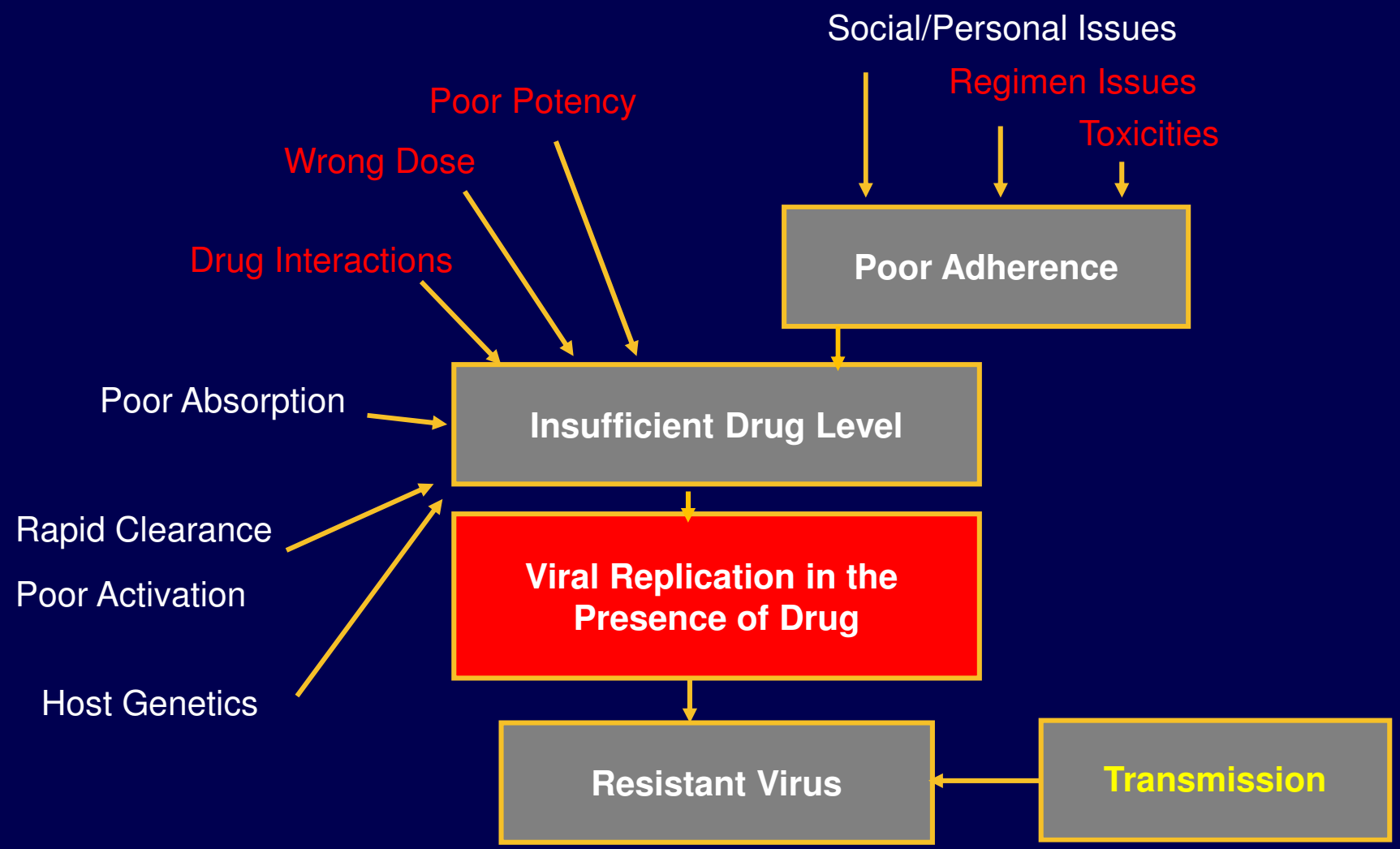
Virologic/Treatment failure

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



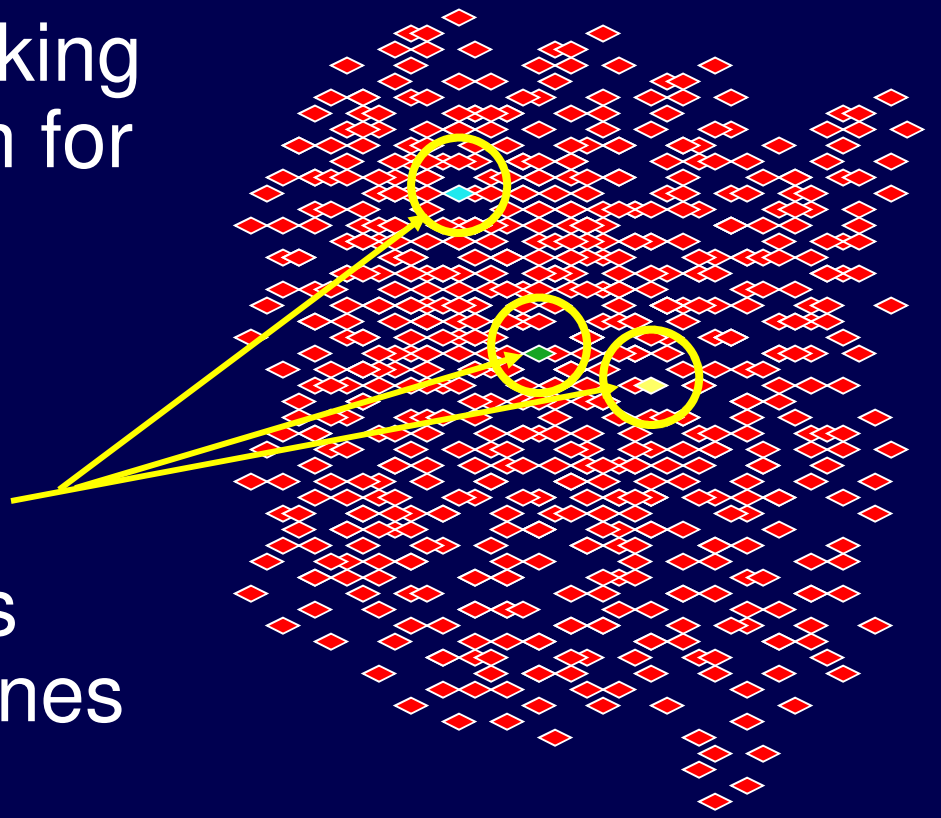
How Resistance Mutations Arise

- HIV replication $\Rightarrow$ error prone:
  - DNA Replication $1:10^9$
  - HIV Replication $1:10^4$
  - RNA Synthesis $1:10^4$
  - Airline Baggage Loss 1:200
  - Good Typist 1:100
- 10^9 viral particles produced/day
  - All possible mutations emerge daily
- Persistence of mutant depends on fitness

Modified from http://hivinsite.ucsf.edu

Growth in the absence of inhibitory pressure

- HIV multiplies freely taking the most optimum form for rapid growth $\rightarrow$ wt.



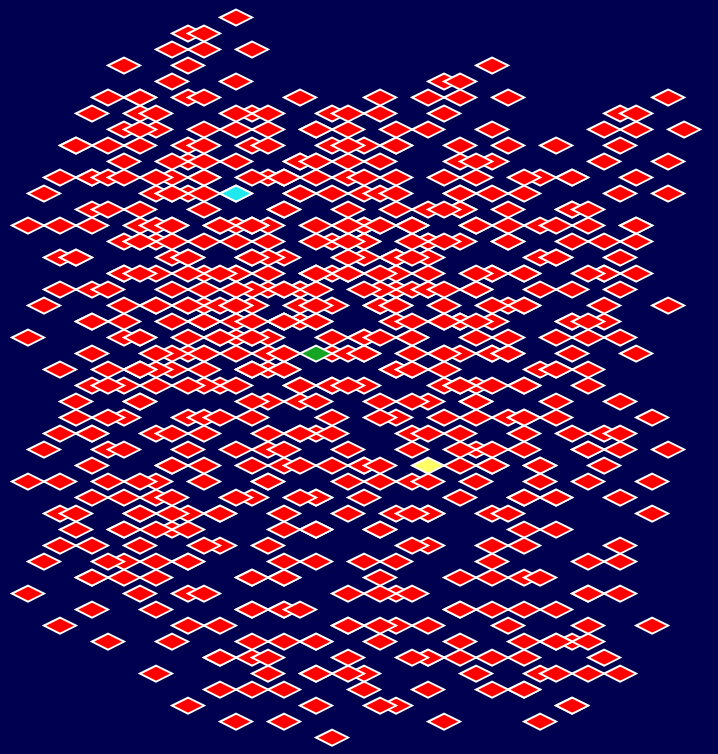
- As it proliferates, HIV undergoes spontaneous mutations in random genes due to error prone RT enzyme.

Growth in the presence of ARV pressure

- ARVs kill all of the original wild type organisms

but

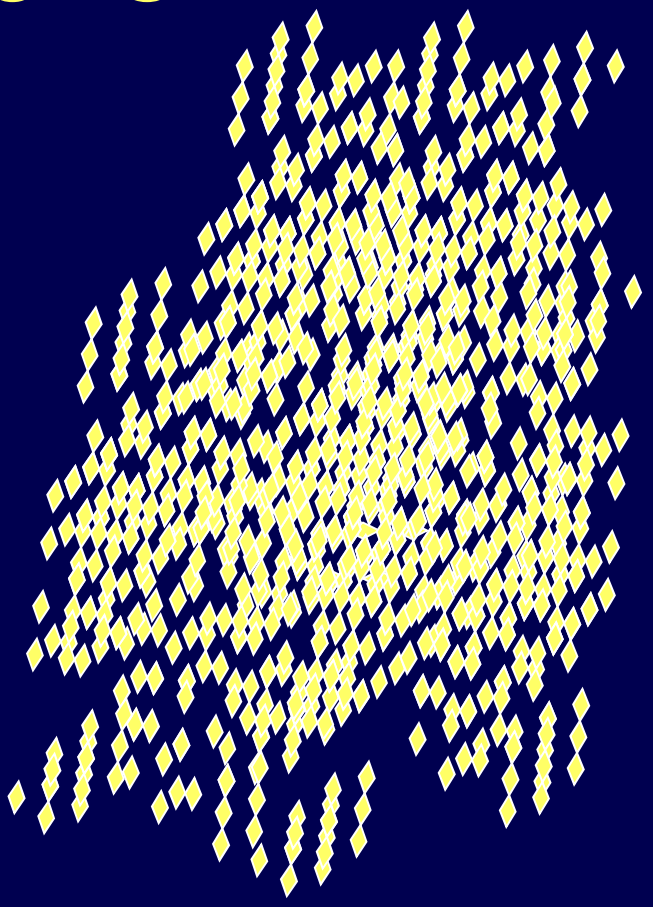
- The mutated virus which is RESISTANT survives.



Growth in the presence of ARV pressure

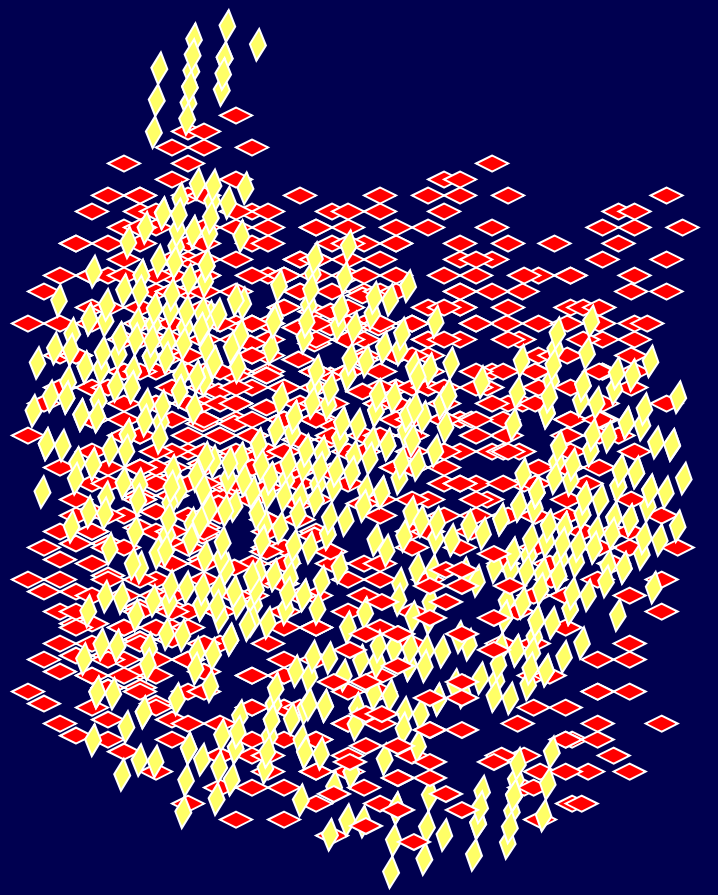
- The mutated HIV grows and multiplies, even in the presence of ARVs.

- 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.

Johnson *et al. XV int. HIV Drug Resistance Workshop,* 2006, #69.
Palmer *et al. PNAS 103 (no. 18) 2006: 7094-7099*

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

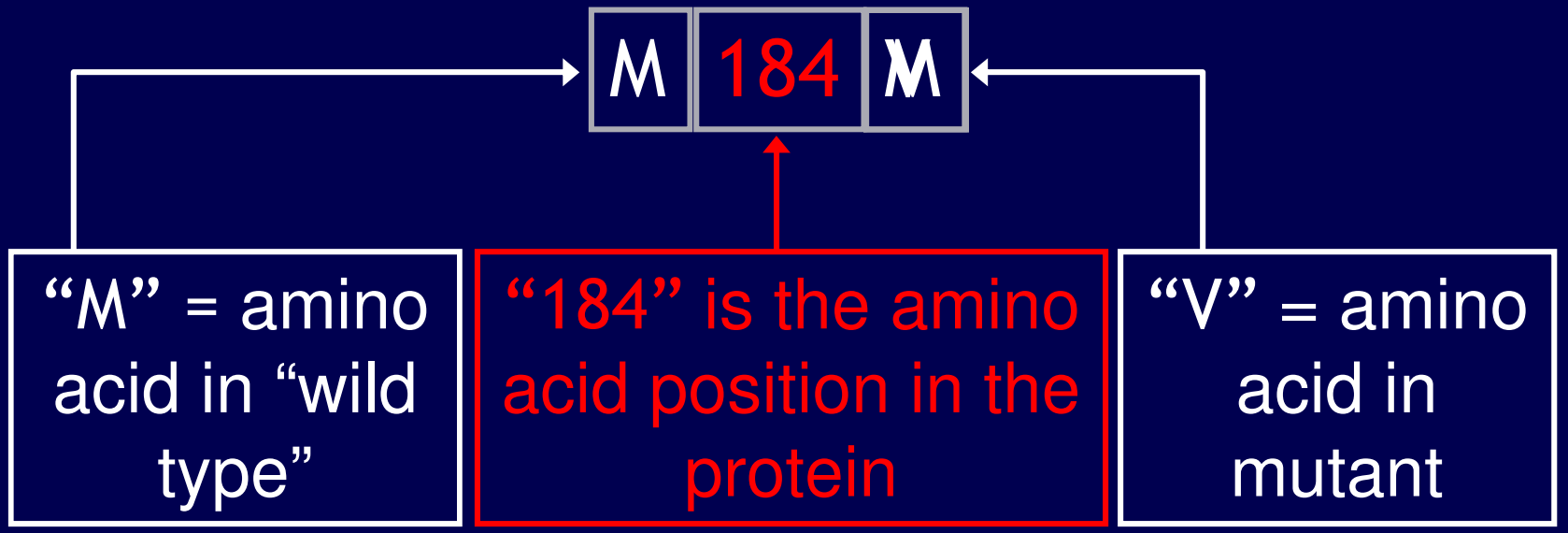
Facts on resistance testing

- 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

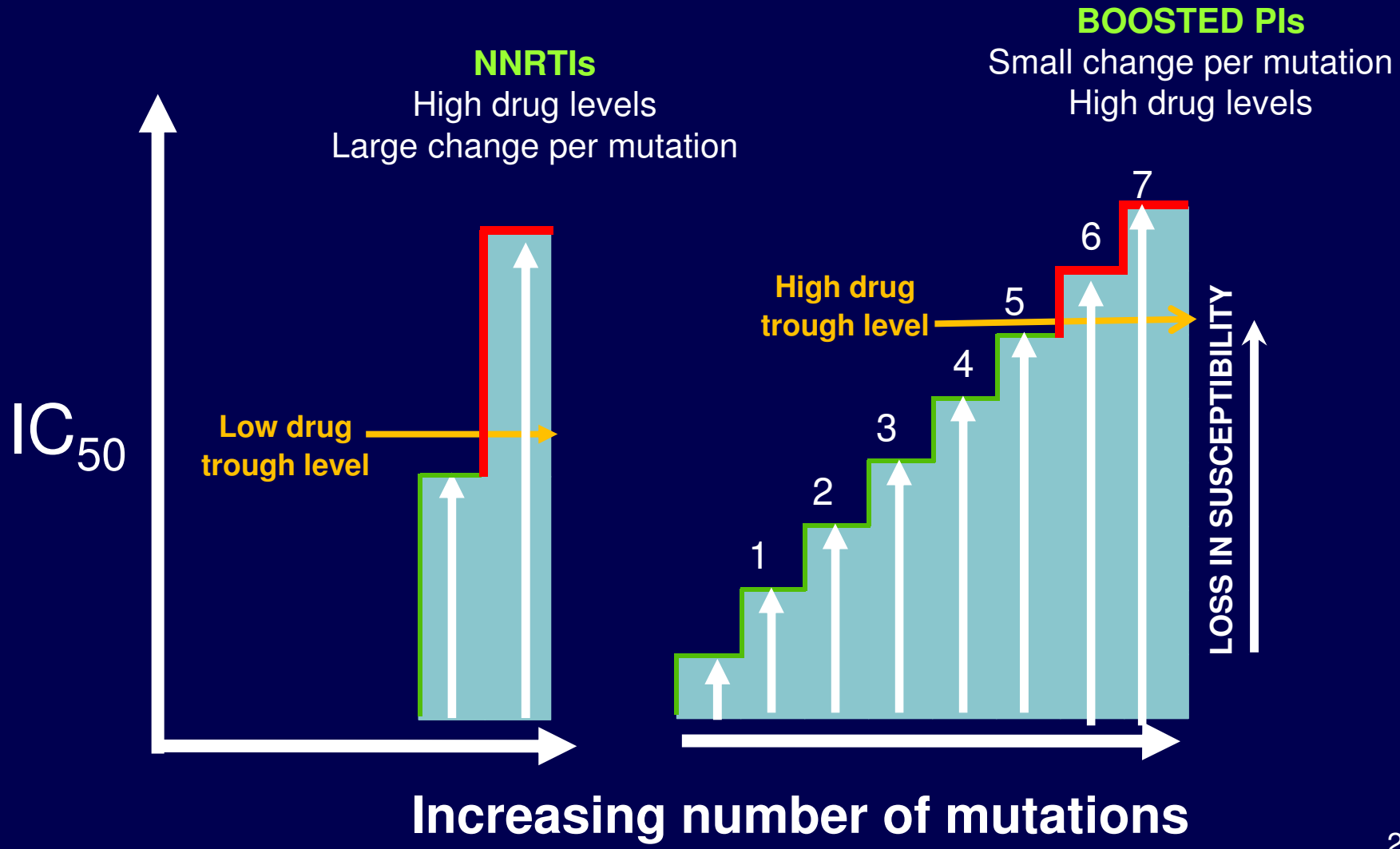
- How do we identify a resistance mutation?



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



Brun S *et al., 8th ECCATH*, Athens, October 2001, #7

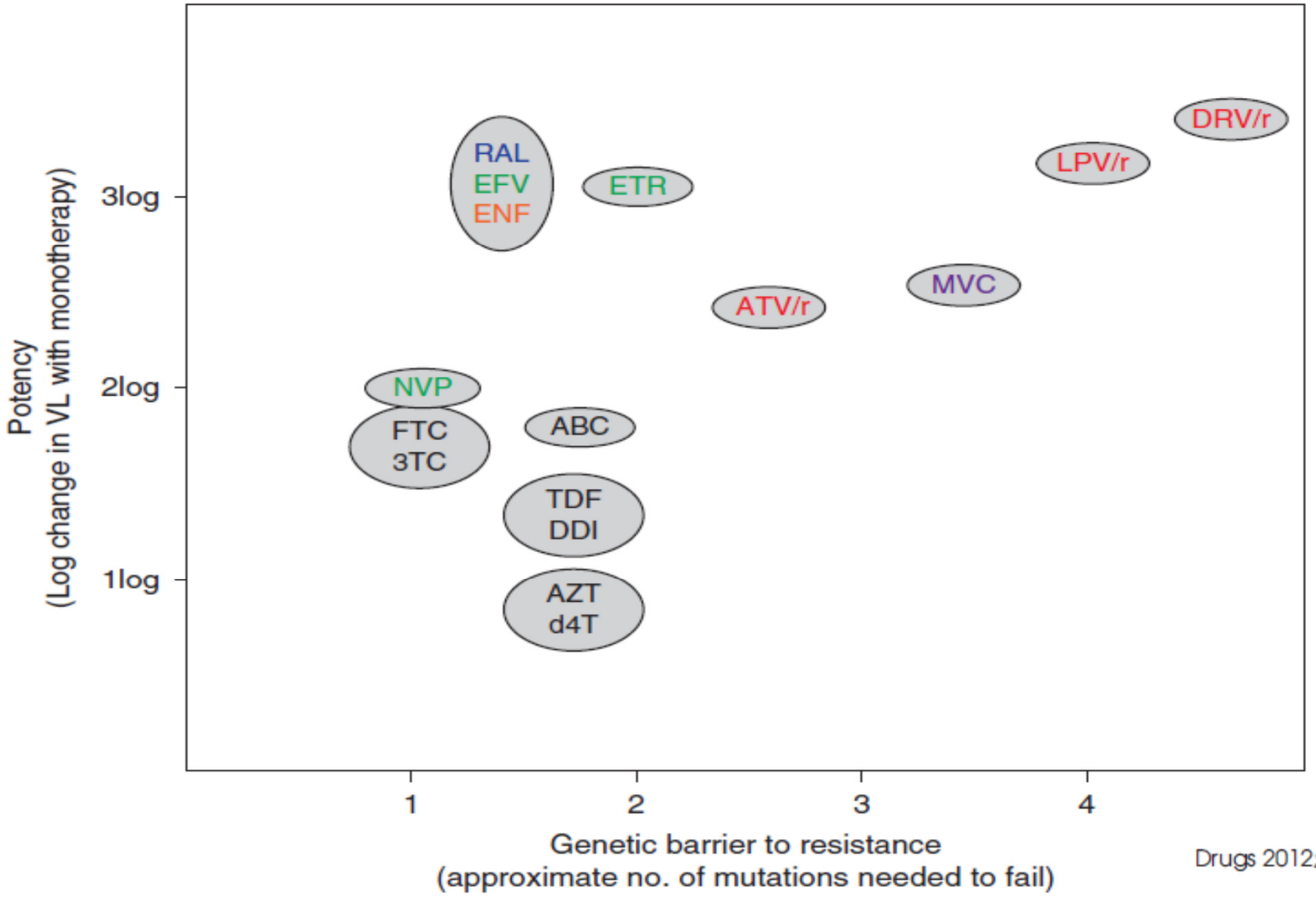
Genetic Barrier of Drug Classes

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**

http:// www.hivfrenchresistance.org/2006/tab2.html *General estimation of approved drugs, Nov 2006

Potency vs. Genetic barrier



Drugs 2012; 72 (9)

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 ⇒ 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)

TAM 1 Pathway

41L
215Y
210W

Higher-level Ω

More cross Ω within Class

Less likely to be sensitized by M184V

67N
70R
219Q/E

TAM 2 Pathway

Lower-level Ω

Less Cross Ω within Class

Likely to be sensitized by M184V

"ARV mutations" presentation: http://www.clinicaloptions.com/HIV.aspx

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 ⇒ high level cross- Ω to other NNRTIs
- Mutations do <u>NOT</u> ↓ replicative fitness
- Do not continue NNRTI if VL not suppressed ⇒ additional mutations will compromise 2nd generation NNRTIs

Gallant J., Topics in HIV medicine

NNRTI Resistance Mutations

- K103N ⇒ 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

Johnson et al, 2007

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
Cons	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**	V**A**		**V**	VTA**LM**	**V**	ATS**F**	**V**		**M**
IDV/r		I	**IL**	V			**VTA**LM	**V**	**AFTS**	**V**	S	**M**
LPV/r		**I**	IL	V**A**	VM	**V**	**VTALM**	**V**	**AFTS**	**V**		M
NFV	**N**		**IL**	V	**VM**		**VTALM**		**AFTS**	**V**	**DS**	**M**
SQV/r					**VM**		**VTA**LM		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

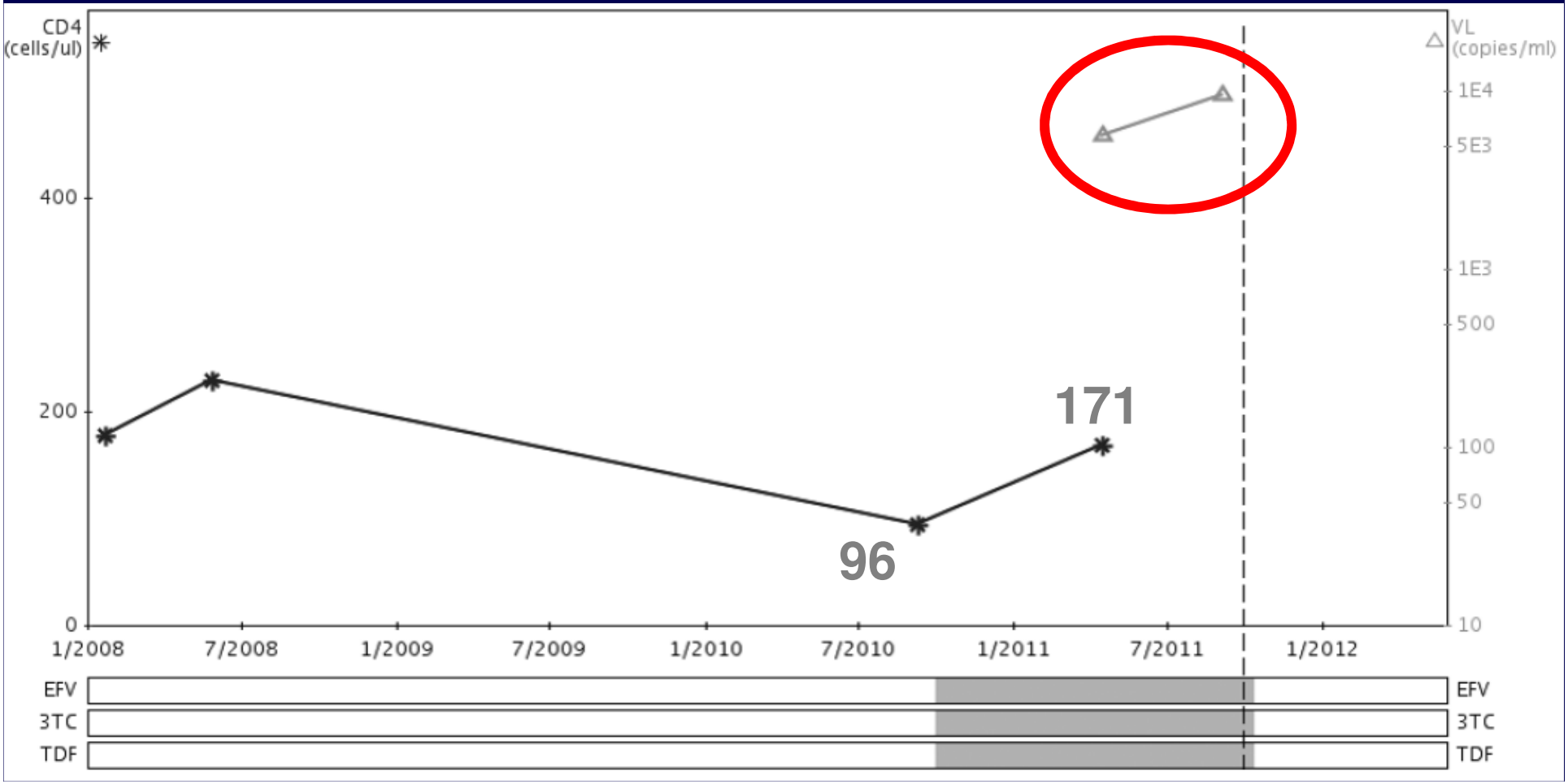
*WHO recommends (http://www.who.int/hiv/topics/drugresistance/indicators/en/index.html)

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 phenobarbitol 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 genotypicaly susceptibility

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
- Montior renal function at baseline & 3mnths – 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

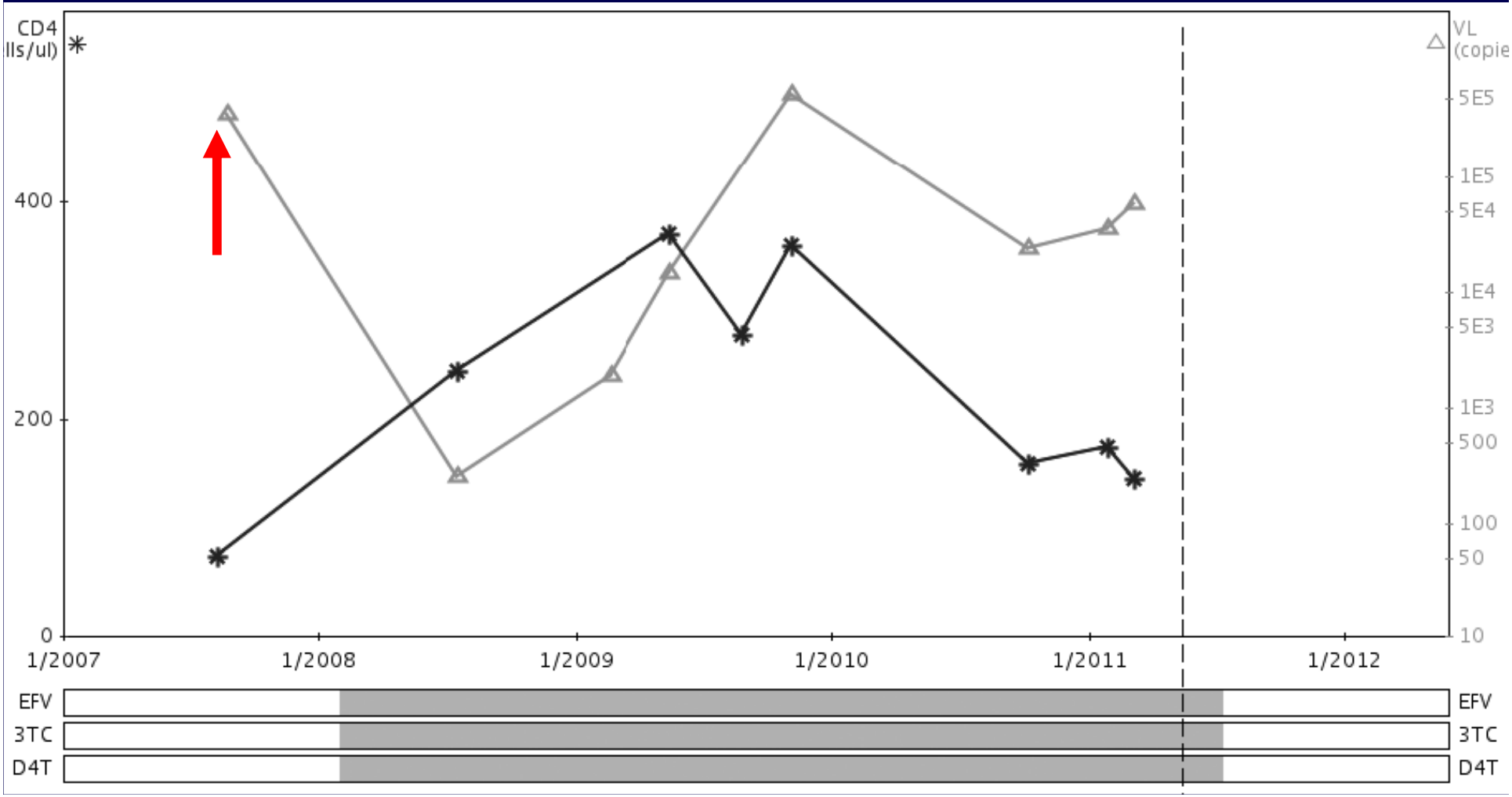
- Switch antiepileptic Rx

Case 2

- 17yr ♀ on d4T/3TC/EFV since age 14 (2005).
- Baseline CD4 77cells/µl
- 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 maintaine
regimen for a lor
develop mutatio
from the same c
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 ⇒ diagnosed smear negative TB⇒ 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 etraverine, 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