Principles of HIV Drug Resistance: Resistance to New Drug Classes

Mark A Wainberg
McGill University AIDS Centre
Montreal, Quebec, Canada

Why Is It Important to Understand HIV Drug Resistance?

1. Resistance is an important factor in treatment failure
2. Resistance limits drug activity
3. Cross-resistance can limit the use of therapies
4. An understanding of resistance may permit the sequencing of drugs within the same class

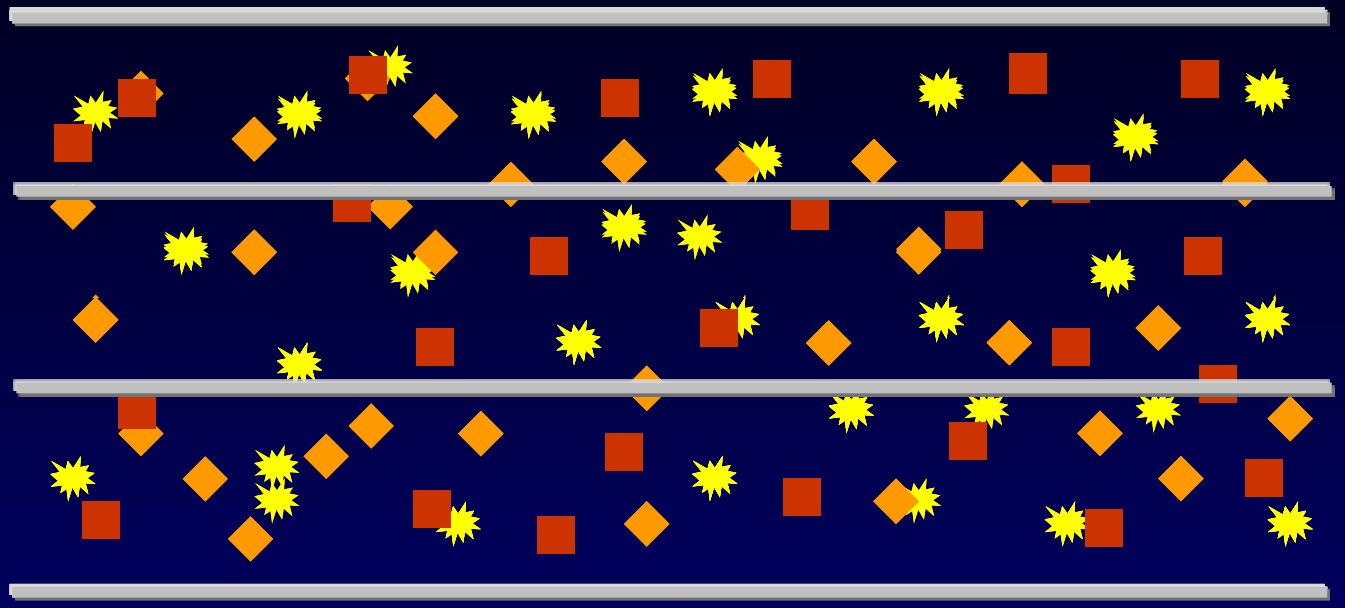
Hirsch MS, et al, *Clin Infect Dis*. 2003 Jul 1;37(1):113–28. Epub 2003 Jun 23.

Drug Resistance

- Approximately 20 billion mutations can occur each day in an infected person
- All possible point mutations including those responsible for drug resistance occur daily
- Minority mutated populations are generally less “fit” than wild-type viruses but can be selected by antiviral drugs, if viral replication is not fully suppressed

Hammer SM, et al, International AIDS Society-USA panel. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *JAMA*. 2006 Aug 16;296(7):827–43.

Resistance Is a Result of HIV Replication in the Presence of Drug



Outgrowth of mutated viruses

Drug B
pressure
No drug pressure

- Wild type
- Drug-resistant virus
- Double drug-resistant virus

Hammer SM, et al, *JAMA*. 2006, Aug 16; 296(7):827–43.
Wainberg MA, et al, *JAMA*. 1998 Jun 24;279(24):1977–83.

HIV Drug Resistance Is Influenced by:

1. The genetic barrier for a drug, i.e.: number of mutations needed for resistance to occur and the speed with which they are likely to occur
2. Ability of a resistant virus to grow out and to become predominant in the presence of drug (i.e.: increased fitness compared to wild-type virus in the presence of drug)
3. Development of additional compensatory and/or secondary mutations

Martinez-Cajas JL, et al, *Antiviral Res*. 2007 Dec;76(3):203–21. Epub 2007 Jul 16.

The Enzymes of HIV
All are now targets of therapy

- Reverse Transcriptase – Converts the HIV RNA into HIV DNA
- Protease – Cleaves HIV polyproteins into functional protein pieces
- Integrase – Processes this HIV DNA and incorporates it into the host genome

Thus, Clinical Resistance

a. Begins with a mutation (or a change in the genotype) that increases the IC_{50} for a given drug, i.e.: establishment of a resistant phenotype.
b. This, in turn, leads to increased plasma viral load or virological failure.

Hammer SM, et al, *JAMA*. 2006, Aug 16; 296(7):827–43.

Role of Host Factors

- Maintenance of adequate drug levels both in plasma and/or inside cells is key to prevention of drug resistance.
- Individual variations may occur in regard to drug levels. These may be influenced by host genetic composition.

Martinez-Cajas JL et al, *Antiviral Res*. 2007 Dec;76(3):203–21. Epub 2007 Jul 16.

Prevalence of Resistance

1. Between 20 and 30% of patients who start triple therapy may develop resistance to one, two,
   or three classes of drugs within a few years.
2. Transmission of drug-resistant viruses
   is a major problem in regard to both public health and care.

Harrigan PR, et al, *J Infect Dis*. 2005 Feb 1;191(3):339–47. Epub 2004 Dec 22.
Wainberg MA, et al, *JAMA*. 1998 Jun 24;279(24):1977–83.

Some Examples of Transmission of MDR Viruses in Primary HIV-1 Infection

Patient	Codon substitutions associated with drug resistance		
	NRTI mutations	NNRTI mutations	PI mutations
1	41L; 67N; 69N; 70R; 74V; 184V; 215F; 219Q	100I; 103N	10I; 36I; 54V; 63P; 71V; 73S; 82V; 90M
2	41L; 184V; 215Y	103N, 179E	48V; 63P; 71V; 73S; 77I; 82A; 90M
3	None	103N	10I; 54V; 63P; 71V; 82T; 84V; 90M
4	184V; 215Y	103N	77I
5	184V	108I	20R, 77I
6	41L; 67N; 210W; 215Y	None	63P; 71V; 73S; 90M
7	41L; 215Y	101E	None
8	41L; 215Y	101E	None

Brenner B, et al, *AIDS*. 2004 Aug 20;18(12):1653–60.

Independent Predictors of Drug Resistance in Individuals after Beginning HAART

- High baseline plasma HIV RNA
- Low CD4 cell count
- Moderate levels of adherence

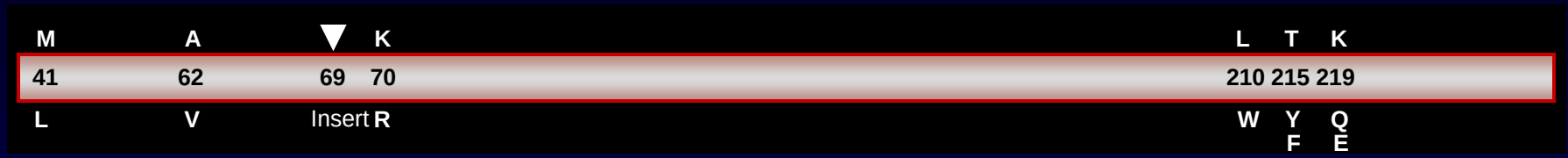
Harrigan PR, et al, *J Infect Dis*. 2005 Feb 1;191(3):339–47. Epub 2004 Dec 22.

Mutations Selected by NRTIs

	41	65	67	70	74	115	184	210	215	219
Abacavir		K65R			L74V	Y115F	M184V			
Didanosine		K65R			L74V					
Emtricitabine		K65R					M184V/I			
Lamivudine		K65R					M184V/I			
Stavudine	M41L		D67N	K70R				L210W	T215Y/F	K219Q/E
Tenofovir		K65R		K70E						
Zidovudine	M41L		D67N	K70R				L210W	T215Y/F	K219Q/E

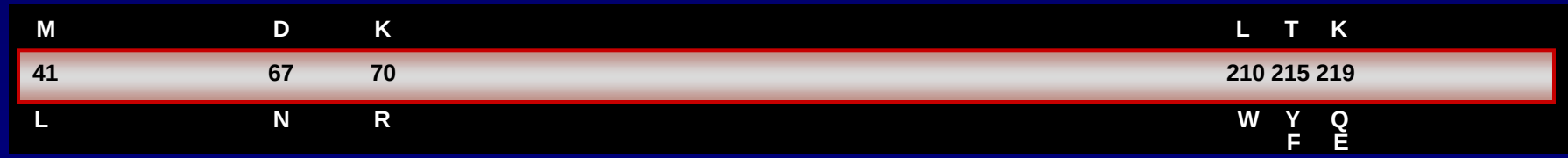
Mutations Selected by NRTIs

Multi-NRTI Resistance: 69 Insertion Complex (affects all NRTIs currently approved by the US FDA)



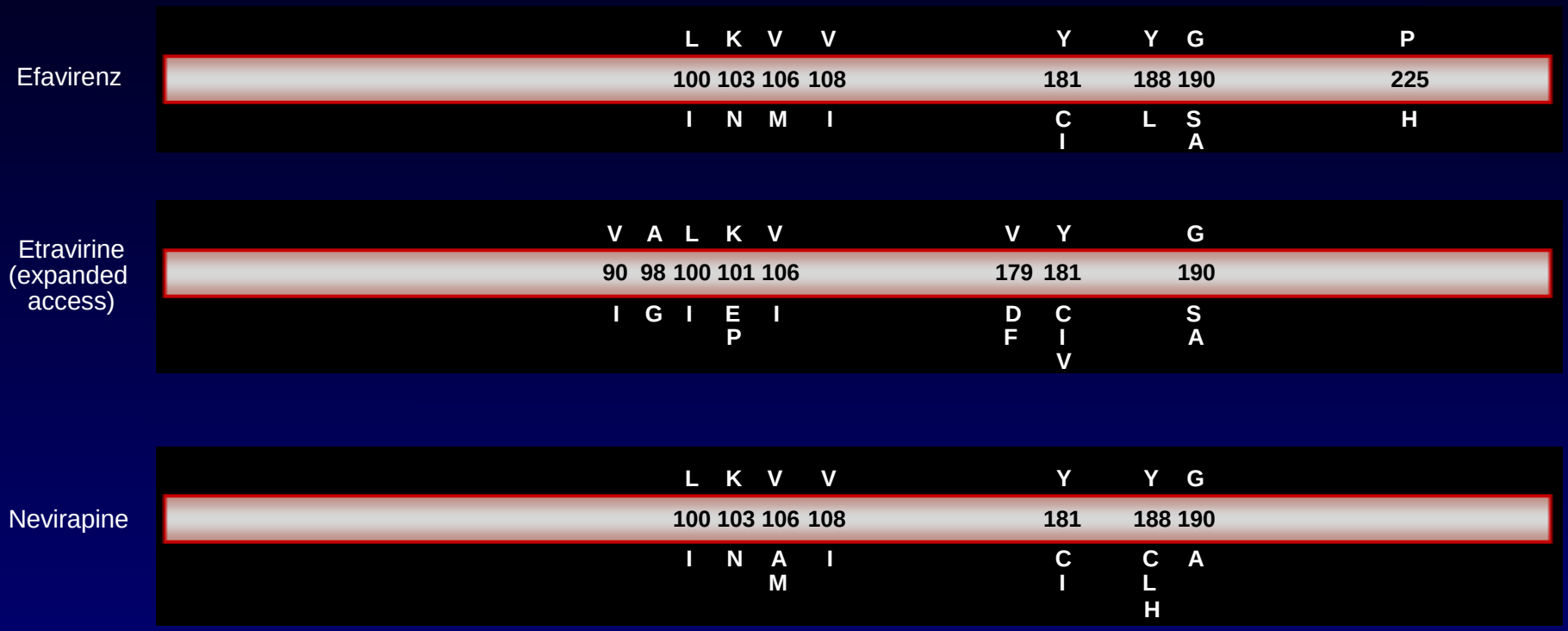
Multi-NRTI Resistance: 151 Complex (affects all NRTIs currently approved by the US FDA except tenofovir)

Multi-NRTI Resistance: Thymidine Analogue-associated Mutations (TAMs; affects all NRTIs currently approved by the US FDA)



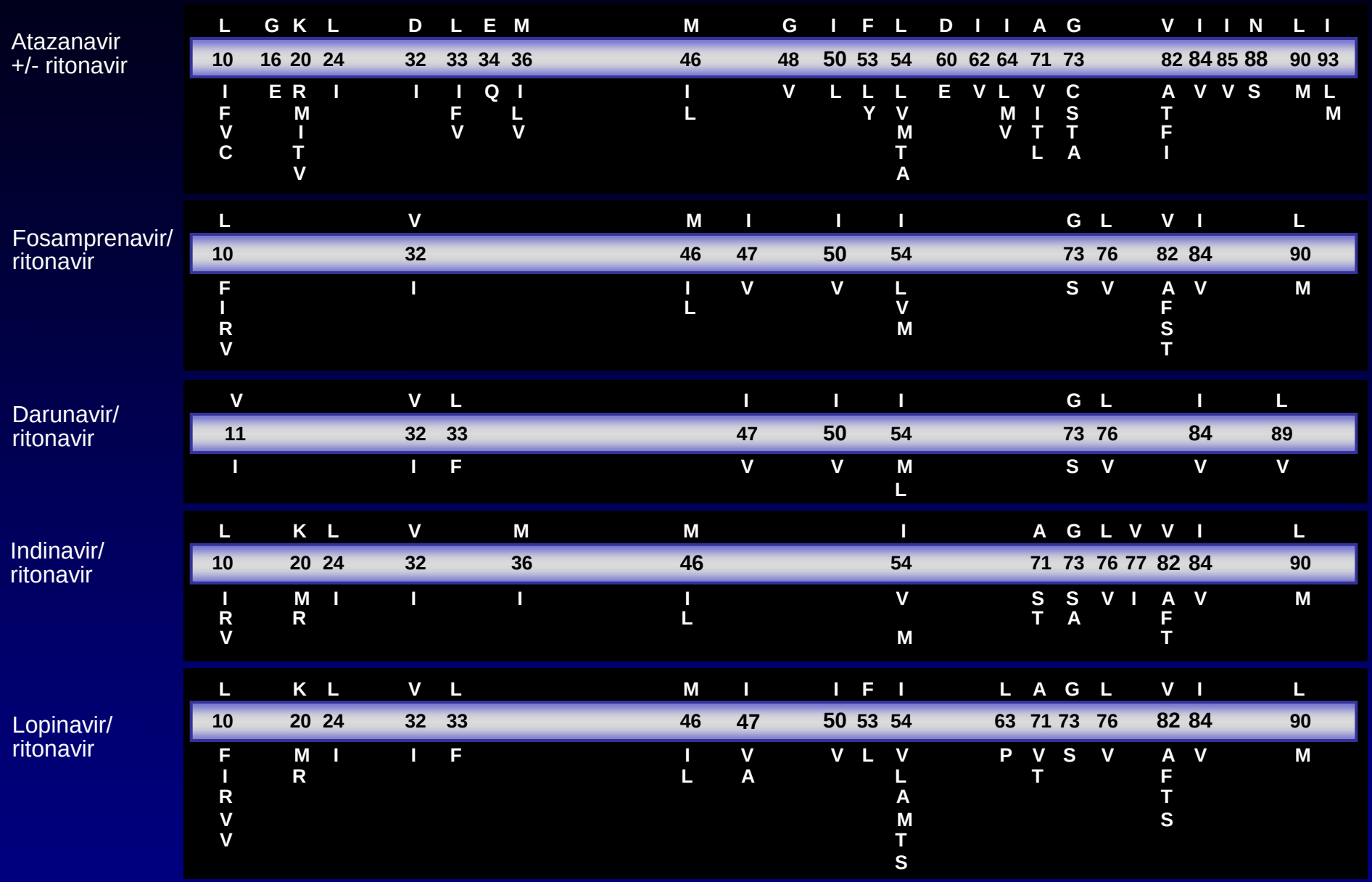
Johnson VA, et al, 2007. *Top HIV Med*. 2007 Aug-Sep;15(4):119–25.

Mutations Selected by NNRTIs

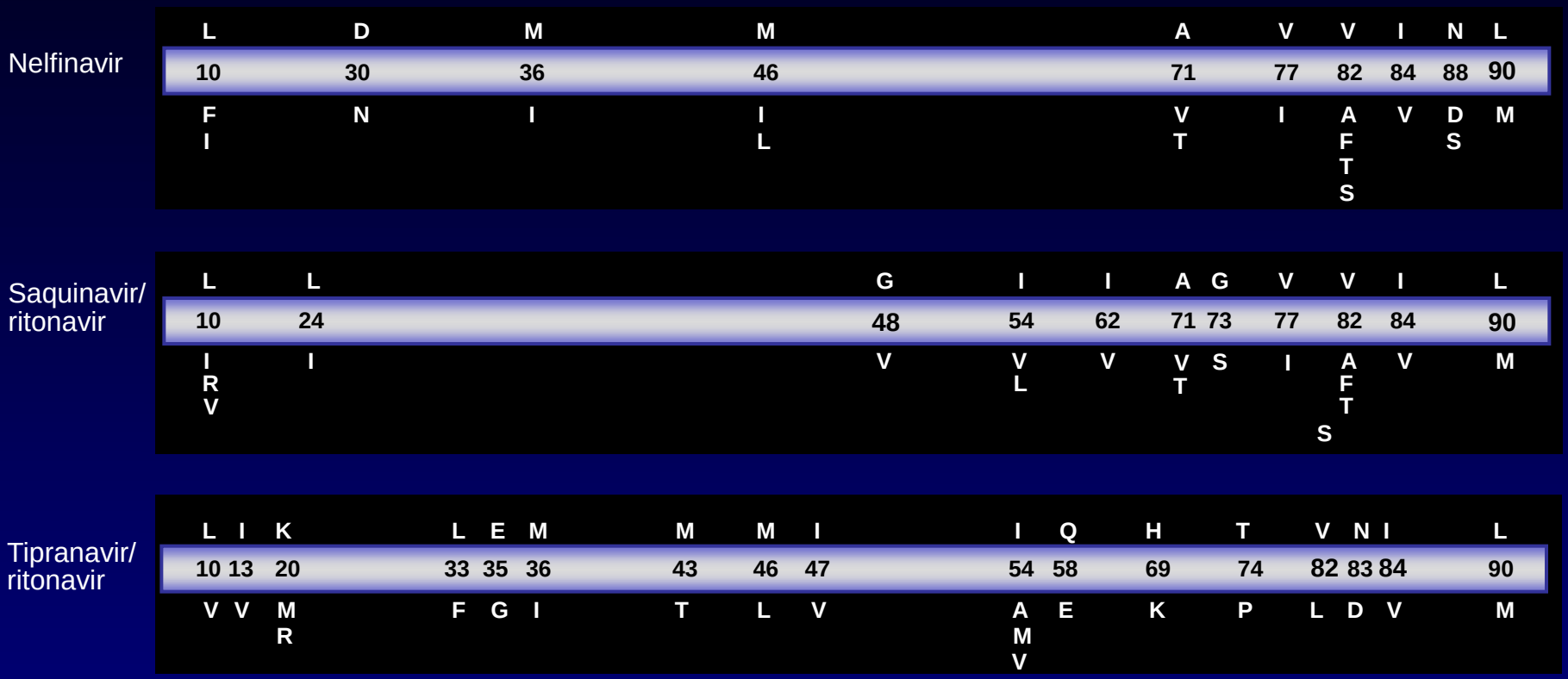


Johnson VA, et al, 2007. *Top HIV Med*. 2007 Aug-Sep;15(4):119–25.

Mutations Selected by PIs



Mutations Selected by PIs (cont)



Johnson VA, et al, 2007. *Top HIV Med*. 2007 Aug-Sep;15(4):119–25.

	Drugs		Fold change[1]	Cut-off[2]		Resistance analysis[3]
NRTI/NtRTI	**NRTI/NtRTI mutations[4]: 41L, 184V, 210W, 215Y**					
	Retrovir®	Zidovudine	5.6	1.5	11.4	Reduced response
	Epivir®	Lamivudine	45.4	1.2	4.6	Minimal response
	Videx®	Didanosine	1.1	0.9	2.6	Reduced response
	Zerit®	Stavudine	1.0	1.0	2.3	Maximal response
	Ziagen®	Abacavir	2.8	0.9	3.5	Reduced response
	Emtriva®	Emtricitabine	43.5	*3.1*		Resistant
	Viread®	Tenofovir DF	1.2	1.0	2.3	Reduced response
NNRTI	**NNRTI mutations[4]: 103N**					
	Viramune®	Nevirapine	43.3	*6.0*		Resistant
	Sustiva®, Stocrin®	Efavirenz	16.4	*3.3*		Resistant
PI	**PI mutations[4]: 10I, 32I, 54L. 71T, 77I, 84V, 90M**					
	Crixivan®	Indinavir	5.8	1.0	5.4	Minimal response
	Crixivan®; boosted	Indinavir / r	5.8	2.3	27.2	Reduced response
	Viracept®	Nelfinavir	27.4	1.2	9.4	Minimal response
	Invirase®; boosted	Saquinavir / r	9.7	3.1	22.6	Reduced response
	Lexiva®, Telzir®; boosted	Fosamprenavir / r	12.8	1.5	19.5	Reduced response
	Kaletra®	Lopinavir / r	7.8	6.1	51.2	Reduced response
	Reyataz®; boosted	Atazanavir / r	16.2	2.5	32.5	Reduced response
	Aptivus®; boosted	Tipranavir / r	0.9	1.5	7.0	Maximal response[1]
	Prezista™; boosted	Darunavir / r	5.3	10.0	106.9	Maximal response

1. Predicted fold change in 50% inhibitory concentration (IC_{50}), relative to susceptible reference virus. 2. Cut-off values for maximal and minimal clinical response (clinical cut-off) or for normal susceptibility range *in vitro* (biological cut-off). Biological cut-offs are printed in italic. 3. Resistance analysis based on the magnitude of the fold change relative to the clinical or the biological cut-offs.4. Mutations printed on page 1 are those reported on public lists (ANRS, Stanford, IAS-USA) or by drug development sponsors. A complete list of all differences from the reference wild type is given on page 2.

Adding New Drugs Is Useful When:

1. A new member of a previously used class can help to suppress mutated viruses, i.e.: sequencing of drugs within a class.
2. The genetic barrier of a newly added drug is higher than that of a previously used member of that class.
3. The new combination suppresses viral replication and can prevent new mutations from arising; this pre-supposes that the drugs in the new combination can reinforce each other so that viral replication and mutagenesis do not occur.

Martinez-Cajas JL, et al, *Antiviral Res*. 2007 Dec;76(3):203–21. Epub 2007 Jul 16.

Lessons in Drug Resistance

1. All drugs select for resistance-conferring mutations
2. Combination therapy with different drugs can select for novel patterns of mutations
3. Availability of new drug classes (i.e.: integrase and CCR5 inhibitors) offer potential for treating people who have resistance to older agents

Harrigan PR, et al, *Clin Infect Dis*. 2000 Jun;30 Suppl 2:S117–22.
Correll T, et al, *Pharmacotherapy.* 2008 Jan;28(1):90–101.
Van Lunzen J, *Eur J Med Res*. 2007 Oct 15;12(9):435–40.

Treatment Failure

1. **Will** first result in resistance to those drugs that have a low genetic barrier (e.g., 3TC, NVP, EFV)
2. **Will** result in the preferential outgrowth of mutated viruses
3. **Will** often potentiate an accumulation of mutations that increase both levels of resistance as well as viral “fitness”
4. **Will not** commonly result in resistance to all of the drugs in a regimen

Harrigan PR, et al, *Clin Infect Dis*. 2000 Jun;30 Suppl 2:S117–22.
Maguire M, et al, *AIDS*. 2000 Jun 16;14(9):1195–201.

Overcoming Drug Resistance

- The use of newer generation boosted PIs (darunavir and tipranavir) can be shown to be useful in treatment of patients resistant to earlier generations of boosted PIs.
- However, an accumulation of limited numbers of additional mutations may result in resistance to these newer agents.
- Thus, the genetic barrier of a new generation boosted PI in the case of PI-experienced patients is not as high as would be the case for a treatment naïve patient.
- Indeed, in some cases involving treatment-experienced patients, only 1 or 2 new mutations might now suffice in order for resistance to occur.

Hammer SM, et al, *JAMA*. 2006 Aug 16;296(7):827–43
Morand-Joubert L, et al, *Antivir Ther*. 2006;11(2):143–54.

Genetic Barrier for Resistance

1. Refers to number of mutations required in order for resistance to develop and the speed with which such mutations are likely to accumulate.
2. A high genetic barrier for resistance is always advantageous for a drug, but is not less of an important consideration than potency.
3. There is a need to protect drugs with a low or moderate genetic barrier for resistance by using other potent drugs in combination with them. Sometimes, long drug half-lives can help to forestall resistance in the event of non-adherence. This is sometimes referred to as the “forgiveness” of a regimen.

Mo H, et al, *J Virol*. 2005 Mar;79(6):3329–38.
Hoefnagel JG, et al, *Antivir Ther*. 2005;10(8):879–92.
Morand-Joubert L, et al, *Antivir Ther.* 2006;11(2):143–54.

Cross-resistance

1. Occurs when mutational changes selected by a given drug also confer resistance to other drugs that are members of the same class
2. Is closely associated with specific mutations or combinations of mutations
3. Can be conferred by some single mutations in the case of NRTIs and NNRTIs but never in the case of PIs

Harrigan PR, et al, *Antimicrob Agents Chemother*. 2002 Mar;46(3):909–12.

Drug Resistance Is Relative and Not Absolute

- In many cases, an increase in IC_{50} may not necessarily preclude use of a drug in therapy
- Some mutations may actually enhance sensitivity to certain drugs, e.g., some NRTI mutations
  can enhance sensitivity to some NNRTIs
- The M184V, L74V, and K65R mutations can enhance sensitivity to ZDV
- Sometimes, CD4 counts continue to rise despite virological failure

Kempf DJ, et al, *Antivir Ther*. 2002. 7: 165–74.
Larder BA, et al, *Science*. 1995 Aug 4;269(5224):696–9.
St Clair MH, et al, *Science*. 1991 Sep 27;253(5027):1557–9.
Parikh UM, et al, *J Virol*. 2006 May;80(10):4971–7.

Treatment Interruptions

1. Can lead to non-detectability of previously detectable mutations and re-emergence of more fit wild-type viruses
2. Do not prevent archiving of resistance mutations and their subsequent reappearance upon introduction of relevant drugs

El-Sadr WM, et al. *N Eng J Med*. 2006.

Allele Specific PCR or Ultrasensitive Resistance Assays

1. Are more sensitive than traditional bulk sequencing for detection of mutated viruses
2. Can sometimes predict treatment failure for certain drug classes, even if minority species of mutated viruses are detected

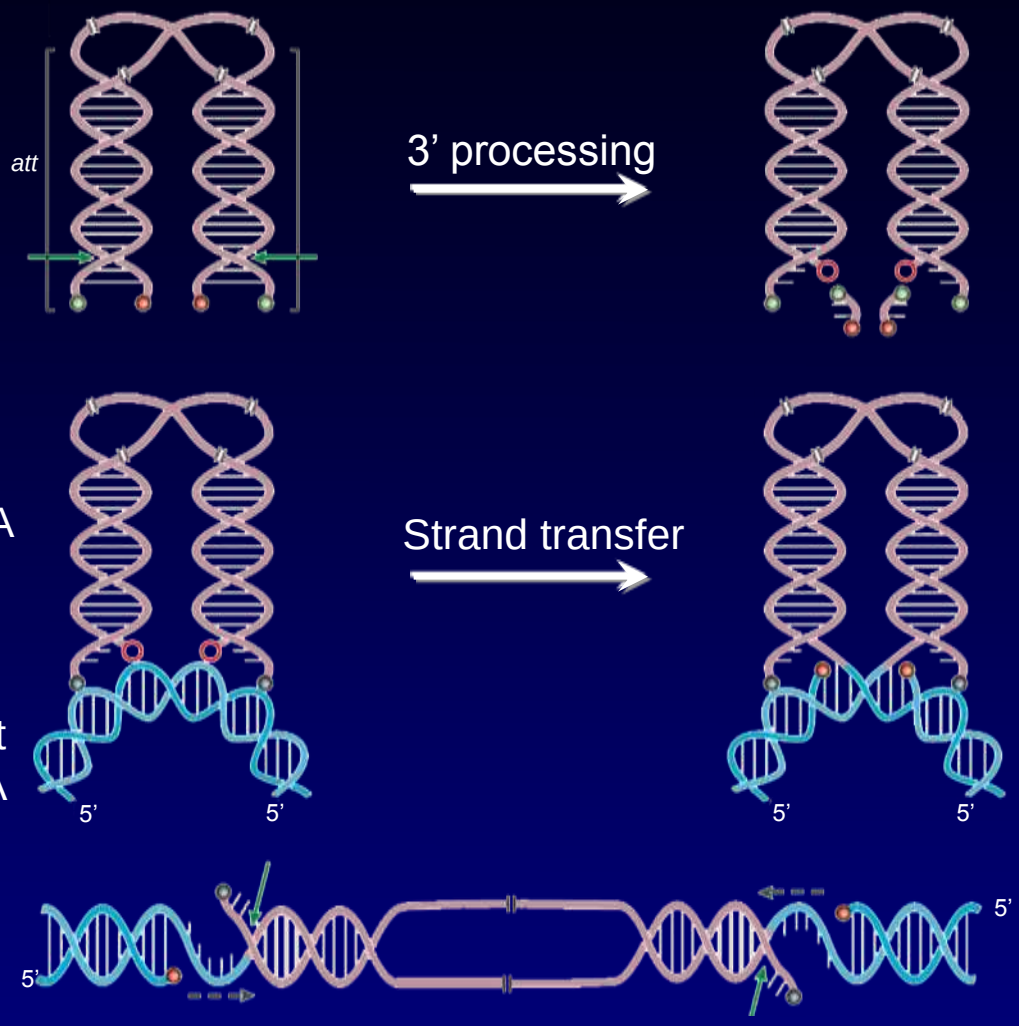
Johnson VA, et al, *Top HIV Med.* 2007 Aug-Sep;15(4):119–25.

New Agents in Existing Classes

- Mutations to older agents are likely to be present
  - Majority variants
  - Minority variants
- Some degree of cross resistance can be anticipated
  - Cross resistance increases with the number and type of mutations
- The activity of a new agent from a new class is likely to be more predictable even with state of the art resistance testing

Hammer SM, et al, *JAMA.* 2006, Aug 16; 296(7):827–43
Martinez-Cajas JL, et al, *Antiviral Res*. 2007 Dec;76(3):203–21. Epub 2007 Jul 16.

Steps of Integrase Activity



1) Endonucleotide specifically cleaves 2 bases from 3’ ends of HIV DNA takes place in cytosol, leaving “CA” ends

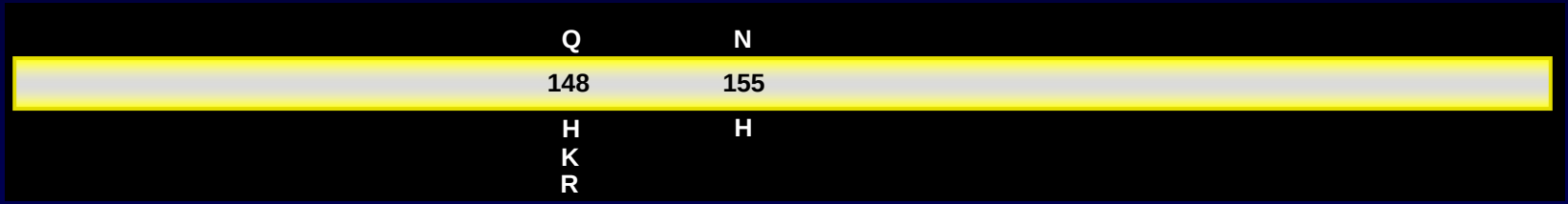
2) HIV DNA strands are transferred onto host DNA at fairly non-specific sites

3) Host DNA gaps are filled and repaired, leaving fully integrated HIV “provirus”

Thomas M, et al, *Trends Biotechnol*. 1997 May;15(5):167–72.

Mutations in the Integrase Gene Associated with Resistance to Integrase Inhibitors

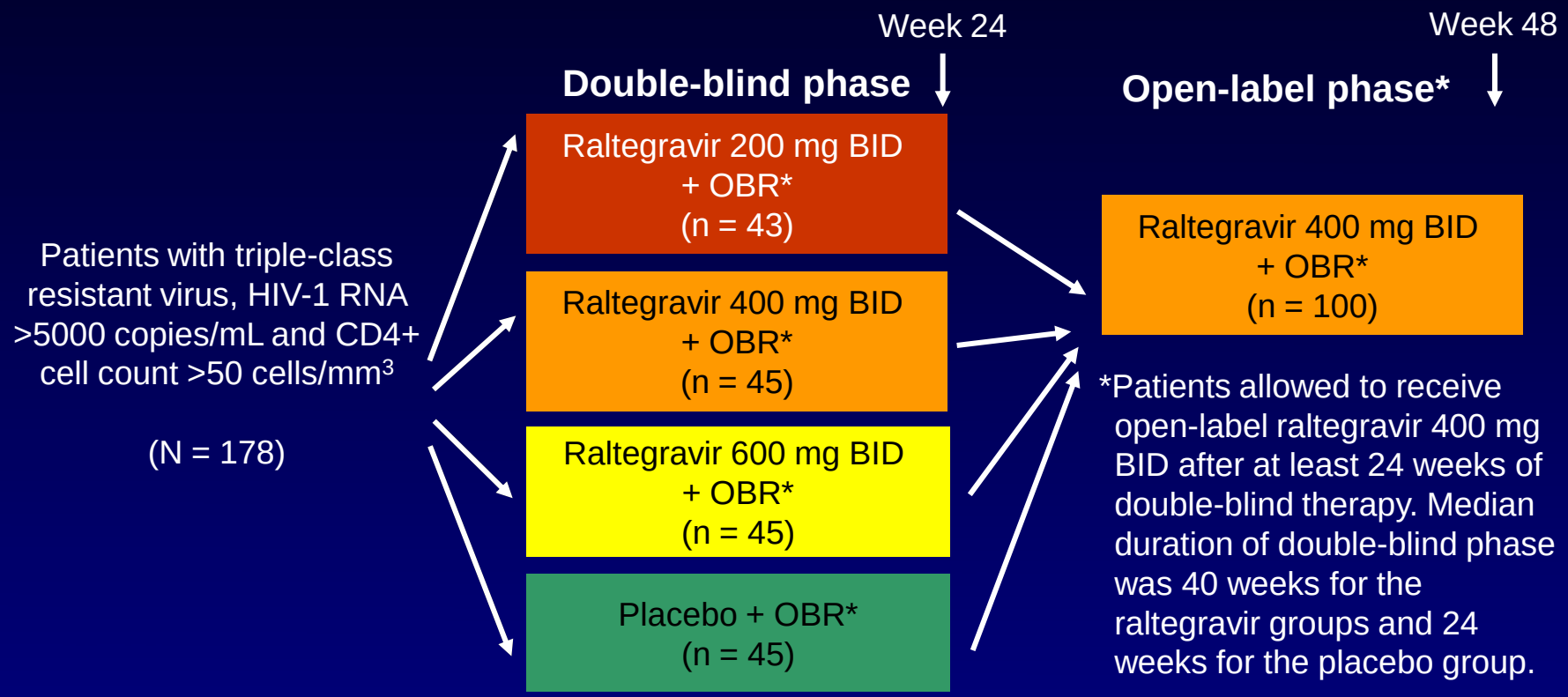
Raltegravir (expanded access)



Johnson VA, et al, 2007. *Top HIV Med*. 2007 Aug-Sep;15(4):119–25.

Protocol 005: Raltegravir + OBR in Patients with Triple Class-resistant Virus

Randomized, double-blind, placebo-controlled, dose-ranging, phase IIb study



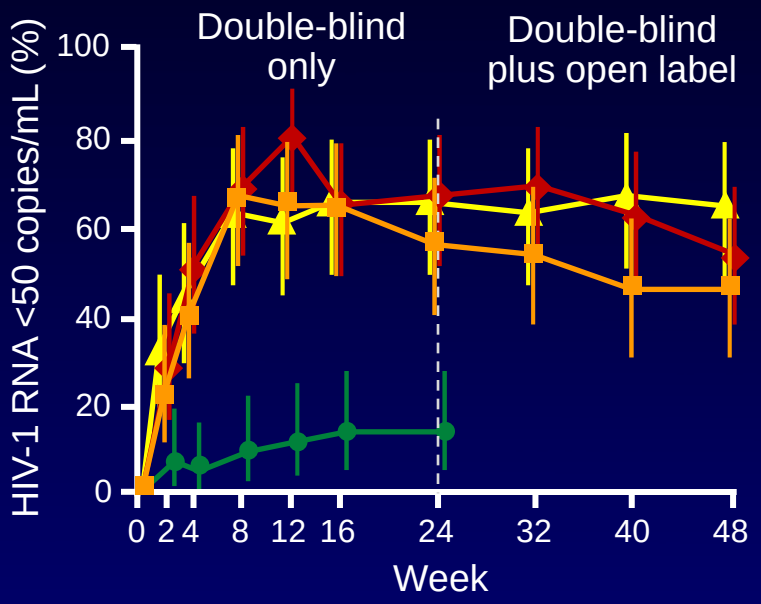
*DRV/RTV was not available for use in OBR

Grinsztejn B, et al, *Lancet*. 2007 Apr 14;369(9569):1261–9.

Protocol 005: 48-Week Results

▲ Raltegravir 200 mg BID ■ Raltegravir 400 mg BID ◆ Raltegravir 600 mg BID ● Placebo

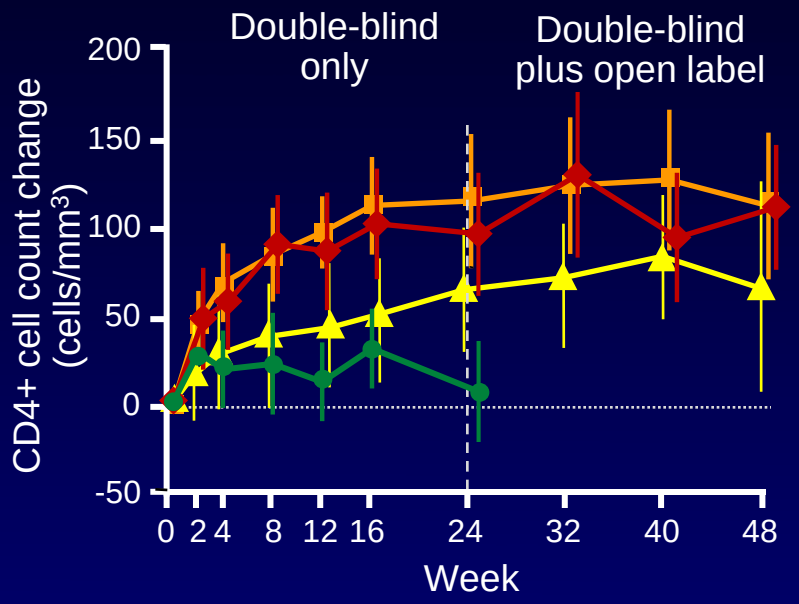
ITT, NC = F



No. of Contributing Patients

▲	43	43	42
■	45	45	44
◆	45	45	45
●	45	45	

Baseline Carried Forward for VFs



No. of Contributing Patients

▲	43	41	37
■	45	43	44
◆	45	42	43
●	45	43	

Grinsztejn B, et al, *Lancet*. 2007 Apr 14;369(9569):1261–9.

Analysis of Raltegravir Resistance in PN005

Phase 2 Study in patients w/triple class resistance:

Genotype of first time point, 24 week results

- Virologic failure was observed in 38/133 (28.6%) patients on RAL
- Integrase mutations were observed in 35 of 38 patients failing RAL
- RAL failure was usually associated with either of two genetic pathways (N155 or Q148)
- Additional mutations were frequently observed with both pathways
  - E92Q, L74M, T97A (predominately N155 pathway)
  - G140A/S, E138A/K (predominately Q148 pathway)
- Q148 containing mutants were more common than N155

N155H pathway Total n=14	Q148 pathway Total n=20	Y143 pathway Total n=1
N155H (n=2) N155H + secondary change (n=12)	Q148H + secondary change (n=19) Q148R (n=1)	Y143R (n=1)

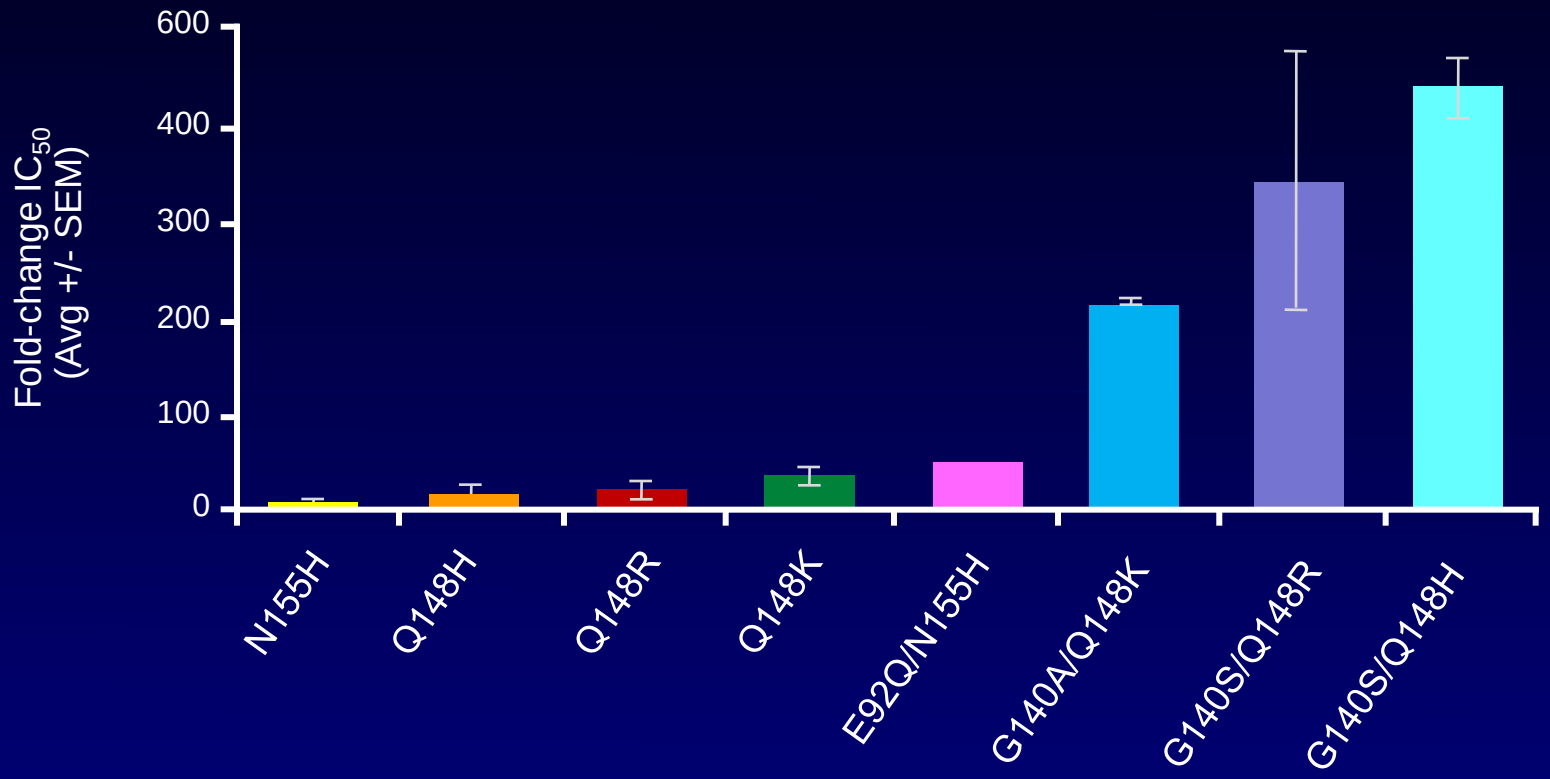
Hazuda DJ, et al, June 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #8.

Raltegravir Resistance Data Protocols 005, 018, and 019

- Paired sequence analysis of baseline and on-treatment samples from 77 subjects with evidence of virologic failure
  - 75/77 (97%) genotypic mutations in the HIV-1 integrase coding region
- 3 key mutations, **Y143C/H/R, Q148H/K/R, or N155H**
  - Observed in 65/75 subjects (87%)
  - ↓ susceptibility in cell culture to raltegravir
    - Q148 H/K/R 24- to 46-fold
    - N155H 13-fold

Grinsztejn B, et al, *Lancet*. 2007 Apr 14;369(9569):1261–9.

Phenotypic Effects of Selected Integrase Mutations on Raltegravir Sensitivity



Hazuda DJ, et al, June 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #8.

Raltegravir Summary

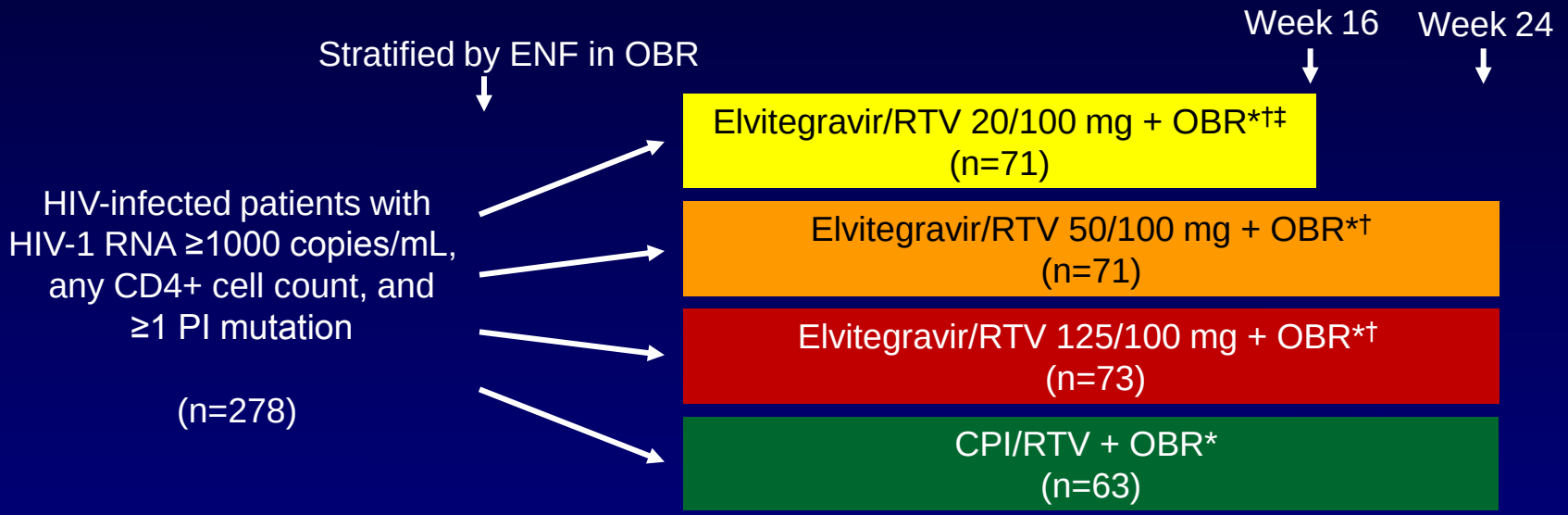
- The highest levels of resistance were seen when three or more mutations were present in Integrase.
- Single mutations were rarely seen and conferred only low-level resistance as well as decreased fitness.
- Data from the Merck 005 study are consistent with tissue culture results.

Hazuda DJ, et al, June 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #8.

Elvitegravir in Treatment-experienced Patients

Randomized, active-control, partially-blinded (dose of elvitegravir), phase II dose-finding study

Primary endpoint: time-weighted average change from baseline in HIV RNA through 24 weeks ($DAVG_{24}$)



*OBR = NRTIs ± ENF (NNRTIs excluded). †TPV and DRV permitted after Week 16.
‡Discontinued at Week 16 by DMSB.

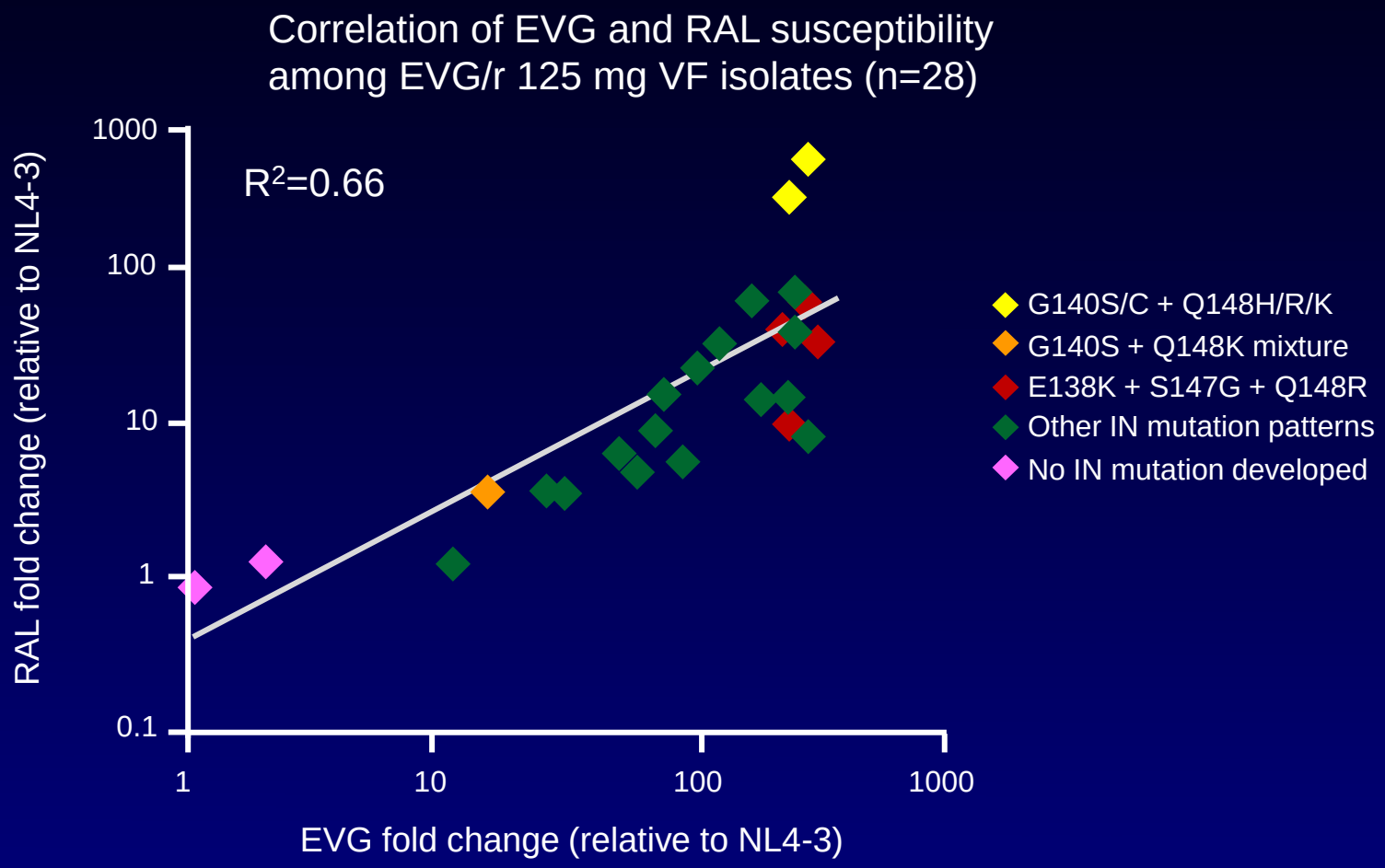
Zolopa A, et al. ICAAC 2007. Abstract H-714.

Resistance to Elvitegravir

- Phase 2 study involving 278 patients
- Most common mutations were E92Q and T66I/A/K
- Other mutations included
  - E92Q
  - E138K
  - Q148R/K/H
  - N155H
- Levels of resistance ≈150-fold

Zolopa A, et al. ICAAC 2007. Abstract H-714.
McColl DJ, et al, 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #9.

Cross-resistance Between Raltegravir and Elvitegravir



McColl DJ, et al, 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #9.

- Levels of resistance associated with failure to elvitegravir seem higher than those associated with failure against raltegravir.
- Cross-resistance between raltegravir and elvitegravir is a reality.

McColl DJ, et al, 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #9.

Resistance to Raltegravir and Elvitegravir: Observations to Date

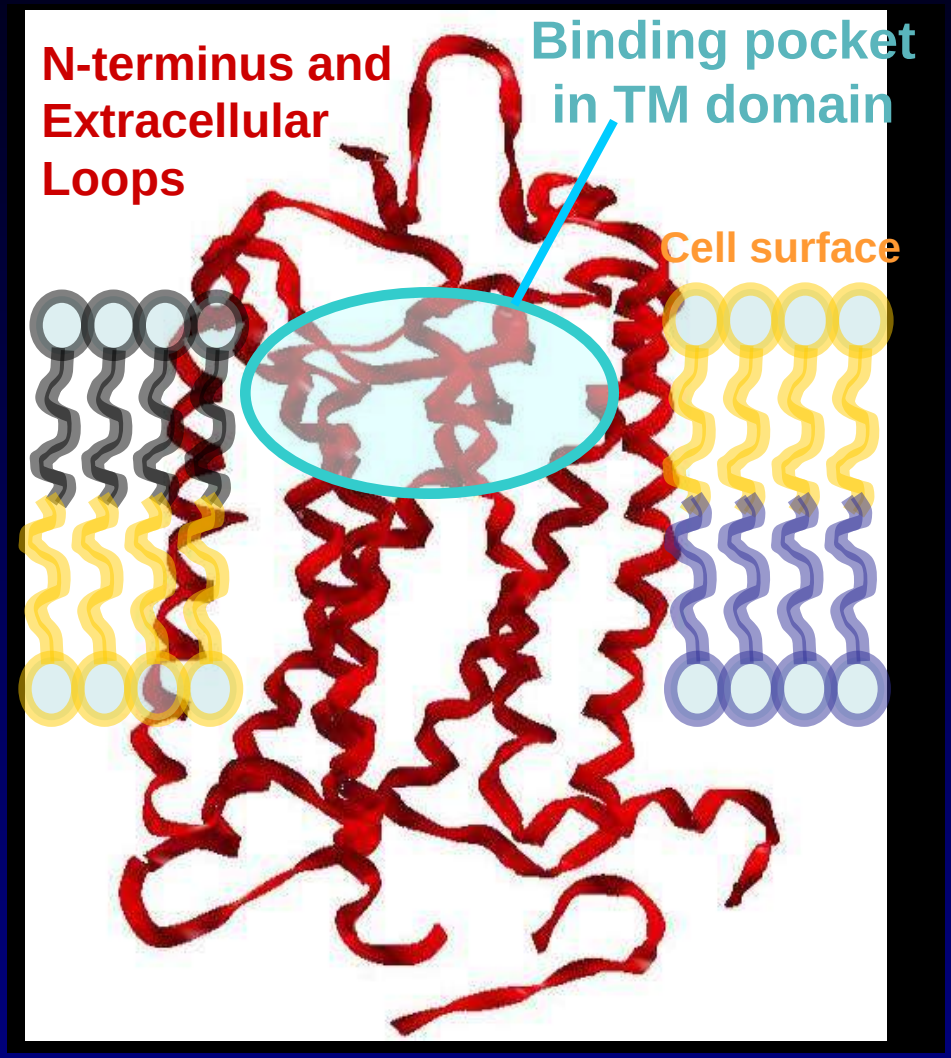
- Virologic failure is generally (but not always) associated with the development of integrase mutations
  - Multiple mutation patterns have been observed
  - For raltegravir, at least three different genetic pathways defined by a specific signature mutation and additional secondary changes have been identified
    - Secondary changes are required to confer high level resistance; consistent with a pharmacologic threshold (clinical cut-off needs to be established)
    - Time to rebound and evolution of high level resistance varies
- Early data suggest significant potential for cross resistance with Elvitegravir, but NOT all integrase inhibitors

Hazuda DJ, et al, 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #8; also McColl DJ, et al, 2007, 16th International HIV Drug Resistance Workshop, Barbados, Abstract #9.

Viral Escape to Maraviroc will be Different from Anything Seen Previously

Maraviroc:

- Binds to a host protein (all other ARV have viral targets)
- Only active against CCR5-tropic strains
- Not a competitive inhibitor

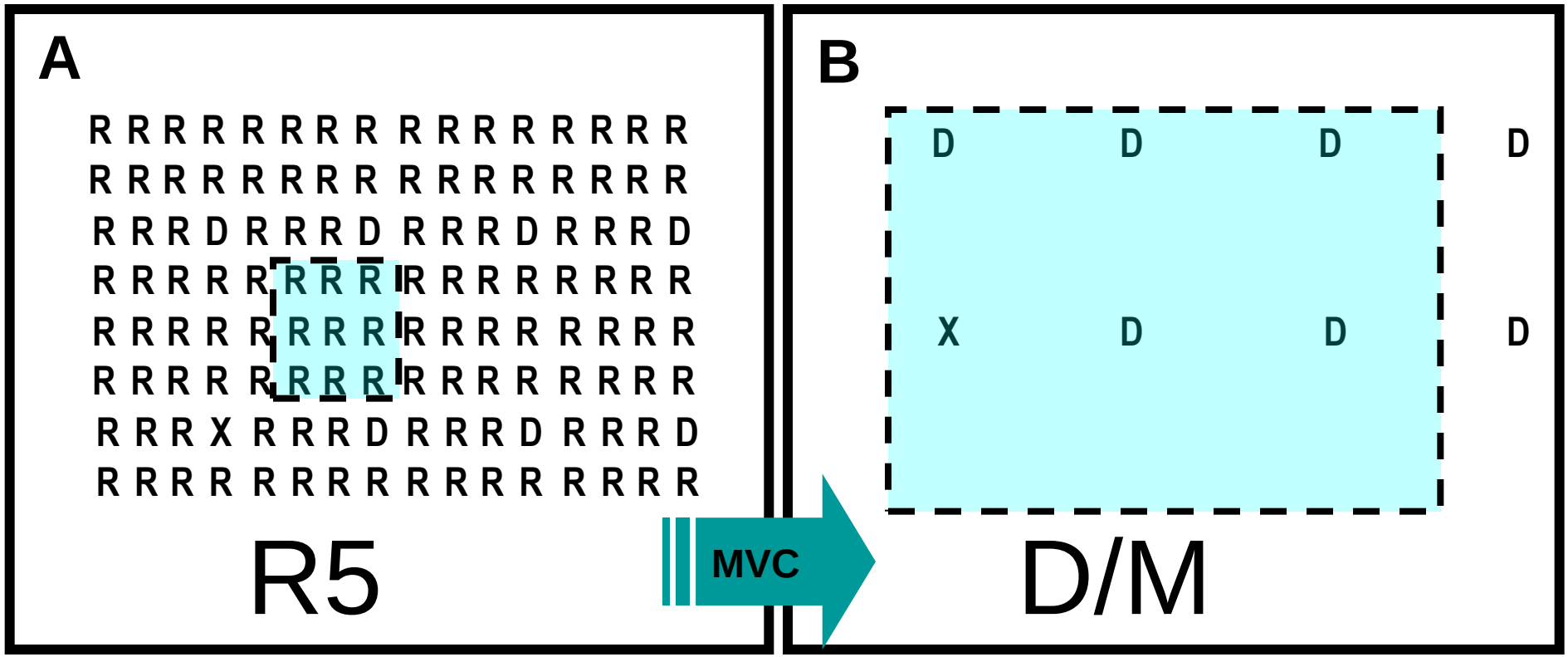


Viral escape to CCR5 inhibitors

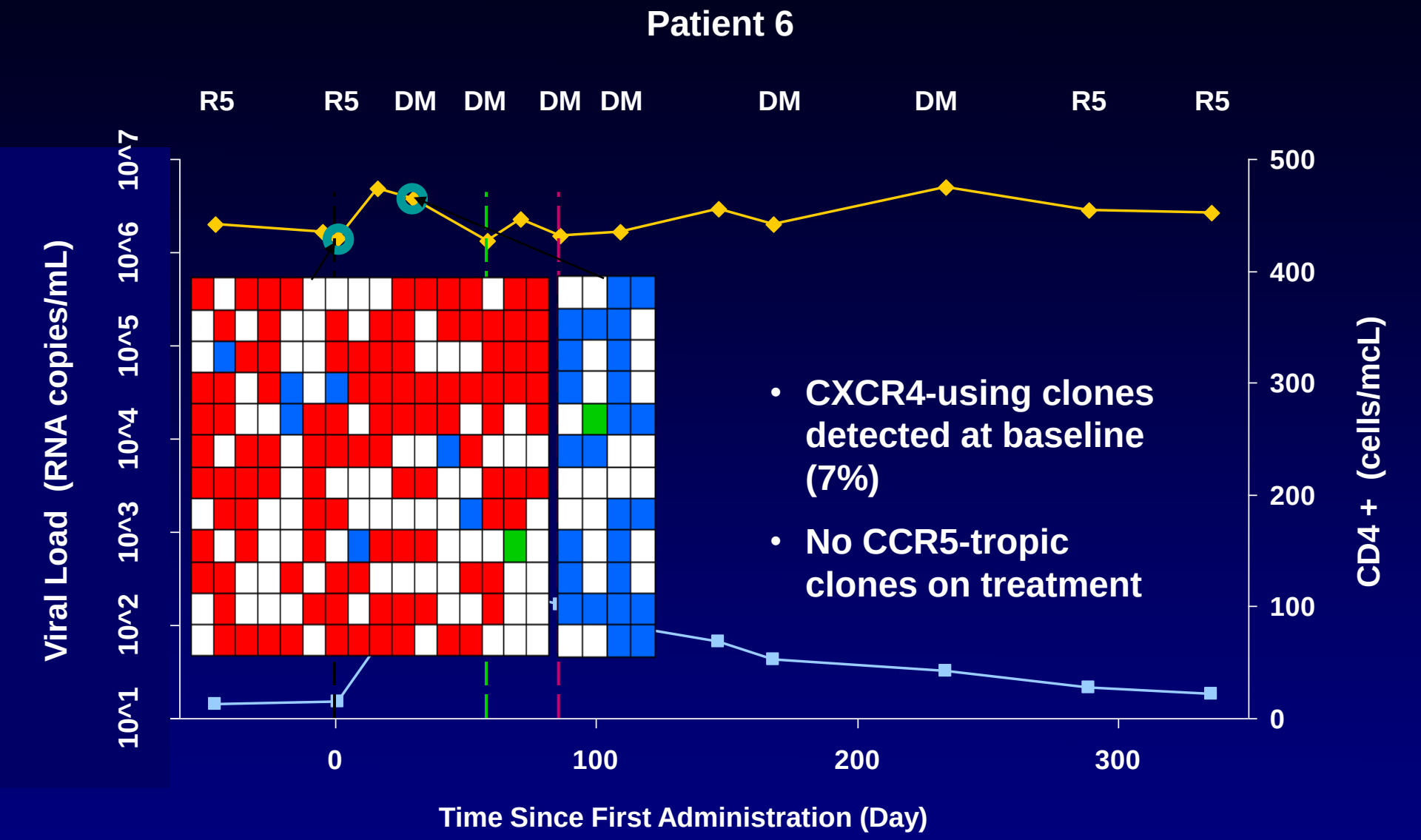
- Change in Tropism
- Phenotypic Markers
- Genotypic Markers

Selective Inhibition of R5 Viruses can Lead to a Change in Tropism Result to D/M or X4

- **Trofile™ (like all resistance tests) measures relative proportions (not absolute amounts) of different viruses (Panel A)**
- **Selective inhibition of a majority virus type, increases the sensitivity to detect the minor variant (Panel B)**



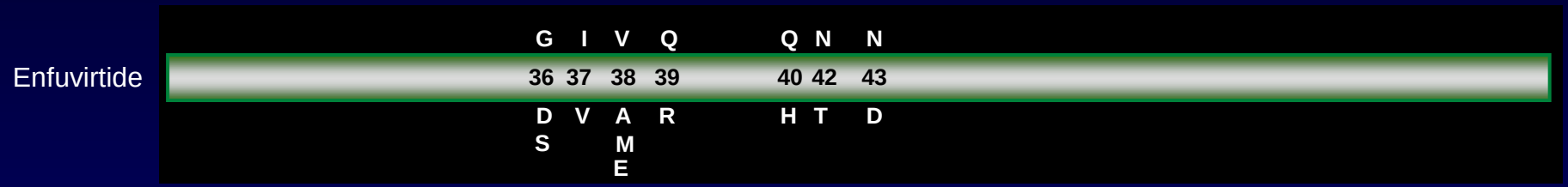
CXCR4-using Env Clones Were Detected at Low Frequency in the Baseline Sample



Resistance to Entry Inhibitors

- What about T-20 or Enfuvirtide, the fusion inhibitor that prevents HIV entry into target cells.
- It is important to understand that the target of this drug is the viral envelope gene. Therefore resistance is encoded by mutations within the env gene, unlike the situation with CCR5 inhibitors.

Mutations in the Envelope Gene Associated with Resistance to Entry Inhibitors



Johnson VA, et al, 2007. *Top HIV Med*. 2007 Aug-Sep;15(4):119–25.
Westby M, et al, 2007. *Journal of Virology*. Vol 81:2359-7.

Summary and Conclusions

- Lack of pre-existing cross-resistance is of significant benefit for new drug classes.
- The recent availability of entirely new drug classes along with newer PIs and NNRTIs is very promising for experienced patients.
- Cross-resistance between integrase inhibitors is likely.
- More information about resistance to these new agents will accumulate with more experience.

Summary

- Complete HIV RNA suppression is now a realistic goal for most ART experienced individuals even with the most complicated resistance patterns.

Conclusions

1. The occurrence of drug resistance mutations is a continuous process, so long as viruses are able to replicate.
2. Drug resistant viruses are likely to become predominant in the presence of drug, which is when they have a growth advantage over wild-type viruses that are drug sensitive.
3. Higher viral loads in patients will favour a more rapid accumulation of mutations, because more extensive viral replication will occur in this circumstance.

MUCHAS GRACIAS