

Report on 8th European HIV Drug Resistance Workshop

*17-19 March 2010
Sorrento, Italy*

Main themes of Workshop

- Managing patients with low/undetectable VLs
- Mechanisms and clinical implications of ARV resistance
- Importance of adherence to combination ARV therapy (cART)
- New diagnostic and interpretation techniques
- Epidemiology of drug resistant HIV strains
- Resistance to anti-influenza and anti-HBV drugs

Managing patients with low/undetectable VLs

- Switching ARVs is common in patients with low/undetectable VLs – because of toxicity, intolerance, incomplete viral suppression, resistance
- Careful analysis of patient's history necessary before switching to maximise regimen success
- Newer ARVs tend to have higher genetic barriers
- Genotyping used to guide ARV choices – can be successful even if VL 500-1,000 copies/mL
- Tropism testing necessary before initiating maraviroc –use plasma HIV-1 RNA if VL >1,000 copies/mL or proviral DNA if VL <1,000 copies/mL

Genetic barrier - an overview

Class	Drugs	Genetic barrier
NRTIs	ZDV/3TC, d4T/3TC	+ / ++
	ABC/3TC, TDF/3TC	+
	TDF/FTC	++
NNRTIs	EFV, NVP	+
	ETV	++
PIs	Boosted	++++
Fusion inhibitors	T20	+
CCR5 antagonists	MVC	++
Integrase inhibitors	RAL, ELV	+

Geretti, A. *Switching therapy due to toxicity in patients with undetectable viral load - the virologist's perspective*

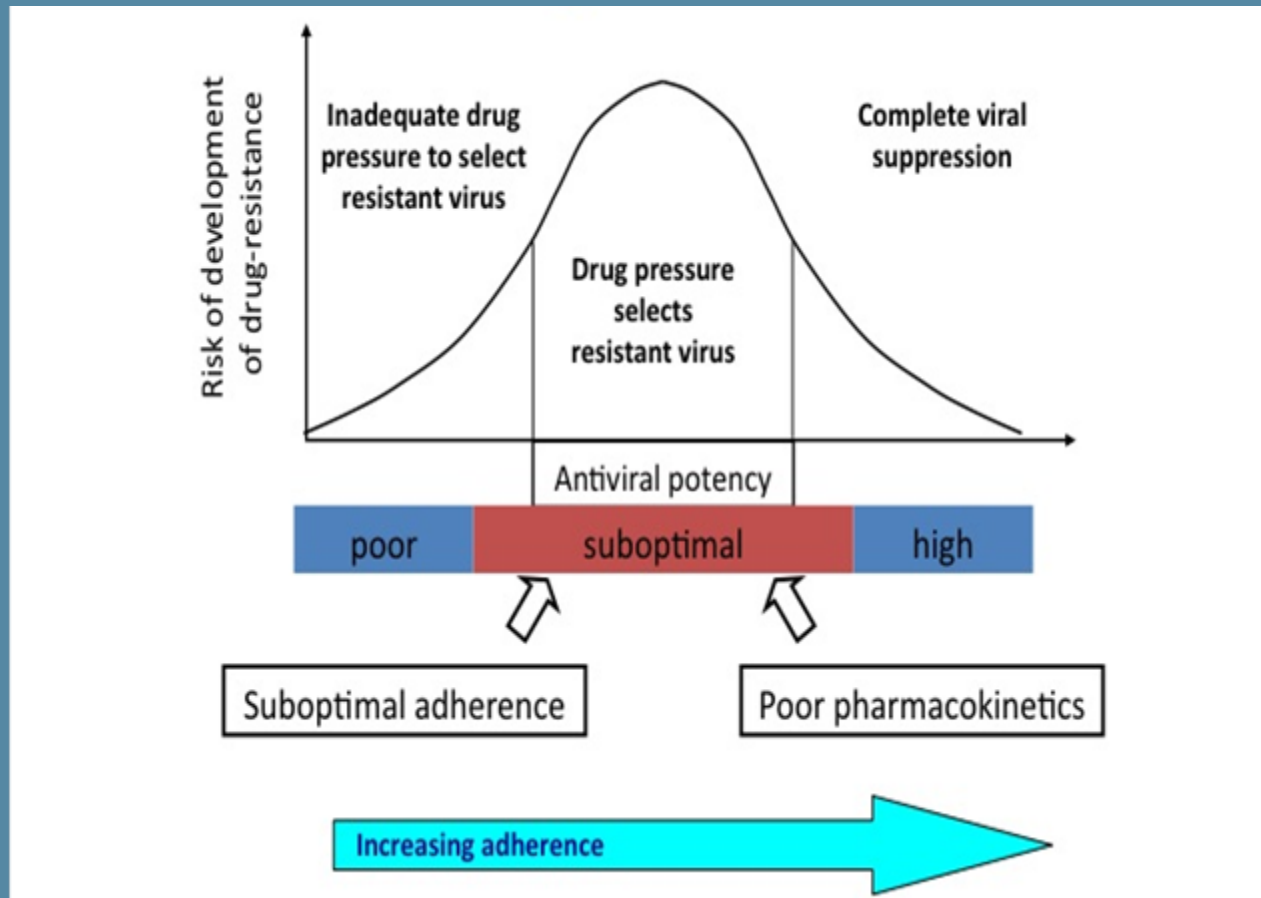
Importance of adherence to cART

- Despite the introduction of new ARVs that are better tolerated and easier to take than first generation ARVs, adherence is still a major challenge for patients
- Education about the need for adherence is not enough – patients need to develop their own adherence support mechanisms
- Ageing can affect adherence: many older patients find regular clinic visits and investigations burdensome
- Adherence rates <95% are associated with higher rates of mortality than >95% adherence

Volny Anne, A. *The patient's perspective.*

De Luca, A. *The clinician's perspective.*

Relationship between antiviral potency, drug resistance and adherence



De Luca, A. *The clinician's perspective.*

Mechanisms and clinical implications of ARV resistance

- Choosing the right backbone in initial therapy may minimise drug resistance at failure
- Risk of transmitted virus being resistant to ≥ 1 ARV ranges 2.2-24% worldwide
- Etravirine has higher genetic barrier to resistance than nevirapine or efavirenz
 - Pattern of etravirine resistance-associated mutations not yet fully defined

Perno, C. *Clinical implications of resistance to the NNRTIs.*

Minority and rare mutations associated with NNRTI resistance

- Ultra deep sequencing identified minority NNRTI resistant variants in 70 samples from patients starting NNRTI based regimen. Primary virological failure (VF) in 3 patients and secondary VF in 2 patients. No association between presence of minority NNRTI resistant variants and VF
- Rare resistance mutations are associated with NNRTI resistance only in presence of specific mutations:
 - 139R affected NNRTI resistance when 103N and 181C or 181C and 188L present
 - 219D and H affected resistance to efavirenz, but not nevirapine, if 103N and 181C present

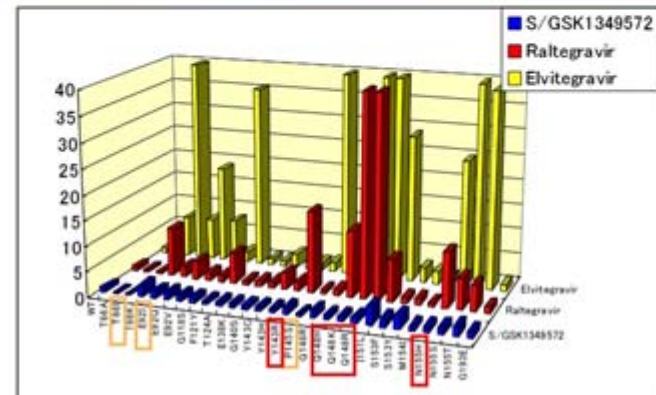
Vandekerckhove, L. *Pyrosequencing of HIV-1 reverse transcriptase to reveal minority populations of resistant virus before start of a NNRTI-based regimen.*

van Houtte, M. *Rare HIV-1 drug resistance mutations exert subtle synergistic and antagonistic effects in the context of the genetic background.*

Resistance to integrase inhibitors

- VF during therapy with integrase inhibitors associated with mutations at Y143, Q148 or N155, usually plus ≥ 1 other mutation
- S/GSK1349572 designed to have better resistance characteristics than 1st generation integrase inhibitors
- Active against virus carrying single mutations associated with resistance to integrase inhibitors

S/GSK1349572, RAL and ELV Mean FC against RAL & ELV-related Single Mutation SDMs

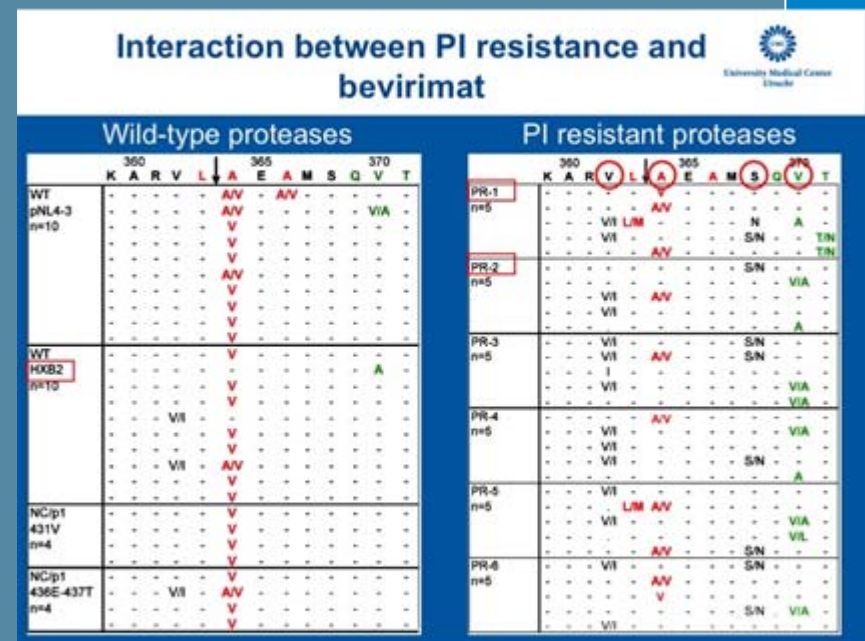


Schapiro, J. *Clinical implications of resistance to the Integrase Inhibitors.*

Sato, A. *S/GSK1349572: a next generation integrase inhibitor (INI) with limited or no-cross resistance to first generation INIs or other classes of anti-virals*

Resistance to bevirimat

- Bevirimat - maturation inhibitor that prevents capsid formation
- Significant accumulation of bevirimat mutations (QVT motif) observed in PI resistant/bevirimat naïve isolates
- Mutations in protease gene shift bevirimat resistance pathway towards QVT motif



Fun, A. Resistance mutations in the viral protease alter the *in vitro* resistance profiles of bevirimat.

New diagnostic and interpretation techniques (1)

- Ultra deep or population sequencing used to determine V3 sequences from triplicate samples of HIV RNA. Sequencing data interpreted with g2p or PSSM algorithms to determine if virus is CCR5, non-R5 or CXCR4
- Analysis of data from MOTIVATE, A4001029 and MERIT trials showed V3 genotyping is as efficient as Monogram's Trofile or ESTA assays in predicting tropism
- CCR5 antagonists most likely to be effective when CD4 cell count >50 cells/mm³ since CCR5 virus predominates in these patients. If CD4 cell count <50 cells/mm³, CXCR4 virus tends to be the majority variant

Harrigan, R. *Clinical implications of resistance to the Entry Inhibitors.*

Harrigan, R. *The influence of PCR amplification variation on the ability of population-based PCR to detect non-R5 HIV.*

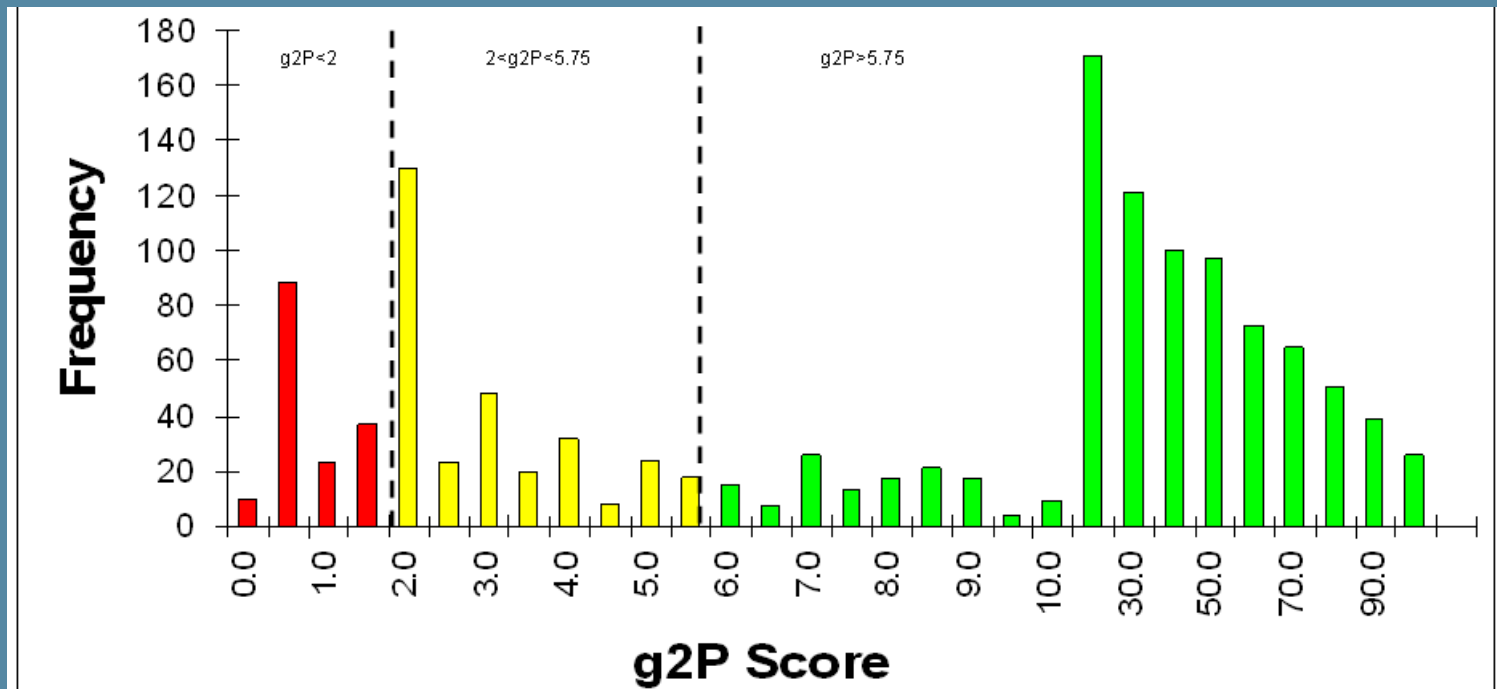
New diagnostic and interpretation techniques (2)

- Replicate PCR reactions should be carried out if testing low VL samples (e.g. <5,000 copies/mL) or proviral DNA samples
- Clinical data must be taken into account when interpreting V3 genotypic data
- False positive rate (FPR) for V3 g2p should be established using clinical data
- Automated base calling software is accurate, efficient, fast and labour/time saving. Eliminates judgement calls

Harrigan, R. *Clinical implications of resistance to the Entry Inhibitors.*

Harrigan, R. *The influence of PCR amplification variation on the ability of population-based PCR to detect non-R5 HIV.*

New diagnostic and interpretation techniques (3)



Viruses with g2p scores <2 likely to be non-R5 viruses while viruses scored >5.75 likely to be CCR5 and susceptible to maraviroc

Harrigan, R. *Clinical implications of resistance to the Entry Inhibitors.*

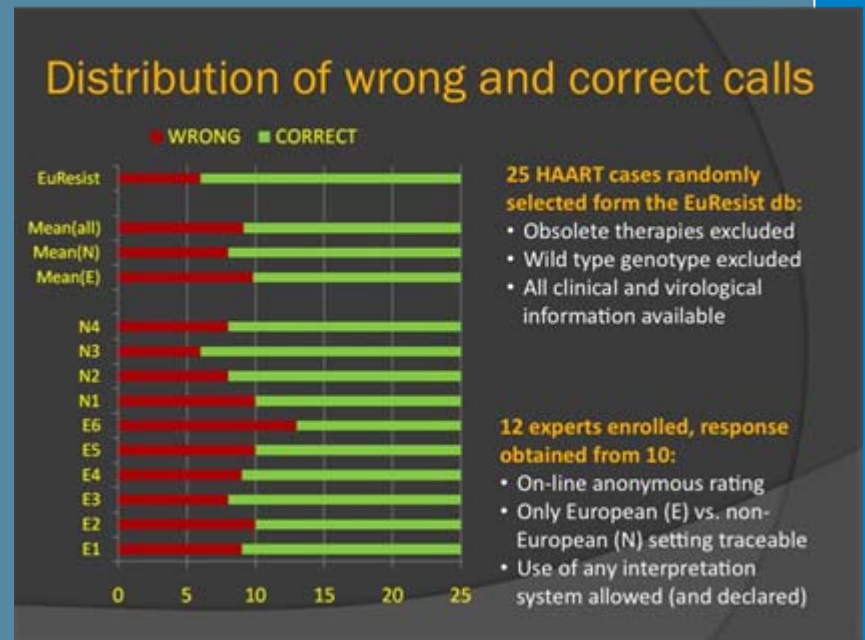
New diagnostic and interpretation techniques (4)

- Ultra deep (454) V3 sequencing evaluated as a sensitive method of detecting the evolution of CXCR4 using variants
- Paired plasma and PBMC samples were obtained longitudinally and tested in ESTA and MT-2 assays, respectively
- Samples scored as positive if $\geq 2\%$ of V3 sequences predicted to be SI/CCR4
- In 6/10 patients, predicted CXCR4-using V3 sequences were detected at $\geq 2\%$ level in samples obtained 3-6 months prior to first detection of CXCR4-using variants with MT-2 assay
- In depth analyses of evolutionary pathways leading to CXCR4 usage are in progress

van 't Wout, A. *Detection of predicted CXCR4-using HIV-1 variants in longitudinally obtained paired plasma and PBMC samples using 454-sequencing.*

EVE Study

- Comparison of experts' and EuResist's ART response predictions
- Overall accuracy of best expert & EuResist: 76%
- Only significant difference between best expert and worst expert
- EuResist and experts all wrong in 4 cases



Zazzi, M. Prediction of response to antiretroviral therapy by human experts and by the EuResist data-driven expert system (the EVE study).

Genotypic tropism testing – Cologne experience

- 61 patients treated with maraviroc in Nord-Rhein-Westfalen (NRW) cohort
 - 21 samples not analysed with Trofile: low VL or sample <3 mL
- 32 patients classified as infected with CCR5 virus using Trofile assay and 51 with g2p assay
- Treatment success rate for CCR5 positive patients: 87.5% (Trofile assay) vs. 88.2% (g2p, 20% FPR)
 - 89.7% therapy success rate if g2p 5% FPR used
- Lower cut off values may be considered in new German-Austrian guidelines

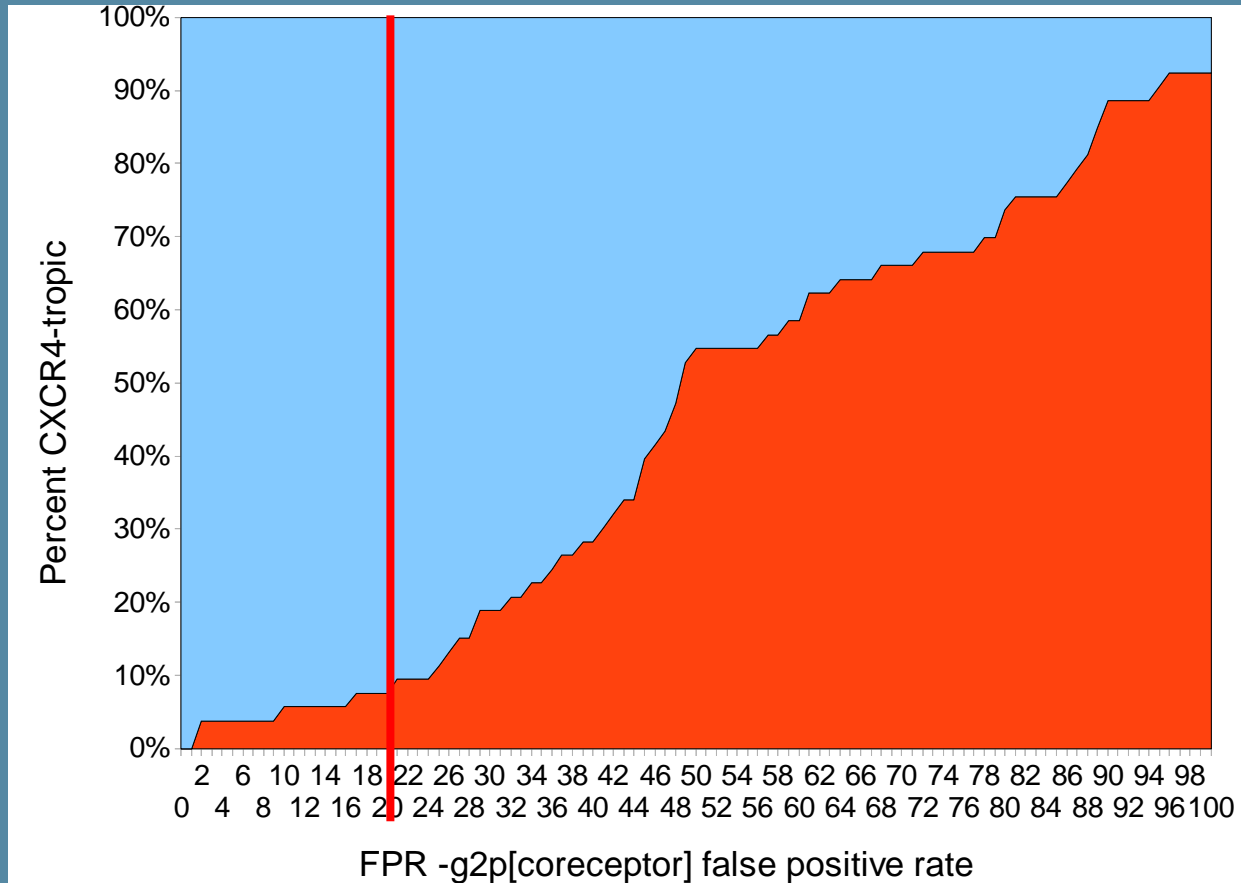
Sierra, S. *Tropism determination and clinical outcome of 61 patients under MVC treatment.*

Genotypic tropism testing – Berlin experience

- 157 patients changed to maraviroc due to VF or AEs
- Tropism testing:
 - 141 (89%) had a genotypic tropism test
 - 95 (60%) had only a genotypic tropism test
 - 53 (32%) had only a genotypic tropism test from proviral DNA
 - 70 (45%) had a Trofile® test (59 Standard Trofile; 11 Trofile-ES)
- Using a 20% FPR, 49 patients were infected with CCR5 virus; 3 with CXCR4 virus
- Even if a lower FPR used, results substantially similar for most patients
- Treatment success at Weeks 12, 24 & 48: 80-89% VL <50 cp/mL

Obermeier, M. *Tropism testing from proviral DNA - analysis of a subgroup from the Berlin Maraviroc cohort.*

Impact of different FPRs – Berlin data



Obermeier, M. *Tropism testing from proviral DNA - analysis of a subgroup from the Berlin Maraviroc cohort.*

Tropism testing – London experience

- Genotypic tropism testing undertaken using g2p
 - FPR of 6% used for clonal model or 15% FPR if clinical information available
- 28 patients switched to maraviroc due to VF (n=13) or AEs (n=15) based on tropism testing
- 7/13 (54%) patients switched to maraviroc due to VF had VL <50 cp/mL at follow up (median FU: 13 wks)
- 14/15 (93%) patients who switched to maraviroc due to toxicity had VL <50 cp/mL at FU (median FU: 19 wks)
- g2p R5 interpretation highly predictive of successful use of maraviroc

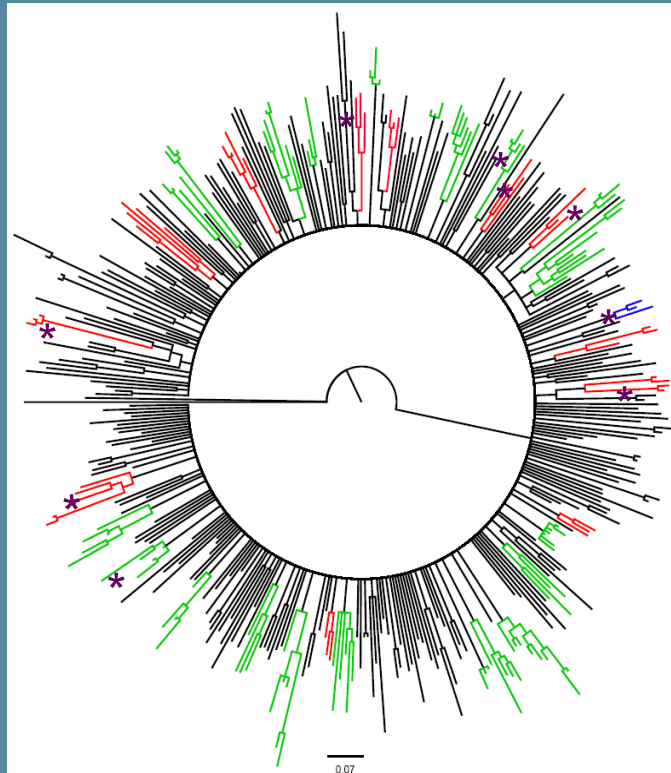
Macartney, M. *Use of a genotypic assay for prediction of HIV-1 co-receptor tropism and guiding the use of CCR5 antagonists in clinical practice.*

Genotypic tropism testing – clinical case

- 33 year old male, admitted 2007 with first episode of left leg weakness. Negative HIV test 6.5 years prior
- HIV test – confirmed HIV Ab+ve.
- pVL 361,837 cp/mL. CD4 cell count: 190 cells/mm³
- Neurological manifestations. No evidence of CNS OIs
- ART commenced: pVL <50 cp/mL; CD4: 360 cells/mm³
- Ongoing CNS HIV replication despite pVL suppression. GTT showed CCR5 virus in pVL and CNS.
- Maraviroc added to regimen. pVL & CNS VL <50 cp/mL

Mackie, N. *Tropism testing on proviral DNA to guide the use of Maraviroc in clinical practice.*

Epidemiology of drug resistant HIV strains



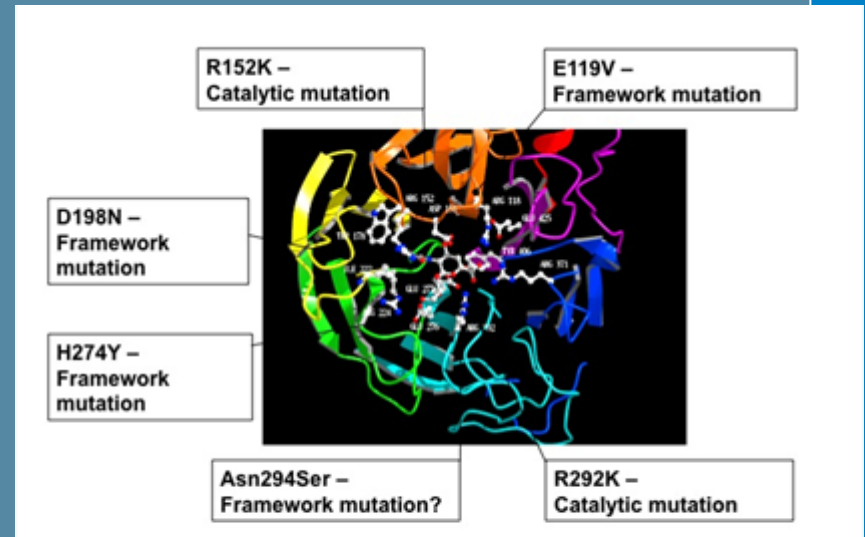
- Phylogenetic analysis of samples from 145 newly HIV infected, urban Italian patients
- 34 clusters: 12 only newly diagnosed (red); 1 seroconverters only (blue); 21 NDs and SCs (green)
- 9 clusters (26.5%) involved resistant strains

Key: ND = newly diagnosed; SC = seroconverter

Lai, A. *High prevalence of epidemiological networks involving primary resistance in chronically infected HIV-1 naive individuals.*

Resistance to anti-influenza drugs

- Several mutations in genes encoding catalytic pocket and framework are associated with resistance to neuraminidase inhibitors (figure)
- Oseltamivir-resistant pH1N1 can emerge quickly and replace wild type virus

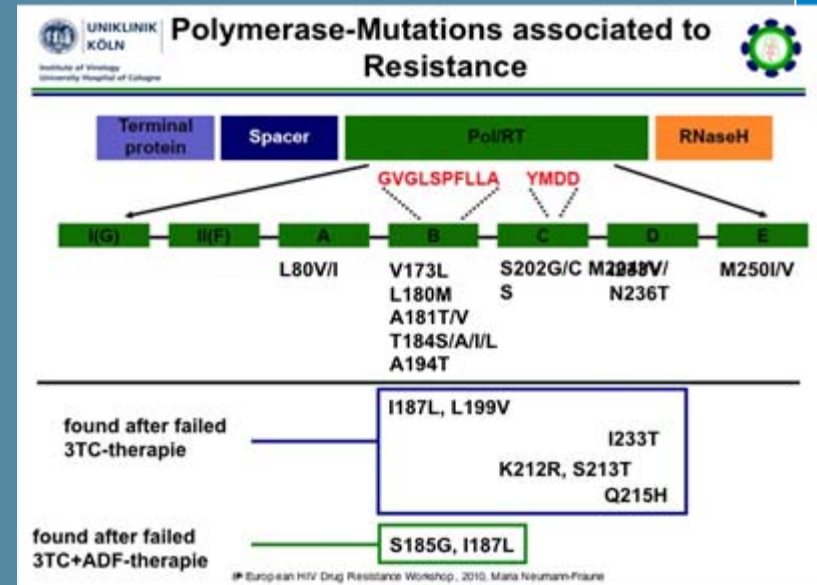


Lina, B. *Influenza A resistance to antiviral therapy*

Foster, G. *Detection and quantification of oseltamivir resistant pandemic influenza A H1N1 in immunocompromised patients.*

Resistance to anti-HBV drugs

- Numerous mutations associated with resistance to anti-HBV drugs identified in polymerase/RT gene of HBV (figure)
- Web based service for HBV drug resistance analysis: www.genafor.org



Fraune, M. *Frequencies of hepatitis B surface-antigen mutations in drug resistant hepatitis B virus isolates.*
Beggel, B. *A web service for HBV drug resistance analysis.*

Conclusions (1)

- Drug resistance is a major and growing problem for management of many infectious diseases – HIV, influenza, TB and HBV
- Emergence of drug resistant strains can compromise treatment efficacy and patient's prognosis
- Careful choice of treatment regimens can minimise development of resistance

Conclusions (2)

- Innovative diagnostic and analytical methods can guide treatment choices e.g. genotypic tropism testing to predict likelihood of maraviroc success
- Maximising adherence to ART minimises risk of resistance to ARVs emerging