

SUMMARY HIGHLIGHTS FROM THE
2ND EUROPEAN RESISTANCE WORKSHOP

FROM BASIC SCIENCE TO CLINICAL IMPLICATIONS

11-13 MARCH 2004, ROME

Advances in HIV treatment experience and the concomitant rise in drug resistance have meant that resistance can no longer be perceived as a specialist cache only of concern to basic scientists. Changing viral landscapes reflective of migration and population mobility, transmission of new infections, a plethora of new treatment agents with novel and shared mutational pathways have all profoundly affected the dynamics of drug resistance evolution. Drug resistance testing and interpretation is now more than ever an essential medical prerequisite for physicians and patients in the critical decision-making process of selecting and sustaining effective treatment strategies.

The 2nd European HIV Drug Resistance Workshop (11-13 March 2004) provided a useful opportunity to share and reflect on progress in HIV drug resistance and its clinical application, particularly the impact on patients across Europe. In the holy milieu of the Catholic University in Rome, 400 scientists, practitioners and community journalists discussed developments in clinical science and updates on regional and pan-European epidemiology. The native Italian ebullience and aesthetic setting of Rome helped to create an industrious but enjoyable environment for peer-learning.

Our thanks to all those who contributed their expert insight and participated in the debates. We look forward to welcoming delegates to next years meeting.

Organising Committee

Charles Boucher, University Medical Center Utrecht, The Netherlands.

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THE GLOBAL EPIDEMIOLOGY OF HIV AND DRUG RESISTANCE

Sally Blower from UCLA began the discussion on the global epidemiology of HIV by highlighting the role of treatment and its potential impact on transmission rates and resistance evolution. One major obstacle often cited for the parsimonious intervention made so far by the developed world towards treatment in resource-poor nations, has been the distracting claim of mass-scale population resistance. Dr Blower's scholarly modelling techniques helped to illustrate two key postulates: firstly that the roll-out of the promised levels of therapy will need to be both substantial and real if we are to effectively inhibit the alarming rates of infection currently raging across the developing world. Secondly, that the alarm at potentially generating an epidemic of drug-resistant strains of HIV is both misplaced and erroneous. Therapeutic benefits she maintained can and should be considered on the enormous impact that it will have at both the individual and the population level. For the second premise, that the use of antiretroviral therapy may serve as secondary prophylaxis to reduce infectiveness and hence limit transmission, she has extensively applied biostatistical methods with her colleagues at UCSF and Harvard to determine the rate of therapy introduced and the consequential impact on rates of transmission. For example, if the 'plans and promises' of the WHO (3 million by the year 2005), the US (2 million over the next 5 years) and the South African government (1 million by 2008-9) were indeed realised, then these combined pledges would address the need of the 6 million currently eligible for treatment in the developing world. This amounts to 5-10% of the 40 million population HIV-infected at present. Variables included in the models relate to response to therapy and factorial determinants of clinical and virological failure that would result in drug resistance. She showed that transmission decreases over time relative to the different fractions of the population being treated with cumulative infections prevented:

Treated population	Cumulative cases of transmitted resistance after 5 years
5%	11,000 – 172,000
10%	23,000 - 300,000
25%	19,000 - 855,000

The epidemiological and undoubtedly, political analysis is clear – whilst the absolute number of infections may be high, the percentage of resistance transmission can be maintained below

the specified 5% WHO threshold. And to strengthen the case, she noted that the majority of drug resistance will be due to acquired rather than transmitted resistance. Finally, Dr Blower presented a critique of the argument for greater surveillance of resistance in the developing world, noting that if resistance is unlikely to be greater than 5% of the population, large-scale surveillance systems may be superfluous and precious resources could be more usefully channelled towards treatment strategies.

This final proposition was in direct contrast to the presentation by the WHO representative Silvia Bertagnolio who maintained the value of global surveillance. Her presentation focused on the disparate and comparatively inconsistent methodologies currently utilised to assess drug resistance prevalence regionally, and the problematic nature of bringing these data together in a uniform and meaningful format. In response, she promoted the WHO resistance surveillance programme that aims to standardise reporting and analysis of resistance evolution at an international level. (abstract 2, S Bertagnolio et al)

The changing viral landscape in Europe

Reports that 10% of the HIV population in Europe were infected with HIV drug resistant strains made headline news internationally last year when findings from the CATCH study were first published. At this meeting, further results were presented from this pan-European study which involves 19 member states and over 2000 antiretroviral-naïve patients. David van der Vijver on behalf of the CATCH study team provided an effective profile of the evolving landscape of viral subtype distribution across Europe. The escalation of non-B subtypes can be linked to the chronological patterns of migration and an increasingly mobile population globally. Whilst subtype B continues to dominate the HIV landscape in Europe, the prevalence of non-B subtypes has increased from 17% in 1996 to 28% in 2002; a 70% increase of non-B subtypes among patients infected less than one year within this timeframe. The breakdown of subtypes include greater prevalence of clades C, G and A variants and interestingly, the largest subtype profile appears to be the recombinant A/G variant. As expected, these are distributed unequally amongst the different transmission groups, notably that only 58% of the heterosexual community harbour clade B virus whilst 72% of injecting drug users and 95% of MSM (men who have sex with men) have been confirmed as B subtype.

Although the overall prevalence of resistance is greater in the B population, there is an observed 3% decrease in resistance transmission. This pattern is reversed in the non-B subtypes which have seen an increase in resistance transmission from 2% to 4% between 1996 and 2002. ⁽¹⁾

TRANSMISSION OF RESISTANCE

The singleton problem

Current resistance guidelines recommend resistance testing for newly diagnosed patients if the incidence of transmission of resistant virus exceeds 10% of the treatment population. Jan Albert from the Swedish Institute of Infectious Disease Control noted however, the distinct absence of a clear definition of resistance transmission and appropriate guidelines to help interpret transmitted or evolving mutations. This was the rationale proposed for the newly formed expert panel that will develop informed guidelines for interpretation of resistance transmission in drug naïve patients.

By way of highlighting the contentiousness of the task ahead, he discussed the difficulty of interpreting sequences that harbour single-resistance-associated mutations reported as evidence of transmission or as defined it, 'singleton problem'. Of the 16 mutations recently reported in JID as drug-selected mutations, identified in 18% of newly diagnosed subjects, 12 of the 16 mutations were singleton, i.e. involved changes at a single position of the reverse transcriptase sequence, namely V118I (3), T69N (3), T69S, E44D, Y115F, K103N, K101E and protease mutation M36I. The key questions posed by the singleton issue is that of the source patient and whether these mutations are natural variants or caused by reversion from more complex mutational patterns. Dr Albert commented on the complexity of retrospectively analysing a pandemic 20 years after it has been introduced with intermittent, sequential and highly inconsistent treatment strategies. The popular perception of transmitted resistance is to consider these to be evidence of prior drug pressure, but that does not explain how it is that the K70R mutation was detected even before AZT was introduced as treatment for HIV. ⁽²⁾

Deenan Pillay from University College London made one of the most important and frequently referenced observations from the workshop - that referring to a group of HIV-1 viruses as simply non-B clades is a tragic misnomer. This anomalous nomenclature does not accurately

reflect the complex phylogenetic of viral dynamics and classifying viruses as B or non-B in fact reinforces a dichotomous misconception. Phylogenetically he described the disparity between clade B and C for example as genetically distant as the range between clades A and G. Understanding and developing a more appropriate lexicon and approach to other viral subtypes will positively influence the study of different viruses as separate genetic phenomenon worthy of independent research.

In his overview of HIV epidemiology, Dr Pillay also commented that the different techniques used to define viruses including sequencing of different parts of the gene (RT, PR, RT-PR), hetero-duplex mobility assays (HMA) and similarity scores complicate any useful meta-analysis and learning from these important studies.

Data presented on prevalence of subtypes

Abstract	Country	N	Time	Char	Non-B	Methods
11	Greece	49	02-03		45	Phylo
14	Den	251	00-01		37	Similar.
17	UK	284	01-03	Risk gp	39	Similar.
20	Italy	63	97-01	Risk gp	9	HMA/Ph
22	Italy	157	94-03		15	Phylo
24	C d'I	206	97-02		100	Phylo
24	Vietnam	200	01-02	Risk gp	100	Phylo
4	p-USSR	265			92	Similar.
7	P-USSR	119	96-02	IVDU	100 (A)	HMA/Ph
13	Germ	580	94-03	sc	8	Phylo
21	Italy	2061	81-03	K d s	7	?
23	Baltic					Phylo

Similarly, he supported the contention that a systematic approach to identifying mutations to assess transmission of drug-resistant HIV for use in epidemiological studies was urgently needed and that acceptance of epidemiology abstracts for scientific meetings and publications should be predicated on uniformity of sampling strategies, risk groups and definition of resistance.

MOTHER TO CHILD TRANSMISSION AND DRUG RESISTANCE

Marleen Temmerman's presentation on prevention of mother-to-child-transmission (PMTCT) contained much salutary advice. Programmes for PMTCT have flourished in the developing world since the realisation that a single dose of nevirapine (NVP) administered to the mother during childbirth could prevent transmission by 16% or 2% when combined with AZT. But more recently concerns have been raised that even a single dose of NVP could confer drug resistance, compromising future therapeutic options for the mother and the child. Of the 455 women treated in South Africa for PMTCT 39% developed resistance as did 45% of the babies who were born infected with HIV. Similarly, in a Thai study, 30% of women treated with NVP acquired resistance and when treatment continued after delivery they struggled to maintain viral control. At 6 months post-delivery only 34% had undetectable viral load compared to 75% of those who had not receive NVP during labour.

She reiterated some of the cautionary lessons of intervening before we have fully understood the dynamics of a population and context that are significantly different to those in the industrialised world. "Without due planning", she noted (from Stevens, BMJ 2004), "antiretroviral therapy is likely to be introduced to Africa in a random and haphazard way, with inconsistent prescribing practices and poor monitoring of therapy and adherence. This risks the rapid development and transmission of drug resistance." Advice based on direct experience such as Dr Temmerman remains invaluable in informing strategies for intervention in the developing world, but can nevertheless be limiting in its scope. Inadequate systems for health care, lack of diagnostic resources and poor adherence pose an inherent danger of becoming obstructive truisms. Recent studies from Africa have in fact shown greater levels of adherence compared to patients in the developed world including a study by MSF using blood levels as a measure for adherence and treatment response found that 95% of patients were 96% compliant at 6 months. Experience in Kenya where rates for HIV testing can range between 50-90% although the return rates for results only total 10-30% of those tested do indeed tell us a great deal about situational complexities.

Nevertheless, the sobering and emphatic conclusion made by Dr Temmerman is that despite our intervention we have in fact learnt very little that is useful. Quite rightly, she advocates for a model of care that does not simply consider drugs as the only solution, but rather commitments towards the availability of quality prenatal care, understanding the lives of

women living with HIV and the associated stigma, violence and social ostracism and importantly, strategies for maintaining the health of the mother and child beyond the concern for transmission as integral components to effective and sustainable care.

Meanwhile, reports from an ongoing Italian study of resistance evolution in pregnancy considered genotypic profile, transmission and viro-immunologic changes as a result of HIV treatment.

The patient profile included:

	Group 1	Group 2	Group 3
Women of European origin	75%	92%	100%
Women of African origin	25%	8%	--
Mother to child transmission	7%	--	9%
GRT	16 pts	12pts	13pts
Caesarean delivery	100%	69%	--
Intrapartum ZDV	100%	100%	85%
Infant 6-wk postpartum ZDV	100%	100%	100%
Key mutations observed >5 pts	L63P,M36I	L63P,M36I	L10I,M36I,M46I/L,A71V,V82A

(Group 1 – ART-naive at conception, Group 2 – interrupted therapy during first 3 months and Group 3 – treatment failure at different time of pregnancy)

The study found minor mutations in treatment-naive patients with a higher number of mutations observed in HAART-failed than in patients who interrupted treatment during the first 3 months of pregnancy.

They concluded that:

- GRT-guided treatment during pregnancy led to a viro-immunologic benefit in patients stopping HAART during first trimester.
- an immunologic benefit was observed in naive patients.

- limited value was observed in patients with HAART failure but despite the high number of mutations in these patients and the low percentage of women delivering with viral suppression, a low frequency of transmission was observed
- despite highly standardised procedures for prevention mother-to-child, transmission can still occur
- after delivery, the motivation to continue treatment is severely decreased leading to a high prevalence of treatment interruption and in patients who continue therapy, poor adherence leading to accumulation of mutations and inadequate virologic control. ⁽³⁾

RESISTANCE TO NEW ANTIRETROVIRALS

In her opening delivery, Anne-Mieke Vandamme from the Rega Institute, Leuven, Belgium alerted delegates to the inexorable rise of HIV resistance in the absence of adequate new drugs susceptible to resistant virus. With remorseful caution, Dr Vandamme outlined the developmental pipeline for newer agents with the observation that these potential therapies were not keeping pace with the level of clinical need. Her observation was not so much that new molecules were not being discovered, but rather that even with the current pipeline, there was little to raise patient and physician enthusiasm. Second generation drugs including the newer PIs, she noted, whilst adding additional potency to treatment regimens may compromise longer-term sustainability with the possibility of conferring cross-resistance. With the rise of 3-class resistance at around 50% in some populations the demand for newer drugs with novel target sites was far exceeding the promise of current supply.

Interfering with entry

As one of the newest candidates on the treatment block and the only compound targeting a different site of viral action, enfurvitide (T20) continues to receive much attention, especially as remains way ahead of its competitors in being currently available in the clinic, albeit at a premium cost. Given the limitations and fragility of constructing sustainable regimens for patients in salvage for whom T20 may be particularly useful, the understanding of emergence resistance to this agent is of great importance.

François Clavel from Claude Bichat, Paris provided an erudite insight into the mechanisms of fusion inhibition and evolution of resistance in the *env* gene. Dr Clavel posed the very pertinent question uppermost in our minds of what may happen when we start to interfere with host responses as opposed to intracellular viral processes. For example, might the blocking of CCR5 co-receptors impact on the dynamics of CXCR4 population and function? And are other issues such as co-receptor density likely to be an important factor in the efficacy of the emerging entry inhibitors? For instance, it was suggested that T-20 may be more active against cells with low CCR5 density and have greater activity against viruses displaying lower *env* receptor affinity. Research from other fields confirm that ethnicity and gender may also influence CCR5 density and it remains unclear whether these issues will be clearly addressed in future clinical development programmes as significant determinants affecting potential inter-patient variability and clinical outcome.

The take home message for entry-inhibitors at this stage may be that we should not consider these agents as “just another therapeutic target” but that HIV entry:

- is a complex, multi-step, highly dynamic and co-operative process
- resistance to entry inhibitors will involve direct mechanisms affecting drug affinity
- resistance will impact on other direct and indirect processes of *env* kinetics
- naïve viral populations may differ in their response to entry inhibitors

Dr Clavel was less forthcoming on the issue of diagnostic technologies, understandably so given the current paucity of validated assays available for profiling *env* resistance and even less clarity on what is being measured at the level of co-receptor action. There are some emerging methodologies including those from his own laboratory for *env* sequencing, but the latter issue of co-receptor dynamics is further complicated by the measure of quantitative CXCR4 (X4) and CCR5 (R5) virus circulating at any given time. Current techniques simply confirm the patient’s status as X4, R5 or dual-tropic, but does not statistically stratify the dominant receptor usage and the emergence of X4 viruses. The need to develop an appropriate diagnostic arsenal is becoming increasingly important as R5 and X4 inhibitors proceed into clinical development.

Continuing the T-20 story, a group from Galicia, Spain reported on an interesting study of naturally occurring resistance to fusion inhibitors including T20, showing that resistance in the

gp41 sequence occurred in over 11% of patients with B, non-B and recombinant subtypes in Spain. The cohort included 170 patients' samples (131 on HAART, 20 with chronic infection but untreated and 19 patients newly diagnosed) – all were T20 naïve. Over 42% harboured polymorphisms in the N-peptide HR1 region. Subtype variations did appear to have an impact on the incidence of mutations observed. For example, a statically significant proportion of polymorphic changes were noted for non-B and CRFs, 72% compared to 28% for B clades. The N42S polymorphism associated with increased susceptibility to T20 was more frequently observed in non-B and recombinant forms. ⁽⁴⁾

Francois Roman from Luxembourg presented an analysis of the impact of the I37V mutation which has previously been reported both in patients' naïve to T20 and in clinical isolates with moderate resistance to T20. The study found that as a single mutation, I37V does not impact on T20 susceptibility, but interestingly its presence does appear to impair the replication capacity of HIV. ⁽⁵⁾

The evolution of genotypic and phenotypic resistance to T20 was considered in a very different patient group, those with long term experience of T20 therapy. Eva Poveda from Madrid assessed 8 heavily treated patients with multiple drug resistance and found that resistance at codons 36 and 43 occurred most frequently leading to high-level phenotypic resistance to T20. ⁽⁶⁾

Finally, findings from the TORO studies have helped to establish the virological and immunological benefits of adding T20 with optimised background maintained to 48 weeks. A presentation from Michael Greenberg of Trimeris confirmed that patient's naïve to fusion-inhibitors do indeed exhibit a range of susceptibility to T20 and that these are not influenced by co-receptor tropism. Additionally, although virologic failure with resistance to T20 has been associated with genotypic changes at positions 36-45 at gp41, it was suggested that these substitutions may in fact confer reductions in replicative fitness. ⁽⁷⁾

Next generation PIs

The resistance profile of the most recently licensed protease inhibitor, atazanavir (ATV) was profiled by Rich Colonno of BMS. Its pathways to resistance include both cross-resistant strains and signature mutations that at present seem to be unique to ATV.

ATV signature resistance profile	Primary	Secondary
	G48	I10
	V82	K20
	I84	L24
	L90	I33
		M36
	I50	M46
		I54
		I63
		A71
		G73

No single substitution or combination of mutations was reported to be predictive for ATV resistance and accumulation of 4 or more mutations resulted in a decreased susceptibility to ATV. 6 or more ATV associated mutations may be required for a greater than 50% clinical failure rate due to resistance, drawing inevitable comparisons with the activity and response to LPV/r treatment. The I50L mutation is known to emerge with ATV treatment in around 30% of PI-experienced patients and 100% of resistant isolates from drug naïve patients and is mostly accompanied with changes at positions 33, 34, 45, 71 and 73. This mutation has been widely reported to impair viral fitness and replicative competence. A more tenuous link has also been made that the I50L mutation may confer increased susceptibility to other PIs in the regimen but this needs further evidential support.

Resistance patterns with ATV treatment

Treatment naïve	ATV 400mg	all reported I50L
Treatment experienced	ATV 400mg	36% acquired I50L
Heavily experienced	ATV/r 300/100mg	21% acquired I50L
Treatment experienced	ATV/SQV	accumulated other mutations

As ATV is the only PI currently licensed that can effectively be prescribed without RTV boosting for some patient groups namely those drug-naïve or wishing to avoid the lipid-related side-effects associated with some of the other PIs, it would be extremely useful to learn whether there is a correlation between plasma concentrations and evolution of pathways to

resistance. Unfortunately, this data has yet not been forthcoming. The emerging data on ATV resistance does seem to confer a fairly distinctive pattern of resistance with I50L as a unique substitution. The critical question for clinicians is whether this genotypic profile preferentially disposes the use of ATV early on in treatment to help preserve options for future therapeutic strategies. ⁽⁸⁾

Finally, the next piece of the TPV puzzle was presented by Luisa Valer from Madrid measuring the qualitative impact of protease resistance against UPAMs (universal protease associated mutations), namely changes at 33, 82, 84 and 90 which confer cross-class resistance. When 2 or more UPAMs are present, the response to TPV seems to be 'abolished'. However, from the analysis of their database of 1389 patients who had failed on at least one PI, they found that whilst UPAMs are frequently observed given the ubiquitous use of RTV as a pharmacokinetic booster, the presence of >2 UPAMs is relatively uncommon (5.5% of individuals); but that all these patients had 10 or more PI-related mutations. The authors conclude that there may still be a utility for the use of TPV in PI-experienced patients but caution that whilst the molecular flexibility of TPV may result in a higher genetic barrier, the actual mechanism for resistance may in fact not safeguard against accumulation or cross-resistance to other PIs. ⁽⁹⁾

New NNRTIs

Currently in Phase IIb of clinical development, the new Tibotec NNRTI TMC125 was profiled by Johan Vingerhoets. Its resistance characteristics include single mutations at positions 101, 179, 181 and possibly 227 and 230 associated with decreased susceptibility to TMC125. When they interrogated their database containing over 7,000 NNRTI-resistant viruses, these mutations appeared at a rate of only >2%. ⁽¹⁰⁾

CLINICAL APPLICATIONS OF HIV RESISTANCE TESTING

Historically, given the extensive use of NRTIs as sequential and now commonly as a backbone to NNRTI or PI therapy, the interest in tenofovir (TDF) as a new nucleotide analogue is exceptionally high. Its promising activity and safety profile has made TDF an increasingly popular choice for patients and clinicians. Resistance to TDF however, has proven to be a more complex issue and explains the level of data generated on the viral

kinetics and impact of its signature mutation the K65R substitution. Two presentations focused on very different patient groups, one considering the role of K65R in patients with no TDF experience and a second clinical cohort including some with extensive histories of different treatment regimens.

Maria Paola Trotta from Rome discussed the genotypic and clinical characteristics of K65R emergence and impact on patients with no prior exposure to TDF. Of the 1392 drug resistance tests performed from 771 patients between 1999 and 2003, only 1.6% or 12 samples were found to have the K65R mutation present. The total patient profile included a range of NRTI exposure and reflected both thymidine analogues and d-drugs.

Previous exposure to NRTIs	K65R
ABC	33.3%
AZT	58.3%
d4T	91.7%
ddl	83.3
ddC	---
3TC	91.7%

The study confirms findings from other research that prevalence of K65R mutation remains relatively low and becomes more apparent with advanced disease. It also confirms the negative relationship between the 3TC associated mutation M184V and the K65R mutation in that one seems to obviate the presence of the other. Longer exposure to 3TC is independently correlated with a lower probability of the K65R emergence and co-administration of TDF and 3TC may accumulatively further impair viral fitness. While the multi-drug-resistant (MDR) mutation, Q151M complex is observed in only 5% of patients on antiretroviral therapy, there is nevertheless a strong correlation between K65R and Q151M, encouraging the need for resistance testing before starting TDF in patients heavily treatment experienced. Finally, in this study, of the patients treated with efavirenz (EFV) or NVP, almost 35% of patients were found to harbour K65R with EFV use compared to no K65R presence in the NVP treated patients, the difference being statistically significant. ⁽¹¹⁾

The contribution by Schlomo Staszewski and his colleagues have provided an invaluable insight into the real-life experiences of clinical decision-making in a diverse range of patients,

many of whom have extensive treatment experience and limited options for switching. In this prospective analysis of 258 experienced patients and 37 drug-naïve patients on their first exposure to TDF-containing regimen, he highlights the factors associated with lack of response to treatment strategies including TDF.

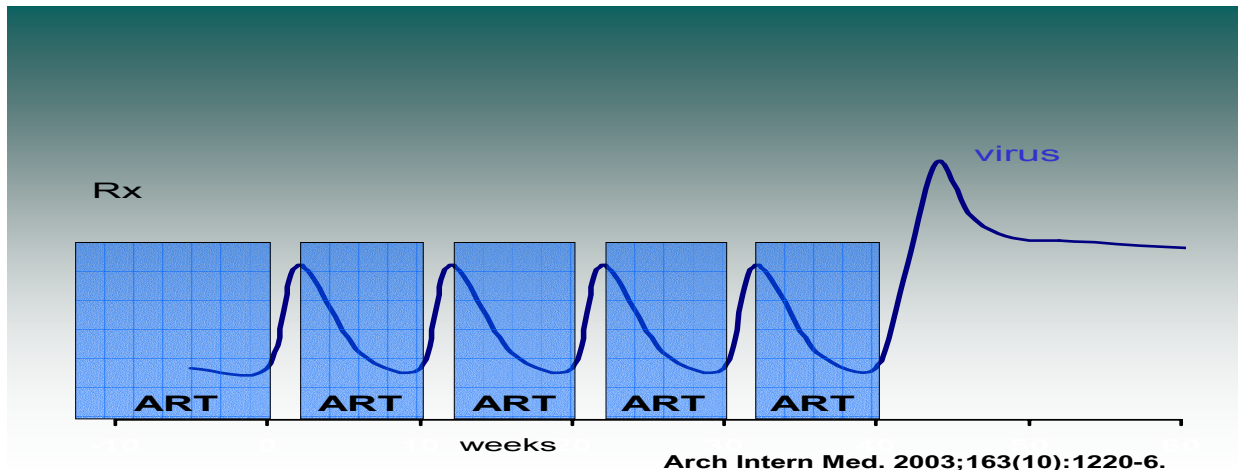
The response rate to TDF in naïve patients was 83.3% and included the following breakdown:

EFV-containing regimens	20 responders versus 3 virologic failures
PI/r regimens	11 versus 2
nucleoside only regimens	1 failure

In patients with prior treatment experience, 57.4% responded with a median time to virologic failure at 16 weeks. In addition to factors previously identified to predict loss of response such as treatment history, NRTI experience and higher baseline viral load, a correlation was also made with past failure of abacavir (ABC) and use of ddI treatment. Statistically significant predictors of K65R amongst the poor responders were ABC treatment, ddI treatment and taking 3 antiretrovirals compared to regimens with more than 3 agents.⁽¹²⁾

Disrupting therapy

It is unlikely that there are significant numbers of clinicians recommending structured treatment interruptions (STIs) as a viable therapeutic strategy for their patients. But despite the obvious benefits of relieving medication fatigue, limiting toxicities and improving cost-effectiveness, the suspicion remains that as with the initial induction-maintenance studies, the utility of STI strategies have been limited by divergent study designs making meta-analysis and coherent clinical application impossible. Indeed, the prolific research into STIs has resulted in scientifically confirming much of what was known intuitively from clinical practice. Under drug pressure viral load decreases and stopping therapy leads to an escalation of viral burden; schematically:



However, these studies have contributed to our understanding of viral kinetics, resistance evolution and host response. Bonaventura Clotet from Barcelona provided a useful overview of the key developments in STI research highlighting preferential selection of mutants during STIs (J Martinez-Picado et al. AIDS 2002), in particular the M184V/I (Perrin L et al. Antiviral Therapy 2002), the emergence of minor species (Metzner KJ et al. JID 2003) and the usefulness of PBMC genotyping to predict resistance (Palmisano L et al. Antiviral Therapy 2003).

More interestingly, recent analyses have discussed the pharmacologic characteristics of drugs involved in STI regimens. In the STOP study reported at CROI this year by Stephen Taylor from Birmingham considered the impact of treatment modalities for drugs with long half-lives such as EFV. The study recommended that based on assessment of serum concentrations, EFV should be stopped 7 days prior to other drugs in the regimen with shorter half-lives or consider using an alternative to EFV for STIs – a conclusion confirmed by the work of Lydia Ruiz from Dr Clotet's laboratory also reported at CROI.

HIV REPLICATIVE CAPACITY AND VIRAL FITNESS

Although the terms replicative capacity (RC) and viral fitness are often used interchangeably, Andrew Leigh-Brown from Edinburgh provided a strong overview of the distinctions between these as well as illuminating the related phenomenon of hypersusceptibility (HS).

Definitions

Fitness

- determined by genome and environment
- defined over entire viral life-cycle but can be measured according to different functions such as gp120 and the entry process
- *absolute fitness* can be defined as the quantitative rate of change
- *relative fitness* is the change in proportion compared to a competitor virus

Replicative Capacity

- can contribute to but does not in itself define fitness *in vivo*
- is determined by changes in RT and PR and measured *in vitro* relative to a reference clone (NL4-3)

Hyper-Susceptibility

- emerges with prior PI treatment, for example NFV has been associated with HS to APV (6%) linked to the N88S mutation
- can emerge in PI-naïve

Dr Leigh-Brown confirmed that the relationship between HS and RC is both real and meaningful in that it provides a statistically significant correlation for PI susceptibility. But conversely, a low RC value does not equate with susceptibility to NRTIs.

An interesting collaboration between the Czech Republic and the French discussed the mechanisms of NFV resistance and more precisely the characterisation of the D30N mutation, the mutant initially selected with NFV exposure. Viruses with changes at L90M and D30N demonstrate severe defect in replication with D30N, D30N+A71V and D30N+N88D exhibiting the highest selective advantage in the presence of NFV. This important exception of D30N+L90M with its associated replicative defectiveness is explained by a possible dimerisation effect, i.e. the combining of these two molecules. This conclusion compelled them to question whether viral fitness does in fact result from a proteolytic effect involving the cleavage of complex proteins into simpler individual protease mutant forms. ⁽¹³⁾

Adding to our understanding of the K103N mutation associated with NNRTI mutation and the first key mutation to appear particularly with EFV, a study by an Italian group at the University

of Milan showed that the K103N continues to persist even once therapy has been discontinued. From the two groups of patients observed, the first continued treatment but without NNRTIs (Group A) and the second stopped all treatment after failure to NNRTI (Group B). As expected all 62 patients had confirmed presence of K103N with use of NNRTIs, 50% selecting this with NVP treatment, 48% with EFV and 2% (1 patient) with delavirdine (DLV). Reversion to wild-type was confirmed in 37% of the patients who continued therapy but without the inclusion of an NNRTI and in 39% of those who discontinued all treatment.

	tested at 12 months	tested 12-24 months	beyond 24 months
Group A	61%	63%	86%
Group B	70%	75%	0% (2 patients)

These findings may prove to be influential in our consideration of selecting or stopping treatment with NNRTIs and does usefully supplement the emerging knowledge on viral kinetics of drugs with long half-lives as presented for example in the STOP study at CROI this year. ⁽¹⁴⁾

INTERPRETATION OF GENOTYPIC RESISTANCE

With the growing proliferation of non-clade B viruses, it is heartening to note the increased focus on resistance evolution in these patients. The importance of expanding knowledge in this field will be extremely important beyond our own geographic borders as treatment starts to become available in the developing world. Martine Peeters reported findings from an international review involving partners from Cameroon, Senegal, Gabon and the Democratic Republic of Congo of genotypic interpretation systems. The comparison included three established interpretation system: Rega 5.5 (Belgium), Stanford database algorithm (USA) and the ANRS national guidelines (France). The study considered which mutations occurred as natural variants in non-B clades and whether these could be associated with resistance as well as discordances observed in interpretation.

Prediction of PI susceptibilities		
Drug	Strains with discordant score (%)	Subtypes with more discordance than B
RTV	39	A, F
IDV	38	A, F
NFV	19	
APV	12	F
SQV	12	F, K, CRF05-DF
LPV	0,6	

Distribution of PI mutations: minor mutations observed at position 10, 20, 36 and 77 and significantly more present as compared to B.

mutation	subtype
L10I, M36I	A
K20R, M36I	C
L10V, K20R, M36I	F
M36I	D, G, J, CRF01, CRF02, CRF06
L10V	CRF05
L10I	CRF11
M36I, V77I	CRF13
Major mutation M46L (ANRS/Rega)	CRF13

Distribution of NRTI and NNRTI is also proving to be different with significantly more presence when compared to B in particular the V118I mutation found in subtype D.

The study found better concordance between the algorithms in their prediction of susceptibility to NRTIs and NNRTIs than to PIs. This is attributed to the presence of minor mutations occurring as natural polymorphisms in certain subtypes of recombinant forms and to the reporting of intermediate scores rather than absolute resistance or sensitivity. ⁽¹⁵⁾

The prevalence of mutations and subsequent response to therapy in non-B patients who had previously failed treatment was the focus of a Belgian study. As would be expected, the patient population in the non-B cohort was overwhelmingly African (81%), heterosexual (84%) and female (78%). The study found that despite similar treatment histories, non-B patients

appeared to be less disposed to thymidine analogue mutations (TAMs). Treatment response showed a trend was observed to improved response to treatment in clade B patients at months 6, but this disposition was reversed at month 12. ⁽¹⁶⁾

VIRAL CO-INFECTIONS AND CO-RESISTANCE

With the growing population of hepatitis and HIV co-infection, the need for effective treatment and surveillance has become ever more important. The global epidemiology of hepatitis infection is ten times that of the HIV epidemic with over 400 million people chronically infected with HBV. With shared routes of transmission, particularly for HCV, the potential for dual infection is significantly high. Furthermore, the management of each viral infection is likely to be complicated by the presence of the different viral aetiology.

Spain has one of the highest hepatitis infection rates in Europe and research from Vincente Soriano's group focused on HBV with a study of 2500 HIV positive patients tested for HBsAg and positive samples further screened for HBeAg and HBV-DNA. Drug resistance was examined in patients with detectable HBV-DNA after 10 months of exposure to anti-HBV drugs by sequencing the HBV RT region. Routes of transmission were strongly correlated with genotype with Genotype A (58%) associated with sexual contact, 74% versus 25% ($p=0.03$) and Genotype D (34%) associated with intravenous drug use, 62% versus 18% ($p=0.03$). The presence of HBeAg was associated with genotype A, 71% versus 22% ($p=0.03$) and 36% of the HBsAg/HIV patients were found to be co-infected with HCV whilst 79% of these were negative for HCV-RNA suggesting a suppressive effect of HBV on HCV.

Their resistance profile including 11 of the 19 patients failing 3TC reported the 3TC resistance genotype (L180M and M204V or M204I). No genotypic changes were potentially linked to TDF resistance including mutations at codon 236. This study serves to confirm the impact of different viral infections and the association between genotype and route of transmission. ⁽¹⁷⁾

Entecavir (ETV) is a new HBV inhibitor from BMS currently in Phase III development. Whilst it does not have anti-HIV activity, it is also not antagonistic when used with other NRTIs and has been shown to inhibit all 3 HBV polymerase functions (priming, RT and DNA-dependent synthesis). Its resistance profile was highlighted by Rich Colonna from BMS in his second presentation, in particular that *in vitro*, viruses with 3TC associated mutations at L180M and

M204V continue to respond to ETV therapy. Study 014 considered patients with 3TC resistance refractory to HBV. ETV dosed at 0.5mg and 1.0mg was shown to produce substantial reductions in viral load independent of 3TC resistance. At 48 weeks, ETV therapy did not result in genotypic consequences of baseline 3TC mutations and therefore no changes in virologic breakthrough or viral load reductions. Interestingly, there were two extensively pre-treated patients who reported viral rebound after prolonged ETV or ETV/3TC therapy. Pol substitutions at I169T+ M250V and I169T, T184G and S202I emerged on ETV therapy for Patient A and B respectively showing that a combination of M250V or T184G/S202I with 3TC signature resistance reduced ETV susceptibility. However, viruses with these mutations but lacking 3TC resistance remained susceptible to ETV. ⁽¹⁸⁾

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