

The e-mailed (pdf version) of HTB is fully hyperlinked, including contents page and referenced websites

July 2005

CONTENTS

EDITORIAL	2
CONFERENCE REPORTS	2
14th International HIV Drug Resistance Workshop (14th IHDRW), 7-11 June 2005, Quebec City, Canada	
• Genetic factors affecting HIV infection and progression rates: immunological factors relating to entry inhibitors	
• Resistance during MTCT strategies: new data on protective benefit of Combivir added to single-dose nevirapine, and impact of treatment and breastfeeding on the infant:	
- Short course of 3TC and AZT reduces the emergence of nevirapine resistance	
- Higher rates of resistance in TOPS using more sensitive resistance tests	
- Avoiding maternal nevirapine and adding AZT to infant prophylaxis	
- Resistance patterns vary by subtype in infants in HIVNET 012 and NVAZ studies	
- Mutations present in majority of women: early analysis suggests a similar rate of resistance in infants	
- Patterns of viral load and resistance in breast milk vary by HIV sub-type	
• Reduced replicative capacity of M184V explains benefit of 3TC monotherapy compared to stopping all drugs	
• Interaction between tenofovir and ddI in triple-nucleoside combinations: role of M184V in explaining resistance on failure	
• Predicting clinical responses to ddI from genotypic resistance	
• Under-detection of transmission of transmitted resistance and impact on treatment response	
• Risk of death in UK after diagnosis of 3-class resistance	
TREATMENT ACCESS	13
• Six Indian generic ARVs are given FDA 'tentative' approval	
• Methadone and buprenorphine added to WHO list of essential medicines	
• MSF's pricing guide to purchasing ARVs: 8th edition online	
• '3 by 5' progress report launched	
ANTIRETROVIRALS	15
• TMC-114 to be submitted for registration based on Phase 2b trial results: expanded access expected in UK in Autumn 2005	
• Tipranavir (Aptivus) approved in US	
• FDA fast-track designation for CCR5 inhibitor vicriviroc (SCH-D)	
• Pfizer discontinues development of capravirine	
PREGNANCY and PMTCT	16
• Use of T-20 in pregnancy: case study shows transmission occurred in highly treatment experienced mother with multiple-drug resistance	
• Further reading on nevirapine resistance	
SPECIAL REPORT: Human Papilloma Virus – a review of advances in the development of HPV vaccines	18
OTHER NEWS	25
• CDC estimate over 1 million HIV-positive people in US	
• Newly diagnosed HIV infection - review in UK and Ireland	
• Re-emergence of syphilis in the UK: the new epidemic phases	
• Prescription of heroin is less costly for society	
ON THE WEB	27
MEETING ANNOUNCEMENTS	28
PUBLICATIONS AND SERVICES FROM i-BASE	29
DONATION FORM	32
FAX-BACK ORDER FORM	33

EDITORIAL

This issue of HTB includes reports from the 14th International Drug Resistance Workshop, which as always included research that has implications of clinical care. Most importantly at this meeting, in terms of impact on the numbers of people receiving treatment, were probably the presentations relating to prevention of mother-to-child transmission (PMTCT).

Also in this issue we include a comprehensive review of recent successful HPV vaccine studies, and our usual round up of ARV and treatment access news and guide to useful resources published online over the last month.

Together with a group of FDA approvals of Indian generic ARVs, the news that data on TMC-114 will be submitted for consideration for approval based on the results from Phase 2 studies is one of the most exciting hopes for people who have resistant to current drugs.

As one of the UK studies at the resistance workshop highlighted, while this may be a situation that affects a growing minority of patients, the clinical impact of treatment resistance is just as difficult as ever.

The inclusion of methadone and buprenorphine on the list of WHO essential medicines is also one of the most significant advances that activists have been demanding. Without this the prospects of access to ARVs was limited or impossible in many of the countries where the epidemic has IDU-affected populations.

As we go into Summer we hope this is all interesting reading.

CONFERENCE REPORTS

14th International HIV Drug Resistance Workshop (14th IHDRW)

7-11 June 2005, Quebec City, Canada

Simon Collins and Polly Clayden, HIV i-Base

This annual Resistance Workshop is primarily a meeting for around 230 specialist researchers to discuss drug resistance. With a few exceptions nearly all the attendees present a poster or oral session, and the research submitted each year is the basis for the programme of the meeting. This meeting is mainly aimed at basic scientists, researchers and clinicians, around one quarter of who are from industry.

Reports in HTB from previous years meetings focus on new clinical implications. At this years meeting it was difficult to find many new surprises. To some degree, this was recognised as a limitation by the organisers, who announced that the priorities for selecting abstracts for the 2006 meeting would be data-driven studies with independent validation, and fewer studies dealing just with methodology.

One of the themes at the meeting was the increasing complexity of interpreting resistance test results. Resistance is an increasingly complex science, but it is not going away. Not on a biological level, because once resistance develops in an individual it remains archived. But also neither as a discipline relating to patient management, because potency of every individual HIV drug is insufficient to stall resistance. Even the most potent combinations fail to suppress virus in perhaps a fifth of treatment naive patients, and this proportion increases with subsequent treatments reducing options for treatment.

Many of these reports included small numbers of patients, and included tentative conclusions. With almost as much time allowed for questions as presentations, virtually no study did not include important caveats to the results, or methodological or validation questions from the audience.

Studies with most important indication for clinical care covered the following subjects and are reported below.

- Genetic factors affecting HIV infection and population differences: immunological factors relating to entry inhibitors
- Resistance during MTCT strategies: new data on protective benefit of Combivir added to single-dose nevirapine, and impact of treatment and breastfeeding on the infant:
 - Avoiding maternal nevirapine and adding AZT to infant prophylaxis optimal
 - Short course of 3TC and AZT reduces the emergence of nevirapine resistance
 - Higher rates of resistance seen with more sensitive resistance tests
 - Resistance patterns vary by subtype in infants in HIVNET 012 and NVAZ studies
 - Mutations present in majority of women: early analysis suggests a similar rate of resistance in infants
 - Patterns of viral load and resistance in breast milk vary by HIV sub-type
- Reduced replicative capacity of M184V explains benefit of 3TC monotherapy over stopping all drugs
- Interaction between tenofovir and ddI in triple-nucleoside combinations: role of M184V in explaining resistance on failure

- Predicting clinical responses to ddl from genotypic resistance
- Under-detection of transmission of transmitted resistance and impact on treatment response
- Risk of death in UK after diagnosis of 3-class resistance

A pdf file of the abstract book from this meeting is available as part of the AEGiS conference database, and will be posted as html web pages at the same site. (Click the meeting name to download this file).

<http://www.aegis.org>

<http://www.aegis.org/conferences/hivdrw/>

Genetic factors affecting HIV infection and progression rates: immunological factors relating to entry inhibitors

Simon Collins, HIV i-Base

The opening session at the meeting was one of only two invited lectures and appropriately for a virology meeting, focused on immunological responses. Entry inhibitors that target human receptors will bring a new awareness of the range of immunological responses in patients. As the newest class of drugs, now in Phase 2/3 studies, this will become an increasing focus for this meeting.

This lecture by Sunil Ahuja from the Veterans Administration Research Center in San Antonio, Texas provided more detail relating to the complexity of viral entry, its relationship to viral fitness on an individual and epidemiological level, and the role played by genetic background. [1]

The larger context of the talk was to provide an explanation for the wide inter-patient variability in terms of exposure and risk of infection. This included why some people appear to be protected from infection, and differences in HIV progression rates in individuals who become infected. It was based on research published earlier this year in Science. [2]

From a virological perspective, factors affecting the risk for infection include viral fitness and viral load levels of the source partner. Viral fitness (and resistance) therefore become important factors for the effectiveness of drugs that target the virus life-cycle. As CCR5 inhibitors are dependent on host responses, it is important to understand new immunological factors that contribute to their effectiveness.

Immunological factors contributing to risk of infection, and speed of progression include differences in CCR5 co-receptors. The double deletion in delta32 is well known for providing protection against HIV-infection, but the importance of other sites in the cis region of CCR5 and changes at 641 in CCR2 in the level of protection have received less attention.

In a similar way, levels of human leukocyte antigen (HLA) varies between individuals and is one of the factors that determine whether an individual is able to mount strong or weak CD8+ CTL immune responses.

Genetic differences in the CCR5 ligand network were the main focus for the talk: principally CC chemokine ligand 3-like1 gene number (CCL3L1, also called MIP-1-alphaP), which affect levels of RANTES which also vary considerably between individuals. A ligand is the term for a molecule that binds to a specific protein or receptor to form a larger molecule, and RANTES and MIP-1-alphaP directly block HIV from attaching to the receptor. Ahuja's research focused on whether higher levels of these chemokines correlated with levels of protection against HIV on both an individual and population level.

Their hypothesis is that these differences explain why some multiply-exposed individuals never become infected, and others are infected after one exposure. They also explain the broad range of responses between the two extremes of rapid progressors and long-term slow progressors and why two people infected with the same strain may have widely different rates of disease progression.

This was tested against data from two large cohorts. The first cohort included 1100 adult HIV-positive patients, half of whom were seroconverting, representing different genetic backgrounds (approximately one third African American) treated at the Wilford Hall Medical Centre, Texas (with a similar number of HIV-negative controls). This is the referral centre for USAF military and is 94% male with median ages at diagnosis of 28 (range 18-70 years). The second cohort included around 800 perinatally-exposed children in an Argentinian cohort (genetically classed as European) half of whom were HIV-positive half were HIV-negative.

The distribution of population differences in gene copy was determined from 1046 samples from the Human diversity genome panel, including humans from 57 populations plus 83 chimpanzees. This showed wide population differences. Approximate mean copy number ranged from 5-7 in Africans, 3-5 in Americans, 2-5 in East Asians and 2-3 in Europeans. Interindividual and interpopulation differences resulted in a median phenotype for African Americans of 3-4 copies compared to 1-2 copies in Non-Africans; ie half the dose in Caucasians has a comparable protective effect. They found no relationship between absolute copy number of CCL3L1 and protective benefit. However, when individual copy number was then compared to the genetic population distribution, they found a clear correlation between CCL3L1 copy number and infection risk in both the Texan and Argentinian cohorts.

Compared to a median of 2 copies, children in the Argentinian cohort with either greater or less than two copies had significantly higher or lower risks respectively of acquiring HIV. Each increase in copy number above the median produced a dose-dependent step-wise decrease in the risk for acquiring HIV.

In HIV-positive individuals, they found a dose dependent correlation between copy number and both viral set point and rate of change in CD4 cells.

When they looked at the phenotypic effect of CCL3L1 dose and CCR5 haplotypes when combined in four mutually exclusive genetic risk groups based on CCL3L1 number relative to population (high or low) and whether the CCR5 genotype was detrimental (det) or accelerating (non-det). If the impact on disease progression was related to both factors, then low CCL3L1 plus detrimental CCR5 genotype should correlate to one extreme of faster disease progression, and high CCL3L1 plus non-detrimental CCR5 genotype would be linked to slow disease progression. Definitions for detrimental CCR5 genotype were derived from previous genetic analysis, that had showed highly statistically significant associations with disease progression, relating to changes in the non-coding region and delta-32 deletion in CCR5, and the CCR2-64I region of CCR2.

Relative to the high+nondet group, low+det genotypes were associated with a >3-fold increased risk of progressing to 8 of 12 AIDS-defining illnesses (ADIs). Relative to the high+nondet group, the two middle risk groups low+nondet and high+det were associated with a less than 3-fold risk of progressing to 3-4 ADIs.

Summaries of three of these analyses are shown in Tables 1, 2 and 3 below. (*EA=European American; AA=African American*).

Table 1: RH (95% CI) acquiring HIV by genetic response group in adult cohort

	CCL3L1	
	low (<2EA, <3AA)	high (>=2 EA, >=3 AA)
R5 det.	3.44 (2.54-4.67)	1.75 (1.26-2.42)
R5 non-det.	1.52 (1.22-1.90)	1.00

Table 2: RH (95% CI) acquiring HIV by genetic response group in children exposed perinatally

	CCL3L1	
	low (<2EA, <3AA)	high (>=2 EA, >=3 AA)
R5 det.	4.03 (2.39-6.69)	1.48 (0.97-2.27)
R5 non-det.	2.36 (1.40-4.00)	1.00

Table 3: RH (95% CI) progressing to AIDS by genetic response group in adults diagnosed during seroconversion

	CCL3L1	
	low (<2EA, <3AA)	high (>=2 EA, >=3 AA)
R5 det.	4.87 (2.82-8.39)	1.76 (1.02-3.02)
R5 non-det.	1.45 (0.94-2.25)	1.00

The lecture only covered part of more detailed research from the earlier Science publication, and referral to that paper with the accompanying notes available online is recommended. [2] The conclusions have particular significance now that new drugs are in development that are likely to vary in activity based on immunological parameters. Gene dosage and response to CCR5-blockers in an obvious area for future study, as are other host genetic factors.

References:

1. Ahuja S. Host genetic determinants of HIV transmission and pathogenesis: should we care? Abstract P1.
2. Gonzalez G, Kulkarni H, Bolivar H et al. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science 307: 1434. 4 March 2005. Published online 6 Jan 2005.

Resistance during MTCT strategies: new data on protective benefit of Combivir added to single-dose nevirapine, and impact of treatment and breastfeeding on the infant

Polly Clayden, HIV I-Base

The frequency and persistence of nevirapine associated mutations after single dose prophylaxis and strategies to preserve this class of drug for future maternal and paediatric treatment generated several reports at this meeting. It was good to see the prominence of this issue this meeting. The first four oral presentations covered this area of research.

Short course of 3TC and AZT reduces the emergence of nevirapine resistance

David Hall from Boehringer Ingelheim presented further analyses from the TOPS (Treatment Options Preservation Study), supporting interim findings that were first presented at the World AIDS Conference in Bangkok in 2004. [1]

TOPS compared nevirapine single dose (HIVNET 012 regimen) to single dose nevirapine plus either 4- or 7-days of Combivir, to mother and infant to cover the nevirapine "tail". In the previous interim analysis of 61 women, resistance was detected in 53.3% receiving nevirapine alone and 9.3% overall in those receiving nevirapine plus Combivir ($p=0.001$) by standard genotyping at 6 weeks. Enrollment in the single dose nevirapine arm was closed following the interim results.

This larger analysis included 6-week follow up from 226 mothers and 228 infants [2]. This presentation by the company was unexpected at this meeting. Full reporting of the 6-week results from this trial will be at the 3rd IAS conference in Rio in July, with separate presentations covering the results in mothers and infants.

All mothers in the trial were subtype C except for one subtype B and one CFR02AG. Population sequencing (Bayer) was performed at baseline (pre prophylaxis) and at 2 and 6 weeks. Maternal results show changes from baseline sequence known to be associated with nevirapine resistance in 41/68 (60%), 8/67 (12%) and 7/68 (10%) of samples in the nevirapine, nevirapine plus 4 days Combivir and the nevirapine plus seven days Combivir arms respectively. The most frequently observed nevirapine associated mutations were: K103N, Y188C, Y181C, V106M, A190G and V106A.

The investigators reported that after single dose nevirapine, mutations emerged at different rates and remained for different durations. Mutations at positions 106 and 181 emerged earliest and were present at 2 weeks. Mutations at codon 190 emerged last, and were not present until after week 2 in over 40% of samples with those mutations. By week 6, the majority of mutations at 181 and at 106A were no longer detectable by population sequencing. The investigators wrote: "The pattern of mutations observed will depend on when the samples are collected. Mutations were observed for the first time as late as 12 weeks." Emergence of resistant virus in mothers receiving single-dose nevirapine was associated with higher baseline viral load.

The addition of Combivir prevented the emergence of detectable resistance in 80% of mothers compared to single dose nevirapine. The investigators also noted that while 30/41 (75%) of the nevirapine group had multiple mutations, only 5/15 (33%) of the mothers receiving additional Combivir had multiple mutations. There was no evidence of 3TC associated mutations at codon 184.

Of the 228 evaluable infants, 8, 7 and 7 children were infected *in utero* in the nevirapine, nevirapine plus 4 days Combivir and nevirapine plus 7 days Combivir arms respectively. Nevirapine resistance was detected in 7/8 (88%) infants in the single-dose nevirapine arm and 1/7 (14%) and 0/7 (0%) in the Combivir 4- and 7-day arms respectively. Mutations observed in the infant samples were: V106A/M, Y181C, Y188C, and G190A in the single dose nevirapine arm (in 5, 3, 3 and 2 samples respectively), and K103N and Y181C in the one sample with resistance in the nevirapine plus 4-day Combivir arm.

There were 2 perinatal infections: one in the nevirapine arm and one in the nevirapine plus 4-day Combivir arm, both without mutations at six weeks.

The investigators wrote: "A short course of Combivir reduces the emergence of nevirapine resistance associated mutations in mothers taking single dose nevirapine to prevent mother to child transmission of HIV-1. A short course of AZT and 3TC reduces the emergence of nevirapine resistance associated mutations in infants taking single dose nevirapine after intrauterine infection."

Higher rates of resistance in TOPS using more sensitive resistance tests

Additionally, Sarah Palmer presented findings from an analysis of samples from the TOPS study, using an allele specific RT-PCR assay that quantifies variants encoding 103N or 181C variants at frequencies $<0.1\%$ [3].

This substudy analysed samples at baseline, and at 2 and 6 weeks post prophylaxis, from 32 women (10 from the single-dose nevirapine, and 11 from each of the nevirapine plus Combivir arms).

The investigators reported that using the more sensitive test, 103N or 181C variants were detected in 75% of week 6 samples from women receiving single dose nevirapine prophylaxis (mutant frequency 1-75%, median 7%) and 6/22 (27%) of women receiving single dose nevirapine plus either 4 or 7 days of Combivir (mutant frequency 0.8-8%, median 1.9% and 0.3%-1%, median 0.9%, 4 and 7 days respectively)

The investigators noted that at 6 weeks, the median fraction of plasma virus that is comprised of 103N (AAC) or 181C variants, appears to be lower in women who receive Combivir in addition to single-dose nevirapine.

They added that additional studies are needed to establish clinical significance of these results and optimum duration of Combivir. The results presented above show a modest trend towards less detectable resistance using population sequencing in mothers receiving 4 vs 7 days of Combivir (12% vs 10%). This had been counter intuitive in the interim analysis (5% vs 13%) but only evaluated a small group of mothers (20 and 23 in the 4 and 7 day Combivir groups respectively).

Avoiding maternal nevirapine and adding AZT to infant prophylaxis

Susan Eshleman presented data analysing nevirapine resistance in mother and infant pairs from the NVAZ study in Malawi, which studied four different MTCT prophylaxis regimens as follows [4]:

- Group 1: Mothers and infants each received single dose (SD) nevirapine according to the HIVNET 012 regimen.
- Group 2: Mothers received SD nevirapine; infants received SD nevirapine plus AZT BID for 7 days;
- Group 3: Mothers (late presenters in labour) no nevirapine; infants SD nevirapine;
- Group 4: Mothers (late presenters in labour) no nevirapine; infants SD nevirapine plus AZT BID for 7 days.

The overall transmission rate at 6-8 weeks was 14.1%, 16.3%, 20.9%, 15.3% in groups 1-4 respectively (so rates were similar for 1,2 and 4 but statistically higher for group 3).

The investigators analysed resistance using the ViroSeq genotype system, in evaluable samples collected at 6-8 weeks from 78 infected infants (23, 21, 19 and 15 in groups 1-4 respectively)..

Dr Eshleman reported detectable nevirapine resistance in 50/78 (64.1%) of the infants (see Table 1). There were significant differences in the frequency in groups 1-4 ($p=0.001$). The highest was in group 1 (as HIVNET 012 regimen) in which 20/23 (87%) infants had nevirapine resistance. The frequency was lower when there was infant but no maternal nevirapine dose, 14/19 (74%) in group 3 or when the infant received AZT in addition to the nevirapine, 12/21 (57%) as in group 2. The lowest frequency of nevirapine resistance was achieved combining the two strategies: no maternal nevirapine and nevirapine plus AZT 4/15 (27%) to the infant, as in group 4.

Table 1: Nevirapine resistance detected in infants by MTCT treatment strategy

Gp 1 (as HIVNET 012 regimen)	20/23	87%
Gp 2 (infant rc'd AZT + NVP)	12/21	57%
Gp 3 (no maternal NVP)	14/19	74%
Gp 4 (no maternal NVP and NVP+AZT to the infant)	4/15	27%
Total	50/78	64%

In a multiple logistical regression analysis, both avoiding the maternal nevirapine dose ($OR=0.20$, $p<0.001$) and adding AZT BID for 7 days to the infant regimen ($OR=0.31$, $p=0.04$) were independently associated with reduced infant nevirapine resistance.

The authors also reported that group 4 not only had the lowest infant nevirapine resistance but that transmission rates were comparable to groups 1 and 2 and lower than group 3. None of the infants developed resistance to AZT.

The authors concluded that of the four mother to child transmission prophylaxis regimens evaluated avoiding maternal nevirapine and adding AZT to the infant nevirapine dose as in group 4 was optimal as maternal nevirapine resistance was avoided and infant nevirapine resistance was reduced.

They also noted that the transmission risk using this regimen was not statistically different to the HIVNET 012 regimen in this analysis (15.3% vs 14.1% for groups 1 and 4 respectively).

Resistance patterns vary by subtype in infants in HIVNET 012 and NVAZ studies

In a previous comparison of frequency of maternal resistance across subtypes - using samples from the NVAZ and HIVNET012 trials - Susan Eshleman and co-workers revealed that resistance was more frequent in mothers with subtype C (45/65, 69%), than with subtype D (37/97, 36%, $p=0.0001$) or subtype A (28/144, $p=0.0001$). [5] This finding was independent of maternal viral load, age, parity or time between dose and resistance testing.

The same group evaluated nevirapine resistance in infants who had become HIV infected at 6-8 weeks in these two trials despite nevirapine prophylaxis being given to the mothers. [6] Plasma samples were available for 24/37 (65%) of infants in HIVNET 012, and 26/59 (44%) of infants in NVAZ. Analysis was performed using ViroSeq HIV Genotyping System. Genotypes were obtained for 24 infants in HIVNET 021 and 23 infants in NVAZ.

Analysis of subtypes in HIVNET 012 infants found 9 with subtype A, 1 with subtype C, 9 with subtype D and 5 with recombinant HIV. All NVAZ infants were subtype C.

The investigators reported that 20/23 (87%) infants in NVAZ had detectable resistance, compared to 11/24 (46%) of HIVNET 012 infants ($p=0.005$). (A similar number of infants had two or more mutations in both groups with 6/23 vs 2/24 in NVAZ and HIVNET respectively ($p=0.49$). A significant difference was reported comparing the 23 NVAZ infants to the infants in HIVNET with subtype A or D only ($p=0.016$); with subtype D only ($p=0.23$) or with recombinant HIV only ($p=0.008$). There was a higher proportion of infants in NVAZ with resistance than HIV 012 infants with subtype A but this did not reach statistical significance due to the small sample size, $p=0.076$. Additionally the infant in HIVNET 012 with subtype C had nevirapine-associated resistance.

The investigators concluded that nevirapine resistance after single dose was more frequent in the Malawian infants with subtype C than in the Ugandan infants of whom the majority were subtype A or D at 6-8 weeks post dose. These comparative results are concordant with those reported for the mothers in these studies. Further analysis is needed to evaluate whether these findings are due to the effect of HIV subtype or other factors.

Mutations present in majority of women: early analysis suggests a similar rate of resistance in infants

Studies reported at CROI this year, suggested that as in that: only a *minority* of women exposed to single dose nevirapine *do not* develop nevirapine resistance; that conventional sequencing genotyping significantly *underestimates* this; and that *resistance persists after it is no longer detectable using conventional genotypic resistance tests*. [7, 8, 9]

At this meeting, Jeffery Johnson presented further analysis from a study evaluating matched samples collected pre and post single dose nevirapine exposure (6-36 weeks, median 7 weeks post partum) from 50 South Africa mothers with subtype C. They compared results using real-time PCR assays that have detection limits of 0.2% for K103N and 0.3% for Y181C to population sequencing genotyping (that requires 10-20% of resistant virus to be present for detection). [10]

The investigators found that 17/40 (43%) mothers who sequenced negative for nevirapine resistance had detectable mutations using real time PCR: 12 with K103N, 1 with Y181C and 4 with both mutations. They noted that Y181C was not found after 13 weeks post dose, but that K103N was detected throughout the 36 week study.

Resistance emerged in at least 65% of women. This was a 62% increase in the incidence reported with conventional genotyping.

Shayne Loubser also presented further sequence genotype and real time PCR results (sensitivity 0.2%) for mothers, analysing samples collected at different time points between 6 weeks and 12 months. [11]

This study found K103N mutations in 16/31 (52%) of plasma samples using population sequencing and in 27/31 (87%) using real time PCR. Standard genotyping failed to detect mixtures of K103N below a frequency of 10%.

In a cross-sectional analysis of plasma RNA samples collected at 6 weeks, 3 months, 7 months and 12 months; 27/31 (87%), 18/27 (67%), 13/37 (35%), 7/ 54 (13%) respectively had detectable K103N variants. DNA analysis from samples collected at 2 weeks and 12 months found that 23/43 (53%) and 2/48 (4.2%) samples had detectable mutations. Longitudinal analysis of plasma RNA in samples collected 6 weeks to 12 months for 15 women showed similar rates of decay, but mothers with higher levels resistance detected earlier had longer persistence of K103N variants.

Dr Loubser's group found an additional 73% ($n=15$) of samples contained K103N variants than identified by standard genotyping, which declined over time but were present in a minority at 12 months.

Finally Dr Troyer presented data from a detailed phylogenetic analysis using a quantitative radiolabelled oligonucleotide DNA ligation assay (OLA) performed on samples collected in an observational mother to child transmission cohort in Uganda [12].

This study looked at mother and infected infant pairs receiving: AZT ($n=90$) or nevirapine ($n=61$) and no prophylaxis at 6 weeks post dose.

This analysis revealed 75% of samples from the nevirapine receiving pairs to have detectable resistance with this assay 6 weeks post dose.

The clinical implications of this very low level of resistance, and its potential to prejudice future NNRTI treatment response, are unclear.

Patterns of viral load and resistance in breast milk vary by HIV sub-type

The majority of breastfeeding transmission takes place in the first six weeks postpartum. Although single dose nevirapine has been much discussed and studied in terms of maternal and infant resistance patterns, less is known about the effect of single dose nevirapine on breast milk viral load, or about the prevalence of nevirapine-associated mutations in breast milk compared to blood plasma.

Dr Lehman presented findings from a sub study of a Kenyan trial comparing single-dose nevirapine (HIVNET 012 regimen) to AZT monotherapy (Thai short-course regimen) [13]. Breast milk samples from 30 women in the nevirapine arm were collected every 2-3 days for the first 6 weeks postpartum, and plasma samples were collected during pregnancy, at delivery and at one-month post partum,.

Breast milk HIV-1 RNA was quantified using the Gen-Probe HIV-1 assay. Blood samples, collected at one month post partum, were quantified using an allele-specific PCR assay (sensitive to 0.1%) to detect the K103N mutation in subtypes A and D.

The investigators reported comparable levels of HIV viral load in breast milk from women receiving single dose nevirapine to those found in women receiving the Thai short-course AZT regimen. 404 breast milk samples were evaluated (a median of 14 samples per woman) collected during the first 6 weeks postpartum.

Women receiving single dose nevirapine had significantly greater log reductions in breast milk viral load during days 3-21 postpartum: 3 to 7 days (-2.42 vs -1.98, $p=0.1$); 8 to 14 days (-2.48 vs -1.78 $p=0.005$); and 15 to 21 days (-2.97 vs -1.90, $p=0.003$).

Additionally they reported that 40% plasma samples collected at one-month post partum had detectable K103N mutation using the allele specific assay. They noted that these are preliminary findings and that breast milk samples are currently being analysed.

The investigators wrote: "Compared to the Thai CDC short-course zidovudine regimen, single-dose nevirapine results in sustained suppression of breast milk viral loads during weeks 2-6 postpartum. However the benefits of this suppression may be counterbalanced with a high prevalence of drug resistance."

This group are currently completing a detailed temporal analysis of nevirapine resistance in breast milk during the first 6 months post partum, from women receiving single dose nevirapine. They will also analyse viral load and resistance from samples from women receiving either short-course AZT plus nevirapine vs short-course HAART during breastfeeding.

C O M M E N T

Although single dose nevirapine was a tremendous step forward it is certainly not the future.

The latest data from TOPS show a significant reduction in nevirapine resistance in the mother if the prolonged maternal exposure following single dose nevirapine is covered by the addition Combivir for 4-7 days. As expected, given the known rate of plasma clearance of nevirapine, the proportion of mothers with resistance, as detected by population based sequencing, was lowest with 7 days cover. This appears to be a sensible strategy to limit the spread of NVP resistance, and to preserve maternal therapy options, in settings where short term HAART for PMTCT is neither available or feasible. Although it is disappointing that neonatal AZT plus 3TC for one week did not effect transmission, this largely reflects the efficacy of single dose nevirapine to reduce transmission during labour and the apparent preservation of treatment options for the infected infants may justify the continuation of this approach.

The alternative approach to avoid maternal nevirapine resistance is to avoid maternal nevirapine exposure. This was first suggested by the MASHI study, in which single dose nevirapine given to the infant, was of equal efficacy, to the dual maternal and infant single dose nevirapine on a background of AZT monotherapy from 34 weeks and continued in the infant for a least 1 month [14]. Although the transmission rates in all four arms are high, the data presented from NVAZ appear to indicate that 7 days of neonatal ZDV 'makes up' for the absent maternal nevirapine dose in terms of transmission, and significantly reduces nevirapine resistance in the infected infants.

But as "What is to be done?" may be becoming clearer there are practical questions around the feasibility of implementation. James McIntyre says that he believes that the addition of a 7 day tail cover would not provide an insurmountable barrier to the use of the TOPS strategy to reduce the selection of NNRTI resistant virus. At current prices the additional cost would be minimal in relation to the benefits, especially as good quality generic AZT/3TC combinations are available. What is required is some rapid innovative thinking to develop ways to achieve this: such as co-packaging of the drugs and access mechanisms.

As we go to press the WHO are meeting with opinion leaders to discuss their pregnancy and MTCT guidelines – we hope that some of these new data will motivate some changes.

References

Unless stated otherwise, all abstracts are from the 14th International HIV Drug Resistance Workshop (14th IHDRW), 7-11 June 2005, Quebec City, Canada.

1. McIntyre J, Martinson N, Boltz V et al. Addition of short course Combivir (CBV) to single dose Viramune (sdNVP) for prevention of mother-to-child transmission of HIV-1 can significantly decrease the subsequent development of maternal NNRTI-resistant virus. XV Intl AIDS Conference, Bangkok. LbOrB09
2. McIntyre JA, Martinson N, Gray GE et al. Single dose nevirapine combined with a short course of Combivir for prevention of mother to child transmission of HIV-1 can significantly decrease the subsequent development of maternal and infant resistant virus. 14th IHDRW. Abstract 2.
3. Palmer S, Boltz V, Maldarelli F et al. Short course Combivir single dose nevirapine reduces but does not eliminate the selection of nevirapine resistant HIV-1: improved detection by allele-specific PCR. 14th IHDRW. Abstract 3.

4. Eshleman SH, Hoover DR et al. Infant nevirapine resistance can be substantially reduced after single dose nevirapine by avoiding maternal dosing and providing infants with nevirapine maternal nevirapine dosing and providing infants with zidovudine in addition to single dose nevirapine after birth. 14th IHDRW. Abstract 1.
5. Eshleman SH, Hoover DR, Chen C et al. Nevirapine resistance in women with HIV-1 subtype C compared with subtypes A and D, after the administration of single-dose nevirapine. JID, 192:30-36; 2005.
6. Eshleman SH, Hoover DR, Hudelson SE et al. Resistance after single dose nevirapine prophylaxis varies by subtype in infants from sub-Saharan Africa. 14th IHDRW. Abstract 10.
7. Johnson J, Li JF, Morris L et al. Resistance emerges in the majority of women provided intrapartum single-dose nevirapine. 12th CROI, Boston, 2005. Abstract 100.
8. Palmer S, Boltz V, Maldarelli F et al. Persistence of NNRTI-r resistant variants after single-dose nevirapine in HIV-1 subtype-C-infected women. 12th CROI, Boston, 2005. Abstract 101.
9. Loubser S, Balfe P, Sherman G, et al. Sensitive real-time PCR quantification of 103N resistance mutants following single-dose treatment with nevirapine. 12th CROI, Boston, 2005. Abstract 102..
10. Johnson JA, Li JF and Morris L et al. Resistance mutations arise in the majority of women provided single dose nevirapine and appear to differ in emergence and persistence. 14th IHDRW. Abstract 11.
11. Troyer R, Lalonde M, Kyeyune F et al. High frequency of nevirapine resistant mutations in the HIV quasi species found in NVP-treated participants of an MTCT Ugandan cohort. 14th IHDRW. Abstract 12.
12. Loubser S, Balfe P, Sherman G et al. Increases sensitivity of detection of K103N resistance variants by real time PCR in RNA and DNA after single dose nevirapine. 14th IHDRW. Abstract 13.
13. Lehman DA, Chung MH, Richardson BA et al. Patterns of viral load and drug resistance in breast milk and blood from women treated with single dose nevirapine to reduce mother to child transmission of HIV-1. 14th IHDRW. Abstract 4.
14. Shapiro R, Thior I, Gilbert P et al. Maternal single-dose nevirapine may not be needed to reduce mother-to-child HIV transmission in the setting of maternal and infant zidovudine and infant single-dose nevirapine: results of a randomised clinical trial in Botswana. 12th CROI, Boston, 2005. Abstract 74LB.

Reduced replicative capacity of M184V explains benefit of 3TC monotherapy compared to stopping all drugs

Simon Collins, HIV i-Base

Last year at the Bangkok IAS conference an Italian E-184V study, of 60 patients with CD4 counts >500 cells/mm³ who had the M184V mutation on HAART, and who wanted to stop treatment, reported lower CD4 reductions and additional clinical benefits in patients who maintained 3TC monotherapy, compared to patients who interrupted all their drugs.

At the Resistance Workshop, Nicola Gianotti and colleagues an analysis of viral replicative capacity from this study. Replicative capacity (RC) from the first 31 consecutive patients in the study was measured as p24Ag productivity from recombinant clones after four days in culture. Median replicative capacity ratio was calculated as RC at week 24 divided by RC at study baseline; this was 11.42 (IQR 2.4-57.1) in the treatment interruption group and 1.14 (IQR 1.00-1.28) in the 3TC monotherapy group (p=0.0006).

Protocol defined failure in the study was a CD4 drop to below 350 cells/mm³. In this analysis patients with protocol failure prior to 24 weeks had greater RC recovery compared to those without early failure. RC ratio also correlated with CD4 and CD4% decreases at week 24 (r=-0.46, p=0.01 and r=-0.55, p=0.001, respectively); but not with baseline CD4, CD4%, viral load, 24-week increase in viral load, or 48-week changes in CD4 and viral load.

RC recovery was consistent with reduction in resistance in protease and RT mutation and with the outgrowth of wild-type virus at position 184, although statistically analysis for this is still ongoing.

Ref: Gianotti E, Menzo S, Danise A et al. E-184V study: immunological and virological correlates of HIV-1 replicative capacity. 14th HIV Drug Resistance Workshop, 7-11 June 2005, Quebec City, Canada. Abstract 160.

Interaction between tenofovir and ddl in triple-nucleoside combinations: role of M184V in explaining resistance on failure

Simon Collins, HIV i-Base

A study presented at the meeting by Douglas Barnas and colleagues, provided an explanation for the 91% virological failure rate before week 24 in patients using a triple nucleoside combination of ddl/tenofovir/3TC in the Jemsek Study.

The mechanism of failure for this and several other triple nucleoside combinations has still not been convincingly demonstrated.

M184V was found in 100% and K65R in only 50% of failures, with standard phenotype test (Phenosense) showing no resistance to tenofovir and moderate resistance to ddl (mean change of 0.6-fold and 1.8-fold respectively).

Barnas used single genome sequencing (SGS) of samples from 9/10 patients in the study who failed with both K65R and M184V by standard genotype. Susceptibility to 3TC, ddl, tenofovir and abacavir and the impact double mutants (where both

mutants are on the same genome; n=3), was compared to mixtures (where both mutations were found, but not on the same genome; n=6).

Total number of genomes was 204 with a mean of 22 samples per patient (range 12-46). Most common genotypes were the K65R/M184V double mutant (50%) and M184V single mutant (38%). M184I occurred in 11% samples and only one genome contained K65R single mutant.

Table 1: Mean fold-resistance by standard genotype

	ddl	ABC	TDF
Mean fold-Rx of double mutant	2.45	5.41	0.86
Mean fold-Rx of mixture	1.49	2.83	0.52
Overall average fold-Rx	1.81	3.69	0.63
P value	0.02	0.02	0.02

Table 2: Mean fold-resistance by SGS

	ddl	ABC	TDF
Mean fold-Rx of 184V only	1.4	2.4	0.6
Mean fold-Rx of double mutant	3.5 *	7.5 *	1.8 *

* $P < 0.01$

The study concluded that when 65R occurs with 184V in the same genome, hypersensitivity to tenofovir is reversed and ddl resistance is increased, giving the double mutants selective advantage, and that prior selection of 184V contributed to this (shown in Table 1 and 2).

Previous phenotypic testing had failed to show resistance to tenofovir, because samples had included single 184V mutations, to which TDF is hypersensitive and this cancelled out the increased resistance from 65R and 184V dual mutations.

Assessment of phenotypic resistance to other nucleosides should also be considered with and without M184V mutations (see report on ddl resistance below).

Ref: Barnas D, Bazmi H, Mellors J et al. Genotypic and phenotypic explanation for failure of triple NRTI therapy with lamivudine, didanosine and tenofovir. 14th HIV Drug Resistance Workshop, 7-11 June 2005, Quebec City, Canada. Abstract 152.

Predicting clinical responses to ddl from genotypic resistance

Simon Collins, HIV i-Base

Shulman and colleagues addressed the difficulty of interpreting resistance to ddl, by analysing stored samples from 444 treatment-experienced patients in three ACTG studies (359, 368 and 398) to test the results against responses to ddl monotherapy in ACTG 175 and ACTG 309.

Paired genotype-phenotype samples were compared to consensus RT and compared with treatment response. 16/444 had >4 fold change (all with either Q151M or substitutions at 69) and these samples were excused from the analysis.

In the remaining 428 cases, 20 codons with $P < 0.01$ in the univariate analysis were used in stepwise regression modeling. This resulted in nine codons in the final multivariate model: 184, 215, 43, 67, 74, 223, 210, and 75 were associated with increased resistance (all $p < 0.001$) while 83 was associated with decreased resistance.

The predictability of these mutations was tested against 52 genotype and 46 phenotype samples from ACTG 175 and ACTG 307. The resulting resistance score (total score = $184 + 215 + 43 + 67 + 74 + 223 + 210 + 75 - 83$) had a strong correlation between with ddl-fold sensitivity change (Spearman $r = 0.67$ ($P < 0.0001$)).

This fitted more accurately than the Jaguar score (M41L + L74V + D67N + K219Q/E + T215Y/F + T69D - K70R - M184V) which is based on 4 week virological response to ddl in patients with a relatively high CD4 count, and correlated with fold-change, $r = 0.38$, $P < 0.0001$.

The low phenotypic change associated with loss of activity with ddl makes predicting response from genotypic results a difficult challenge, for which there is certainly a clinical need. An important suggestion from the audience, similar to other studies, was that cooperation between the researchers in this study and those involved Jaguar should usefully now cross-validate each set of results.

Ref: Shulman N, Bosch R, Fiscus S et al. Mutations associated with didanosine resistance determined from 444 matched genotype-phenotype pairs. Abstract 49. Antiviral Therapy 2005; 10:S54

Under-detection of transmission of transmitted resistance and impact on treatment response

Simon Collins, HIV i-Base

Several studies focused on implications of under-detection of transmitted resistance - both from an individual and epidemiological perspective. Both the limit of sensitivity of genotype tests and the shift to wild-type after infection in the absence of drug pressure are both understood, these reports provided an indication of the level of the problem.

Using real-time PCR point-mutation tests for M184V, D67N, K70R in reverse transcriptase and L90M in protease, Jeffrey Johnson and colleagues analysed 183 samples from treatment naive patients in the US and Canada, with evidence of transmitted resistance by genotype sequence analysis at diagnosis. [1] Although real-time assays are sensitive to detect strains down to 0.05% of the viral population, a cut-off of 0.5% was used for positive screening in this study.

Prevalence for all four mutations was found at a higher level than previously seen with routine genotyping and the results are shown in Table 1 below.

Table 1: Prevalence of mutations in treatment naive pts

Mutation	Sequence	Point mutation	Relative difference
D67N	7%	12%	+71%
K70R	9%	14%	+60%
M184V	10%	12%	+20%
L90M	8%	11%	+35%

Detection of additional mutations resulted in 9/183 (5%) samples showing resistance to a new class. The frequency of 2-class resistance increased by 25% from 12% to 15% and the frequency of three-class resistance doubles from 2% to 4%. MDR resistance generally appeared on the same genome.

The relatively low incidence of M184V was attributed to rapid natural decay. Prior exposure to any ARVs (PEP etc) was ruled out by a detailed patient questionnaire and review of medical notes.

A similar study was presented from Karin Metzner, who detected transmission of drug resistance in around 20% (10 out of 49) of acute seroconvertors at two German clinics between 1999-2003. [2]

The study used quantitative real-time PCR to look for three key mutations: L90M (protease), K103N (NNRTIs) and M184V (RT) which was detected in 1/49 (2%), 5/49 (10%) and 6/49 (12%) of patients. Half of these mutations were present in less than 25% of the viral population and would have been below the limit of the test to be detected by routine genotypic sequencing analysis.

The clinical implications for missing transmitted resistance prior to initiating treatment were indicated by an analysis of treatment response at 3 months, presented by Marie-Laure Chaix and colleagues from the French primary infection and resistance databases. [3]

Of 297 people treated during primary HIV infection (PHI; within 6 months of infection, median 39 days IQR 33-55 days), 35 people had resistance to at least one drug in their combination (RG) and 262 were wild-type (WT). 21 had resistance to one drug, 10 to two drugs and 4 people had resistance to all three drugs in their regimen.

At baseline, the resistance group tended to have a slightly lower viral load and a higher CD4 cell count than the WT (both non-significant), but a significantly poorer response to treatment, detailed in Table 2 below.

Table 2: Baseline and treatment response

	Resistance Gp	Wild-type	p-value
Baseline viral load (log copies/mL)	5.0	5.3	0.11
Baseline CD4 (cells/mm ³)	512	475	0.12
% viral load <400 at month 3	63%	82%	0.02
% viral load <50 at month 3	16%	40%	0.01
% viral load <400 at month 6	81%	95%	0.02
% viral load <50 at month 6	57%	79%	0.02

In multivariate logistic regression analysis, taking into account gender, age, plasma HIV-RNA and CD4 cell count at HAART initiation, people with genotypic resistance to at least one drug were significantly less likely to achieve an undetectable viral load.

This study was performed without clinicians having access to the results. Although treatment in PHI rarely occurs outside a clinical trial, the impact on response is startling, and undoubtedly led to treatment failure and resistance long before the patients

needed treatment. French guidelines now recommend performing resistance test prospectively in patients with PHI with results available soon after HAART initiation.

The sub-optimal treatment response wouldn't surprise anyone in the audience. To have this poorer response carefully documented however is very important. It provides additional support for resistance testing prior to starting treatment and additional caution against starting treatment without confirming sensitivity to all drugs in the regimen. The understanding of archived resistance is such that similar differences in responses may be expected even if treatment was initiated during chronic infection, when detection of resistance would also be more difficult.

Finally, Bruna Brenner and colleagues from McGill Medical Centre looking at transmission of drug resistance in Quebec, using high bootstrap (>99%) and short phylogenetic branch length ($p < 0.015$) of reverse transcriptase and protease regions to look at clustering of recent infections from two Canadian cohort databases. [4]

484 unique subtype-B sequences were obtained from people diagnosed within six months of infection, half of which segregated into 71 clusters (range 2-11 individual per cluster). Mean age was 38, 40 and 36 in transmission groups (MSM, IVU, heterosexual respectively), with no difference in CD4, viral load or cluster size between the groups.

There also found no association between primary resistance (10.4% vs 9.4%) and dual/triple class resistance (3.8% vs 2.9%) in the clustered and non-clustered infections respectively.

This study was useful to show that recent infection are often connected – and support the view that much of the ongoing epidemic is driven by newly infected individuals who are unaware of their change in HIV status.

Another comment from the audience, was that results from this study cannot be extrapolated to actual clustering and behaviour risk as this would require detailed individual patient and partner histories, which by the anonymised nature of this study were not available. The discussion focused on legal implications of this work, although anonymised in this study. In the UK for example similar research was moved for research laboratories by police in recent high profile cases relating to transmission prosecutions.

The results also do not justify the researchers conclusion that this establishes a rationale for earlier treatment. Earlier diagnosis certainly, but not earlier treatment.

References

1. Johnson JA, Li J-F, Brant A et al. Multi-drug resistant HIV-1 are transmitted more frequently than current estimates. Abstract 111. Antiviral Therapy 2005; 10:S124
2. Metzner KL, Rauch P, Wlatter H et al. Detection of minor populations of drug-resistant HIV-1 in acute seroconvertors. Abstract 110. Antiviral Therapy 2005; 10:S123
3. Chaix ML, Desquilbet L, Cottalorda J et al. Sub-optimal response to HAART in patients treated at time of primary HIV-1 infection and infected with HIV resistant strains. Abstract 114. Antiviral Therapy 2005; 10:S126
4. Brenner BG, Roger M, Moisi D et al. Transmission events within risk groups following primary HIV-1 infection (PHI) in Quebec (1998-2005). Abstract 112. Antiviral Therapy 2005; 10:S125

C O M M E N T

SGS screening is probably not appropriate in the UK given the wide range of HIV sub-types that are prevalent (tests need to be recalibrated). However, although BHIVA guidelines recommend resistance testing on diagnosis, this has not been universally adopted in all clinics.

This is unfortunate. Newly diagnosed infection is the ideal time to confirm resistance, and has the practical advantage that laboratories will be seeing patient samples for the first time.

Checking systems between clinicians and labs in some hospitals are finding this a useful way to pick up patients who, for whatever reason, are not receiving resistance test with their initial CD4 and viral load results.

Risk of death in UK after diagnosis of 3-class resistance

Simon Collins, HIV i-Base

An analysis from Deenan Pillay on behalf of the UK Collaborative HIV Cohort Study (UK-CHIC) evaluated predictors of survival after diagnosis with multi-drug resistance (MDR). Of 628 patients 54 (9%) died within two years of their MDR diagnosis, defined as at least one primary mutation to nucleosides, NNRTIs and PIs. The cohort was 85% male, median age 43, with a median CD4 at MDR diagnosis of 238 cells/mm³ (IQR 110-376). This rose to 13% at three years. though far fewer patients had this length of follow-up.

The study was primarily looking at response to strategies including staying on the same treatment, switching to a treatment

with the same, higher, or lower genotypic sensitivity score (GSS) or stopping treatment (with the same or a reduced GSS). Compared to staying on the same treatment, switching to a regimen with a lower GSS score increased the risk of death (RR 1.4 [0.17, 13.0]) while switching to a regimen with a high GSS (RR 0.31, [0.11, 0.89]) or even the same GSS (RR 0.35, [0.16, 0.80]) was protective.

There are several important factors to remember when looking at these results. Firstly, the majority of patients with 2-year follow-up in this cohort were diagnosed with MDR between in 1999, 2000 or 2001 (26% 29% and 20% respectively). The subsequent period was therefore prior to the availability to T-20 and tipranavir. The analysis didn't look at role of individual drugs used in either the maintained or switched combinations, nor the effect of several changes in treatment.

Although success rates to treatment are generally improving, a small percentage are likely to accumulate resistance, and this study provided a sobering reminder of the clinical impact of resistance. Prevalence of MDR in the UK is steady at 4% of patients, although the increasing number of people on treatment mean that absolute numbers are increasing.

Ref: Grover D, Allen L, Pillay D et al. Predictors of death, and response to therapy in patients with multi(three)-class drug resistant HIV in the UK. Abstract 5. Antiviral Therapy 2005; 10:S7.

TREATMENT ACCESS

Six Indian generic ARVs are given FDA 'tentative' approval

On 27 May 2005, FDA granted tentative approval to Ranbaxy Laboratories, for lamivudine tablets, 150 mg. This was followed a few week later, on 15 June, by tentative approval for generic lamivudine manufactured by Aurobindo Pharma.

On June 20, 2005, two applications for nevirapine (one from Ranbaxy, one from Aurobindo) also received tentative approval.

Four days later the FDA granted tentative approval for efavirenz tablets manufactured by Aurobindo Pharma. This product is the first tentatively approved generic version of efavirenz tablets.

Finally, on 1 July FDA granted tentative approval for d4t (stavudine) manufactured by Aurobindo Pharma.

A Tentative Approval means that FDA has concluded that a drug product has met all of the agency's quality, safety and efficacy standards required for marketing in the US, even though it may not yet be marketed in the U.S. due to existing patents and/or exclusivity. It does, however, make the product eligible for use under the President's Emergency Plan for AIDS Relief (PEPFAR) program outside the United States.

An archive of FDA list serve announcements is available on the FDA web site at:

<http://www.fda.gov/oashi/aids/listserve/archive.html>

Source: FDA list serve

Methadone and buprenorphine added to WHO list of essential medicines

Gregg Gonsalves, GMHC

In November 2003, a small group of people living with AIDS, drug users, women and gay men met with the Director General of the World Health Organization Dr. Jong Wook-Lee and the new head of the HIV/AIDS Department, Dr. Jim Kim. This was the first meeting in the history of the epidemic between a Director General and a delegation of PLWHAs from around the world.

One of the priorities for the activists at that meeting was the inclusion of methadone on the WHO's Model List of Essential Medicines, since this drug is life saving for those struggling with addiction and is a key adjunctive therapy with antiretroviral treatment for drug users.

The push for methadone came from the activists from Eastern Europe and the Newly Independent States and from Southeast Asia.

On 30 June 2005, in what is a victory for drug users, people with AIDS, and all who care about them, the WHO put methadone (and buprenorphine as a medicine with a similar clinical performance within a pharmacological class) on the WHO Model (Complementary) List of Essential Medicines.

"Complementary" list is used for medicines when specialised diagnostic or monitoring facilities and/or specialist medical care and/or specialist training are needed.

Another significant achievement is introduction of a new section in the WHO Model List of Essential Medicines, namely - Medicines used for substance dependence (programmes). This is an opening for other medicines used for treatment of substance dependence, including alcohol.

Source: Letter from Vladimir Poznyak, M.D., Ph.D. Department of Mental Health and Substance Abuse (MSD), WHO, Geneva.

MSF's pricing guide to purchasing ARVs: 8th edition online

On June 28 Médecins Sans Frontières (MSF) published the 8th edition of "Untangling the web of price reductions: a pricing guide for the purchase of ARVs in developing countries".

The report shows that while generic production has brought down the prices of most first-line antiretrovirals (ARVs) from over \$10,000 in 2000 to as little as \$150 per patient in June 2005, prices of newer ARVs and formulations for children are up to 12 times higher.

It focuses on the political issues relating to drug pricing and trade agreements that threaten access to generic medication; and the importance of access to newer drugs and affordable second-line therapy for management of treatment failure and drug toxicity.

The full report is available at:

<http://www.accessmed-msf.org/prod/publications.asp?scntid=286200519752&contenttype=PARA&>

WHO '3 by 5' progress report

The latest 3x5 progress report from WHO was published on 29 June and is available online.

This interim report highlights progress to date in scaling up HIV treatment and prevention in low- and middle-income countries. The momentum achieved has been the result of a broad range of local, national and international efforts including, first and foremost, those of many of the most highly affected countries. These efforts have been reinforced by financial and technical support from many multilateral and bilateral institutions and donors.

The report focuses primarily on understanding the reasons for the successes and failures of scaling up HIV/AIDS interventions in different settings and on the need for sustainable financial mechanisms and improved harmonisation of efforts by partners at country level. A comprehensive report and country-specific analysis of access efforts and obstacles that remain will be released at the end of 2005.

A breakdown on numbers of people on currently receiving treatment is shown in Table 1.

Table 1. Estimated number of people receiving ARV therapy, people needing ARV and percentage coverage in developing and transitional countries by region, June 2005

Geographical region	Est. no. people receiving ARV therapy, June 2005 (low – high estimate) (x1000)	Est. no. people 15–49 years old needing ARVs, 2005 (x1000)	ARV therapy (%) coverage, June 2005	Est. no. people receiving ARVs, Dec 2004 (x1000)
Sub-Saharan Africa	500 [425–575]	4,000	11%	310 [270-350]
Latin America & Carib.	290 [270–310]	425	62%	275 [260-290]
East, South & SE Asia	155 [125–185]	1,200	14%	100 [85-115]
Europe and Central Asia	20 [18–22]	150	13%	15 [13-17]
North Africa & Middle East	4 [2–6]	55	5%	4 [2-6]
Total	700 [630–780]	5,800	15%	700 [630-780]

The report is available electronically in English, French and Japanese.

Executive summaries are available in Spanish and Russian.

<http://www.who.int/3by5/progressreport05/>

C O M M E N T

The goal of 3 million people on treatment from low- and middle-income countries will not be met by the end of the year, but this is still the world's largest scale up for treatment of a chronic disease programme. One of the most useful comments in the context of this report is from Stephen Lewis who noted that while the goal won't be met it 'has unleashed an irreversible momentum for treatment'.

When launched in 2002 many people thought the target was too ambitious – and many more that the target was by definition too little – that everyone who needs treatment should be the only goal to fix on. Universal treatment itself is now on the political agenda and a recent Labour Party statement recommended this as a goal by 2010.

This will depend on support from G7/G8 countries and the outcome from the summit held in Edinburgh as this issue of HTB went to press. Funding is still a central problem, both now and in the future – it will cost an estimated at \$22 billion annually to sustain. Access to second-line treatments and management of toxicity such as peripheral neuropathy due to the choice of drugs that are now rarely used in Western countries are also highlighted as new concerns for the programme.

In press calls to the launch of this report, Dr Jim Kim said that the WHO will focus on extending and building on the current programme. The call discussed both the difficulty and accuracy of the systems used to gather these updated figures and the increased access to treatment since the last progress report in December 2004, most of which has occurred in South East Asia and Africa.

Figures from UNAIDS showed that 2004 had the highest number of new infections and the highest numbers of deaths (5 and 3 million respectively), and it is unclear where the treatment programme will impact on this. The number of people who need treatment now is estimated at over 6.6 million, and this now includes 650,000 children who are included for the first time in the figures.

ANTIRETROVIRALS

TMC-114 to be submitted for registration based on Phase 2b trial results: expanded access expected in UK in Autumn 2005

Simon Collins, HIV i-Base

Based on the results from Phase 2 studies, and supported by safety data in a rapidly enrolled group of 300 additional patients, Tibotec announced on 15 June that it plans to submit its new protease inhibitor (TMC114) for early approval. This is the first time in ten years (since indinavir) that Phase 2 data have been sufficiently impressive, in a population with currently unmet treatment needs, to justify accelerating approval even more quickly than current Fast Track system. TMC-114 has activity against a broad range of currently PI-resistant virus.

Importantly, an expanded access programme will start in the Autumn 2005, that will provide guaranteed access to TMC-114 for people in greatest need of life-saving treatment. This will be an international programme (initially 24,000 places worldwide) for heavily treatment-experienced adults, with CD4 count <100 cells/mm³. The planned phase 3 studies will continue to recruit and enroll patients with higher CD4 counts.

The results from the 24-week combined interim analysis of the phase IIB trials of TMC114 were presented at the 12th Conference on Retroviruses and Opportunistic Infections (CROI) in February this year and were covered in HTB April 2005. [1]

Patients in the highest dose group, 600mg/100mg BID, plus optimised background regimen, experienced a mean reduction in plasma HIV RNA of -1.85 log, compared to a reduction of -0.27 log in the control group. These studies will continue to 144 weeks.

Based on these 24-week results, the selected dose of TMC114/RTV for treatment-experienced patients in phase III trials is 600mg/100mg BID. TMC114 will be studied in both treatment-experienced and -naïve patients in phase III trials.

Source: Tibotec PR

Reference:

1. McKerrow G. 24-week efficacy of TMC114: dramatic early activity in heavily-experienced patients, HIV Treatment Bulletin Vol6No4, April 2005. [12th CROI, Boston, 2005. Abstract 164LB.]
<http://www.i-base.info/htb/v6/htb6-4/24.html>

Tipranavir (Aptivus) approved in US

On June 22, 2005, the US Food and Drug Administration (FDA) granted accelerated approval of a new protease inhibitor, tipranavir (Aptivus). Tipranavir, co-administered with 200 mg of ritonavir, is indicated for adult patients who are highly treatment-experienced or have HIV-1 strains resistant to multiple PIs.

The approval of tipranavir/r is based on 24-week results from of two controlled phase III studies previously reported in HTB

(RESIST 1 and 2). A statistically greater percentage of HIV-positive patients taking tipranavir/r achieved treatment response versus the comparator group (40% vs 18%). Treatment response was defined as a confirmed 1 log or greater decrease in HIV RNA from baseline.

The approved dose of tipranavir is 500 mg taken with 200 mg of ritonavir, twice daily with food. Taking the drug with food improves absorption.

Source: FDA listserve and Boehringer Ingelheim PR

FDA Fast Track designation for CCR5 inhibitor vicriviroc (SCH-D)

The U.S. Food and Drug Administration (FDA) granted Fast Track designation to Schering-Plough's CCR5 receptor antagonist vicriviroc (SCH-D, SCH-417690). FDA Fast Track programs are designed to facilitate the development and expedite the review of new drugs that are intended to treat serious or life-threatening conditions and that demonstrate the potential to address unmet medical needs.

Vicriviroc is currently being studied in two Phase II clinical trials, one in the United States and one in Europe and Canada. The U.S. Phase II trial, conducted by ACTG, is currently open for enrollment. The European trial is fully enrolled. Phase III clinical studies are expected to start this summer.

Source: Schering Plough Press Release

Pfizer discontinues development of capravirine

On July 1 Pfizer announced that it has discontinued clinical development of capravirine, an NNRTI with activity against NNRTI-resistant HIV, that was in phase 2 development. This came as no surprise given the complicated drug interactions that make it difficult to use with other PIs or NNRTIs.

Results reported earlier this year at the 12th Annual Conference on Retroviruses and Opportunistic Infections (CROI) indicated no benefit compared to placebo.

Source: Pfizer

PREGNANCY and PMTCT

Use of T-20 in pregnancy: case study shows transmission occurred in highly treatment experienced mother with multiple-drug resistance

Polly Clayden, HIV i-Base

In a letter in the 10th June issue of AIDS, Deborah Cohan and co-workers describe a case in which an infant was infected with multi-drug resistant HIV, despite undetectable plasma HIV-1 RNA levels in the mother. [1] The authors note that the mother was receiving a regimen in which the drug to which the patient was fully sensitive, T-20, may have limited distribution to the genital tract.

This was the patient's eighth pregnancy and she had two children. She presented for prenatal care at the clinic at 7 weeks gestation. Since 1996 she had been intermittently adherent to several combinations of antiretrovirals and was experienced in all the then available three classes. When she presented she was receiving 3TC, abacavir and lopinavir/ritonavir and had a viral load of 29,712 copies/ml and a CD4 cell count of 323 cells/mm³.

Genotypic resistance testing revealed multidrug resistance (reverse transcriptase: M41L, L74V, K103N, M184V, and T215Y; protease: L10F, L33F, I54V, L63P, A71V, V82A, I84V). Phenotypic resistance testing confirmed high level resistance to most antiretrovirals. The patient was admitted to hospital for directly observed therapy at 33 weeks. Her treatment was changed to 3TC, abacavir, tenofovir and T-20. When labour was induced at 37 weeks, the patient's HIV-1 RNA level was undetectable. She received Intravenous AZT during labour, and the baby received AZT, 3TC, lopinavir/ritonavir and nevirapine after delivery.

The infant's DNA PCR at birth was indeterminate but at 8 days the baby's HIV-1 RNA level was 6,240 copies/ml. Genotyping revealed the presence of multiple protease and reverse transcriptase mutations but no known T-20 associated mutations.

The authors speculate that transmission could be explained by several mechanisms:

- Transmission may have occurred before the initiation of the T-20 regimen but the HIV-1 DNA result suggesting transmission just before labour;

- Transmission may have occurred at the time of delivery despite the patient's undetectable HIV RNA levels. Although T-20 containing combinations have been shown to produce undetectable levels in plasma, its activity in other compartments are undefined but it does not appear to cross into male genital fluids. Theoretically T20 resistant replication may have occurred within the genital tract leading to transmission at delivery.

The authors note that T-20 should be an ideal candidate for preventing mother to child transmission (and success has been described in another case study. [2, 3] Testing these hypotheses will require longitudinal assessments of HIV-1 in multiple compartments in patients receiving salvage regimens that contain T-20.

They write: "As the role of the genital compartment in perinatal transmission becomes further elucidated, decisions regarding the preferred delivery route may be guided by genital viral load measurements, and, as suggested here, knowledge of the relative capacity of a given drug regimen to penetrate the genital compartment."

This clinic has provided prenatal care for 165 women since 1995 and has pioneered treatment of HIV positive women with antiretrovirals during pregnancy many of whom have had multi drug resistant HIV-1 at presentation. This is the first case since then in which they were unable to prevent mother to child transmission of HIV.

References

1. Cohan D, Feakins C, Wara D et al. Perinatal transmission of multidrug-resistant HIV-1 despite viral suppression on an enfuvirtide-based treatment regimen. *AIDS* 19(9): 989-990, 2005.
2. Meyohasa MC, Lacombe K, Carbonne B, et al. Enfuvirtide prescription at the end of pregnancy to a multi-treated HIV-infected woman with virological breakthrough. *AIDS* 2004, Vol 18 No 14. Research letters 1966.
3. See report in HIV Treatment Bulletin Volume 5 Number 9/10, October/November 2004.

C O M M E N T

This single case study highlights the difficulty of treating multi-drug resistant HIV in any setting.

The previously reported case study was effective, but with no comment can really be made on use of T-20 during pregnancy on such low numbers.

Further reading on nevirapine resistance

Polly Clyden, HIV i-Base

Three studies from Johnson et al, Flys et al and Eshleman et al, looking at nevirapine resistance and persistence which we covered in our HTB CROI report are published in the *Journal of Infectious Diseases*. [1, 2, 3]

These are accompanied by an editorial commentary from Scott Hammer entitled (and in danger of becoming the new nevirapine resistance catch phrase): "The more you look the more you find", in which he provides an overview of the more recent data and its implications. [4]

And in the *Journal of Acquired Immune Deficiency Syndrome*, Kagaayi et al report an encouragingly low rate of transmission (7.5%) and high rate of adherence to maternal (85.2%) and infant (84.8%) self administered nevirapine doses in an underserved rural setting in Rakai, Uganda, with considerable and readily accessible community support. [5]

The authors write: "Mothers can be empowered to self-medicate themselves and their newborns and to reduce perinatal HIV infection. In circumstances where access to or utilisation of supervised delivery care is poor, as is the case in most rural areas of sub-Saharan Africa, there is an urgent need to replicate this study in a more conventional service setting." These results are consistent with those reported in a trial setting and conflict with less efficacious results from a field setting such as those reported from Mombasa. [6]

It seems there will be an urgent need to replicate this level of success with more complex interventions in the near future.

References

1. Johnson J, Li Jin-fen, Morris L et al. Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. *J Infect Dis* 2005;192:16-23.
2. Flys T, Nissley D, Claasen CW et al. Sensitive drug resistance assays reveal long-term persistence of HIV-1 variants with the K103N nevirapine (NVP) resistance mutation in some women and infants after the administration of single dose nevirapine: HIVNET 012. *J Infect Dis* 2005;192:24-29.
3. Eshleman SH, Hoover D, Chen S et al. Nevirapine (NVP) resistance in women with HIV-1 subtype C, compared with subtypes A and D, after the administration of single dose nevirapine. *J Infect Dis* 2005;192:30-36.
4. Hammer SM. The more you look, the more you find. *J Infect Dis* 2005;192:1-3.

5. Kagaayi J, Dreyfuss ML, Kigozi G et al. Maternal self-medication and provision of nevirapine to newborns by women in Rakai, Uganda. *J Acquir Immune Defic Syndr*. 2005. 39: 121-124.
6. Quaghebeura A, Mutungab L, Mwanyumbac F et al. Low efficacy of nevirapine (HIVNET 012) in preventing HIV-1 transmission in a real life situation. *AIDS*: 18(13) Research letters1855.

SPECIAL REPORT

Human Papilloma Virus Vaccines – a review of advances in the development of HPV vaccines

Leighton Davies MD, MSc for HIV i-Base

It is now clearly established that individuals infected with HIV are at increased risk of human papillomavirus (HPV)-related anogenital neoplasia.

HIV-positive women have a higher prevalence of HPV infection of the cervix and anus as well as high- and low-grade squamous intraepithelial lesions (HSIL and LSIL) at these sites compared to HIV-negative women, matched for age & risk factors. Similarly HIV-positive men who have sex with other men (MSM) have a higher prevalence of anal HPV infection and anal SIL and carcinoma than HIV negative MSMs. During the last decade it has been shown conclusively that HPV infection is implicated in more than 99% of cases of invasive cervical carcinoma. [1]

The prevalence of anogenital SIL as well as the proportion infected with multiple HPV types increases with decreasing CD4 count. This may reflect the failure of the impaired immune response to deal with HPV antigens or may possibly be due to local interactions between HIV and HPV at the tissue or cellular level.

Now in the third decade of the HIV pandemic, we are seeing increasing numbers of SIL progressing to invasive carcinoma. This is despite HAART, which by prolonging survival may increase the risk of progression of SIL to invasive carcinoma if these lesions do not resolve spontaneously or remain untreated.

There are now over a 100 different HPV subtypes with new types being identified annually. Broadly speaking most types can be divided into two groups: low risk (non-oncogenic) types which are rarely, if ever implicated in neoplastic lesions; and high risk (oncogenic) types.

Of the low risk types (**6, 11, 42, 43** and **44**), types **6b** and **11** are responsible for the majority of cases of condyloma acuminata (genital warts) and are infrequently associated with cases of cervical intraepithelial neoplasia (CIN) type 1. Of the high-risk types, types **16** and **18** (and to a lesser extent types **31** and **45**) are found in 50-80% of CIN 2 and 3 and up to 90% of invasive cervical cancer. [2, 3]

Many other types are also associated with cervical neoplasia, though with much less frequency, including types **33, 35, 39, 52, 56, 58, 59** and **68**.

Papilloma viruses are members of the papovavirus family: double stranded DNA viruses that replicate in their hosts' nuclei. The virion's icosahedral capsid comprises an outer protein coat, that consists of two different proteins; a major (L1) and a minor (L2) capsid protein, which encloses a circular 7900 base-pair genome divided into three groups of (1) early, (2) late and (3) control genes. Additionally, there is a non-coding region called the Long Control Region (LCR), which regulates the expression of the open reading frames (ORFs). Papilloma viruses with less than 90% sequence homology in E6, E7 and L1 ORFs to any of the known HPV types are classified as a new type. The function of these three genes is crucial to the virus' oncogenicity. E6 is responsible for cell transformation through p53 degradation, as is E7 through its binding to the retinoblastoma protein (pRB). The L1 gene is responsible for the coding of the major capsid protein. [4] The E6 protein of high-risk HPV interferes with p53 function and deregulates the cell cycle. E6 binds to p53, forming a stable complex that undergoes proteolysis by an ubiquitin-dependant ligase known as E6AP. [5] The degradation of p53 targets the transcriptional co-activator CBP/p300, which has a role in cell cycle and differentiation. [6]

Meanwhile, the E7 protein forms inactivating complexes with the pRB anti-oncoprotein through competitive binding with the "retinoblastoma pocket". This binding releases a transcription factor E2F, which accelerates DNA synthesis and cell cycle progression. [7] Thus E6 has an anti-apoptotic effect, whilst E7 promotes cell proliferation. In high-risk HPV, viral gene integration occurs in the E1/E2 region, disrupting the E2 gene, which represses the promoter from which E6 and E7 genes are transcribed. This leads to accelerated expression of E6 and E7, ultimately leading to the accumulation of damaged DNA and the development of the cancer phenotype over an extended period of time.

It should be noted, however, that infection with a high-risk HPV by itself is insufficient to trigger the chain of events that ultimately leads to the development of invasive carcinoma. Other co-factors have been proposed, including becoming sexually active at an earlier age, number of sexual partners (increasing likelihood of exposure to an oncogenic HPV type), smoking, the number of children that a woman has given birth to, duration of use of oral steroid contraceptives, and co-infection with other sexually transmitted organisms (particularly chlamydia trachomatis).

HPV Vaccines

Whilst attempts to arrest SIL from progressing to invasive lesions, through the use of immunomodulating drugs such as Imiquimod are progressing, exciting advances in the development of vaccines directed towards oncogenic HPV types are also in development.

Prophylactic HPV Vaccines

Initial attempts using recombinant vaccines based on the major capsid protein L1, were largely unsuccessful in animal models, as these proved to be of insufficient immunogenicity to augment the body's natural immune response. Whilst compelling evidence from animal studies showed that neutralising antibodies against L1 were able to block new infection, it was deemed important to obtain a source of conformationally correct L1 protein. A breakthrough was made by Kirnbauer and colleagues, and later by Zhou and colleagues, who discovered that L1 self-assembles into virus-like particles (VLPs), when expressed at high levels in cultured insect cells. [8] Moreover these VLPs induced the production of neutralising antibodies to conformational epitopes. HPV VLPs can now be produced using several expression systems including vaccinia viruses, baculoviruses and yeast systems.

Several groups are using VLPs against HPV types 6, 11, 16 and 18 in clinical trials.

Phase I and II studies have shown VLP vaccines to be well tolerated, safe and capable of inducing high titres of both binding and neutralising antibodies. These are often 50 times higher than titres induced by naturally occurring infection. In some studies, T-cell responses were also reported, suggesting possible therapeutic as well as prophylactic uses.

In 2002, Koutsky and colleagues published the results of a large randomised double-blind study to establish the efficacy of a HPV-16 VLP vaccine, developed by Merck, in preventing infection in women aged 16-23. [9]

In this study, 2392 women were eligible, but nearly 36% were excluded because they were HPV-16 seropositive or had HPV-16 DNA detected in the genital tract either at enrolment or at the last vaccination. This shows not only the need for a vaccine, but raises the issues of providing early protection against a sexually transmitted infection, long before young people actually become sexually active. This clearly raises issues of moral and political ethics that will vary by country.

Vaccinations of HPV-16 VLP (40 micrograms per dose) were given at day 0, month 2 and month 6. Follow-up at 6-monthly intervals, looked for evidence of persistent HPV-16 infection, defined as detection of HPV-16 by PCR at two visits, at least 24 months apart. An interim analysis was performed after a fixed number of persistent HPV-16 cases were detected, although all women will be followed up to 48 months following completion of the vaccination regimen. The results were impressive. In the placebo group (n=765), 41 cases of persistent HPV-16 including nine cases of HPV-16 associated SIL. With no cases in the vaccinated group (n=768) this established 100% efficacy (95% CI 90-100; p<0.001). Furthermore, an additional 33 women (6 in the vaccine group and 27 in the placebo group) were positive for HPV-16 DNA at a single visit. However, none of whom went on to develop SIL suggesting the possibility that this vaccine may provide sterilising immunity. With the median time of follow-up was 17.4 months, it is impossible to assess the durability of protection. To be completely efficacious the vaccine would need to provide protection from adolescence for several decades.

More recently, Harper and colleagues published results of a trial of the efficacy of a bivalent VLP vaccine developed by GlaxoSmithKline, to prevent infection with HPV-16 and HPV-18. [10]

This was a randomised, double blind, placebo-controlled trial in women aged 15-25 years old, performed at numerous centres in North America and Brazil. In addition to the primary trial objective, efficacy against cytological abnormalities and CIN as well as vaccine immunogenicity, safety and tolerability were assessed.

Two study phases were evaluated: an initial phase for vaccination and follow-up to 18 months, and a blinded follow-up extension phase that ended at month 27. Inclusion criteria for the initial phase of the study included previous history of no more than six sexual partners, no history of an abnormal cervical smear or ablative or excisional treatment of the cervix, no ongoing treatment for external condylomata, and who were cytologically negative, seronegative for HPV-16 and HPV-18 antibodies by ELISA and HPV-DNA negative by PCR for 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) within 90 days of study entry. Women who did not have any surgical treatment of the cervix or uterus were eligible to participate in the extension phase.

The bivalent vaccine contained 20mcg of HPV-16 VLP and 20mcg of HPV-18 VLP combined with an AS04 adjuvant (500mcg aluminium hydroxide and 50mcg 3-deacylated monophosphoryl lipid A) to stimulate dendritic cells. The placebo, identical in appearance to the vaccine contained only 500mcg of aluminium hydroxide. All study participants received a 0.5ml dose of either vaccine or placebo at 0, 1 and 6 months.

Cervical smears for cytology and HPV-DNA testing were obtained at screening and months 6, 12 and 18. The study participants self-obtained cervicovaginal samples at months 0 and 6 and every 3 months thereafter for HPV DNA testing.

Colposcopy was recommended after two reports of atypical squamous cells of undetermined significance (ASCUS), or after one report of atypical glandular cells of undetermined significance, LSIL, HSIL, squamous cell carcinoma, adenocarcinoma *in situ* or adenocarcinoma.

Incident cervical infection with HPV-16 or HPV-18 was defined as at least one positive PCR result. Persistent infection was defined as at least two positive PCR assays, for the same viral genotype, separated by at least 6 months. Serological testing was carried out by ELISAs to HPV-16/18 VLPs, with seropositivity defined as a titre greater than or equal to the assay cut-off titre (8 ELISA units/ml for HPV-16 and 7 ELISA units/ml for HPV-18). These were compared to the typical natural titres obtained from women in a previous epidemiology study, found to be seropositive for HPV-16/18.

Of the 1113 women enrolled and randomised, 560 received the vaccine and 553 the placebo. Demographic characteristics were similar between both groups, including similar patterns of risk factors for HPV acquisition (smoking, number of sexual partners and age at sexual debut). Reasons for elimination from the according-to-protocol (ATP) efficacy analysis were abnormal cytology, high-risk HPV DNA positivity or seropositivity for HPV-16 or 18 at enrolment.

A total of 958 women (85%) completed the initial phase, with similar proportions of women from both groups dropping out of the study.

In the ATP analyses, vaccine efficacy was determined to be 91.6% (95% CI 5-98.0) against incident infection (not statistically significant) but was found to be 100% (47.0-100) against persistent infection (statistically significant). In the intention-to-treat analyses vaccine efficacy was 95.1% (63.5-99.3) against persistent cervical infection with HPV16/18 and 92.9% (70.0-98.3) against cytological abnormalities associated with HPV-16/18 infection.

The vaccine was generally determined to be safe with only a few reports of injection site symptoms, which were transient and mild. Among the vaccinated women in the ATP cohort from month 0 to month 7, 100% seroconverted to HPV-16 positive and 99.7% seroconverted to HPV-18 positive after 3 doses of vaccine. By 18 months 100% of women had seroconverted to HPV-16 and HPV-18 positive. Geometric mean titres for vaccine-induced antibodies to HPV were over 80- and 100-fold greater than those seen in natural infections with HPV-18 and HPV-16 respectively. These titres remained substantially raised at 18 months, being 10-16-fold higher than those seen in natural infection with HPV-16 and HPV-18 respectively. This suggests that the immune responses induced in vaccinated women may provide a longer duration of protection than natural HPV infection. A protective antibody level has however not yet been established.

SanofiAventisPasteur are currently conducting trials of a tetravalent vaccine directed against HPV subtypes 6,11,16 and 18. The results of which are eagerly anticipated, as this vaccine would not only confer protection against the neoplastic subtypes but also against the subtypes most commonly responsible for condylomata acuminata.

Therapeutic HPV vaccines

The purpose of a therapeutic HPV vaccine is to eradicate or reduce the number of infected cells by inducing specific cell-mediated immunity (CMI) that prevents the development of lesions and eliminates existing lesions or even malignant neoplasms. There are many strategies to generate cytotoxic T lymphocytes (CTLs), which all involve causing antigen presenting cells (APCs) to process the tumour or viral antigen and present it in the context of an MHC receptor, along with adhesion and co-stimulatory molecules to stimulate anti-tumour lymphocytes. The induced specific CMI can directly target HPV viral products, HPV-induced cellular products or a combination of both.

Although HPV VLPs can induce L1-specific CMI responses, in addition to inducing high titres of neutralising antibodies, L1 capsid proteins are not expressed at a detectable level in the proliferating basal keratinocytes of virus producing lesions, or in the dedifferentiated cells of HPV-induced dysplasias and carcinomas. It is therefore unlikely that CMI responses to L1 proteins will induce regression of established lesions; HPV VLPs have been generated in which polypeptides of non-structure viral proteins are incorporated into the VLPs as fusion proteins of L1 or L2. Since E6 and E7 are consistently expressed in most cervical cancers and their precursor lesions, but are absent from normal tissues, most efforts have focussed on eliciting CTLs directed against these viral oncoproteins. While most tumour-specific antigens are derived from normal or mutated proteins, E6 and E7 are completely foreign viral proteins and may therefore harbour more antigenic peptides/epitopes than a mutant cellular protein. Furthermore, since E6 and E7 are required for the induction and maintenance of the malignant phenotype of cancer cells, cervical cancer cells are unlikely to evade an immune response through antigen loss.

Finally animal studies suggest that targeting oncoproteins such as E7 can generate therapeutic as well as preventive effects.

Potential therapeutic vaccine strategies

It is useful to now provide a short overview of the following eight vaccine approaches:

- Vector-based vaccines
- Peptide- and protein-based vaccines
- DNA vaccines
- Chimeric VLP vaccines
- Dendritic cell-based vaccines
- Tumour cell-based vaccines
- Self-replicating RNA vector vaccines
- HPV pseudovirion vaccines

Vector-based vaccines

Using viral vectors to introduce genes for vaccination is an effective way to stimulate many arms of the immune system. Vaccinia virus vectors have the advantage of being able to accommodate large recombinant gene insertions, and do not persist in the host. They also offer high efficiency of infection and high levels of recombinant gene expression. Finally vaccinia virus is a lytic virus and thus the chance of integration into the host genome is extremely small. One downside is that older people may have pre-existing antibodies to vaccinia virus that limits the elicited immune response. A recombinant vaccinia virus comprising mutated HPV-16/18 E6 and E7 genes (to remove their oncogenic potential) was created and in an initial study the vaccine was reported to be safe when administered to nine patients with late-stage cervical cancer. Most of these patients were immuno-suppressed and only one developed CTLs in addition to a clinical remission. [11]

In a more recent trial, 29 patients with stage Ib or IIa cervical cancer were vaccinated. After a single vaccination 4 patients developed CTLs and 8 developed serological responses to the HPV proteins. [12] This approach may offer some promise for future developments, however it emphasises the difficulties in eliciting therapeutic responses in immuno-compromised individuals.

More recently a vaccinia virus has also been utilised to explore tumour vaccine strategies employing intracellular sorting signals. Endosomal and lysosomal compartments, associated with MHC-II processing and presentation are characterised by the presence of a number of compartment-specific membrane proteins, including the lysosomal associated membrane protein (LAMP-1). In one study the sorting signals of LAMP-1 were linked to the HPV-16 E7 antigen to create the Sig/E7/LAMP-1 chimera. It was found that expression of this chimera with a recombinant vaccinia virus targeted E7 to endosomal and lysosomal compartments and enhanced MHC class II presentation to CD4+ T cells compared to vaccinia virus expressing wild-type E7. Furthermore, the Sig/E7/LAMP-1 vaccinia virus vaccine cured established tumours containing the E7 antigen whilst the wild-type E7 vector showed no effect on the established tumour. These experiments demonstrate that modifications rerouting cytosolic antigen to the endosomal/lysosomal compartment can profoundly improve the in vivo therapeutic potency of recombinant vaccinia vaccines. Phase I/II clinical trials using intramuscular administration of attenuated Sig/E7/LAMP-1 are underway at the Johns Hopkins Hospital in the USA (13).

Other viral delivery systems include recombinant adenoviruses and RNA-based alphavirus vaccines that have been constructed to express E7 or polyepitope proteins and are in early clinical trials.

Bacteria can also be used to deliver recombinant gene products. *Listeria monocytogenes* has recently emerged for use as a recombinant vaccine for human cancers due to its ability to elicit both CD8+ and CD4+ responses and induce regression of established tumours expressing a model antigen. This gram-positive intracellular bacterium usually infects macrophages, whereupon it is phagocytosed and taken up into a phagosome. Unlike other intracellular bacteria it escapes into the macrophage cytoplasm by secreting a factor – listeriolysin O, that disrupts the phagosomal membrane. Because of its presence in both endosomal compartments and cytoplasm it can deliver its antigens or carry foreign antigens into both the MHC-I and MHC-II pathways, thus inducing strong CMI responses. A recombinant *L. monocytogenes* secreting HPV-16 E7 has recently been shown to lead to regression of pre-existing E7-expressing tumours and is currently undergoing phase I/II trials (14). Other bacterial carrier systems have been investigated for delivering HPV vaccines, including *Salmonella*, *Shigella* and *Escherichia coli*.

Peptide- and protein-based vaccines

The characterisation of many CTL-defined antigenic determinants has opened the possibility of developing antigen-targeted vaccines. Several HPV-16 E7-specific CTL epitopes have been characterised for the HLA A-0201 haplotype and clinical trials have been conducted on patients possessing this genotype and whose HPV tumour type matched the viral peptide epitopes. Mixed results however, were obtained from these trials. Preclinical data has suggested that longer peptides that contain a helper T-cell epitope linked to the CTL epitope are more efficient at eliciting CTLs than the minimal epitope. The potency of HPV-16 E7 peptide based vaccines can be further enhanced by the use of a dendritic cell activating adjuvants such as immune stimulatory complexes (ISCOMs) and immunostimulatory carriers (ISCARs). Other strategies include modifying the CTL epitopes using lipid conjugation to form an immunogenic lipopeptide vaccine. An additional problem with using peptides is that one must know the HLA haplotype of the patient and the HPV genotype of the tumour. This has prompted many investigators to consider full-length E6 and/or E7 proteins, or fusions with other proteins e.g an E6/E7 fusion protein in a saponin-based adjuvant. To increase the immunogenicity of the E7 protein, one group has fused E7 to the BCG heat shock protein 65 (HSP65), which stimulates immunity through engaging the Toll-like receptor 4. This fusion has been used to immunise men with anal HSIL, some of whom, also had condylomata acuminata, in an open-label trial. [15]

Of 14 patients with genital warts, three had a complete resolution of symptoms and ten had a reduction in size of 70-95%. At least 95% of the men with HSIL showed a reduction in histopathological findings to at least LSIL with 44% having complete remission. These results obviously need to be confirmed in double-blind randomised controlled trials but are nevertheless encouraging and suggest that HSP65-E7 fusion protein elicits cross-reactive immunity.

Not all peptide based vaccines generate CTL responses and tumour protection; although interestingly the same epitopes loaded onto dendritic cells (DCs) can generate protective immunity, indicating that it might not be the peptides *per se*, but rather

the method of presenting the epitope to T-cells that determines the outcome of vaccination with peptide based vaccines. It is therefore important to choose the appropriate adjuvants and route of administration for peptide based vaccines in order to determine their immunising or tolerising properties *in vivo* before clinical use. It is evident that the application of peptide-based vaccines is limited by MHC restriction and the necessity to define specific CTL epitopes. In fact, most epitopes of HPV-16 E6 and/or E7 in patients with HLA other than HLA-A0201, remain undefined, making it difficult to use peptide-based vaccines in such situations. As intimated above, the use of protein-based vaccines can present all possible epitopes of a protein to the immune system, thereby bypassing the MHC restriction. Additionally, with a protein vaccine, serious side effects such as insertional gene activation and transformation (a possible concern with the use of recombinant viruses and DNA vaccines), are not an issue.

Several strategies devised to increase the potency of protein-based vaccines include:

- i) Association of the E7 protein with various adjuvants to enhance E7-specific CTL activities.
- ii) The fusion of antigen with heat shock protein (*vide supra*) represents another strategy for enhancing CTL priming.
- iii) Linking GM-CSF to an antigen, can target the antigen to DC and other GM-CSF responsive cells, after the chimeric molecule binds to the GM-CSF receptor, generating enhanced immune responses in these cells. In the context of HPV immunotherapy it has been possible to take peripheral blood monocytes (PBMCs) of cervical cancer patients, differentiate the cells in culture using IL-4 and GM-CSF into dendritic cells, mix the DCs with an HLA-A0201 E7 epitope and sensitise the autologous PBMCs from the cancer patients. [16]

A case report, of a woman with an HPV-18-containing adenocarcinoma, treated over 10 months with DCs that had been pulsed with an HPV-18 E7 protein, suggested an inhibition of metastatic disease for at least three years. [17]

DNA vaccines

DNA vaccines can be prepared inexpensively and rapidly on a large scale, and allow for expression of antigen for a sustained period of time. Consequently, the availability of antigen to be processed and presented as MHC-peptide complexes is likely to be more prolonged than peptide-based vaccines. Furthermore, DNA transduced into antigen inside APCs, enables the synthesised peptides to be presented by the patient's own HLA molecules. DNA vaccines targeting many different types of HPV can be mixed and effectively administered together, thus providing an efficient method of treating a variety of HPV-associated infections and tumours. Although the efficacy of DNA vaccination is important, safety is also a critical issue. DNA present in the vaccine may integrate into the host genome, potentially inactivating tumour suppressor genes or activating oncogenes, ultimately inducing malignant transformation of the host cells. Fortunately, it is estimated that the frequency of integration is much lower than that of spontaneous mutation, and integration should not pose any real risk. [18]

Other potential risks are associated with the presence of HPV-16 E7 oncoprotein in host cells. It is feared that the presence of E7 in the host nuclei may lead to accumulation of genetic aberrations and eventual malignant transformation of the host cells. Strategies such as employing the endosomal/lysosomal-targeting Sig/E7/LAMP-1 DNA vaccine, may be sufficient to divert E7 away from the nucleus to regions such as the endosomal and lysosomal compartments. This would physically separate E7 from pRB, thus abrogating the transformation activity of E7.

Ultimately DNA vectors employed in human clinical trials could utilise a minimally mutated E7 gene in which critical epitopes are preserved whilst eliminating potential oncogenic transformation.

Chimeric VLP vaccines

In order to create a preventive and therapeutic VLP-based vaccine, several E7 chimeric VLPs consisting of the L2 minor capsid protein plus the E7 protein or the n-terminus of E7 fused to L1 have been created. These E7 chimeric VLPs have been shown to generate significant E7-specific CTL activities and E7-specific anti-tumour effects. Furthermore E7 chimeric VLPs are indistinguishable from parental VLPs in their ability to elicit high titres of neutralising antibodies in murine models.

The anti-tumour immune response to the chimeric VLPs appears to be primarily mediated by CD8+ cytotoxic lymphocytes. *In vitro* E7-specific CTL activity was detected in lymphocytes from chimeric VLP-vaccinated mice. Furthermore, good protection was observed in MHC class II knockout or natural killer cell-depleted mice, but no protection was seen in beta-2-microglobulin or perforin knockout mice. It is unclear how the VLPs are routed for class I presentation – it might involve an endocytic pathway that the virus normally uses to enter the cell during the infectious process.

L1 and L2 chimeras for E7 produced similar results in mice, so it is unclear whether L1 or L2 chimeric VLPs would be preferable for testing in humans. L1 chimeras have the theoretical advantage of delivering more copies of the target antigen per VLP than L2 chimeras (360 for L1 versus 12 for L2). L2 chimeras on the other hand have the theoretical advantage of being able to incorporate larger polypeptides and thereby increasing the number of epitopes for immune recognition. It would seem reasonable to continue testing both types of chimeras. Currently, clinical grade HPV-16 L1/L2-E2-E7 fusion chimeras, which contain four HPV-encoded proteins (L1, L2, E2 and E7) as target antigens are undergoing phase I/II clinical trials. [19]

Chimeric HPV VLPs containing polypeptides of non-HPV targets are also being investigated in pre-clinical studies. One approach is to incorporate polypeptides of other sexually transmitted infections (STIs). With the provision that induction of

neutralising antibodies is sufficient for protection against genital HPV infection, this strategy could produce a vaccine that provides protection against both HPV and another STI at little or no increase in the cost of production or administration. A second approach involves incorporating cellular tumour antigens into the VLPs. This strategy was recently shown to induce therapeutic anti-tumour immune responses in a murine model. [20]

Immunisation of mice with an immuno-dominant peptide derived from the P815 tumour-associated antigen P1A induces specific T-cell tolerance, resulting in progressive outgrowth of a normally regressing P815 tumour line. In contrast immunisation with an L1 chimera that contained the same P1A peptide did not induce tolerance – rather it protected mice from lethal challenge with a progressor P815 line. Vaccination with this chimeric VLP also functioned therapeutically to suppress the growth of established tumours and to increase survival of the tumour-bearing mice.

Cell-based vaccines

Cell-based vaccines for cancer immunotherapy can be conceptually divided into two broad categories: DC-based vaccines, and cytokine-transduced tumour cell-based vaccines.

DCs are the most potent professional APCs, specialised to prime helper and killer T-cells *in vivo*. *Ex vivo* preparation and modification of DCs, therefore represent an attractive vaccine strategy that is capable of enhancing T-cell mediated immunity against tumours. The understanding that DCs can be generated from haematopoietic progenitors in the setting of various cytokines, mainly GM-CSF and Flt3-ligand, has created the opportunity to use a tumour cell-based vaccine transduced with GM-CSF or Flt3-ligand cytokines to expand and prime DCs *in vivo* (see under Peptide- and Protein based vaccines, point 3 above).

Dendritic cell-based vaccines

A lack of information about DC maturation and their lineage-specific markers, which define their cellular differentiation state, previously hindered the generation of a large number of DCs. Recent advances have revealed the origins of DCs, their antigen uptake mechanisms, and the signals that stimulate their migration and maturation into immuno-stimulatory APCs (21, 22). DCs derived from cultured haematopoietic progenitors appear to have an APC function similar to purified mature DCs. Broadly speaking; two strategies are employed to generate DCs *ex vivo*.

Dendritic cells pulsed with peptides/proteins.

Syngeneic spleen DCs pulsed with E7-specific T cell epitopes can generate protective E7-specific anti-tumour T CTLs. Treatment of tumours with peptide-pulsed DCs had resulted in sustained tumour regression in several different tumour models. [23] Similarly DCs pulsed with whole E7 proteins are able to elicit potent E7-specific CTL responses *in vivo*, which are associated with protection against a challenge with syngeneic HPV-16 induced tumour cells. [24] Another study demonstrated that DCs derived from patients could be pulsed with fusion proteins such as E6/E7 and used to generate E6/E7-specific CTLs *in vitro*. [25]

Dendritic cells transduced with HPV E6 and/or E7 genes.

Gene-transduced DC-based vaccines represent an attractive alternative to peptide pulsed DC-based vaccines since MHC restriction may be bypassed by directly transducing genes coding for E6 and/or E7 inside DCs, allowing synthesized peptides to be presented by any given patient's HLA molecules. Gene transfer into DCs can be accomplished by a variety of methods involving either naked DNA or the use of viral vectors. The major limitation of naked DNA transfer into DCs is poor transfection efficiency using various physical methods. Various studies indicate that the potency of DC-based vaccines may ultimately depend on their route of administration be it subcutaneous, intramuscular or intravenous. [26]

Tumour cell-based vaccines

The use of tumour cell-based vaccines may not be suitable for the treatment of early-stage, pre-cancerous HPV-associated lesions, because of the risks and controversy associated with administering modified tumour cells to these patients.

Tumour cell-based vaccination is therefore better reserved for patients with advanced HPV-associated carcinomatous lesions. Transduction of tumour cells with genes encoding co-stimulatory molecules or cytokines may enhance immunogenicity leading to T-cell activation and anti-tumour effects after vaccination. [27]

In murine studies strong anti-tumour effects have been demonstrated with tumour cells transduced with IL-2 and IL-12 genes that indicate that tumour cell-based vaccines may be useful for the control of minimal residual disease in patients with advanced HPV-associated cervical carcinomas.

Self-replicating RNA vector vaccines

Nucleic acid vaccines using RNA replicons have recently been shown to significantly enhance vaccine potency. [28] RNA replicon vaccines are self-replicating and self-limiting and may be administered as either RNA or DNA, which is then transcribed into RNA replicons in transfected cells or *in vivo*. [29] The self-replication allows the expression of the antigen of interest at high levels for an extended period, optimising vaccine potency. Since such replicons ultimately cause lysis of

transfected cells, the concern associated with naked DNA vaccines of integration into the host genome is lessened, particularly important when the oncogenic E6 and E7 proteins are targeted.

The potency of a self-replicating RNA vaccine can be further enhanced by applying the LAMP-1 targeting strategy, creating a Sig/E7/LAMP-1 RNA replicon. [30]

DNA based RNA replicons – also known as “suicidal” DNA - share the advantages of both the RNA replicons and naked DNA vaccines, being both stable and easily prepared. Furthermore they are significantly more potent than conventional DNA vaccines. Since cells transfected with DNA-launched RNA replicons are eventually lysed (hence the term “suicidal”) there is little concern for malignant transformation commonly associated with naked DNA vaccines.

Recently a DNA-launched RNA replicon vaccine demonstrated significant E7-specific CTL activity and anti-tumour effects. [31]

HPV pseudo-virion vaccines

The encapsulation of naked DNA by HPV capsids forming HPV pseudovirions has been achieved using various expression systems including recombinant vaccinia viruses, Semiliki Forest virus, baculoviruses and even in yeast systems. The target DNA can be packaged into HPV-16 VLPs expressed for example in yeast cells and transduced into different primary and established cells in culture and in vivo via receptor-mediated endocytosis, establishing a quantitative system to assess HPV-16 VLP infection, thus providing a safe, nonreplicative and improved delivery of therapeutic DNA vaccines to target cells. The enhanced delivery of DNA vaccine to professional APCs may be due to several reasons.

The capsid can protect the DNA from nuclease activity and may also act as an adjuvant. Additionally alpha-6-integrin has been proposed as the cell surface receptor for HPV and is highly expressed by DCs of the skin (Langerhans cells) and lymph nodes. Hence HPV pseudovirions may represent an ideal method to deliver therapeutic DNA vaccines to DCs to prime MHC-I-restricted CD8+ cytotoxic T cells and MHC-II-restricted CD4+ helper T cells, and these are the most potent effector cells in anti-tumour immune responses. Pseudovirions may therefore act as ideal prophylactic and therapeutic vaccines.

Conclusion

In the rapidly expanding fields of immunology, molecular biology and vaccinology there is clearly an abundance of on-going research into delivering the most potent, effective and inexpensive prophylactic and therapeutic Human Papilloma Virus vaccines. Later this year, two or more licensed prophylactic HPV vaccines are likely to be approved. It is hopefully only a matter of time before the “ideal” therapeutic vaccine is produced capable of eradicating early HPV-associated malignant disease, as well as curing established advanced carcinomatous disease.

From the evidence on prophylactic vaccines, and the exclusion rate during screening for these trials, if these vaccines are approved, then deciding when and how to offer the vaccination and in which populations will introduce new challenges for public health programmes.

It is as yet unclear how these vaccines will benefit adults already infected with HPV, and specifically those coinfecting with HIV. If they have a therapeutic role, then optimal time for successful vaccination is likely to be linked to CD4 count and possibly CD4 nadir.

References

- Fontaine et al. High levels of HPV-16 DNA are associated with high-grade cervical lesions in women at risk or infected with HIV. *AIDS*, 2005, 19 (8) 785-794.
- Munoz N et al. Current views on the epidemiology of HPV and cervical cancer. In Lacey C et al. *Papilloma virus reviews: current research on papillomavirus*. Leeds. Leeds University Press, 1996: 227-37
- Schiffman MH et al. Epidemiological evidence showing that human HPV infection causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst* 85, 958-64, 1993.
- Furomoto H et al. Human Papilloma Virus and cervical cancer. *J Med. Invest.* 49, 124-133, 2002
- Scheffner M et al. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 75, 495-505, 1993
- Zimmerman H et al. The HPV-16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional co-activator CBP/p300. *J Virol* 73, 6209-6219, 1999
- Dyson N et al. The HPV-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science*, 243, 934-937, 1989.
- Zhou J et al. Expression of vaccinia recombinant HPV16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV VLPs. *Virology* 185, 251-7, 1991
- Koutsky L A et al. A controlled trial of a human papillomavirus type 16 vaccine, *NEJM*, 347,21,1645-1651
- Harper D et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet*, 2004, 364, 1757-1765.
- Borysiewicz L K et al. A recombinant vaccinia virus encoding HPV types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. *Lancet*, 1996, 347,1523- 1527.
- Kaufmann AM et al. Safety and immunogenicity of TA-HPV, a recombinant vaccinia virus expressing modified HPV 16 and 18 E6 and E7 genes in women with progressive cervical cancer. *Clin Cancer Res.* 2002,8, 3676-3685.
- Wu T C et al. Engineering an intracellular pathway for MHC class II presentation of antigens. *Proc Natl Acad Sci USA*, 1995, 92, 11671-11675.

14. Pan ZX et al. Regression of established tumours in mice mediated by the oral administration of a recombinant *Listeria Monocytogenes* vaccine. *Cancer Res*, 1995, 55,4776-4779.
15. Goldstone SE et al. Activity of HspE7, a novel immunotherapy in patients with anogenital warts. *Dis Colon Rectum*. 2002, 45, 502-507.
16. Santin AD et al. Induction of HPV-specific CD4 (+) and CD8 (+) lymphocytes by E7-pulsed autologous dendritic cells in patients with HPV-16 and -18 positive cervical cancer. *J Virol*. 1999, 73, 5402-5410.
17. Santin AD et al. Vaccination with HPV-18 E7-pulsed dendritic cells in a patient with metastatic cervical cancer. *NEJM*, 2002, 346, 1752-1753.
18. Nichols WW et al. Potential DNA vaccine integration into host cell genome. *Ann NY Acad Sci*, 1995, 772, 30-39.
19. Schafer K et al. Immune response to HPV-16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumour protection. *Int J Cancer*, 1999, 81, 881-888.
20. Nieland JD et al. Chimeric HPV VLPs induce a murine self-antigen-specific protective and therapeutic anti-tumour immune response. *J Cell Biochem*, 1999, 73, 145-152.
21. Cella M et al. Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol*, 1997, 9 10-16.
22. Hart DN. Dendritic cells: Unique leucocyte populations, which control the primary immune response. *Blood*, 1997, 90, 3245-3287.
23. Mayordomo Ji et al Bone marrow derived dendritic cells serve as potent adjuvants for peptide-based anti-tumour vaccines. *Stem Cells*, 1997, 15, 94-103.
24. De Bruijn ML et al. Immunisation with HPV-16 oncoprotein-loaded dendritic cells as well as protein in adjuvant induces MHC class I-restricted protection to HPV-16 induced tumour cells. *Cancer Res*. 1998, 58, 724-731.
25. Murakami M et al. Induction of specific CD8+ T-lymphocyte responses using a HPV-16 E6/E7 fusion protein and autologous dendritic cells. *Cancer Res*. 1999, 59 1184-1187.
26. Arthur JF et al. A comparison of gene transfer methods in human dendritic cells. *Cancer Gene Ther*, 1997,4, 17-25.
27. Chen CH et al. Experimental vaccine strategies for cancer immunotherapy. *J Biomed Sci*, 1998, 5, 231-252.
28. Ying H et al. Cancer therapy using a self-replicating RNA vaccine. *Nat Med*, 1999, 5 823-827
29. Berglund P et al. Enhancing immune responses using suicidal DNA vaccines. *Nat Biotechnol*, 1998, 16, 562-565.
30. Wang TL et al. A LAMP-1 targeting strategy enhances the anti-tumour immunity of Semliki Forest virus self-replicating RNA vaccines against E7-expressing murine tumours (manuscript in preparation)
31. Hsu KF et al. Enhancement of suicidal DNA vaccine potency by linkage of antigen gene to an HSP70 gene (manuscript in preparation)

OTHER NEWS

CDC estimate over 1 million HIV-positive people in US

- *New data indicate continuing impact on African Americans and gay and bisexual men*
- *HIV prevention interventions for at-risk and HIV-positive populations show effectiveness*
- *Rapid HIV testing efforts increase number of people receiving counseling and test results*

Data presented at the 2005 National HIV Prevention Conference in Atlanta, Georgia, indicate that roughly one million Americans were living with HIV *at the end of 2003* and that HIV prevalence remains extremely high among African-American men who have sex with men (MSM) in several U.S. cities. Other data show that while HIV diagnoses reported among adolescent and young adult females have declined steadily, diagnoses in males have increased in recent years. Data presented also show that some prevention programs are substantially reducing sexual risk behavior among people with HIV and those at risk for infection, and that voluntary rapid testing efforts are increasing the number of people who find out their HIV status.

African Americans and MSM Most Affected

New Centers for Disease Control and Prevention (CDC) estimates of HIV prevalence in the United States indicate that between 1,039,000 and 1,185,000 people were living with HIV in December 2003. The estimates provide the clearest picture to date of the scope of the U.S. epidemic overall and among specific racial and ethnic and risk groups.

The new estimates indicate that HIV continues to have the greatest impact among African Americans and MSM. At the end of 2003, blacks accounted for 47 percent of people estimated to be living with HIV in the US; whites accounted for 34 percent and Hispanics for 17 percent. Asian/Pacific Islanders and American Indians/Alaska Natives each represented roughly 1 percent of the HIV-positive population. By transmission category, MSM remained the most heavily affected group, accounting for 45 percent of people living with HIV. Individuals infected through high-risk heterosexual contact comprised 27 percent, and those infected through injection drug use accounted for 22 percent of the HIV-positive population. Roughly three-quarters (74%) of Americans estimated to be living with HIV are male.

A separate CDC analysis suggests that undiagnosed HIV infection continues to play a significant role in the extremely high rates of infection among African-American MSM. Consistent with earlier research, black MSM in a new five-city study were more than twice as likely to be infected with HIV as other MSM, and were less likely to be aware of their infection. Forty-six percent of black MSM in the study were HIV-positive, compared to 21 percent of white MSM and 17 percent of Hispanic MSM. Among HIV-infected MSM, 67 percent of black men, 48 percent of Hispanic men, and 18 percent of white men were unaware of their infection before study participation, underscoring the need to reach MSM with testing and prevention services. The study surveyed 1,767 MSM over age 18 at public venues in Baltimore, Los Angeles, Miami, New York City, and San Francisco

between June 2004 and April 2005 (Plenary session, "New Approaches to Tracking the HIV Epidemic in the U.S.").

Other CDC data point to the continuing impact of HIV on young African-American MSM across the nation. Researchers examined trends in new HIV diagnoses (with or without AIDS) among persons 13 to 24 years of age between 1994 and 2003 in 25 U.S. states with longstanding, name-based HIV reporting. Results indicate that new diagnoses declined significantly among young women, but rose among young men. Among 13- to 24-year-old females, new HIV diagnoses fell 20 percent over the 10-year period. HIV diagnoses also declined among young men for the first few years of the period (by 30% from 1994 to 1998); but the decline was offset by a 41 percent increase from 1999 to 2003. The increase among young men was driven by a 47 percent rise in diagnoses among MSM ages 20-24, 60 percent of whom were black. While researchers were unable to determine if the increases in HIV diagnoses among young men were the result of increased testing or an actual increase in new infections, the findings are consistent with other recent data suggesting a possible resurgence of HIV among young MSM.

Source: CDC Press Release 13 June 2005

Re-emergence of syphilis in the UK: the new epidemic phases

To characterize the re-emergence of infectious syphilis in the United Kingdom between 1997 and 2003, the authors conducted a retrospective analysis of surveillance data from genitourinary medicine clinics and additional data collected through enhanced surveillance.

Results of the analysis showed that from 1997 through 2002, syphilis diagnoses (primary, secondary and early latent) were up by 213 percent in heterosexual males, 1,412 percent in men who have sex with men (MSM), and 22 percent in females. A series of outbreaks have driven the increases through October 2003, chiefly in Manchester (528 cases) and London (1,222 cases). The majority of cases were MSM, and all the outbreaks were geographically localized. HIV co-infection was reported in a high percentage of cases. Oral sex was often reported as a route of transmission.

"Syphilis has re-emerged in response to behavior change, probably driven by changes in the HIV epidemic," the authors concluded. "The future course of the epidemic is difficult to predict and control remains elusive."

Source: CDC HIV/STD/TB Prevention News Update. April 11, 2005

Ref: Simms I, Fenton KA, Ashton M et al. The re-emergence of syphilis in the United Kingdom: the new epidemic phases. *Sex Tran Dis* 32(4) 220-226 (04.05.05)

Newly diagnosed HIV infection - review in UK and Ireland

Nicola Pocock, BMJ online

BMJ early online have published a case review of new HIV diagnoses in the UK and Ireland, looking at the occurrence of late diagnosis and associated features and to determine if patients had prior presentations that may have been related to HIV infection.

Data was collected via questionnaires, which were sent to adult HIV care providers in the UK and Ireland. Data on a total of 977 patients presenting with new diagnosis of HIV infection in January-March 2003 was collected.

A total of 301 patients (33%) presented late - this was more common in both older patients (adjusted odds ratio per increase in age group 1.68, 95% CI 1.42-1.98, $p=0.0001$) and in black Africans (1.66, 1.05-2.62, $p=0.03$). Overall, 401 (41%) were diagnosed via routine screening (e.g. sexual health, genitourinary or HIV clinic) - diagnosis in this way was associated with a lower chance of late diagnosis. A high proportion of patients (17%) sought medical care with symptoms in the preceding 12 months but remained undiagnosed.

The authors conclude that this study provides further evidence of the late diagnosis of HIV infection, following national trends reported by the Health Protection Agency. They say that improving the offering and uptake of HIV testing both as part of routine screening and as indicated by associated medical conditions should reduce the number of undiagnosed infections.

<http://bmj.bmjournals.com/cgi/rapidpdf/bmj.38398.590602.E0v1>

Prescription of heroin is less costly for society

Prescribing methadone plus heroin to chronic, treatment resistant addicts is less costly than methadone alone because it reduces criminal behaviour, finds a study in this weeks BMJ.

The study involved treatment resistant heroin addicts taking part in methadone maintenance programmes in six cities in the Netherlands. Prior to study entry, the heroin addicts frequently engaged in illegal activities to acquire money or drugs.

They were randomised to treatment with methadone plus heroin (experimental group) or with methadone alone (control group). After one year, data from 430 patients were analysed.

Co-prescription of heroin was associated with better quality of life measures. Although the costs of co-prescription were considerably higher, they were offset by lower costs of law enforcement and reduced costs of crime against property. The average total net savings amounted to 12,793 per patient per year.

From a societal perspective, supervised medical prescription of methadone plus heroin to chronic, treatment resistant addicts is very efficient.

Source: BMJ online

Ref: Cost utility analysis of co-prescribed heroin compared with methadone maintenance treatment in heroin addicts in two randomised controlled trials.

<http://bmj.com/cgi/content/full/330/7503/1297>

ON THE WEB

Medscape full text articles

(Medscape requires one-time free online registration)

From AIDS

http://www.medscape.com/viewpublication/744_index

- Interactions between antimalarial and antiretroviral drugs
- Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow Impact of protease inhibitor exposure
- Micronutrients: current issues for HIV care providers

Hepatitis coinfection links

Management of anemia and fatigue in an HIV-HCV-coinfected patient - Jeffrey Nadler

<http://www.medscape.com/viewprogram/4267?src=hp1.lead>

Improved treatments for chronic Hepatitis C

http://www.hivandhepatitis.com/hep_c/news/2005/ad/070105_a.html

Table providing a snapshot of over 30 new anti-HCV therapies now in clinical development in the US.

Community newsletters and journals

HIV inSite Knowledge Base

Updates April and May 2005

Malaria and HIV

<http://hivinsite.ucsf.edu/InSite?page=kb-05-04-04>

Pneumocystosis and HIV

<http://hivinsite.ucsf.edu/InSite?page=kb-05-02-01>

The science of HIV vaccine development: a collection of material about policy and scientific issues in HIV vaccine development

<http://hivinsite.ucsf.edu/InSite?page=kbr-02-01-06>

Adherence to HIV antiretroviral therapy

<http://hivinsite.ucsf.edu/InSite?page=kb-03-02-09>

Histoplasmosis and HIV infection

<http://hivinsite.ucsf.edu/InSite?page=kb-05-02-06>

PRN notebook - June 2005

http://www.prn.org/prn_nb_cntnt/current.htm

- **Ulcerating STDs and HIV: a cause for concern** - Susan Blank
- **Integrating rapid HIV testing into fast-paced private practice settings** - Kevin Armington
- **View from the pipeline: 2005 review of experimental antiretrovirals** - Roy Gulick
- **Women and HIV** - Judith Aberg
- **Treatment of HCV in HIV/HCV coinfection: what are the new questions?** - Francesca Torriani

Community resources:

New Resources from the International AIDS/HIV Alliance.

Care and treatment publications

<http://www.aidsalliance.org/sw7418.asp>

ARV treatment factsheets

<http://www.aidsalliance.org/sw19588.asp>

Community education referral: Supporting adherence to ARV treatment and prevention for people with HIV in Zambia

<http://www.aidsalliance.org/sw23263.asp>

Understanding and challenging HIV Stigma: Toolkit for action

<http://www.aidsalliance.org/sw23480.asp>

The Toolkit was developed from a two year research project on stigma which took place in Zambia, Tanzania and Ethiopia. Tools were developed with the involvement of over 50 NGOs from the three countries who developed exercises, shared stories and experiences and tested some of the tools.

It contains over 100 participatory exercises which can be adapted to fit different target groups and contexts. There are different sets of picture codes which help to identify stigma, discuss the rights of PLHAs and help to stimulate discussions around gender, sexuality and morality issues which link to stigma.

MEETING ANNOUNCEMENTS

EACS - Third Advanced HIV course

29-31 August 2005, Montpellier, France

The European AIDS Clinical Society (EACS) are running their third course on 'Antiretroviral therapy and comprehensive care' focused on the clinical management on HIV.

Tuition fee is 100 euros and includes accommodation for four nights, lunches for the three-day course and course material.

Please contact the EACS office for details:

sylvie-chatelain@eacs.ws

<http://www.eacs.ws>

International Association of Physicians in AIDS Care

European sessions 2005, 6-7 October 2005

Co-Chairs: Bernard Hirschel and Joep MA Lange

This meeting is intended for HIV-treating physicians and is limited to 100 delegates. Registration is on a first-come first-served basis. Please post and share this information with physicians in your department.

Programme includes:

- Controlling lipid levels in the HIV clinical setting - *Peter Reiss*
- Predictors and Management of HAART-Related Morphologic Changes - *Esteban Martinez*

- Maximizing HAART - *Brian Gazzard*
- Moving Forward... New Antiviral Targets - *Schlomo Staszewski*
- Reconciling the Impact Migration on HIV/AIDS Care Delivery in Europe - *Mark Nelson*
- Stemming the Tide of HIV Infection Among Europe's Immigrant Populations - *Angus Nicoll*
- Emerging Treatment Options for HIV/hepatitis-Coinfected Patients - *Stefan Mauss*
- Toxicity of HAART in HIV/Hepatitis-Coinfected Patients - *José R. Arribas*

Workshop costs: If you are a member of IAPAC, registration fee of \$100 USD. If you are not a member, your fee is US\$200 and will include a one-year membership to IAPAC.

For more information and registration please contact:

Carrie Scharrer <cscharrer@iapac.org>

or 312-795-4935 (in the U.S.)

or click on the IAPAC European Sessions 2005 banner visit on our website at:

<http://www.iapac.org/>

PUBLICATIONS AND SERVICES FROM i-BASE

i-Base website redesigned

<http://www.i-Base.info>

The website has been totally redesigned so that it is faster and easier to use, and is more accessible for those with impaired sight. For those who understand these matters, all pages conform to at least the W3C-WAI Level A and most to level AAA.

There is a new section on Education and Training with treatment training for advocates. i-Base has developed a training manual with eight 2-hour modules that include questions and evaluation. Subjects start from the basics including CD4, viral load and other monitoring tests, combination therapy and side effects, to brief overviews of the main opportunistic infections. There is a module on pregnancy and another module on IV drug users and treatment

All i-Base publications are available at our website, including 2005 editions to three treatment guides. The site gives details about i-Base, the UK Community Advisory Boards (UK-CABs), our phone service and meetings, as well as access to our archives and an extensive range of links. It can be used to order publications and regular subscriptions to be delivered by post or email (as pdf files).

UK-Community Advisory Board

The UK-Community Advisory Board (UK-CAB) is a network for community treatment workers across the UK. Each meeting includes two training lectures and a meeting with a pharmaceutical company.

<http://www.i-base.info/ukcab/index.html>

Transcriptions and slides of training sessions from previous meetings are included on the site:

World CAB: International drug pricing

Report from a meeting in February 2004 of community advocates and three major pharmaceutical companies that focussed on pricing issues and global access to treatment.

Available to download as a pdf file. See website below

Introduction to Combination Therapy

June 2004 edition

This non-technical patient guide to treatment is available in 12 languages. It explains what combination therapy is, how well it works, who can benefit from it, when to start taking it, some differences between treating men and women, side effects, the best combinations, changing treatment, taking part in drug trials, your relationship with your doctor, the importance of adherence, and how to avoid drug resistance.

Printed and pdf versions of this booklet are available in Bulgarian, Chinese, English, French, Georgian, Italian, Latvian, Macedonian, Portuguese, Russian, Slovak, and Spanish.

Guide to HIV, pregnancy & women's health

New Spring 2005 edition

Updated and revised in April 2005, this patient guide helps women get the most out of HIV treatment and care before, during and after pregnancy. It should help whether you are on therapy or not and includes information for your own health and for the health of your baby.

The guide gives information on medication, Caesarean section and breastfeeding, as well as details of other sources of help. It is aimed at people in a wide range of circumstances including positive women thinking about having children and pregnant women who have recently been diagnosed HIV-positive.

Guide to changing treatment: what to do when your treatment fails

New April 2005 edition

Also updated and revised in April 2005, this is a non-technical patient guide to changing treatment and what to do if your treatment fails.

This booklet helps patients in discussions with doctors, and covers what you can do if your viral load starts to rise, and the importance of considering or finding out why your current combination failed, treatment strategies and new and pipeline treatments.

Guide to avoiding & managing side effects

New February 2005 edition

This is a comprehensive 44-page guide that is aimed at helping anyone using HIV drugs to get the most out of their treatment, the most out of their relationships with their doctor and other health professionals, to get better medical care to improve their health and, most importantly, to enjoy a better quality of life.

It is written by people who are HIV-positive, who have been on most of the treatments, who have had many of the side effects and who have learnt to negotiate their own healthcare.

New sections are included on heart disease, lipodystrophy, and information relating to newer drugs including T-20, atazanavir, tenofovir, FTC and fosamprenavir.

Chinese, French, Italian and Spanish translations of the previous edition are still available.

Treatment 'Passports'

These popular booklets are for HIV-positive people – whether newly diagnosed or positive for a long time - to keep a record of health and treatment history. Like all i-Base publications, they are available free as single copies, or in bulk.

HIV Treatment Bulletin (HTB)

This is the journal you are reading now: a review of the latest research and other news in the field. HTB is published 10 times a year in a printed version, in a pdf file that we can email to you, and on our website.

The printed version is available at most HIV clinics in the UK and is available free by post.

Treatment information request service – 0808 800 6013

i-Base offers specialised treatment information for individuals, based on the latest research.

We can provide information and advice over the phone, and we can mail or email copies of the latest research studies relevant to the caller.

For details, call the i-Base treatment information free phone line on 0808 800 6013. The line is usually staffed by positive people and is open Mondays, Tuesdays and Wednesdays from 12 noon to 4pm. All calls are in confidence and are free within the UK.

Find HTB on AEGiS

AEGiS.org - the longest established and largest global resource of online HIV information - includes HTB in the regular journals that it puts online. You can find us at:

<http://www.aegis.org/pubs/i-base/2004>

The AEGiS daily email news service also carries i-Base conference reports.

Order i-Base publications via the internet, post or fax

People with internet access can use our site to order and receive publications. You can access our publications online or subscribe to receive them by email or by post; and you can order single copies or bulk deliveries by using the forms at:

<http://www.i-base.info/forms/index.html>

Copies of publications can also be ordered by post or fax using the form on the back page of HTB. These methods of ordering are suitable for all our publications: HIV Treatment Bulletin (HTB), Positive Treatment News (PTN), Treatment 'Passports' and all our treatment guides and reports.

h-tb

HIV Treatment Bulletin

HTB is a monthly journal published in print and electronic format by HIV i-Base. As with all i-Base publications, subscriptions are free and can be ordered directly from the i-Base website:

<http://www.i-base.info>

by sending an email to:

subscriptions@i-base.org.uk

or by fax or post using the form on the back page.

Editor in Chief: Paul Blanchard

Editor: Simon Collins

Commissioning Editor: Polly Clayden

Medical Consultants:

Dr Sanjay Bhagani, Royal Free Hospital, London.

Dr Karen Beckerman, Bellevue Hospital, New York.

Dr Gareth Hardy, Royal Free Hospital, London.

Dr Saye Khoo, University of Liverpool Hospital.

Prof. Clive Loveday, International Laboratory Virology Centre.

Dr Graeme Moyle, Chelsea & Westminster Hosp, London.

Dr Stefan Mauss, Düsseldorf.

Dr Graham P Taylor, Imperial College, London.

Dr Stephen Taylor, Birmingham Heartlands Hospital.

Dr Gareth Tudor-Williams, Imperial College, London.

HTB is a not-for-profit community publication that aims to provide a review of the most important medical advances related to clinical management of HIV and its related conditions as well as access to treatments. Comments to articles are compiled from consultant, author and editorial responses.

Some articles are reproduced from other respected sources and copyright for these articles remains with the original authors and sources, as indicated at the end of each article.

We thank those organisations for recognising the importance of providing widely distributed free access to information both to people living with HIV and to the healthcare professionals involved in their care. We also thank them for permission to distribute their excellent work and we encourage HTB readers to visit the source websites for further access to their coverage of HIV treatment.

Articles written and credited to i-Base writers, as with all i-Base originated material, remains the copyright of HIV i-Base, but these articles may be reproduced by community and not-for-profit organisations without individual written permission and reproduction is encouraged. A credit and link to the original author, the HTB issue and the i-Base website is always appreciated.

HIV i-Base receives unconditional educational grants from Charitable Trusts, individual donors and pharmaceutical companies. All editorial policies are strictly independent of funding sources.

HIV i-Base
Third Floor East
Thrale House
44-46 Southwark Street
London SE1 1UN
T: +44 (0) 20 7407 8488
F: +44 (0) 20 7407 8489

<http://www.i-base.info>

HIV i-Base is a registered charity no 1081905
and company reg no 3962064.

HTB is also known as DrFax



HIV i-Base

All publications are free, including bulk orders, because any charge would limit access to this information to some of the people who most need it.

However, any donation that your organisation can make towards our costs is greatly appreciated.

STANDING ORDER DONATION

THANK YOU FOR YOUR SUPPORT

Title: _____ First Name _____ Surname _____

Address _____

_____ Postcode _____

Email _____ @ _____

Telephone (s) _____

Please pay HIV i-Base £ _____ each month until further notice

Please debit my account number _____

Name of account (holder) _____ Bank sort code ____/____/____

Starting on ____/____/____ (DD/MM/YY)

Signature _____ Date ____/____/____ (DD/MM/YY)

To: Manager: (Bank name, branch and address)

Please complete the above and return to: HIV i-Base, 44-46 Southwark Street, London SE1 1UN

(Our bank details for donations: NatWest, Kings Cross Branch, 266 Pentonville Road, London N1 9NA, Sort Code 60-12-14. Account Number: 28007042)

ONE-OFF DONATION

I do not wish to make a regular donation but enclose a one-off cheque in the sum of _____ instead.

GIVE AS YOU EARN

If your employer operates a Give-As-You-Earn scheme please consider giving to i-Base under this scheme. Our Give-As-You-Earn registration number is **000455013**. Our Charity registration number is 1081905

Since many employers match their employees donations a donation through Give-As-You-Earn could double your contribution. For more information on Give-As-You-Earn visit www.giveasyouearn.org

REFUNDS FROM THE TAX MAN

From April 2005 the Inland Revenue is operating a system whereby you can request that any refunds from them should be paid to a charity of your choice from the list on their website. If you feel like giving up that tax refund we are part of this scheme and you will find us on the Inland Revenue list with the code: **JAM40VG** (We rather like this code!) Any amount is extremely helpful.

Whichever of the above schemes you might chose to donate to i-Base we would like to thank you very much for your support.

HIV i-Base

Third Floor East, Thrale House, 44-46 Southwark Street, London SE1 1UN
T: +44 (0) 20 7407 8488 F: +44 (0) 20 7407 8489



Subscription Fax-Back Form

Please use this form to amend subscription details for HIV Treatment Bulletin (DrFax) and to order single or bulk copies of other publications. *All publications are available free, but if you would like to make a donation please use the form on the inside back page.* i-Base currently receives no health authority or statutory funding.

Name: _____ Position: _____

Organisation: _____

Address: _____

Tel: _____ Fax _____

E-mail: _____

I would like to make a donation to i-Base - **Please see inside back page**

HIV Treatment Bulletin (HTB) by Email (PDF format) by Post

HIV Treatment 'Passports' - Booklets for patients to record their own medical history

1 5 10 25 50 100 Other _____

Guide To HIV, Pregnancy and Women's Health (Spring 2005)

1 5 10 25 50 100 Other _____

Introduction to Combination Therapy (June 2005)

1 5 10 25 50 100 Other _____

Earlier versions available in FRENCH, ITALIAN, SPANISH, PORTUGUESE, CHINESE, and GREEK as pdf files on the i-Base website

Changing Treatment - Guide to Second-line and Salvage Therapy (April 2005)

1 5 10 25 50 100 Other _____

Guide To Avoiding and Managing Side Effects (February 2005)

1 5 10 25 50 100 Other _____

Earlier versions available in SPANISH as a print version and in FRENCH, SPANISH, ITALIAN, CHINESE as pdf files on the i-Base website

Paediatric HIV Care - March 2001 - Report from i-Base Paediatric Meeting

This 44-page comprehensive report is now only available in pdf format and on the i-Base website.

Adherence planners and side effect diary sheets - In pads of 50 sheets for adherence support

1 Sheet 1 pad 5 pads 10 pads Other _____

Please fax this form back or email a request to HIV i-Base:

020 7407 8489 (fax) subscriptions@i-Base.org.uk

Office use: