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- Marc GIRARD
- Luc PERRIN
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So... we decided to publish the magazine again this year! Here is a resume of the 3-day ISHEID symposium which took place in Toulon from June 21-23, 2006. Of course, the articles cannot replace active participation in the symposium or take every aspect of it into account, but they are a valuable complement to the book of abstracts, and offer an unbiased vision of the debates that took place. The members of the "Sequences & Strategies" editorial committee are all independent practitioners who have been involved in HIV management for years; here you will find their personal perception of the various issues addressed.

We are delighted to note that the magazine is receiving increasing support from our pharmaceutical partners, who are as strongly attached to the notion of free and high-quality information as we are. Many thanks to them!

The ISHEID symposium has become a regular date in the diaries of practitioners dealing with HIV infection and viral hepatitis, who are also interested in learning more about the infectious challenges of tomorrow. In today’s 'jungle' of meetings on these issues, ISHEID continues to stand out thanks to its original and attractive design. Its human-scale framework means participants can meet face to face with international 'stars' come to report on the state of the art in their specialist fields, or present their own work for the first time. Our symposium also aims to break down barriers and renew dialogue between researchers and practitioners, biologists and clinicians, and North and South… In keeping with this vision, we have already chosen the motto for the 2008 edition, which will be entitled "Challenges Without Borders"!

Alain Lafuillade
HIV virology data presented at the 14th edition of ISHEID were distributed during several oral and poster presentations and symposia.

**Luc Perrin** (Geneva, Switzerland) first talked on the main stakes of virology research in coming years. One of our priorities is to evaluate treatment efficacy in developing countries, where 3 million people are treated without having access to plasma viral load tests. Therapeutic follow-up is currently based on clinical evolution and CD4 measurement. The percentage of nonresponders is estimated at 25-30%. In addition, 30% of women of child-bearing age are likely to transmit resistant viruses to their babies, whose actual virologic status can only be assessed after 15 to 18 months.

We will therefore have to develop new, simpler and less expensive tests, and determine the status of children born from HIV-positive mothers at an early stage.

Several virologic issues must be resolved:

- Evaluating the size of the reservoir of infected cells, which appears to form from the first days of infection, is of prime importance. To what extent can the quantity of infected cells predict the speed of disease progression? We should also evaluate the capacity of the latest molecules to reduce the cellular reservoir. Our eventual hope is to obtain a treatment capable of minimizing reservoir size, and obtain a clinical cure comparable to that achieved for HCV infection.

- Another problem relates to the evolution of strain virulence over time: are recombinant viruses more or less pathogenic? Why does subtype C tend to supplant all the other subtypes?

Do treatments and the acquisition of resistance mutations make the viruses less pathogenic?

- We should also aim to better identify replication mechanisms in order to develop new drugs.

- Another question: are resistant mutants a real threat?

- Lastly, we need to better evaluate the consequences of co-infections.

Another research path is the relationship between genetics and infection: approximately 1% of Caucasian subjects have an allelic mutation (deletion of 32 base pairs) of CCR5, which, in its homozygote form, causes the synthesis of a non-functional protein. In this case, cells cannot be infected by a virus using CCR5.

- Molecules inhibiting binding to CCR5, or the function of CCR5, are currently being developed. We can also attempt to achieve blocking or molecular inhibition of the gene itself (siRNA, blocking on chromosome). The development of DNA chips enabling the detection of multiple genetic polymorphisms (associated with the risk of progression, resistance to infection, metabolism, etc.) could be envisaged, although millions of points would have to be analyzed!

- Preventive and therapeutic vaccine development is continuing, but despite major efforts and substantial means, the results are disappointing. We will have to coordinate efforts on an international level.

The prerequisites for vaccine development are as follows:

1) Identify protection mechanisms;

2) Induce broad spectrum neutralizing antibodies;

3) Induce active T cells at a systemic level (in particular in mucous membranes);

4) Control viral diversity (quasi-species);

5) Protect from cross infections;

6) 1)Take the genetic environment into account.

22 different candidate vaccines are currently undergoing clinical trials (Table 1).

**Anti-HIV-1 Vaccines in trials in 2005**

<table>
<thead>
<tr>
<th>22 candidates</th>
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<tr>
<td>- 7 DNA vaccines: naked, multiclade, adjuvanted, etc.</td>
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<tr>
<td>- 8 viral vectors: Adeno, AAV, VEE, MVA, Fowlpox, Canarypox, Vaccinia, NYVAC</td>
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<tr>
<td>- 3 subunits or peptides: V1-V2 deleted envelopes, lipopeptides, adjuvanted protein</td>
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<tr>
<td>- 4 prime-boost combinations:</td>
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<tr>
<td>● DNA = Viral Vectors (MVA, Fowlpox, Adeno)</td>
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<tr>
<td>● Viral Vector + heterologous viral vector</td>
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<tr>
<td>● Viral vector + lipopeptides</td>
</tr>
<tr>
<td>● DNA + protein</td>
</tr>
<tr>
<td>- 1 phase III trial in Thailand of canarypox-gp120</td>
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Mark Wainberg (Montreal, Canada) reported that among 22 patients from Botswana infected by subtype C and treated using d4T+ddI+NVP, HIV-1 subtype C viruses rapidly select (after 12 weeks) a K65R resistance mutation to TDF (12 out of 22 patients). This is not the case for other HIV-1 subtypes (B,CRF-02) or HIV-2 subtypes after 28 to 33 weeks of culture (Figure 1).

In fact, the variation of a single nucleotide is enough to provoke a subtype C transition (AAG-->AGG), causing a 6.5 to 10-fold increase in resistance to TDF, and a 5 to 25-fold increase in resistance to ABC, 3TC and ddI. This observation has practical consequences on therapeutic or preventive strategies incorporating TDF for patients infected with subtype C HIV-1 virus.

François Simon and J.-C. Plantier [Poster PP1-15] (Rouen, France) have been experimenting a genotyping technique using filter paper used in Tunisia and sent by standard mail to France in a sachet containing a dessicator. The time lapse between sampling and sending to France was 5 to 10 days. 35 Tunisian patients infected with HIV-1 with plasma viral loads of 2.2 log to > 5.9 log were studied: The protease gene was amplified in 77% of cases, and the reverse transcriptase (RT) gene in 71% of cases. When viral load was above 4 log, the protease gene was amplified in 100% of cases, and the RT gene in 92% of cases. Phylogeny indicated that all viruses were subtype B. The sequences showed mutation profiles compatible with patients' therapeutic history. As a viral load above 5,000 copies is currently necessary, the team is working on improving the sensitivity of this technique.

Philip Lawrence [Poster PP2.1] (Saint-Etienne, France) presented an interesting study on subtype CRF-02 protease and RT gene polymorphisms in treatment-naive infected subjects in Saint-Etienne (France) versus subtype B: CRF-02 virus sequences showed far more polymorphisms on both genes than subtype B sequences. However, the analysis of virologic and CD4 response after treatment initiation in 22 patients infected with CRF-02 and 45 patients infected with a subtype B was not significantly different 6 months after the start of treatment.
The Virco laboratories symposium focused entirely on virology.

Vincente Soriano (Madrid, Spain) gave a general presentation on the importance of resistance tests for the follow-up of infected patients, the continual propagation of multi-resistant viruses and the difficulties encountered in selecting a relay treatment. According to various European and American studies, primary resistance mutation prevalence is 10% overall. Resistance to nonnucleosidic inhibitors (NNRTIs) and the prevalence of non-B subtypes are on a constant upward trend in subjects infected in Spain between 1997 and 2005 (de Mendoza et al. CID, 2005). Between 2000 and 2005, the same study reported 15% primary resistance to nucleosidic inhibitors (NRTIs), 10% resistance to NNRTIs and 5% multidrug-resistant (MDR) strains.

First line therapeutic strategies using 3 NRTIs are mainly selected because they facilitate adherence, but these triple therapies are less powerful than solutions associating 2 NRTIs +1 PI or NNRTI.

Vicente Soriano then recapped resistance mechanisms to NRTIs: reduction in NRTI incorporation in viral DNA during synthesis due to K65R, L74V and M184V mutations, or excision of the inhibitor mediated by ATP due to Thymidine Analog Mutations (TAMs). Certain mutations have a decisive impact on the choice of relay treatments:

a) TAMs: selected by d4T and AZT, whose accumulation leads to wide cross resistance, without affecting replicative capacity.

b) M184V: selected by 3TC and ABC. Does not incur significant cross resistance and has highly invalidating impact on virus replicative capacity.

c) K65R mutation: selected by TDF, ABC, ddI and ddC. This mutation influences NRTI sensitivity in various ways and, similarly to the M184V mutation, it decreases virus replicative capacity. The occurrence of this mutation increased from <0.5% in 1999 to >11% in 2004 in the Madrid cohort (Valer et al, AIDS, 2004).

d) NNRTI resistance: incurs wide cross resistance (mediated by K103N, V108I and Y181C); the genetic barrier to resistance to these compounds is low.

e) Resistance to ritonavir-boosted PIs only occurs when numerous resistances accumulate, as this optimized solution provides longer exposure to the molecule, a longer drug half-life and a higher genetic barrier to resistance.

Soriano’s team demonstrated that the I47A mutation incurs a high level of resistance to LPV/r, cross-resistance to APV and hypersensitivity to SQV (De Mendoza et al., AIDS, 2006).

The interpretation of genotypic resistance to PIs is mainly based on mutation scores: multiple scores have already been established for LPV/r (Kempf et al, J Virol, 2001 ; Isaacson et al., 9th CROI 2002 ; Parkin et al., AIDS, 2003 ; Wong et al., J Infect Dis, 2003): Certain common mutations are present in all LPV/r resistance scores: L10F/I/V, K20M/R, I50V and V82A/F/T/S. These PI/r resistance scores are presented in Table 2.

<table>
<thead>
<tr>
<th>Qualitative</th>
<th>Quantitative</th>
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<tr>
<td>Primary Mutations</td>
<td>Mutations in the Score</td>
</tr>
<tr>
<td>Pox-APV</td>
<td>150W</td>
</tr>
<tr>
<td>ATV</td>
<td>150L</td>
</tr>
<tr>
<td>LPV</td>
<td>147A</td>
</tr>
<tr>
<td>TMC-114</td>
<td>?</td>
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Table 2: resistance scores for ritonavir-boosted PIs

Efavirenz is undoubtedly the most powerful NNRTI compound.
These different resistance scores allow us to predict response (defined by a viral load <50 copies or a reduction of 1 log in viral load after 24 weeks of treatment). They do however have limits, related to:

- The limited size of patient groups;
- An absence of cross-validation in the various databases;
- The presence of mutations on different genomes, which may limit the effects of interactions between mutations (antagonism, re-sensitization, etc.).

The genotype also has its own limits, related to:

- The presence of minority mutants;
- The selection of new RT or protease mutations;
- The selection of mutations in other areas of the genome (RNase H for NRTIs; Gag and Gag cleavage sites for PIs).

Jürgen Rockstroh (Bonn, Germany) gave a talk on therapeutic management based on clinical cutoff threshold interpretation. The Vircotype® provides a quantitative evaluation of resistance using a very large database of viral genotypes, corresponding to phenotypes expressed in terms of sensitivity reductions (IC50): these reductions are translated into clinical cut-offs based on virologic response in various clinical trials (contrary to biological cutoff, which is based on the distribution of sensitivity thresholds in clinical isolates from naive subjects). Numerous conclusive clinical cases (from the "T20 cohort") were presented.

A low clinical cutoff is associated with a 20% drop in sensitivity versus virologic response observed in a wild reference.

A high clinical cutoff is associated with an 80% drop in sensitivity versus virologic response observed in a wild reference.

Laurence Bocket (Lille-Tourcoing, France) presented a retrospective analysis conducted at the Lille-Tourcoing General Hospital on 66 multiple-treated patients in therapeutic failure. The relay treatment was selected according to genotype results, interpreted using the ANRS v13 algorithm.

Virologic response was estimated 12 weeks after treatment change. Virtual phenotyping was performed retrospectively. Genotype and virtual phenotype corresponded to the predicted NNRTI response in 100% of cases and 23% of cases for predicted NRTI response, with 32% minor discordances (sensitive virus versus intermediate resistance, or intermediate resistance versus resistance) and 45% major discordances (sensitive virus versus resistance). Correspondence with virologic response to PIs was 30%, with 38% minor discordances and 32% major discordances.

Globally, major discordances for PIs and NRTIs were observed in 18% of patients.

Jorge Villacian (Mechelen, Belgium) presented data on virtual phenotype and clinical cutoffs: the HIV-1 Vircotype® combines data on the genotype, the phenotype predicted on the basis of the genotype, and a clinical cutoff value to optimize therapeutic choice prediction. Phenotype is predicted from genotype using a large databank of genotypes and phenotypes determined on the basis of clinical isolates and biocomputing techniques. The TM-LM Virtual Phenotype uses multiple linear regression models (one for each molecule) capable of predicting the phenotype of a sample. Linear regression models are derived from a data bank containing the genotypes (all mutations), and phenotypes (expressed in measured "Fold Changes") of 6,143 to 41,958 clinical isolates. This enables the analysis of individual mutations, mutation associations, their synergistic or antagonistic interactions and their phenotypic consequences. Contrary to biological cutoffs, clinical cutoffs provide a more efficient prediction of ritonavir-boosted PI resistance.
Catherine Tamalet: You have defined a number of priorities for virology research. Can you recap them for us?

Luc Perrin: First and foremost, I would like to talk about the actual definition of a "priority": this is a crucial research issue and as such should be tackled collectively.

For example, I consider viremia measurement in developing countries to be a priority issue, because these countries don't have our measurement techniques. As a result, cross-resistance to second line drugs is even more frequent. The problem is further exacerbated in women of child-bearing age, as resistant viruses can be transmitted to the baby. We must not allow resistance to accumulate. Just as a reminder, the WHO recommends 2 NRTIs + Nevirapine (due to its low cost) for the first line of treatment.

We will therefore need to set up new, inexpensive tests, focus on technical staff training, and provide efficient team follow-up.

Regarding limits of detection, we will probably have to be less demanding than in Northern countries: a reliable viral load test at 200 copies should be adequate.

C. T.: You have insisted on the importance of measuring the quantity of infected cells...

L. P.: One of our priorities is measuring the size of the viral reservoir: Christine Rouzioux's research in this field has taken us a big step forwards. What we need to know is whether the speed of disease progression is related to the formation of a more or less abundant reservoir of infected cells from the outset of infection.

With the emergence of today's new molecules, we will need to assess whether a highly diminished reservoir goes hand in hand with clinical cure. We must ensure that the reservoir measured in peripheral blood genuinely reflects the reservoir in lymphoid tissue and the digestive tract.

C. T.: In your opinion, should we measure all types of DNA (integrated and nonintegrated)?

L. P.: The question of integrated or nonintegrated DNA measurement has sparked a quarrel between the experts, and its clinical interest is unproved. In fact, we mainly measure integrated DNA, as is the case with the ANRS technique. The importance of this measurement has been confirmed with the arrival of integrase inhibitors, which block viral integration in the genome of the host cell. Patients who have been treated for over 15 years have highly-diminished reservoirs; the question is, can the immune system control this extremely low viral production alone at this stage? We must also check if the reservoir forms again after 5 years of effective treatment.

C. T.: Do you think we should also measure infected cells in other reservoirs such as sperm, cervicovaginal secretions or CSF?

L. P.: Measurement of these reservoirs is not easy; in my view, it is more interesting to focus on the distribution of various molecules in these compartments.

C. T.: You were the first scientist to demonstrate the harmful impact of cross infection. Do you think cross infections are frequent?

L. P.: First of all, we must stress that "cross infection" is a rare phenomenon: cross-infected subjects are defined as people who develop a subtype B infection for over 2 years, then are infected again with a subtype B or other subtype virus (there is a significant time lapse between infection by the primary virus and the secondary virus). For example, in Switzerland we detected a drug addict infected with a slow-progressing subtype B; this subject was then cross-infected with CRF-11, which accelerated disease progression (Yerly et al., AIDS, 2004).

Co-infection is different from cross infection: it is defined as the simultaneous coexistence of 2 viruses in subjects...
infected for less than one year, and is mainly due to multiple sexual contacts: this explains the emergence of recombinant viruses in Africa where 5% of primary infected subjects show co-infections. In principle, there are few co-infections in Northern countries as HIV infection is less prevalent; this phenomenon is more related to risk behaviour.

C. T.: Can you specify the consequences of co-infections and cross infections?

L. P.: They are numerous:
1) As we already said: faster disease progression;
2) A vaccine manufactured using subtypes B will not protect against reinfection with a non-subtype B (this situation is comparable to HCV infection, where reinfection with another genotype may occur);
3) Some cross infection with the same subtype have been observed with subtype B, but they are only detectable using highly sensitive techniques and are generally transient.

C. T.: What do think about the increase in the prevalence of subtypes C, which tend to supplant other subtypes?

L. P.: Evolution is undoubtedly different in South Africa and Ethiopia, and could be related to a more efficient transcription factor, and better viral fitness. In Western Africa, subtype C could theoretically soon replace CRF-02.

As to the clinical effects of recombinants on pathogenicity and treatment sensitivity, none of the data published so far are conclusive, and a lot of work remains to be done.

C. T.: How should we handle multiple-treated patients infected with MDR viruses who have a low viral load (1,000-1,500 copies)?

L. P.: In Western cohorts, this case represents 5 to 6% of patients, 1 to 2% of whom have low viremia. No real evaluation exists with regards to possible strategies. But if we have at least 2 active drugs available (to which the virus has never been exposed and to which it does not have intermediate sensitivity), I would recommend intensification. If this is not the case, I think it is advisable to continue the same treatment.

C. T.: Do you think the question of minority resistant viruses is a priority issue?

L. P.: No, because we already know they exist. Douglas Richman was the first to highlight the presence of minority NVP-resistant mutants in subjects previously unexposed to NVP. Perhaps it would be useful to quantify them – in particular in Africa where few relay treatments are available – prior to selecting a first or second line of treatment. But it is not a research priority.
Marc Girard is an honorary Professor of virology at Paris 7 University, a former Professor and Head of Department at the Pasteur Institute, and honorary Chairman of the Mérieux Foundation in Lyon. He is also a consultant for the WHO. His involvement in anti-HIV vaccine research dates back to the outbreak of the epidemic.

You have been a witness and party to anti-HIV vaccine research for the 15 last years. Can you tell us the main reasons why a preventive vaccine is still unavailable?

Marc Girard: AIDS is a persistent chronic viral disease. New vaccine concepts and new types of vaccine are needed to attempt to impede it via adapted immune response. But the virus shelters from the immune system by investing the memory T cell lymphocyte chromosomes in the form of silent "provirus": this creates an unshiftable viral reservoir in the body which can rekindle itself spontaneously at any time, as we can see when antiviral chemotherapy is stopped. The vaccine must be powerful enough to prevent this reservoir forming at all costs.

The virus is also characterized by its enormous potential genetic variability, which has led to the emergence of a genuine constellation of sub-types, variants, recombinants and strains; it is highly unlikely that these could all be covered by just one vaccine. No one knows how many vaccines could actually be needed.

Lastly, we are particularly disarmed by the fact that most viral strains seem to be virtually insensitive to neutralization by antibodies. We don't know what type of vaccine could induce antibodies capable of neutralizing these strains efficiently, even though we know this type of antibody exists.

A. L.: Have all these years of research been in vain? Are we back to square one?

M. G.: Although we have suffered a lot of failures, they have been fairly useful in that they have taught us what not to do. We now understand the physiopathology of HIV infection much better and have considerably improved the vaccinating capability of the candidate vaccines under development. Potential vaccines are currently being studied on human subjects and the results are expected in 2008-2009.

A. L.: If we adopted less ambitious goals for vaccination, i.e. protection against disease progression in HIV-infected subjects rather than protection against infection, aren't we likely to create immunologic pressure leading to eventual pandemic viruses which are even more resistant to human defences?

M. G.: This risk is totally theoretical and probably non-existent, because viral mutants that manage to "escape" an individual's immune system are systematically less aggressive and develop slower than the original virus; in fact, in the event of transmission to a 2nd individual, these mutants are rapidly supplanted by wild virus clones which are still present in the viral population, or which reappear spontaneously due to mutation interplay.

A. L.: What vaccine trials are currently in progress and what can we expect of them?

M. G.: The trials are:

1. A Phase III study on 16,000 volunteers in Thailand, combining a first vaccination of ALVAC-HIV (Sanofi-Pasteur) and gp120 (Vaxgen) boosters;
2. A Phase IIb study on several thousand volunteers in the US and South America using the ADV-5 adenovirus vaccine (Merck);
3. Over 20 Phase I or Phase I/II studies on various candidate vaccines using viral vectors (attenuated MVA strain of vaccinia virus, virus combined with adenovirus (AAV), adenovirus other than serotype 5, avian variola virus (FPV), vaccine strain of measles virus, Venezuelan equine encephalitis virus, etc.) or bacterial vectors (in particular BCG);
4. Several Phase I/II studies on plasma DNA-based vaccines, alone or combined with the above-mentioned vectorised vaccines, some of which are associated with various additives such as interleukins.

As you can see, many paths are currently being explored or remain to be explored!
This original session focused on patient care.

Denis Lacoste (Bordeaux, France) began by reflecting on the role of health carers and their need to adapt to the political and social environment. He recapped the evolution of doctors' attitudes to the disease: their helplessness at the outset of the epidemic in the face of a total absence of medical solutions, and the instrumental role of health carers in accompanying patients. Today, in the light of the many lessons we have learned, doctors form part of a medical team and have acquired multidisciplinary skills. Their role now includes prevention and therapy-oriented education.

Whatever the patient's profile, his hospital and home care program should ideally incorporate a blend of medical and family care. Although antiretroviral treatments have led to a decrease in AIDS-related events since 1995, therapeutic progress has also had some perverse effects, with less media coverage of the disease, trivialization of the phenomenon and a general feeling that the battle is lost in terms of mobilization and prevention of HIV infection (figure 1).

Figure 1: decrease in the number of cases of AIDS-related deaths with the arrival of effective therapies in 1996.

Discrimination, racial and penalization phenomena associated with the disease also emerged. In addition, to further trouble minds and handicap progress, the new medical policy-makers appeared to want to belittle patients, considering them solely in terms of cost.

How much human and financial investment should be granted to meet the demands of Southern countries and immigrants from them? We should not let our Western conceit get in the way of helping the Southern hemisphere.

Caring for HIV patients requires an increasingly sophisticated and specific approach; the edifice we have built remains a fragile house of cards.

Christian Saout (Paris, France), President of the association "AIDES", reviewed the current and future role of the associative environment. He reminded us that all French associations have their own specific commitments, and are governed by the French non-profit law of 1901. "AIDES" has adopted a militant line, although it remains largely dependent on the French State and public health policies. In Christian Saout's view, a militant approach is still required on three essential points: access to antiretroviral treatments, contamination prevention and discrimination against HIV-positive individuals.

In the South, two crucial milestones have impacted patient care in underprivileged countries: the creation of an international fund to fight AIDS, tuberculosis and malaria plus, more recently, easier access to drugs. However, no battles have been won yet, and today's main concern is the construction or modernization of health systems in Southern countries, with entirely free access to antiretroviral treatments. Local activists play a vital role in achieving this objective. Thanks to their extensive practical experience they are ideally positioned to pinpoint priorities and health care choices.

Here in the North, we are faced with the challenge of therapeutic education. In France – where the public health system is supposedly continuing to progress, the association "AIDES" organizes a variety of initiatives such as "treated patient universities and therapeutic action weekends", intended to facilitate exchanges between carers and patients. Partnerships have already been set up with the research community via the ANRS, and will soon be formed with France's official care organization bodies via regional commissions for the fight against AIDS/HIV (COREVIH). But these partnerships must now be extended to the international care community and associative action must be upgraded according to patient needs. "AIDES" has published a booklet on assistance to handicapped adults and, in collaboration with the association TRT5, information on long-term disabilities.
Regarding transmission prevention, we have been managing for over 20 years with a little piece of rubber... The militant's role is to encourage prevention, and provide education and sustained preventive action in the long term. Associations are capable of relaying current and future international initiatives such as the development of vaccines and microbicides. Their ability to deal with ethical issues, fund collecting and initiate collective action means they play a vital pivotal role.

Accepting HIV-positive people implies changing our representations and mentalities. Three years ago, "AIDES" launched a survey which, at the time of the last analysis in 2005, highlighted widespread local discrimination and the distress of sero-divergent couples. Worryingly, it also pinpointed the discrimination suffered by carers and already-vulnerable categories such as drug addicts, foreigners and women. An investigation initiated by "SIDA-info-droit" discovered specifically discriminating attitudes towards homosexuals. Only associations are capable of operating this "alert" network; they are also essential in encouraging dialogue and tackling major issues such as, in France, that of life insurance, disability allowances or access to financial loans. But their victories remain fragile and must be preserved through democracy and a militant approach.

France Lert (Saint Maurice, France) reminded us of the contribution of social science researchers to our approach to HIV-positive patients during the HAART era, and their synergistic role with regards to various players, associations, and clinical and fundamental researchers.

The social sciences were mobilized rapidly after the emergence of AIDS, in an attempt to facilitate understanding of social attitudes, standards and practices, slow down disease progression and expansion, facilitate access to care, and fight exacerbated discrimination against minority populations such as homosexuals and drug addicts. Prior to the HAART era, priority issues were diagnosis and the announcement of HIV-positivity to the patient's family; major research was conducted into death management, which considerably improved our palliative care system. The attitude of carers vis-à-vis to this particular patient population was studied to help overcome the various difficulties encountered in the relevant hospital departments, although this educational action was not unfortunately extended to other departments attended by HIV patients.

With the advent of HAART, HIV infection became a chronic disease in industrialized countries, prompting new stakes and research questions, i.e. the problem of late diagnosis, adherence to antiretroviral treatments, holding down a job, marital and parental projects, and the prevention of partner contamination.

France Lert presented the results of the VESPA study instigated by the ANRS, conducted on 3330 urban patients in the Caribbean and French Guyana. This study was designed to investigate the social aspects of chronic HIV disease, the impact of social aspects on the disease and its treatment, and its repercussions on social life and life projects.

In France, the late diagnosis of HIV infection is frequent – around 33% of cases. This population mainly consists of ageing French heterosexuals who have been living in a couple for several years, rather than people with multiple partners and a minimal income. These observations challenge France's testing and prevention campaigns, which mainly target risk populations; in fact, all sexually active people, whatever their risk situation, should be encouraged to take regular tests. The late diagnosis of heterosexual immigrants during the year following their arrival in France is common and single people are less well diagnosed.

In the Caribbean, HIV-positivity is far less frequently announced to the family than elsewhere, especially among men, due to strong homophobia and entrenched notions of virility. Haitians are in a highly unfavorable situation, and suffer from both racial discrimination and HIV discrimination.

The study also reveals that in the general population, people with the least education are the most intolerant,
and, lastly, that older people are hostages of their family role, hence impeding diagnosis and announcement to the family.

In France, just 3% of HIV-positive patients fail to inform their partners, one-third of whom do not protect themselves.

Prior to HAART, a portion of HIV-infected people did not manage to hold down a job, but divergences versus the general population diminished after 1994, and we even observed an absence of negative impact on people with a high level of education. The desire to have children is expressed by 20% of men and 33% of women, and is related to the partner's HIV status. The desire is stronger among young populations, Africans and North Africans and stable couples.

This quantitative study draws up a social picture of HIV-infection in French territories. It opens the way to effective management based on realities, and must be supplemented with qualitative research in order to tackle psychosocial aspects and the additional input they will bring.

Stéphanie Mulot (Toulouse, France) reported the difficulties in caring for Haitian immigrants in the French West Indies revealed by the "CORES" study. These islands benefit from post colonialisit French assimilation, and their societies still function according to a socio-racial hierarchy, with color ideology and a xenophobic attitude to foreigners and French metropolitan citizens. The rate of unemployment is 35%, and the French care system provides free testing, care and treatment. According to French law, foreigners are granted residency to obtain treatment and welfare if anti-retrovirals are not accessible in their own country. The epidemic is largely characterized by heterosexual transmission, a high percentage of poor patients (70%), foreign patients of mainly Haitian origin (sometimes > 50%), advanced age (one-third > 50 years), delayed diagnosis, little associative action and high social stigmatization of the disease.
Disease management is characterized by two approaches, often affecting access to and quality of care. The first stigmatizing approach is based on cultural differences. Most HIV-positive individuals are clandestine Haitians, emigrating for economic survival. In the Caribbean, they are held responsible for the AIDS epidemic, feared for their Voodoo culture and generally held responsible for all social evils. Due to their low level of education, these patients mostly accept the incomprehension and lack of care they receive. They are made to feel guilty, perceived as dangerous, scorned and threatened. This approach can lead to refused residency and access to drugs, increased precarity and social exclusion. Eviction from the country may compromise future access to care, hence endangering the patient and his family. The second, caring approach is illustrated by the case of a 30 year-old clandestine Haitian woman who was found to be HIV-positive when she contracted cerebral toxoplasmosis. Efforts were undertaken to instigate confidence, announce the disease to the family at the appropriate time, reduce guilt and provide practical information. Administrative and social integration procedures were undertaken and the patient achieved autonomous treatment management followed by rapid therapeutic success. She then returned regularly to Haiti, and will not generate any future problems.

The failure factors revealed by the "CORES" study are ultimately universal, and revolve around racism, culturalism, social exclusion, and legal and therapeutic laxity. The future of care for these migrating patients in a situation of poverty should be cause for concern if we relate it to the current evolution of our health system, and the re-emergence of stigmatizing ideologies.
Undetected: discussion around clinical cases

This interactive session, organized under the aegis of the Roche Laboratories with the participation of Doctor Isabelle Poizot-Martin (Marseille, France) and Jean-Claude Tardy (Lyon, France) was a resounding success. It aimed to encourage a practical discussion based on actual clinical cases, to confront the opinions of participants.

Prior to the presentation of clinical cases, Jean-Claude Tardy recapped some essential criteria for the follow-up of HIV-infected patients.

Plasma viral load is an essential marker for prognosis and efficient therapeutic follow-up. We should systematically continue to refer to the "Mellor curves" established 10 years ago (figure 1).

Isabelle Poizot-Martin presented the first clinical case, entitled "Virologic Success?" This case related to a 66 year-old man who tested HIV-positive in April 1996 during a pre-op medical, and who was living between Hong Kong and France. He was successively treated with AZT, then 2 NRTIs + 1 PI, then with a triple combination of NRTIs (side effect: lipodystrophy).

The patient's evolution was "classic", with two mirrored curves: a decrease in viremia and an increase in CD4 cells (figure 2).

Although CD4 progression is clearly visible, total lymphopenia also remained significant.

Evolution was therefore satisfactory, but optimism was moderated when proviral DNA dosing revealed 118 copies/million PBMC (Peripheral Blood Mononuclear Cells) (threshold = 10 copies), indicating a certain quantity of persistent intracellular nonreplicative HIV.
Lessons learned from this case were:

- The necessity of maintaining therapeutic pressure;
- The instrumental role of initial viral decrease kinetics in obtaining lasting undetectability;
- The importance of CD4 proliferation kinetics;
- The difficulties associated with CD8 level interpretation.

Jean-Claude Tardy referred to two publications corroborating the lessons learned:

- Polis MA et al. (Lancet 2001; 358: 1760-5): a close relationship exists between the speed of viral kinetics and long-term response, and in particular between the drop in viral load obtained at month 6 and clinical evolution.
- Bartlett JA et al. (JAIDS 2006; 41: 323-31): we can minimize the consequences of virologic failure after an initial triple combination by opting for a combination of + boosted PI + 1 NNRTI rather than a triple NRTI association.

The second clinical case was entitled "Persistent Viremia". It concerned a 60 year-old man, found to be infected with subtype B HIV in 1990, currently on his 13th line of treatment (including 9 lines of triple combinations), with a CD4 nadir of 88 and viral load zenith of 4.8 log (approximately 63,000 copies/ml): figure 3.

In the face of these accumulated mutations, and the difficulty in finding a therapeutic solution, we can legitimately challenge the interest of obtaining an undetectable plasma viral load at all costs given that:

- a satisfactory CD4 level guarantees basic immunity;
- low viral replication, equal to a few thousand copies, undoubtedly implies decreased viral fitness (replicative capacity).

Further to these reflections, most participants opted for a therapeutic "status quo".

However, the envisaged treatment (TDF + FTC + T-20 + TMC 114 or TMC 125) offered the possibility of obtaining an undetectable plasma viral load, which was more satisfactory from a purely virologic viewpoint.

The questions that followed illustrated the practical difficulties involved in implementing this type of combination. For example, an expert from Guadeloupe, where TMC 114 is not yet available, asked whether he should wait for the French "Temporary Authorization for Use", as his center was not taking part in the ongoing protocol.

Various comments followed on the notions of "blips" and "persistent viremia".

Almost all (90%) of patients experience "blips" (Nettles RE et al. JAMA 2005; 293 : 817-29); they last for an average of 3 days, reach an average titer of 79 copies/ml, and do not appear to correlate with demographic factors, concurrent infections, vaccinations, etc. However, they are often associated with poor compliance. They do not incur new resistance selection.

"Persistent viremia", studied in particular by Karlsson AC et al (AIDS 2004; 18: 981-9) has varying consequences (figure 4).
In this study, 24% of patients had intermittent viremia ranging from 50 to 1000 copies/ml and 85% had persistent viremia ranging from 50 to 1000 copies/ml.

**Antiretroviral-treated individuals with persistent low-level viremia exhibit a substantial risk of subsequent treatment failure**

![Diagram depicting differences in the evolution of blips (in green) and persistent viremia (in yellow).](image)

Persistent low-level viremia is far more commonly responsible for therapeutic failure at 36 months. It reduces immune restoration, allows the emergence of new resistances, and eventually exacerbates morbidity and mortality.

Lastly, on the question of whether or not blips should be genotyped, Jean-Claude Tardy said he felt this was feasible and interesting for virologists, but would only be useful for detecting certain mutations.

This lunch debate was extremely lively and fruitful, and could well have lasted longer without our tight schedule!
François Simon, a former Professor at the University of Rouen, then Director of the Pasteur Institute in Dakar, has just been appointed Head of Department at Saint-Louis Hospital in Paris. A specialist in HIV diversity, he pinpointed the existence of HIV-O, discovered HIV-N and has conducted lengthy research on HIV-2 in conjunction with Francoise Brun-Vezinet.

Catherine Tamalet : Can you explain the principle and interest of measuring HIV-1 and HIV-2 DNA?

François Simon : Current treatments are increasingly powerful and make HIV RNA plasma viral load undetectable. It was therefore crucial to create an accessible and measurable marker of therapeutic efficacy. To do this, we assessed whether there was a difference between HIV-1 and HIV-2 proviral loads.

C. T. : Can you specify what you mean by "proviral" load?

F. S. : This term is a bit abusive. After penetrating the cell, RNA is retrotranscribed into DNA, whose vocation is to integrate the cell genome, follow its movements and mitoses, or control the synthesis of new virions. But retrotranscription is not always effective: there are complex forms of DNA: although linearized DNA corresponds to more or less complete genome forms, a majority of circular forms exist simultaneously in the cell. These are either overlapping and superimposed 2-LTR circular forms or 1-LTR forms, with twisted noncoding DNA. It is extremely difficult to quantify these various forms, and the literature shows contradictory results, but we can estimate integrated DNA at around 15 to 20%. Up to now, it was impossible to quantify the various forms of DNA.

C. T. : And has that become possible using your technique?

F. S. : Yes, we can now measure total DNA and 2-LTR circular DNA in vitro.

Initially, we compared HIV-1 and 2 DNA by infecting the cells of 5 HIV-negative donors, to avoid individual inter-donor variability. Very rapidly, from the 6th hour, we observed a major difference between the production of HIV-1 and HIV-2 DNA (around 1.5 log in favour of HIV-1) using standardized inoculums of HIV-1 and HIV-2, followed by HIV-1 and 2 load rebalancing after 2 to 3 days.

C. T. : Have you conducted any studies on patients?

F. S. : We conducted a transverse study on 44 HIV-1 patients and 41 patients from the national HIV-2 cohort. Three groups of patients were defined according to CD4 strata: >500, 300-500, and <300.

The results showed a very significant difference in the quantities of HIV-1 and HIV-2 DNA (difference of 0.7 log) in patients with over 300 CD4. Below 300 CD4, HIV-2 viral load was similar to HIV-1 values. As HIV-2 viral load is always initially lower, we were able to correlate our experimental observations with the natural history of HIV-2 disease.

C. T. : Can you explain this phenomenon?

F. S. : We believe various blocking mechanisms exist, either on the level of entry receptors, or less efficient retrotranscription, or integration. We are currently exploring the theory of decreased intracellular RNA messenger expression in HIV-2 patients.

HIV-2 is probably also subject to constraints related to cellular restriction factors type TRIM or APOBEC 3G (capable of restricting viral replication).

In addition, HIV-2 is not as well adapted to human cells as HIV-1. Genetically-speaking, HIV-2 resembles SIV SM (simian immunodeficiency virus infecting the West African Sooty Mangabey monkey): it multiplies on a cellular system which is genetically far removed from ours, whereas HIV-1 is similar to SIV CPZ (simian immunodeficiency virus infecting the chimpanzee), which replicates on a cellular system largely resembling human cells. So it is not surprising that HIV-2 is less efficient at all levels, whether entry, replication or exit.

In parallel to total DNA quantification we quantified 2-LTR forms; these are the only forms which are easily accessible using current techniques.

During the first hours of culture, we found more 2-LTR forms in cells infected by HIV-1, followed by a progressive accumulation of 2-LTR in cells infected by
HIV-2 in following days (difference of over 1 log in favour of HIV-2).

The in vivo technique is not sensitive enough to detect these 2-LTR forms, but we are currently working on increased sensitization to enable its use in clinical practice.

**C. T. : Do you think this marker will become valuable with the arrival of new molecules such as integrase inhibitors?**

F. S. : Yes, we must imperatively have a pertinent marker. The quantification of DNA 2-LTR forms will be extremely useful for measuring the efficacy of integrase inhibitors.

The quantification of integrated forms is a complex issue: we need to find a trigger that can hybrid on both HIV and the cellular genome; we need to position ourselves on the so-called Alu sequence and place a trigger on the HIV genome. Our biggest problem is that the position of this Alu gene varies a lot, resulting in the amplification of fragments of different lengths. There are undoubtedly alternative possibilities, such as real time PCR read software to semi-quantify integrated DNA while working on the start points of the amplification curves. Another possibility is to isolate genomic cellular DNA in which HIV is integrated by separating it from the other forms while working on the chemical separation of DNA of varying lengths.

**C. T. : Is DNA quantification a genuine prognostic marker of evolution?**

F. S. : I think we first need to improve the sensitivity of this marker. We are currently evaluating DNA amplification using CD4 lymphocytes, as total DNA is very hard to interpret: for example, some patients have low quantities of DNA and CD4 collapse; we must therefore re-express total DNA versus CD4 cells (after separation).

Up to now, DNA quantities have been generally low and no highly significant differences have been found in the quantities of HIV-1 DNA (average 3.1 log) and HIV-2 DNA (average 2.3 log), whereas epidemiologic and clinical differences between the 2 viruses are major. The sensitivity of this marker therefore needs improving: it must be re-expressed according to micrograms of CD4 cells so we can monitor whether DNA variations are significant.
Several sessions took stock of progress over the last year in terms of HIV management, and opened the debate on coming research priorities. Immunologic and therapeutic aspects are reported briefly here; virologic aspects are reported in a different paper.

Marie-Lise Gougeon (Paris, France) defined current research priorities in the field of immunology. Over 20 years after the start of the AIDS pandemic, the candidate vaccines tested in human efficacy trials have proved ineffective for the prevention of HIV infection and the suppression of viral load, although proof of concept has been achieved on nonhuman primate models.

In order to develop an effective vaccine, we must determine the correlates of protection arising during natural infection and after vaccination.

The host is a determining factor in virus pathogenicity: SIVagm, SIVmnd and SIVsm do not induce immuno-deficiency in their natural hosts, respectively the African green monkey, Mandrill and Sooty Mangabey. They do however induce AIDS if they are injected into a Rhesus Macaque. Similarly, HIV-1 is not pathogenic in chimpanzees – the natural host of SIVcpz –, but does induce immunodeficiency in man. The correlates of protection identified in the framework of certain viral infections reveal that the immune responses induced by attenuated live vaccines are similar to those induced by natural infection, including neutralizing antibodies which block the infection of target cells, and cellular immunity which destroys infected targets. This applies to smallpox, measles and chickenpox vaccines. Vaccines prepared using dead viruses or synthetic proteins (against rabies, influenza, and hepatitis A and B) induce neutralizing antibodies and CD4+ T helper (Th) cell responses, but do not induce CD8+ cytotoxic T lymphocyte responses. Lastly, viral vectors with defective replication, or combined with DNA, preferentially stimulate Th and CTL cellular responses rather than neutralizing antibodies. It is now accepted that neutralizing antibodies play a crucial role in preventing chronic infection, whereas cellular responses control chronic infection (CMV, EBV, HCV) and consequently impede the disease.

Which are the correlates of protection in HIV infection?

Passive immunization and depletion experiments conducted on monkeys suggest that a combination of neutralizing antibodies and cellular responses is necessary to offer protection against HIV infection and AIDS. Recent studies on cellular response have shown that the efficacy of antiviral memory T cells depends on both the quality and quantity of the antigen's specific T cells: "functional signatures" have been correlated with levels of viral replication and disease stage. For example, the type of cytokines produced by CD4 T-cells, and their ability to proliferate in response to viral antigens, represents a correlate of the efficacy of virus control (figure 1): infections associated with viral clearance show a predominant production of IL-2 by CD4 T-cells, and the coproduction of IL-2 and IFN-\(\gamma\) by CD8 T-cells. In infections characterized by persistent low viral replication (EBV or CMV infections), or during chronic HIV infection with controlled viral load, CD4 and CD8 T-cell responses are multifunctional: some T cells produce IL-2 only, some produce IL-2 and IFN-\(\gamma\) and, finally, some produce IFN-\(\gamma\) only. In addition, CD4 T-cells remain capable of proliferating in response to viral antigens. Conversely, the persistence of a high viral load in untreated patients, patients subjected to therapeutic interruption, or during the acute phase of the infection, is characterized by the predominance of an IFN-\(\gamma\) response by CD4 and CD8 T-cells, and the loss of CD4 T-cell proliferative capacity. In view of these data, the "immuno-monitoring" of T-cell response induced by HIV candidate vaccines should not be limited to the detection of T cells producing IFN-\(\gamma\); it should also include the detection of IL-2 producing cells. Regarding the role of humoral immunity in the control of HIV infection, experiments conducted years back demonstrated that passive immunization with neutralizing antibodies prevents the establishment of chronic infection in chimpanzees infected with HIV-1. The role of neutralizing antibodies in controlling chronic infection in man is not proven, and their limited effect may be due to the rapid mutations of HIV, which enables it to escape neutralizing antibodies.

If we are to continue efforts to identify correlates of protection and develop a vaccine capable of inducing a protective response, we must find answers to the following questions:
1- How can we induce high levels of neutralizing antibodies, and which immunogene should we choose?

2- How can we prompt a vaccine cellular response capable of suppressing viral load and hence hindering infection progression?

3- What type of CD4 or CD8 T-cell response (mono or polyfunctional) should be induced by the vaccines currently being developed, and how diverse should this response be in order to counterbalance the emergence of mutants?

4- What protection mechanism is conferred by the attenuated SIV vaccine that protects vaccinated monkeys against pathogenic SIV infection?

5- What is the most efficient vaccine strategy for stimulating immune response and offering protection against infection?

Figure 1:

Another correlate of protective immunity during natural infection with HIV or SIV is the absence of immune activation: for unknown reasons, chronic HIV infection prompts general immune system activation, which is a significant factor of infection progression. General immune activation is characterized by: 1- the expression of activation markers by T cells and the accumulation of memory phenotype cells; 2- a high production of proinflammatory cytokines; 3- an accelerated turnover of all lymphocytary populations, including lymphocytes T and B, NK cells and accessory cells. This activation only relates to a small fraction of HIV-specific T cells. It results in viral replication through an accumulation of activated targets, irreversible alteration of the immune system's regenerative capacity, exhaustion of antiviral immunity and the emergence of variants which precipitate immune collapse.

The relationship between immune activation and disease progression has been suggested through observations showing that an absence of HIV pathogenicity in chimpanzees is associated with an absence of general immune activation; this situation is also observed in long-term non-progressing patients. This was corroborated in a recent study, showing that differing sensitivity to the pathogenicity of injected SIVagmVer90 in macaques and Vervet monkeys is represented by an absence of immune activation in the latter species, despite a very high viral load. How can we explain the sensitivity of certain hosts to immune activation induced by the HIV/SIV? A partial answer to this question may have been provided by a study on CD33 Siglec (Sialic acid-binding Ig-like lectins) molecule expression, which controls immune activation by inhibiting activation signals received by lymphocytes and inducing apoptosis. A comparison of Siglec expression was done (Varki et al.; PNAS 2006) to identify the factors of protection of large monkeys against AIDS, chronic hepatitis B and C, and rheumatoid arthritis. The study showed that human expression of this molecule is very weak versus chimpanzees, bonobos and gorillas. If Siglec expression represents a significant factor of resistance to immune activation, and therefore to infection progression (which remains to be proved), we could envisage the development of therapies aimed at inducing Siglec and hence controlling general immune activation associated with the chronic expression of persistent viruses.

Many questions remain unresolved on the subject of AIDS pathogenesis:

1- What is the consequence of the rapid and persistent depletion of CD4 T lymphocytes in the intestine and mucous membranes? The majority of lamina propria CD4
T-cells and epithelial cells are rapidly destroyed in the weeks following infection by HIV/SIV, and are not restored during the chronic phase of the infection or by antiretroviral treatment. Why does the quantity of CD4 T-cells remain low in the intestine? Does this massive depletion influence the speed of infection progression? Does it impact the immune system’s regenerative capacity?

2- Following on from (1), what causes CD4 T lymphocyte depletion in lymphoid organs, mucous membranes and blood? Which is the contribution of chronic immune activation (premature death of uninfected cells, increase in the number of infected and killed cells), defective lymphocytary regeneration, and the alteration of new T cell production by the thymus?

3- Which impact does HIV have on effectors of innate immunity (NK and dendritic cells in particular)? Viremia affects the phenotype and functions of NK cells, which are essential components of innate immunity against viruses, parasites, bacteria and certain tumors. Dialogue between NKS and DCs is essential for DC maturation and NK differentiation. Mature DCs play an instrumental role in the presentation of viral antigens to naive T lymphocytes and their maturation into effector cells. The nature of this dialogue, the molecules involved (receptors, cytokines) and the mechanisms of these two cellular populations remain to be determined.

In conclusion, Marie-Lise Gougeon feels that successful therapeutic and/or preventive vaccine development depends on a solid understanding of the immunologic mechanisms regulating immune activation and the homeostasis of T lymphocytes, in physiological conditions and during acute or chronic infection.

Guido Poli (Milan, Italie) had collected the latest data on immunology and HIV. He first stressed that we should never forget that HIV infection induces both immune deficiency and chronic immune activation. He then reiterated the importance of the CD8 response, whose quality is related to nonprogression (Koup R el al. CROI 2006 Denver, abstract 3). One of the main reasons for the continued action of CD8 cells in "nonprogressors" is their persisting proliferative capacity and Perforine expression.

We now know that the induction of certain powerful and broad spectrum neutralizing antibodies is possible. However, some of these antibodies directed against the external proximal region of gp41 show a poly-specific reactivity against the organism’s phospholipids and proteins, making them subject to a negative control (Haynes B el al. CROI 2006, Denver, abstract 112). The negative role of Nef on immunoglobulins’ switch from M to A and G was demonstrated recently (Qiao X el al. Nature Immunology 2006; 7: 229-30). Lastly, regarding compartments, Guido Poli recapped the main works conducted by the teams of Mario Roederer and Ashley Haase, which highlighted the importance of the lymphoid reservoir associated with the digestive tract. He nevertheless listed 3 points which are not explained by the model:

- the fact that if a tiny number of memory CD4 cells survive viral invasion, this can suffice to protect the patient for years;

- the fact that the circulating pool of CD4 is an excellent predictive marker of opportunistic infections, despite the fact that this compartment is negligible from a physiopathological viewpoint;

- the fact that R5 viruses do not switch to X4 earlier, despite the fact that R5 viruses exhaust their targets rapidly.
The ensuing discussion aimed to establish a link with therapy, in order to define the possible consequences of the early introduction of CCR5 inhibitors. According to the speaker, this strategy should be tested in patients with very efficient immune reconstitution; the advent of CXCR4 strains should be considered as an indicator of disease progression rather than a disease mechanism.

José Gatell (Barcelone, Espagne) exhaustively reviewed the latest data on antiretroviral drugs. He considers study 934 to be the most important recent trial, as it established the superiority of the TDF/FTC combination versus AZT/3TC. He stressed that no patient with virologic failure at week 96 in this study had selected the K65R mutation. Thanks to today's antiretroviral drugs, we are now in a position to initiate powerful and well-tolerated combinations; a better option than this seems unlikely in the near future. However, some situations remain to be explored, i.e. a head to head confrontation of TDF/FTC and ABC/3TC, and a direct comparison of EFV with a boosted PI. The role of future molecules (TMC278, integrase inhibitors) also remains to be defined.

The biggest recent progress in terms of antiretroviral treatment simplification in controlled patients is the accomplishment of switches from one PI to a different and hopefully better-accepted and/or tolerated PI, such as ATV. This was done in study BMS047, with favourable results. The relay to a monotherapy strategy using a boosted PI such as Kaletra®, must still be evaluated through clinical trials.

One of the lessons learned in the past year is that interactions between certain PIs such as ATV and proton pump inhibitors must be kept in mind at all times, as these drugs are taken by many patients unbeknownst to doctors. The risk of ATV subdosing and ensuing virologic failure in this case is significant as a result of pharmacological interactions.

Progress has also been made in the treatment of advanced patients, thanks to the arrival of new drugs such as TMC114 (“Power” studies), especially when it can be used in conjunction with at least one other active molecule. We also have improved knowledge on TMC114 interactions (figure 2). Professor Gatell recalled that 4 years ago, at the time of the Barcelona international conference, we were highly enthusiastic about obtaining almost 20% virologic response <50 copies/ml in advanced patients with multiple treatment failure thanks to the use of T-20; we have since achieved 40% response through the use of TPV plus T-20, and now have nearly 65% response with TMC114 plus T-20. The arrival of integrase inhibitors should further improve these figures.

Jean-François Delfraissy (Paris, France) presented the state of the art of antiretroviral treatments in Northern countries, and looked at future prospects. We now possess over 23 different antiretroviral molecules. This choice of combinations means that a viral load <50 copies/ml is now achievable in over 80% of patients at 48 weeks, in both clinical trials and "real life" situations. However, this is no longer sufficient, as the associated treatments must now be made acceptable in the long term. Progress has been made in terms of the number and frequency of units to be ingested. Clinicians have gradually come to a consensus on the best time to initiate an antiretroviral treatment: at around 350 CD4/mm³, and in all symptomatic patients. However, wide variations have been observed among closely-monitored patients on antiretroviral therapy in terms of proviral DNA titers and specific immune response. Additional research...
is needed to enable us to use these markers in the framework of stoppage and simplification strategies.

Patients with severe virologic failure have been the major target of research efforts in recent years, e.g. T-20, TPV, TMC114 trials, etc. Programmed therapeutic interruptions were clearly challenged in 2006 (SMART study) but questions remain unanswered as to the interest of a strategy based on set windows versus a strategy based on CD4 levels. Recent approaches associating just 2 molecules, as in the "BIKS" trial, or using monotherapy (using Kaletra®) constitute interesting research paths.

Future research will particularly focus on the role of coming compounds, i.e. TMC125, integrase inhibitors, TMC278, PA-457, etc. Conversely, the latest results on CCR5 inhibitors are rather disappointing, and several trials have been stopped due to toxicity or failure.

The best time to introduce treatment still remains a major issue. We have delayed initiation in latter years due to risks of toxicity and side effects. However, we now know that the risk of side effects is higher in patients who start treatment late, and today’s molecules are increasingly well tolerated... Will our treatment initiation strategies for HIV infection continue to sway with the tide?

The authors would like to thank Marie-Lise Gougeon for her contribution to this article.
The session took the form of a "Pros" or "Cons" debate on 4 current issues, with two speakers defending their opinion on each topic. The participants voted before and after each presentation, and the results of the votes were announced at the end of the session.

The first question was: can we now avoid lipodystrophy and metabolic complications through an oriented choice of antiretroviral drugs?

Christine Katlama (Paris, France) argued in favour of this, and in particular the idea that these disorders can now be largely minimized. Studies by Australian teams (Nolan D et al. AIDS 2003; 17: 1239-38) have shown that the impact of antiretroviral drugs on fat tissue varies according to products, with a more marked depletion in mitochondrial DNA with d4T. Fat loss therefore depends on the chosen NRTI (Mallal SA et al. AIDS 2000; 14: 1309-1316); the new-generation RT inhibitors such as TDF seem to limit this loss. This was highlighted in studies 903 (d4T versus TDF), 934 (AZT/3TC versus TDF/FTC), and ABCDE (ABC versus d4T), in relation to both fat tissue loss and rise in serum lipid levels. According to certain studies, the occurrence of lipid disorders could even play a predictive role in lipodystrophy. In addition, we can now classify PIs on a lipid toxicity scale (figure 1). It therefore seems that lipid disorders can be efficiently limited by selecting nonthymidine analogues and PIs with the least harmful metabolic side effects.

![Figure 1: Impact of PIs on lipids](image)

Isabelle Poizot-Martin (Marseille, France) then took up the gauntlet, arguing that it is impossible to guarantee patients will never develop these complications. The HIV-infected population is getting older and, in addition, when an effective antiretroviral treatment is found it is normally maintained for years, during which time even minor side effects may be accentuated. Admittedly, the choice of available molecules is increasing and some are less toxic than others, but therapeutic strategies have to be modified in the event of resistance, with increasingly restricted choices in terms of molecules. We cannot therefore guarantee decades of treatment using the least toxic products. In addition, HIV itself can incur lipid disorders and have a negative impact on endothelial cells. Lastly, metabolic disorders and lipodystrophy are also influenced by genetic inequalities; we are powerless to change this.

56% of participants were in favour of Christine Katlama's opinion before the talks, and 57% after.

The second question was: are triple combinations of nucleoside analogues now obsolete?

Jan van Lunzen (Hamburg, Allemagne) "attacked" this question face on, asserting that his opponent could not honestly defend the contrary as most of the arguments were actually put forward by his team. He recapped the criteria defining a "gold standard" treatment:

- viremia below the limit of detection in over 65% of patients at 48 weeks;
- virologic failure rate < 15%;
- a rise of at least 50% in CD4 at 48 weeks;
- less than 15% stoppage due to side effects;
- over 95% adherence;
- a "favourable" resistance profile;
- few pharmacological interactions;
- acceptable long-term toxicity.

As regards power, a meta-analysis of 49 clinical trials (Bartlett J et al. 12th CROI Boston 2005: abstract #586) showed that in terms of virologic success, only 40 to 60% patients reached <50 copies/ml 48 weeks after the introduction of a strategy combining 3 NRTIs. The failure of the AZT/3TC/ABC triple combination was highlighted in study ACTG5095, with 21% failures against 10% in the Efavirenz arm. Other combinations including TDF rather than AZT also gave high failure rates (figure 2). Even induction strategies including one nNRTI or one PI,
subsequently simplified by the prescription of 3 NRTIs (e.g. the "NEFA" study) demonstrate the inferiority of these combinations as "maintenance" solutions (Martinez E et al. N Engl J Med 2003; 349: 1036-46).

Figure 2: Virologic failure of triple therapies comprising NRTIs and TDF

Bartlett’s meta-analysis also pinpointed the inferiority of these strategies in terms of immune reconstitution. Regarding side effects, NRTIs are clearly pinpointed as a cause of lipoatrophy. Lastly, in terms of pharmacological interactions, we possess little knowledge on what happens when we associate several NRTIs. Nevertheless, even if the association of 3 NRTIs is not a valid option, its combination with TDF could be interesting, as demonstrated in the "TIMS" study combining Trizivir® and TDF versus Combivir® + Efavirenz.

Mark Nelson (London, UK) initially asked participants who had no patients on a combination of 3 NRTIs to raise their hand… no hands were raised! He then argued that although the triple combination of NRTIs is obviously not today’s premier choice, it is still widely used in clinical practice. He reiterated that in studies CNA3005 and CNA3014, the combination of Combivir® + ABC worked just as well, or better, than Combivir® + Indinavir. Switch studies such as "TRIZAL" have proved the efficacy of Trizivir® in this situation. In the "ESS40013" trial, where naïve patients initially received Trizivir® + Efavirenz relayed by Trizivir® alone versus full treatment continuation, the "simplified" arm benefited from identical virologic and immunologic efficacy. Mark Nelson finished his presentation by stressing that there is no proof that observance is improved by once-daily versus twice-daily administration if the number of units to be ingested remains low.

41% of participants were in favour of Jan van Lunzen’s opinion before the talks, and 51% after.

The third question was: should the treatment of patients with detectable viremia be changed at an early stage?

Yazdan Yazdanpanah (Tourcoing, France) first recapped current recommendations, i.e. if alternative options are available, antiretroviral treatment must be changed if viral load is measured at >400 copies/ml twice consecutively. He then developed arguments pointing to the fact that maintaining a therapeutic regimen in the presence of viral replication leads to an increased selection of resistant mutants and reduced therapeutic options. In a study on 106 patients (Kantor M et al. AIDS 2004; 18: 1503-11) with viremia >400 copies/ml genotyped twice at an interval of 14-months without therapy change, 75% of patients selected new resistance mutations in all therapeutic classes during this period. Other studies have found a similar risk of resistance selection with low and stable viremia (Aleman S et al. AIDS 2002; 16: 1039-44; Nettles R et al. Clin Infect Dis 2004; 39: 1030-7). The presentation wound up with the analysis of a personal case illustrating this risk.

Rita Murri (Rome, Italie) opposed this. Basing herself on a Spanish study (Badia X et al. Antiviral Ther 2004; 9: 979-85) she first of all underlined the fact that changing an antiretroviral treatment may have a negative impact on quality of life. Moving on from this, she argued that although CD4 rise and viremia evolution were frequently dissociated, a number of studies have shown a paradoxical response despite persistent viremia. Several authors have suggested that the clinical and immunologic benefits of a treatment are maintained in the event of partial viremia control (Tenorio AR et al. JAIDS 2003; 34: 491-6). This is based on the theory that low viremia maintains a certain degree of anti-HIV immunity - in particular cellular immunity (Karlsson AC et al. AIDS
2004; 18: 981-9) -, which would contribute to viremia control. A study recently published by the speaker (Murri R et al. JAIDS 2006; 41: 23-30) tends to show that clinical progression does not speed up with detectable viremia <10,000 copies/ml versus undetectable viremia. Rita Murri then tackled the subject of increased resistance selection, arguing that the replicative capacity of these viruses is altered and that although some patients acquire new mutations, others “lose” some (Hatano H. et al. 13th CROI, Denver 2006, #615).

68% of participants were in favour of Yazdan Yazdanpanah’s opinion before the talks, and 71% after.

The last question addressed the use of entry inhibitors and suggested to spare them for advanced stages of the disease.

This proved to be a delicate and controversial issue; instead of a simple debate on the pros and cons of early T-20 initiation, the speakers chose to expand on this topic...

Jürgen Rockstroh (Bonn, Allemagne) first stressed that the twice-daily sub-cutaneous administration of T-20 is a limiting factor for use, despite its particularly good tolerance profile. Earlier use of this product could however decrease the risk of resistance and would enable its association with more effective molecules: according to the TORO study, this is a paramount success factor. Nevertheless, with the arrival of more active PIs such as the TMC 114 compound, the addition of T-20 may potentially offer only minor benefits (figure 3).

Finally, only ongoing studies such as the “Intense” study will be able to determine whether or not the addition of T-20 subsequent to first or second viral failures is likely to be beneficial. Here again, its role is likely to be constantly questioned by the arrival of new, highly-active products such as Integrase inhibitors.

Giuseppe Tambussi (Milano, Italie) chose to focus on CCR5 inhibitors rather than the indication of T-20. He reiterated that the viral variants using CXCR4 mainly emerge during advanced stages of the disease. The studies conducted on the ‘Vicriviroc’ anti-CCR5 compound produced by Schering-Plough were suspended last year due to failure in advanced patients and the incidence of lymphomas in groups receiving the product. Other tests using the ‘Aplaviroc’ compound by GSK were also suspended due to cases of toxic hepatitis. Lastly, the development of ‘Maraviroc’ is currently being pursued, despite a case of toxic hepatitis in a Thai patient which necessitated a liver transplant. The speaker concluded that CCR5 inhibitors will have a role to play in the early stages of HIV infection provided their long-term safety is ensured...

At the end of the debates, the chairpersons stressed that all of the principles discussed remained general, and that nothing could replace individual strategy adaptation.

55% of participants were in favour of Jürgen Rockstroh’s opinion before the talks, and 40% after.

This session’s interactive format gave lively and concrete results, and will be renewed in coming years.
For the second time in a row, the second day of ISHEID started with a 2-hour session entirely dedicated to the latest antiretroviral data. This session proved to be as successful as in 2004.

Stéfano Vella (Rome, Italie) began with a general review of new antiretrovirals in the 2006 pipeline.

TMC 125 (Etravirine) is a very promising NNRTI, acting on strains which are resistant to other products of the same class. The preliminary results of ongoing trials show a decrease in viral load of -1 log in the presence of 2 NNRTI mutations (figure 1).

Another NNRTI - TMC 278 - is currently in phase IIb of a double blind, randomized, comparative trial versus EFV on 300 patients, intended to assess the efficacy of various doses. Preliminary results are encouraging.

Regarding entry inhibitors, tests on first class "binding inhibitors" kicked off very recently. Apart from Maraviroc (Vicriviroc trials were stopped due to toxicity), several other anti-CCR5 and binding inhibitors are currently being developed.

Two integrase inhibitors are in the advanced stages of development:

- GS-9137: very well tolerated. Powerful antiviral activity, with an average reduction of 2 log at doses of 400 mg and 800 mg BID, or 50 mg QD with 100 mg of ritonavir.

- MK-0518: a phase IIb, multicentric, double blind, randomized trial on 167 heavily pretreated patients has shown this new antiretroviral to be particularly well tolerated and highly effective in multiple-failing patients (figure 2).

Susan Cox (Richmond Victoria, Australie) gave a progress report on the development of Apricitabine (ATC).

This new NRTI has a promising in vitro profile in terms of impact on viral isolates incorporating NRTI resistance mutations, i.e. M184V, TAMS and K65R (figure 3).
A clinical study entitled "AVX-201", conducted on patients with a M184V virus mutation is currently under way. Resistance to ATC develops slowly and no additional mutations were selected by viruses with pre-existing NRTI mutations after 17 weeks.

From a pharmacokinetic viewpoint, this drug’s profile is simple and predictable. It is eliminated by the kidneys and can be taken with any type of food. Trimethoprim significantly increases the AUC of ATC and decreases kidney flushing but, as with 3TC, no therapeutic adjustments are required. Intracellular level measurement suggests a twice-daily dosage would be most suitable, although an ongoing study showed similar efficacy with once-daily dosing after 10 days.

Diego Miralles (Mechelen, Belgique) reported on the development of TMC 114.

Two trials have been conducted to date on this new PI: "Power 1" and "Power 2". These phase IIb, multicentric, randomized and controlled trials, conducted on multitreated patients, were designed to assess the efficacy and tolerance of TMC114. At 24 weeks, the optimal dose was found to be 600 mg + 100 mg ritonavir twice daily (figure 4).

TMC 114 acts as a powerful antiretroviral on multi-resistant strains, and reinforces the notion of a high genetic barrier of resistance. Additionally, Tipranavir-resistant strains remain sensitive to TMC 114 and vice-versa. However, these two antiretrovirals cannot be combined due to the inductive character of Tipranavir and the resulting side effects. Observed undesirable events and biological anomalies were of light to moderate intensity. Finally, the non-randomized Power 3 trial, initiated after trials 1 and 2 and involving 327 patients receiving TMC 114 at 600/100 mg BID is tending to confirm these data.

David Martin (Gaithersburg, USA) reviewed the latest data on the very first maturation inhibitor, Bevirimat (PA-457). Phase Ia and Ib trials, conducted respectively on 38 and 36 subjects, show a good tolerance with no metabolic anomalies, good bioavailability with once-daily dosing by oral route, and a 3-day half-life.

In September 2000, a phase Ila, randomized, double blind trial was conducted on 25 multilibrated patients, who were administered once-daily doses of Bevirimat at 25, 50, 100 or 200 mg for 10 days. Optimal antiviral efficacy was achieved at 200 mg, with a reduction in viral load of 1.8 log at 10 days (figure 5). Side effects were comparable to those of the placebo group.

A phase IIb trial is scheduled for launch in June 2006 to confirm this efficacy and tolerance data, followed by a phase III trial in 2007.

Shani Waninger (San Diego, USA) presented Thiovir™, a new nucleoside analog (Pyrophosphate analog) similar to Foscarnet, with a dose-dependent antiviral impact on HIV (figure 6) and on HSV1, HSV2, Influenza A and B viruses. Complementary studies have highlighted the efficacy of Thiovir™ on NNRTI and NRTI-resistant
strains, a synergistic action with AZT and a resistance profile resembling that of Foscarnet.

**Thiovir efficacy against HIV-1 and -2**

![Graph showing the viral inhibition of Thiovir and Foscarnet](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Virus</th>
<th>IC50 (pM)</th>
<th>r² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiovir</td>
<td>HIV-1 IIIb</td>
<td>5.2</td>
<td>.97</td>
</tr>
<tr>
<td>Foscarnet</td>
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<td>1.8</td>
<td>.90</td>
</tr>
<tr>
<td>Thiovir</td>
<td>HIV-2 KRA</td>
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<td>.94</td>
</tr>
<tr>
<td>Foscarnet</td>
<td>HIV-2 KRA</td>
<td>1.9</td>
<td>.94</td>
</tr>
</tbody>
</table>

*Figure 6: impact of Thiovir on HIV*

This session wound up with a presentation by Dong Xie (Chongqing, China) on the preclinical development of Albuvirtide, a new HIV-1 binding inhibitor.
DEALING WITH HIV: WHAT KEYS FOR LASTING SUCCESS?

The symposium began with a presentation by Gilles Peytavin (Paris, France) on the new Kaletra® tablet formulation. Each tablet contains 200 mg of Lopinavir and 50 mg of Ritonavir. Several excipients thought to be partly responsible for the gastro-intestinal side effects experienced with the capsule format have now been excluded. This formulation was developed thanks to Meltrex® technology (Breitenbach J. Eur J Pharm Biopharm. 2002; 54: 107-17) which uses hot extrusion to favour solid, homogeneous and uniform dispersion of liposoluble active ingredients in an absorbent polymeric matrix. Pharmacological studies have shown that the tablet and capsule forms produce equivalent areas under curve, with approximately 20% more bioavailability in the tablet form, little impact of administration with food and less interindividual variability. Moreover, clinical data currently available on the use of the tablet form confirms that cases of diarrhoea are halved (Klein C. et al ; 10th EACS, Dublin, Ireland, 2005, #PE4.3/2). In terms of drug interactions, Kaletra® posology in tablet form will not necessarily have to be increased in the presence of Efavirenz; in this case, pharmacological monitoring will be of utmost importance. During pregnancy, Kaletra® subdosing is standard practise from 3 months, and led to increased posologies of the capsule form, which may prove unnecessary with the tablet form. Lastly, if we envisage a combination with Tipranavir, which decreases the areas under curve of Lopinavir by 45%, it will certainly be necessary to increase the dose of Kaletra® in tablet form by one tablet in the morning and evening. However, globally speaking, these various dosage adjustments will not concern all patients and will need to be supported by plasma dosages.

Jacques Izopet (Toulouse, France) focused on the resistance challenge. He first pointed out the very high variability of HIV, and our increasingly improved knowledge on the impact of viral recombination. The most efficient strategy for delaying the selection of resistant mutants is to define a treatment capable of eliminating viral replication while offering a high genetic barrier to resistance. Significant progress has been made in recent years in terms of detecting and quantifying minor resistance-associated mutations. These techniques have sensitivity thresholds between 0.01 and 0.1%, and demonstrate that the resistance "reversion" occasionally observed after therapeutic stoppage is only a relative phenomenon, caused by the better fitness of wild strains.

In addition, genotype interpretation can now been improved through the use of "Inhibitory Quotients" and the development of algorithms for non B sub-types.

On an epidemiologic level, the prevalence of resistant virus transmitted to recently infected patients is stable in France (12% of resistance to at least 1 molecule; 3% to at least 2 classes). It concerns NRTIs (prevalence: 6%), nNRTIs (prevalence: 5.9%) and PIs (prevalence: 3.4%). Resistance is frequent among chronically infected patients in a situation of therapeutic failure, (88%) but varies according to class (figure 1).

Several studies suggest that the existence of resistant mutants in primary and chronically infected patients negatively impacts immuno-virological treatment response. A recent meta-analysis of several assays (Barlett J. et al. JAIDS 2006; 41: 323-31) demonstrated the positive impact of a rapid introduction of boosted PIs in the strategies; although initial therapeutic schemes comprising an nNRTI are similarly effective, the "cost" of resistance induced in terms of number of active drugs left is higher.

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Jean Luc Meynard (Paris, France) presented the cardiovascular risks affecting HIV-infected patients. He initially reiterated the fact that the benefits of HAART remain far higher than the disadvantages. Most of the numerous studies published in the past years reveal the potential role of PIs in increased myocardial infarction.
risk. Nevertheless, the statistics remain low (DAD cohort: 3.7/1000 patient-years against 2.8 in the Monica study involving non HIV subjects) and have decreased in recent years (figure 2). Moreover, far less deaths are due to cardiovascular accidents than to complications related directly to HIV or co-infections. In the "SMART" study, cardiovascular accidents were significantly higher in the therapeutic interruption arm, hence demonstrating that antiretrovirals are not the sole cause. Cardiovascular risk factors are now increasingly well known, and in particular the importance of low HDL-c levels, which were shown to be < 0.35 g/l in a quarter of treated and untreated patients in the DAD study. The impact of PIs on lipid risk factors varies according to molecules. In the CONTEXT study, which compared an LPV/r strategy versus a FPV/r strategy, no difference was observed in TG, total cholesterol or LDL-c increases in either group, whereas HDL-c tended to increase in the LPV/r arm. In the BMS045 study, total cholesterol and TG increase was lower in the ATV/r arm but the LPV/r arm showed a 7% increase in HDL-c, whereas the ATV/r arm showed a drop of 3%. The BMS049 study allow us to appreciate the impact of a ritonavir "boost" on lipid anomalies (Malan N et al. 13th CROI, Denver 2006, #107LB): in this study, total cholesterol and TG increase was significantly higher in the ATV/r arm than in the ATV-only arm. Several studies, including the "Kaletrig" assay conducted by the speaker, confirmed that the use of LPV/r was correlated with a regular rise in HDL-c.

Alain Lafeuillade (Toulon, France) put these data into perspective. He first reiterated that our physiopathological knowledge on HIV infection has not changed in the last 10 years, i.e. constant and rapid viral production and CD4 destruction, and the early establishment of a latent viral reservoir preventing eradication. Despite this knowledge, we have tested therapeutic strategies designed to delay the introduction of antiretrovirals, or have administered them intermittently, in the aim of finding the best benefit / risk ratio and decreasing long-term toxicities.

Nevertheless, several assays published this year at the CROI, including "SMART" and "TRIVACA N", have clearly demonstrated the higher risk incurred by these intermittent strategies, with a 2.5 fold increase in clinical progression or deaths versus continuous treatment. We must therefore explore alternative ways of managing long-term antiretroviral treatments in the most acceptable way for patients. Obviously, we all dream of new molecules capable of reaching new targets, which are easier to manage and better tolerated. But we should not forget that for every 1.5 million molecules screened, and the colossal financial investment involved, just one will arrive on the market. Objectively, in the near future we can only hope to see the arrival of integrase inhibitors, and new products belonging to existing classes. It is therefore of utmost importance to optimize what we already have. In this perspective, the arrival of Kaletra® in tablet form is a genuine progress; in addition to the fact that it no longer requires refrigeration, we can hope for improved tolerance due to reduced digestive side effects and the number of pills to be taken. For some patients, once-daily administration may also facilitate treatment adherence. The M02-418 study comparing LPV/r 800/200 mg OD with LPV/r 400/100 mg BID, in association with TDF/FTC, demonstrated the immuno-virological equivalence of these 2 modes of administration. The ongoing M05-730 study will also compare once-daily and twice-daily administration of Kaletra® capsules and tablets.
Finally, various studies are currently evaluating the possibility to use a monotherapy strategy. Although no dogma exists with regards to triple therapy, we need to find adequate power coupled with a high genetic barrier to resistance; Kaletra® combines all of these qualities. This monotherapy strategy could correspond to 3 situations:

1. Genuine monotherapy: naive patients treated by Kaletra® only;
2. Induction/maintenance: patients that have reached undetectability with combined treatment who are prescribed Kaletra® only;
3. Simplification: continuation of Kaletra® only after stoppage of other associated antiretrovirals.

Analyses of the assays or cohorts published to date show a success rate of around 70-80% using this approach (figure 3), in subjects with no prior PI resistance selection. This is comparable to the results of other simplification strategies using Abacavir or Efavirenz. Several studies are still in progress, including the French "MONARK" study, whose initial results are expected in the coming months. There is obviously no question of making this monotherapy strategy the rule, but it could be an efficient solution in particular cases.

Studies on LPV/r Monotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>pts</th>
<th>ITT Week 24</th>
<th>ITT Week 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMAN1</td>
<td>Naive</td>
<td>30</td>
<td>N/A</td>
</tr>
<tr>
<td>Campo</td>
<td>Naive</td>
<td>6</td>
<td>83% &lt;400 c/mL</td>
</tr>
<tr>
<td>CK</td>
<td>No PI failure</td>
<td>21</td>
<td>81% &lt;50 c/mL</td>
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<tr>
<td>Pierne</td>
<td>P naive</td>
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<td>71% &lt;75 c/mL</td>
</tr>
<tr>
<td>Ruzane</td>
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<td>19</td>
<td>69% &lt;75 c/mL</td>
</tr>
<tr>
<td>Waters</td>
<td>Experienced</td>
<td>35</td>
<td>N/A</td>
</tr>
</tbody>
</table>

To conclude, this symposium showed that Kaletra® remains the cornerstone of antiretroviral treatment thanks to its power and low risk of resistance selection. The new tablet formulation is an unquestionable progress in terms of acceptability. Moreover, current research paths are uncovering additional properties of Kaletra®, such as its beneficial effect on HDL-c.
Antoine Cheret: what are the strong and weak points of Maraviroc™ (anti-CCR5)?

Stefano Vella: The strong points are:

- the fact that it forms part of a new therapeutic class: we need these drugs to treat patients with multiresistant viruses;
- the fact that it appears to be fairly well tolerated, unlike previous CCR5 inhibitors which had toxicity problems;
- the fact that it does not cause drug interactions and does not need boosting with ritonavir. However, we should be cautious as data is limited and as with all new drugs possible short or long term toxicity must be monitored.

There are two weak points:

- Initially, the virus uses two co-receptors, meaning that if we block one, the virus may develop a greater affinity with the other. The emergence of a CXCR4 tropic virus has already been observed in clinical studies on CCR5 inhibitors, but this is actually due to the selection of pre-existing viruses in these patients. This raises concerns as to the evolution of patients infected with CCR4 tropic virus, as observed in advanced stages of the disease, but it could be even more aggressive. The question can only be answered by patient follow-up.

- Additionally, if a patient has a majority of CXCR4 tropic viruses, anti-CCR5s are useless. We must therefore identify the tropism beforehand - which will not make things any easier!

A.C. : According to you, what will be the role of Maraviroc™ in current antiretroviral arsenal?

S.V. : From a pharmacological viewpoint, this product does not have any specificities.

From a pathogenic viewpoint, it would need to be used early on, as CCR5 tropic viruses are found in patients in the early stages of AIDS. But as it is a new molecule, its main interest is paradoxically for multiresistant patients, who are generally at an advanced stage of the disease.

Future clinical studies will therefore allow us to define when to use it, with what strategy and with which combination of antiretrovirals, in order to achieve optimization and limit the emergence of resistant viruses.

A.C. : Two integrase inhibitors are currently being developed. What are their specificities?

S.V. : These two molecules are completely dissimilar and open up different doors.

The development of the Gilead inhibitor (GS9137) is less advanced, so we have less data on it. It is a quinolone (modified) which seems to be very well tolerated and very powerful. It has to be boosted with ritonavir which is an advantage as it only needs to be taken once daily, but also means the patient is exposed to the adverse effects of ritonavir.

The second product under development, the MK0518, is the most powerful antiretroviral (in phase I) I have seen in my career! It has an excellent tolerance profile and all patients are still included in the studies, which are already up to phase 3. Associated with a new PI, this could be the right product for building a very powerful "salvage" combination. I think this is the little revolution we have been waiting for. For the last 10 years we have failed to block new targets, and a lot of molecules have been rejected due to their toxicity and inefficiency. After a morose period, we are now seeing the emergence of new and very promising drugs.

A.C. : What will their role be with regards to protease inhibitors?

S.V. : If integrase inhibitors prove to be very well tolerated, they could become the "new PIs" tomorrow… without the side effects!

I think they could even be used to replace PIs in initial therapy. We hope to be able to combine them with the
new NRTIs, NNRTIs and PIs, but of course we need to wait for results on naive patients first.

We will also need to study combinations with Maraviroc™. So tomorrow's "backbone" combinations could be different.

A.C. : What do you think about the arrival of these new molecules?

S.V. : Well, a number of other drugs we didn't mention today are also about to arrive, which are directly derived from pathogenesis, and there are also other targets, such as maturation inhibitors. They work a bit like PIs but differently – they act on a different viral protein. There are also what we call chaperone molecules. These molecules enable compartmental endocytosis of the virus inside the cell... but there is still some way to go – the future will be very complicated.

The arrival of these new molecules will enrich the therapeutic arsenal, but we must be extremely cautious and vigilant to avoid repeating the mistakes we made with antibiotics. We will need to determine clear strategies for the use of antiretrovirals and define the specificities of each drug as objectively as possible.

In this aim, we are currently developing a European network of public clinical trials, financed by the European Community, to complement the studies conducted by the pharmaceutical industry.
Albert Faye (Paris, France) tackled the issue of the fate of HIV-infected children by presenting the current pediatric situation.

In industrialized countries, mother to child (MTC) transmission has regressed strongly and infant morbidity and mortality has decreased thanks to antiretroviral multitherapy. But, as in adults, HIV infection in children is a chronic disease and the long-term impact of current therapeutic strategies remains poorly evaluated.

Among the 2.3 million children living with HIV, Africa accounts for nearly 2 million, and MTC transmission accounts for over 90% of child infection in the world.

In France, the rate of MTC transmission is lower than 1% and less than 10 newborns are infected every year: these are generally children born into immigrants families, where the mother is not always tested in time.

A survey conducted on 10 French pediatric centers (n = 701) collected data on therapeutic practices in recent years: 88% of infected children receive an antiretroviral combination and most are 6 to 18 years old. Early treatment initiated prior to the age of 6 months has been shown to favor survival, with no encephalopathies or AIDS-related events by the age of 2. This benefit persists with a 6-year follow-up (Ciappini et al. AIDS 2006).

The French perinatal cohort provides a good picture of the fate of teenagers infected at the outset of the epidemic. Among 293 children born between 1986 and 1993, 32% have died and 58% are still alive and regularly followed-up; out of the remaining 171 patients, 19% have developed a stage C, 18% are untreated, 8% have less than 200 CD4, and only 40% have undetectable viral load below the threshold of 50 copies/ml. Long-term non-progressors are rare: after 12 years, only 9 patients out of 300 do not require treatment.

The issues inherent to adolescence and requiring specific management are therapeutic compliance, announcement of the diagnosis and handling the mother’s guilt, the issue of sexual relations and the importance of using of a condom to protect partners, the passage to adulthood and envisaging future projects.

Between 2001 and 2003, the Necker University Hospital team evaluated the prevalence of antiretroviral resistance in children versus adults: overall prevalence was globally slightly higher, especially for PIs. Determining factors of failure were related to pharmacokinetic problems and the difficulty in accessing tablet forms adapted to children.

Long-term treatments incurred 25% lipodystrophies and 42% metabolic disorders (Beregszaszi et al. AIDS 2005). Recently, bone density anomalies were reported with TDF (Gafni et al. CROI 2006). Hindsight on this molecule still appears inadequate to recommend its use in pediatrics.

Despite this, thanks to the 18 years of data provided by the French perinatal survey, we can see that the cohort’s survival curve has reached a plateau in the last 8 years; the life expectancy of followed-up and, if necessary, treated children is currently regarded as normal.

Privileged relationships between Herpes Simplex Virus (HSV) and HIV were reported by Anna Maria Geretti (London, UK).

Chronic genital herpes has been described and recognized as a symptom of AIDS since 1981. A two-way relationship exists between the two viruses.

Immunosuppression related to HIV increases the frequency and duration of herpetic reactivation, with a risk of severe disease and antiviral resistance. It also increases the risk of HSV transmission. In addition, HSV promotes HIV replication, accelerates disease progression, and favors HIV genital replication and transmission. A meta-analysis performed on sero-divergent couples showed that infection with HSV-2 doubles the risk of HIV transmission. In order to appreciate the impact of HSV infection on HIV, 140 women took part in a study randomized against placebo in Burkina Faso (Nagot et al. CROI 2006, abstract 33LB). Half received an antitherpetic treatment for 3 months. Viral replication was measured in plasma and in vaginal lavage, and a positive treatment impact was found on plasma viral load and genital HIV load with the antitherpetic treatment.

A seroprevalence survey was conducted in London relating to new cases of HIV diagnosed between 1986 and 2001: the survey showed a high percentage of HSV infections, with 88% HSV-1-positive subjects and 63% HSV-2-positive subjects affecting a majority of black African heterosexual women. In the same population, sero-incidence of HSV-2 was 10% in the 5 years following the diagnosis of HIV infection; infection by HSV-2 has been strongly associated with other sexually transmitted diseases and reflects risk taking (Ramaswamy et al. Sex Transm Dis 2006).

Specific anti-HSV immune response is affected by HIV infection; HAART slowly improves response, and it is estimated that 33 months of immuno-virologic success are necessary to restore a similar immune response to
that of healthy references. The only predictive factors of an effective specific immune response are CD4 count and a long-term non-progressor status (Posavad et al. J Inf Dis 2004).

Valerio Tozzi (Rome, Italy) familiarized us with the problems of neurocognitive deficiencies associated with HIV infection in the HAART era.

Three clinical forms exist: an asymptomatic form, documented using neuropsychological tests, which incurs no deteriorations to everyday life; a moderate form, and a severe form or HIV-associated dementia.

HIV infection of macrophages, astrocytes and microglial cells incurs the production of neurotoxins, cytokines (TNFa, PAF, MCP1) and chemical substances which increase the permeability of the hematoencephalic barrier and induce a neuronal apoptosis of white matter, the frontal cortex, hippocampus and basal ganglia.

Over the years, thanks to progressively improved treatments, we have seen a decline in the incidence of HIV-related dementia. However, moderate cognitive disorders have shown an upward trend. The initial clinical implication of this is a negative impact on treatment adherence, viral replication control and survival (Tozzi et al. AIDS Res Hum Retroviruses 2005). If neurocognitive deficiencies are diagnosed, a neuropsychological evaluation is required to highlight psychomotor deceleration, mnemonic disorders and attention disorders; a brain MRI can be used to diagnose cerebral atrophy and a periventricular white matter hypersignal; lumbar puncture can be useful for excluding other diagnoses: HIV RNA is detected at varying levels in SF, and is accompanied by an increase in immunologic activation markers (MCP-1, TNFa, neopterine, ß2 microglobulin).

The evolution of dementia in patients on HAART is variable. At best, HIV replication is suppressed, and symptoms are fully reversed. Sometimes the patient can evolve to a latent chronic form, characterized by optimized treatment and partial, stable recovery from cognitive disorders. In extreme cases, virologic control may be incomplete and dementia may worsen.

The treatment of HIV-related dementia involves suppressing viral replication in CSF. However, drug penetration in this compartment does not always accurately reflect impact on dementia. The intracerebral penetration of antiretrovirals is crucial, but we still know little about it; available data relate to CSF/plasma ratios rather than cerebral tissue (figure 1).

Many studies have demonstrated the positive impact of HAART on HIV-related dementia, although some patients on HAART continue to have detectable HIV RNA in their CSF. In certain patients, the results of psychomotor and memory tests diverge; CSF/plasma compartmentalization could explain the difficulties in obtaining dementia regression. Treatment using HAART alone appears to be inadequate for controlling HIV-related dementia. Complementary therapies have been tested, and improvements in cognitive functions have been reported with high doses of nimodipine (calcic inhibitor), valproic acid, neuroprotective antioxidants (OPC 14117, selegiline), minocycline in monkeys, and lexiparfant (PAF antagonist) (McArthur et al. Lancet Neurol 2005).

Comparative testing would be required to assess the relative efficacy of these drugs. Jeanine Ohl (Strasbourg, France) gave a progress report on the issue of procreation in sero-divergent couples and recounted her experiences at the Strasbourg center for medically assisted procreation.

In France, among the HIV-positive subjects diagnosed in 2003-2004, 42% were women, mostly aged between 20 and 30 years. Mortality and mother-child transmission have now been vastly reduced thanks to HAART (currently 0.8%), resulting in a keener desire to have children which must be taken into account by the medical profession. Taking into account the problems of possible contamination of the partner, suggested solutions are insemination by donor sperm, adoption or medically assisted procreation, which enables the transmission of genetic heritage.
A qualitative and quantitative improvement in sperm from HIV-positive men is observed with HAART treatment. HIV may be found in sperm, but its level does not correlate exactly with plasma viral load. Opinions diverge on the subject of sperm infection with HIV. In HIV-positive women, HIV is found in follicular fluid but not in ovocytes, and its correlation with plasma RNA titles is also imperfect. Opinions also diverge on the subject of corona radiata infection with HIV.

Medically assisted reproduction techniques prevent contamination of the partner and treat infertility. They were first implemented using the sperm of HIV-positive men in Italy in 1992, then in Spain and France on significant cohorts, with no contaminations. The French government only authorized medically assisted procreation in HIV-infected patients in May 2001.

In order to benefit from these techniques, HIV-positive men must have a CD4 lymphocyte count > 200/mm3 and a stable viral load. An antiretroviral treatment is not essential; the sperm is prepared using two successive techniques - density gradient centrifugation and spontaneous migration. The final fraction is frozen.

Various insemination techniques exist according to the initial viral load found in the seminal liquid (artificial insemination, in vitro fertilization, or intra cytoplasmic sperm injection).

A serologic follow-up of the partner before and after insemination is required until childbirth.

In the case of HIV-positive women, the treatment is considered in terms of risk of mother-child transmission and side effects during the pregnancy.

The CREAT network consists of 9 centers in 6 European countries; 3390 medically assisted procreations were performed there between 1993 and 2003, resulting in 463 births, with no contaminations.

In the tests conducted at the Strasbourg center on 87 men, 3% had positive final fractions associated with negative plasma RNA. The antiretroviral treatment was then changed and a new sample was taken.

Using ovocyte collection, the success rate was 50% if the male partner was HIV-positive but only 20% for HIV-positive women; ovarian response is often altered and an increased number of infertility factors are found in women. A single embryo was transferred to avoid multiple pregnancies and the risk of neonatal contamination. Despite these difficulties, the desire to become a parent can now become a reality for HIV-positive couples.

Although AIDS-defining cancers are declining thanks to HAART, what is the current incidence of non AIDS-defining cancers (NADCs)? According to Nancy Crum (San Diego, USA) the death rate ascribable to NADCs has risen from 1 to 13% in the last 10 last years (Burgi et al. Cancer 2005).

This is assigned to various factors, i.e. the reduction in competitive mortality factors, increase in life expectancy, risk behaviors and the role of alcohol, smoking and illegal substances. Human Papillomavirus and Epstein Barr Virus have been shown to play an oncogenic role, but HIV is not directly implicated. There is no relation to CD4 count, except perhaps in the case of Hodgkin's disease and anal cancer. NADCs are more common in HIV subjects than in the general population (ratio of 12.8 versus 1.9); these cancers are generally aggressive, affect young subjects and respond poorly to therapy. One of the most common - anal cancer - is frequent in the homosexual population and probably favored by HPV infection, although results diverge as to the influence of the patient's immune status (Piketty et al.).

HIV AIDS Reports 2005). Hodgkin's disease is strongly associated with HIV infection and a deteriorated immune status; EBV contributes to its pathogenesis. Lung cancers account for 15% of total cancer deaths: most occur with a high CD4 count and controlled HIV replication, the affected subjects are relatively young – age 40 years on average – and this cancer is significantly related to smoking. Skin cancers are the most common and are currently on the increase; they are generally basocellular carcinomas and occasionally melanomas, unrelated to CD4 count, with traditional factors of risk.

They are aggressive and relapse frequently after treatment. Conjunctival cancer with squamous cells attacking the ocular mucous membrane is common in Africa and related to immunosuppression (Mbulaiteye et al. Cancer 2006). HAART has no specific effects on most of these cancers - it simply prevents HIV disease.
progression and offers better chemotherapy tolerance. In view of the increasing occurrence of NADCs and their particularly aggressive character – resulting in a very pessimistic prognosis - it is important to adopt screening strategies for cancers of the prostate, the colon, the breasts, the cervix and the lungs, adopt a preventive attitude towards alcohol and tobacco consumption, give advice on solar protection, and prevent and treat hepatitis B and C.
The symposium began with a presentation by Professor Pierre Dellamonica (Nice, France) on "lessons learned in the last 10 years". He reviewed the very first treatment lines, their evolution conditioned by major clinical trials, and the lessons they have taught us.

CD4 lymphocyte levels and viral load are the two key parameters for dictating patient fate and interpreting and evaluating clinical trials. Similarly, the first treatment line conditions the patient's future evolution.

An analysis of the "NADIS" database (figure 1) shows that unless the target of undetectability with a satisfactory immunologic gain is achieved within the first two years of treatment, it will never be reached at all.

- The "Abbott 863" study on 653 subjects, which significantly favored LPV/r as first line therapy (75% plasma viral load < 400 copies/ml) versus NFV (63%).

- The ACTG 384 study on 620 subjects, which demonstrated the superiority of the CBV + EFV arm versus d4T/ddI + EFV or NFV.

- The more recent randomized, double blind 903 study on 600 subjects, targeting <400 copies/ml at week 48, which compared TDF/3TC + EFV versus d4T/3TC + EFV. On inclusion, median plasma viral load was 4.9 log, with CD4s at 276/mm³. In ITT at 3 years, no significant difference was found between the TDF/3TC + EFV arm (73%) versus the d4T / 3TC + EFV arm (69%). However, this study did reveal a very high percentage of lipodystrophy in the group treated with d4T (19% versus 3%) and a better lipidic evolution with TDF versus d4T.

- The randomized, double blind A5095 study on 1147 subjects, which was one of first studies to seriously question the use of the AZT/3TC/ABC triple nucleoside analog combination, due to a higher proportion of failures versus arms comprising EFV.

- The 934 randomized, open, noninferiority study on 509 subjects, targeting less than 400 copies/ml at week 48 with TDF/FTC + EFV versus AZT/3TC + EFV. On inclusion, median plasma viral load was 5 log, with 233/mm³ CD4 cells. This test clearly highlighted the virologic and immunologic superiority of the TDF/FTC + EFV combination (80% undetectable patients at week 48) versus AZT/3TC + EFV (70% undetectable patients).

Ten years ago, the advent of PIs was our first major breakthrough, subsequently superceded by PI "boosting". Among the key trials conducted between 1999 and 2006, we can underline:

- the open randomized Dupont 006 study, which aimed to achieve less than 400 copies/ml at week 48 in 350 subjects, with an initial median plasma viral load of 4.7 log and CD4s at 345/mm³. In ITT at 48 and 144 weeks, this study achieved 55% success in the CBV + EFV arm versus 34% in the EFV + IDV arm and 34% in the CBV + IDV arm.

Professor Dellamonica then looked at long-term lessons learned. Certain studies, such as the "Abbott M97-720" testing LPV/r in naive patients, have benefited from a follow-up of up to 252 weeks. At 168 weeks, the DMP-006 study showed that a combination of AZT/3TC + EFV was superior to other arms.

When study 903 switched to open phase after 144 weeks, results proved to be consistent with those obtained at 48 weeks (figure 2).

In the last 10 years, the panel of available molecules has considerably broadened and we must use the experience gained to properly assess the efficacy, sustained potency, tolerance and adherence of coming therapeutic options.
Mark Nelson (Londres, UK), tackled the theme of "differentiating various therapeutic options". He underlined the unquestionable advantages of HAART, while reiterating the toxicity of antiretrovirals, their negative side effects (dyslipidemia, lipodystrophy), the fact that they require large daily doses and quantities of pills, and their consequent negative impact on adherence. These factors must imperatively be taken into account, along with the cost/efficacy ratio of these treatments and the long-term results of various studies. More precisely, Mark Nelson examined the current choice of nucleosides for first line treatment, focusing on the virologic efficacy of various combinations.

In 2000, Squires et al (AIDS 2000; 14: 1591-600) demonstrated that d4T + 3TC + IDV had a similar virologic efficacy to an AZT / 3TC+IDV combination. In 2006, study 903 showed similar virologic efficacy for TDF + 3TC + EFV versus d4T + 3TC + EFV. Study CNA30024 also showed ABC and AZT to have similar potency (with 3TC and EFV).

Mark Nelson presented study 934 again, insisting on the immunologic and virologic superiority of a TDF/FTC combination versus AZT/3TC (Figure 3). Although TDF + FTC combinations sometimes incur M184 V and K65R mutation selection, the viruses remain sensitive to alternative nucleosides.

Also of note is the positive impact of the M184V mutation on the virologic efficacy of TDF, as revealed in studies 902 and 907. This mutation was rarely found at week 48 and 96 in clinical studies 903, 418 and 934, possibly due to the long half-life of FTC.

Regarding metabolic problems and dyslipidemia, the 903, 934 and COMET studies (switch from CBV + EFV to Truvada® + EFV) have demonstrated the superiority of TDF arms versus d4T or AZT arms in terms of lipidic disorders and lipodystrophy. According to the speaker, the relative bone toxicity of the virus and nucleosides is hard to assess; study 903 did not reveal any variations in bone mineralization with d4T and TDF.

Roland Landman (Paris, France) presented future therapeutic options, specifying that several studies are currently aiming to better assess certain bitherapy combinations (NNRTI + PI) and PI monotherapy options.

Various ongoing studies are focusing on existing drug classes. Gilead is developing a new nucleoside analog (GS 9148), and preliminary in vitro data show excellent potency coupled with very low mitochondrial toxicity. This drug remains effective in the presence of K65R, M184V and L74V, and maintains excellent sensitivity in the presence of TAMS. Once-daily dosing appears feasible. Roland Landman also presented trial C223 on TMC 125; the results demonstrate the efficacy of this product in patients who are already resistant to NNRTIs and PIs, versus an optimized treatment (Figure 4).
Regarding new formulas, Roland Landman feels that an EFV and Truvada® combination in single tablet form will be a big step towards treatment simplification.

A new form of Kaletra™ will also be available shortly; it should offer improved tolerance and does not require refrigeration.

The speaker then put forward the question as to whether or not class sparing strategies were a valid future option. We should pursue studies on nucleosides and nucleotides despite the failures encountered in previous combination trials, as these combinations can prove highly useful in certain situations (Africa, tuberculosis treatments), as demonstrated by the "DART" trial.

Strategies comprising four RT inhibitors have now been abandoned. Several PI monotherapy studies have given encouraging results, in particular using LPV/r.

Lastly, in addition to new formulas, new molecules for new targets are currently being studied. These will need to be incorporated in highly upgradeable therapeutic strategies, in which pharmacogenomics will play an increasingly significant role.
In her introduction, Christine Katlama (Paris, France) stressed that "ageing well with HIV" will be tomorrow’s challenge for clinicians, after years focusing on survival then on quality of life. Moreover, physiologically speaking, HIV patients are often regarded as being 10 years older than their actual age. Risks relating to cognitive deterioration, cardiovascular accidents and cancer development are major concerns of ageing patients.

Jacques Gasnault (Le Kremlin Bicêtre, France) developed the topic of "The Brain and HIV". He first pointed out that the notion of "ageing" HIV-infected subjects applies to patients over the age of 50, as HIV is still a "young" epidemic affecting young subjects. According to data published by the Centers for Disease Control, HIV-infected subjects over 50 years of age represent more than 100,000 people in the USA, and this figure has risen 6-fold since 1990. Figures vary according to region, with over 25% of patients aged 50 or over in Hawaiian cohorts. According to the French DMI2 cohort, 19.7% of subjects who had recourse to French hospitals in 2004 were in this age bracket.

A foremost issue is ascertaining whether or not HIV infection in this population is more severe. The answer is probably "yes" due to immune system senescence, witnessed by poorer CD4 reconstitution with age in patients on HAART (Grabar S et al. AIDS 2004; 18: 2029-38) notwithstanding initial viral load. This is also accompanied by an increased risk of clinical progression in subjects aged over 50 years compared to other subjects.

Is cognitive risk also higher? To answer this question, several studies measured the "cognitive reserve" of HIV infected and uninfected patients, and found a similar decline with age, although this decline probably occurred earlier in HIV patients. These disorders are the result of subcortical changes, and may range from characteristic dementia to slight cognitive deficits affecting 15-30% patients with less than 200 CD4/mm3. In the Euro-AIDS cohort, the risk of dementia rose by 19% every 5 years (Grabar S et al. AIDS 2004; 18: 2029-38) notwithstanding initial viral load. This is also accompanied by an increased risk of clinical progression in subjects aged over 50 years compared to other subjects.

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more frequent, partly due to incomplete immunorestoration and combined risk factors. Since the start of the epidemic, it has been widely acknowledged that cancer forms part of the clinical picture of AIDS. Since the advent of triple therapy, Kaposi’s sarcoma and non Hodgkin lymphomas - in particular cerebral - have been less widely observed. Regarding non AIDS-defining cancers, evolution is more or less stagnant, or on an uptrend. Several international studies have shown a relative increase in cancer risk during HIV infection. Similarly, in the DMI2 database, the incidence of lung cancer in men and Hodgkin’s disease in men and women was higher after 1996 than previously (Herida M et al J Clin Oncol. 2003; 21: 3447-53). This highlights the problem of associated co-factors (smoking for lungs) or co-factors favoured by immuno-depression (EBV and Hodgkin’s disease). In addition, anorectal cancer is rising in the male homosexual population in conjunction with infection by HPV 16 and 18.

The above data has been confirmed by an analysis of American cohorts involving several thousand patients (Patel et al. CROI 2006; abstract 813), which reveals post-1996 reduction in cases of Kaposi’s sarcoma and cervical cancer, but not of non Hodgkin lymphomas, plus an increased relative risk (RR) of lung cancer (RR=2.13), Hodgkin’s disease (RR=4.58), anorectal cancer (RR=10.13) and melanoma (RR=2.99). A similar analysis conducted on the Swiss cohort (Clifford JM et al. J Natl Cancer Inst 2005; 97: 877-8) also highlighted an increased incidence of Hodgkin’s disease in the post-HAART era. In the French “Mortality 2005” study, cancers came in second position and accounted for 15% of deaths (figure 2).

The talk ended with a presentation of the French "ONCOVIH" study, which aims to take stock of all cases of malignant tumours reported in HIV patients in 2006. This study should become a reference if the maximum possible numbers of cases are reported. 202 cases had already been reported by June 15, 2006, with, in order of frequency, non Hodgkin lymphomas, followed by Hodgkin’s disease and lung cancers.

The symposium ended with a general discussion and the conclusion that, in the face of an ageing treated HIV population, preventive action is required in terms of "controllable" risk factors at the origin of complications, i.e. reduced smoking and selection of antiretrovirals with the most favourable metabolic profile.

L'exposé s'est terminé par la présentation de l'étude française "ONCOVIH" destinée à recenser tous les cas de tumeurs malignes survenant en 2006 chez les patients VIH. Le signalement de cas le plus exhaustif possible fera de cette cohorte une référence. Au 15 Juin 2006, 202 cas avaient déjà été signalés avec, par ordre de fréquence les lymphomes non hodgkiens, suivis de la maladie de Hodgkin et des cancers pulmonaires.

The symposium ended with a general discussion and the conclusion that, in the face of an ageing treated HIV population, preventive action is required in terms of "controllable" risk factors at the origin of complications, i.e. reduced smoking and selection of antiretrovirals with the most favourable metabolic profile.

The symposium ended with a general discussion and the conclusion that, in the face of an ageing treated HIV population, preventive action is required in terms of "controllable" risk factors at the origin of complications, i.e. reduced smoking and selection of antiretrovirals with the most favourable metabolic profile.
The theme of chronic hepatitis B was tackled by Stephanos Hadziyanis (Athènes, Grèce).

Treatment ambition for chronic hepatitis B is the elimination of Ag HBs, with seroconversion and the appearance of Ac anti-HBs, but this goal is currently achieved in less than 10% of treated patients. The most realistic objective remains the long-term suppression of viral replication, which can reduce necrosis and hepatic inflammation and improve fibrosis. However, the impact of this strategy on hepatocarcinomia remains ambiguous. The issues of cost, toxicity, long-term tolerance and resistance related to antiviral drugs remain to be solved.

Two types of therapeutic strategies can be envisaged using approved drugs:

• a "fixed-term" treatment, intended to provoke a prolonged response after treatment stoppage. This type of response requires immunologic control, primarily using interferon α (INF-α);

• an "indefinite-term" treatment, aimed at normalizing ALATs and maintaining suppression of viral replication, without the emergence of resistance. Nucleoside analogs are used for this in the framework of mono or bitherapy.

Therapeutic strategy and drugs are determined according to the stage of the disease and other variables such as Ag Hbe / Ac anti Hbe status, viral replication rate, ALAT level, complications and co-morbidities. The choice of currently-approved drugs includes conventional INF-a and pegylated INF-α, lamivudine, adefovir and entecavir.

In his study (Annals Intern Med 1993), Wong found 7.8% HBs seroconversions, 33% loss of Ag HBe, and 37% of patients with controlled viral replication after treating with conventional INF-α. In an evaluation of peg INF-α versus lamivudine versus peg INF-α plus lamivudine (Lau et al. 2005), bitherapy was shown to offer better virologic control, but a lower rate of HBe seroconversion in the lamivudine arms. The speaker had compiled several studies on traditional INF-a in HBe antigen-negative patients, with posologies varying from 3 to 10 MU x 3/week. Prolonged response was doubled with 1-year treatment versus 6 months. In Marcellin’s study (N Engl J Med 2004), a 1-year treatment showed 20% sustained virologic responses at 72 weeks with peg Inf-α monotherapy or lamivudine bitherapy.

In conclusion, a 48-week monotherapy treatment with peg INF-α appears to offer the greatest chance of prolonged response in patients with positive or negative Ag HBe.

However, as the majority of the patients cannot expect a sustained biological and virologic response, what is the impact of alternative drugs? Sufficient exists data on the efficacy and short-term resistance of approved drugs at all stages of the disease; the best long-term data relate to adefovir (5 years), lamivudine and entecavir (2 years).

After 30 months of monotherapy with lamivudine, the risk of emergence of a resistance mutation is evaluated at over 50%.

Virologic response at 48 weeks is as high as 69% with entecavir, against 38% in the lamivudine arm in Ag HBe+ patients (Shouval et al. Hepatology 2004). Entecavir also performs best on Ag HBe negative patients at one year: -5.2 log versus -4.7 for lamivudine (Manns et al. EASL 2005). In naive patients, no entecavir resistance is found at 2 years. Conversely, 10% of strains are lamivudine-resistant. Entecavir therefore appears to be the most effective analog for patients with positive or negative Ag HBe.

With adefovir at 10 mg/day, maximum viral suppression is reached late on, after one year of treatment, and resistance can be feared in the event of incomplete response to M12. Primary nonresponse is higher in Ag HBe + patients, and is related to a high initial viral load.

In Ag HBe+ patients, Marcellin (Hepatology 2004) found 56% undetectability at 3 years, 81% normalized ALAT, 51% loss of Ag HBe and 43% HBe seroconversion. A sustained response is associated with early seroconversion during the first year; prolonged response is more random if it occurs late, if the adefovir treatment is of limited duration and with genotype C virus.

Hadziyanis reported his long-term follow-up study on Ag HBe negative patients on adefovir (AASLD, 2005). Viral undetectability was maintained at 96 weeks in the arms receiving adefovir, and ALATs were stabilized in over 75% of patients after at least 1 year of treatment. Virologic and biological response increased with treatment time.
Serologic cure was 5% after 4 to 5 years. At 5 years, there was an improvement in necrosis and hepatic inflammation in 80% of cases, and fibrosis score in 71% of patients. The cumulated probability of genotypic resistance was 29% at 5 years. The rate of viral replication after 1 year of treatment was predictive of resistance emergence at 3 years, and was 67% in the event of HBV DNA over 6 log, 26% between 3 and 6 log and 4% with under 3 log. Two-thirds of patients remained undetectable after 4 to 5 years of treatment.

Among the 33 patients from the cohort followed up 15 month after treatment stoppage, two-thirds were in biological remission and one-third remained virologically undetectable at the threshold of 104 copies/ml (unpublished).

In response to the questions put forward, Professor Hadziyanis commented on the lack of interest of nucleoside analog/interferon combinations. He reiterated that interferon is a nonspecific stimulator of the immune system, requiring the presence of viral antigens to develop an adapted response. In his opinion, we should favor alternative therapeutic paths, using vaccine stimulation, rather than attempt to stop viral replication when the objective of cure is required.

Regarding the controversy relating to analog mono or bitherapy, the speaker reminded us that a combination of two drugs with different resistance profiles is of proven interest in the event of lamivudine resistance and prevents, as with HIV infection, the emergence of drug-resistant strains. This could be an efficient future strategy, even for naive patients.

**Stanislas Pol** (Paris, France) advised that optimized HCV infection treatment currently means administering peg INF-α and ribavirin bitherapy at the right time, at the right doses and for the right length of time. It also means improving adherence, and knowing how to recognize and abandon ineffective treatments.

Hepatitis C can be treated at any time. The natural evolution of this infection includes the emergence of hepatotropism and lymphotropism, with their string of complications at the extreme stages of the disease, when therapeutic efficacy is reduced, but its need is strongest (figure 1).

Very few cases of acute hepatitis C are currently recognized, they occasionally occur during kidney dialysis, in the event of accidental exposure to blood, or during homosexual transmission in HIV subjects. It is currently necessary to administer bitherapy for 24 weeks – this is the best moment to achieve efficacy for hepatitis C. Given the possibility of spontaneous cure, which can reach up to 30 to 40% in mono-infected subjects, virologic evolution in icteric symptomatic patients could be monitored for up to 12 weeks. In the event of co-infection with HIV, the spontaneous rate of cure is lower than 15%. Therefore, in the future, it would be preferable to treat all cases of acute hepatitis C.

Mono-infected subjects should be administered a minimum posology of 800 mg/day ribavirin with a standard dose of peg INF-α for 24 weeks for genotypes 2 or 3; other genotypes should ideally be treated for 48 weeks, with at least 13 mg/kg ribavirin and a standard dose of peg INF-α. Considerable progress has recently been made on early viral kinetics and test sensitivity, enabling rapid forecasting of the chances of therapeutic success (figure 2).
After treatment initiation, we can distinguish three general situations:

- **Therapeutic nonresponse**, which represents nearly 10% of patients; in this case, treatment continuation is useless;

- **Rapid response**, with 64% of patients neutralizing HCV RNA within three months; certain studies prone a reduction in treatment duration from 24 to 12 weeks for genotypes 2 or 3, and from 48 to 24 weeks for other genotypes in patients with undetectable RNA at week 4. Two studies supporting of this strategy have already been published (Zeuzem et al. J Hepatol 2005; Von Wagner et al. N Engl J Med 2005). However, the number of test subjects remains low and, conversely, an as yet unpublished ongoing study has shown an increased risk of relapse if treatment duration is reduced. This practice should be reserved for very bad tolerance situations, and genotypes 1 or 4 with a viral load below 250,000 IU/ml prior to treatment.

- **In the event of slow response**, treatment prolongation is recommended and the concept of undetectability duration begins to emerge (figure 3).

The study conducted by Berg (Gastroenterology 2006), evaluating prolonged response after 48 and 72 weeks of treatment does not show any advantage in intent to treat analysis further to therapeutic stoppage due to poor tolerance. In “per protocol” analysis however, an advantage was shown for genotypes 1 in case of high initial viral load and in overweight patients. Conversely, Spanish studies clearly favor treatment prolongation for prolonged response in patients with still-detectable HCV RNA at week 4. In the Sanchez-Tapias study (Gastroenterology 2006), prolonged response for genotypes 1 was 28% in the group treated for 48 weeks versus 44% in patients treated for 72 weeks.

### Therapy duration according to viral kinetics

![Figure 3: concept of undetectability duration](image)


After initial bitherapy failure, it may be advisable to double the posology of peg interferon-α, as in the Diago study (AASLD 2004, abstract 522): this doubled the chances of success in initial nonresponders (i.e. 37.5%), and particularly those with high viral loads. Moreover, no impact on tolerance was observed. A similar but larger study was conducted by Gross (AASLD 2005, abstract A60) using interferon α2b. He found a mediocre 17% prolonged response, although this remained higher than the 12% achieved with traditional posologies. The other alternative is to increase ribavirin posology from 13 to 15 mg/kg. This molecule has undeniable dose effect, and its combination with EPO reduces hematologic side effects and enables the initial ribavirin posology to be maintained. Many studies have proved the interest of this strategy, which is accompanied by a lower rate of relapse after treatment stoppage: 8% versus 40% in the studies of Shiffman et al (AASLD 2005, abstract A55).

Treatment adherence also modulates the chances of therapeutic success; a significant reduction in prolonged response occurs with adherence of less than 97% (Reddy, EASL 2005). Protecting patients against adverse effects with the help of psychotropics, antidepressants, EPO and anti-inflammatories will improve the chances of...
cure. The use of EPO maintains effective ribavirin at 83% at doses above 800 mg/day (Afdahal, Gastroenterology 2004). Moreover, hematopoietic growth factors do not have a negative virologic impact. France’s Official Drug Agency is expected to publish a law allowing the use of EPO in the framework of secondary anemia during treatment of hepatitis C, which is outside its recognized uses.

The follow-up of 184 Irish women contaminated after the injection of anti-D immunoglobulins in 1977 revealed a low rate of progression over 20 years later. 18% underwent a 2-point increase in inflammation score, 27% underwent a 1-point increase in fibrosis score, and the remainder of the cohort is stable or regressing (Levine et al. AASLD 2005, A586). Treating patients properly also requires patience.

Various means of evaluation are currently at our disposal, such as hepatic biopsy, the FIB 4 or the Fibrotest® (which assess fibrosis score according to biological parameters), elastometry, or Fibroscan®: these tests all have different sensitivities according to the stage of the disease and can be used in a complementary manner.

In essence, Stanislas Pol stressed that we must know how to optimize current treatments - we already possess the necessary means and tools to achieve this goal. In the medium term, we will not be able to bypass the INF-ribavirin combination, although new and very promising molecules should offer alternative solutions in the future.

Marc Bourlière (Marseille, France) offered us his vision of future options for nonresponding or relapsing HCV patients. He reminded us that nonresponse is defined as being the persistence of positive viremia after treatment stoppage, due either to defective therapy – in which case a different bitherapy must be introduced using the criteria developed by S. Pol, or a triple combination – or to genuine resistance of the viral strain. In the latter case, strategy should be more subtle: waiting is recommended if fibrosis score is weak or moderate; if not, an alternative therapeutic scheme could be attempted (figure 4).

Among the molecules which could play a future role in the treatment of difficult cases, Marc Bourlière highlighted:

- **alpha thymosine**: a pilot study conducted by Poo et al. (Hepatology 2004), evaluating a standard bitherapy plus alpha thymosine, obtained 48% virologic response at the end of the treatment;

- **interferon alpha consensus**: after a standard 12-week bitherapy, nonresponding patients were treated with alphacon and ribavirin (Leevy et al, Hepatology 2004). 43% were undetectable after 48 weeks and 37% had sustained virologic response… But this molecule will unfortunately not be developed in France!

- **albuferon**: this drug was evaluated on nonresponding genotypes 1 (Rustgi et al, EASL 2006). It is an interferon alpha 2b binding on human albumin. The best results were found with posologies of 1500 and 1800 µg every 2 weeks. Albuferon gave 15 to 20% benefit versus pegylated interferon;

- **valopicitabine** (NM283) shows virologic efficacy of above 1 log at 24 weeks versus a pegylated bitherapy (Afdhall et al, EASL 2006). However, its development will probably be suspended due to overly-frequent digestive side effects.
Two protease inhibitors administered by oral route are currently in the advanced stages of development:

- **SCH 503034** SCH 503034 at 400 mg x 3/day, combined with peg INF-α2b: viral load drops by 3 log by day 12;

- **VX 950** VX 950 at 750 mg x 3/day has proven virologic efficacy above 4 log by day 14. However, problems of early resistance have been pinpointed in the event of discontinuous or imperfect response, related to weak plasma concentrations of the drug. Its combined use with peg INF-α gives a faster and more regular virologic decrease. In this case virologic efficacy is higher: -5.5 log by day 14.

Using ritonavir at 5 mg/kg to boost these two protease inhibitors improves pharmacokinetic parameters (Kempf et al, EASL 2006); it increases the area under curve 8 times for VX 950 and 20 times for SCHC 503034. Their combination with peg INF-a plus a ritonavir boost offers higher virologic efficacy and should avoid the selection of resistant viruses.

- **Cpg 10101** is an HCV entry inhibitor. Its efficacy is dose-dependent on relapsing genotypes 1 (McHutchison et al, EASL 2006). In a bitherapy context, this product is comparable to ribavirin; in triple drug combinations with peg INF-a and ribavirin, virologic efficacy is above 3 log at week 12.

Maintenance treatments are intended to prevent or slow down the progression of fibrosis, and reduce morbidity and mortality related to liver sclerosis and its complications. Colchicine and IL 10 should not be used for maintenance therapy; the only interesting candidate with proven clinical benefit is INF-α. Three major studies (EPIC, COPILOT and HALT-C), scheduled to last for 4 years, will evaluate the anti-fibrosing power of currently-available interferons. Intermediate results at 2 years have been published by the COPILOT study (Curry et al, EASL 2005) comparing 800 patients including 80% cases of sclerosis divided into 2 arms: colchicine versus peg INF-α2b (0.5 µg/kg). Complications related to portal hypertension were respectively 27% and 11%. The Kaiser study (EASL 2006) is assessing a 36-month maintenance treatment using peg INF-α2b (0.5 to 1 µg/kg) versus no treatment; three liver biopsies were performed on day 0, at 18 months on therapy and 6 months after treatment stoppage. The ISHAK score is statistically lower in the interferon arm. For these critical patients, treatment maintenance must be associated with the fight against co-morbidities such as alcohol, tobacco and obesity. Insulin resistance must be corrected.

The management of antiretroviral treatments in patients suffering from liver sclerosis was covered by **Dominique Salmon-Céron** (Paris, France)

HAART has been shown to reduce liver-related morbidity and mortality: the 2003 Qurishi study published in The Lancet showed a statistically significant benefit on overall mortality and hepatic-related mortality in patients on HAART versus patients receiving a nonoptimal antiretroviral treatment or no treatment. The hepatotoxicity of these drugs is particularly frequent in the event of severe hepatic lesions, although this carries little weight in terms of positive/negative impact. Antiretroviral clearance is decreased, with a risk of hepatic decompensation or toxic attack on other organs. A correlation exists between toxicity and plasma levels of certain antiretrovirals. Antiretroviral drugs must therefore be specifically selected in the event of liver sclerosis.

The intrinsic hepatotoxicity of each drug is judged in the short term. The Aranzabal study (Clin Inf Dis 2005; 40, 588) found 15% overall hepatotoxicity in patients co-infected with HCV F1/F2 and 38% for stages F3/F4. NNRTI toxicity rose according to the degree of fibrosis, but remained lower than in combinations without an NNRTI: respectively 11% and 19% for stages F3/F4. The TARGET cohort found a significant relative risk with patients aged over 60 years, HBV/HCV co-infections, ritonavir at full dose and NNRTIs.

Long-term hepatotoxicity is represented by steatosis, with microvesicles typical of mitochondrial toxicity and macrovesicles. Steatosis affects 40 to 67% of co-infected patients on HAART, and is severe in 7% of cases (Sulkowski et al, AIDS 2005). Risk factors are obesity, genotype 3, a high fibrosis score and Caucasian ethnic background, plus, as regards the antiretroviral treatment itself, insulin resistance, lipodystrophy, the use of d4T, and the cumulative use of PIs. D-drugs (d4T, ddi, ddC) have a clearly negative impact on lactate levels and mitochondrial hepatic DNA, which is reduced by 47% (Walker et al, Hepatology 2004).
Antiretroviral pharmacokinetics are disturbed in the event of sclerosis. Protein synthesis decreases, free-form molecules increase, the hepatic metabolism is diminished by P450 cytochrome enzymes, and hepatic flux and clearance drop. This results in a rise in maximum concentrations (Cmax) and area under curve (AUC).

What results have been obtained with the various molecules currently under test?

- **Nelfinavir**: 3-fold increase in Cmax and AUC in case of sclerosed versus nonsclerosed patients in both HCV positive and HCV negative populations;

- **Lopinavir**: increase in the AUC and a significant increase in the fraction unrelated to proteins; little impact on overall level (Arribas et al, EACS 2003).

- **Amprenavir**: relationship between APV level and Child score. The GSK laboratory has now established a dose correspondence for this product:
  - in the event of normal liver functioning: 1200 mg x 2/day,
  - for Child scores between 5 and 8: 450 mg x 2/day,
  - for Child scores between 9 and 14: 300 mg x 2/day.

- **NNRTIs**: the Nevadose study (Dominguez et al, Croi 2005) was based on a fibrotest and nevirapine Cmin dosing in co-infected patients versus mono-infected HIV patients. Respectively 66% against 27% of F4 patients had nevirapine levels above 6000 ng/ml.

- **NRTIs**: only AZT and ABC are metabolized by hepatic enzymes; the other molecules of this class are 50 to 80% excreted by the kidneys. The reduction in AZT clearance is related to the Child score (Taburet et al, CPT 1990). The GSK laboratory recommends reducing ABC to 150 mg/day in sclerosed patients with a Child score above 6. This product is contra-indicated in case of decompensated sclerosis.

- **Tenofovir**: no impact. This product is virtually unrelated to proteins; it is eliminated by the kidneys in unchanged form.

However, we must not forget that the metabolism of antiretrovirals is prone to inter and intra-individual variability, genetic polymorphism and compensation mechanisms, which can develop even in the event of severe liver disease…

To conclude, the advantages of HAART are far greater than the hepatotoxic risk these drugs represent. Standard posologies should be used for patients with moderate hepatic deterioration, and pharmacological dosing is recommended when possible. A number of recommendations exist for APV and ABC.

To minimize the risk of increased fibrosis, antiretroviral combinations should however be selected according to the safety of their metabolic profile. D4T, ddi and triple nucleoside combinations should be avoided, atazanavir should be the premier PI choice, metabolic disorders should be treated along with hepatitis viruses, and obesity avoided.
Dr. D’Souza is head of the HIV Vaccine development program at the NIH – NIAID (National Institute of Health - National Institute of Allergy and Infectious Diseases), Bethesda (USA)

Alain Rieu : Can you tell us briefly about your role at this prominent NIH Research Centre?

Patricia D’Souza : The NIH believes that a variety and a breadth of approaches are needed to combat the HIV-AIDS epidemic. Specifically, the mission of the Division of AIDS at NIH is to help ensure an end to the HIV/AIDS epidemic by increasing basic knowledge of the pathogenesis and transmission of HIV, supporting the development of therapies for HIV infection and its complications and supporting the development of vaccines, including other prevention strategies such as topical microbicides, and education and behavior modification research. With regards to vaccine research, the NIH has now conducted or initiated approximately 80 Phase I and two Phase II vaccine clinical trials on nearly 50 vaccine candidates, individually or in combination, and in collaboration with our partners in academia and industry.

A. R. : How many types of vaccine are currently on trial and in which countries?

P. D. : HIV vaccines are done in three phases:

- Phase 1 trials involve a small number of healthy, low risk individuals to test the safety of the vaccine. Currently we have nine different products that are in phase 1 trial, ranging from HIV-1 recombinant proteins expressed either by DNA plasmids or by viral vectors, to prime-boost strategies that test combinations of products.

- Phase 2 trials are done on a larger number of individuals and evaluate safety, route, dose and immunogenicity. At the moment we have two phase 2 trials, and one that's going to start at the end of this year. One trial is using a recombinant viral vector vaccine; the other is using a DNA prime followed by the recombinant viral vector boost.

- Phase 3 trials involve several thousand volunteers to determine efficacy. One prime-boost product, a recombinant viral vector and a recombinant protein are currently on efficacy trial in Thailand.

All the vaccine trials are conducted within a global network. For example, the HIV Vaccine Trials Network (HVTN), supported by the NIH, has trial sites in over 27 cities on 4 different continents.

A. R. : What are the hindrances to the development of HIV vaccines?

P. D. : There are many impediments to vaccine development; I’ll discuss the main ones:

The first is the fact that HIV is notoriously diverse: there are many subtypes of the virus, and within a single subtype and in a single infected person, the virus also changes constantly, so a vaccine will need to protect against multiple subtypes.

The second is that HIV infects CD4+ T cells - these are the specific immune system cells necessary to make and orchestrate an immune response.

The third is that HIV can actually evade an immune response: it has a lot of sugars and variable loops which actually act like a mask - they cover the epitopes that are really critical for inducing a good neutralizing antibody response. In fact, generating broad and potent neutralizing antibodies to a vaccine candidate is one of the most difficult problems in the field today. A number of exciting strategies are being pursued to address this problem, such as trying to molecularly or structurally mimic the envelope protein in a form that is similar to how it appears on the virus particle, deleting various regions of the envelope protein, and/or stripping off the sugar residues to reveal hidden epitopes. None of these approaches have yielded a product that is able to elicit broad, cross-neutralizing antibodies as yet.

- The fourth is that we don’t know what elements are necessary to protect against HIV infection. There is no documented case where an infected individual has been able to completely clear the virus from the body, which means that uniquely among viruses, HIV evades the body’s immune system. Consequently, a protective immune response to a vaccine will have to be better than the natural immune response.
**A. R.** : By sugar-shields you mean glycosylation sites?

**P. D.** : Yes, sites which have sugars attached to them.

**A.R.** : One of my colleagues wanted to ask you about the usefulness of the database of multiple epitopes - based at the Los Alamos Centre I believe - provided partly by long-term non-progressors (LTNPs) and partly by exposed, non-infected patients, I mean sex-workers in the Nairobi program for example. Are these epitopes used for future vaccines or not?

**P. D.** : There are vaccine strategies based on “protective epitopes” identified from studies on commercial sex workers in Kenya and Gambia. These vaccines express a string of minimal epitopes and have been tested for their ability to generate broad T cell protective responses. There are also multi-epitope combinations of peptides, fusion proteins and lipopeptides in an early stage of clinical development, either alone or in prime-boost with live vector-based recombinant vaccines. A multi-epitope DNA vaccine, followed by a boost of multi-epitope recombinant fusion protein or a recombinant viral vector encoding the same epitopes has been developed by a company called Pharmexa-Epimmune and is currently undergoing testing. To date, these types of epitopic-based vaccines are poorly immunogenic in early human clinical trials, and newer concepts are incorporating cytokine plasmids as a potential way to enhance the immunogenicity of these vaccines.

**A. R.** : The fourth question relates to the tat gene, which appears to be a good target for improving HIV vaccine efficacy. Are any vaccines being developed with this tat gene as a compound to obtain anti-tat antibodies?

**P. D.** : The concept of using tat in a vaccine is based on the fact that tat is expressed very early in the life cycle of the virus and therefore antibodies to tat might lead to rapid elimination of infected cells. Studies in animal modes have suggested that immunization with tat can protect against subsequent viral challenge. A Phase I vaccine clinical trial of a tat subunit has been carried out in Italy. The vaccine was safe and immunogenic and a Phase II trial is currently being planned. A tat-nef fusion protein that was combined with a recombinant gp120 in a novel adjuvant, developed by Glaxo Smith Kline was recently tested in humans. Although the vaccine was able to induce CD4+ T cell responses, it did not induce CD8+ T cell responses.

**A. R.** : I remember Richard Koup spoke to me two years ago about a multigene of multiclades in his VRC vaccine, but not about the tat gene.

**P. D.** : Yes, tat is not present in the VRC vaccine. The VRC vaccine is designed as a DNA prime followed by an adenoviral 5 boost; both of these vaccines express envelopes from clades A, B, C and gag, and pol and nef genes from clade B. Early data on this vaccine look very promising: it is capable of eliciting CD4 and CD8 responses as well as antibodies. This vaccine has been called a “global” HIV vaccine because it is targeted to multiple HIV subtypes found worldwide. It has currently moved into Phase II of clinical testing.

**A. R.** : About vaccine cost: we know that DNA vaccine has a very high production cost. Is this systematically the case?

**P. D.** : Vaccine development and manufacture requires scarce technical resources and lots of money. There are two major barriers to manufacturing HIV vaccines. The first is that most HIV vaccine developers work in academic institutions and lack the appropriate expertise and specialized facilities to do the preclinical vaccine research and manufacture clinical grade material for human clinical trials. The second is that investments in large scale manufacturing capacity need to be catalyzed and incentivized. Pharmaceutical companies are unwilling to engage fully in vaccine development because many scientific barriers still exist to HIV vaccine development and therefore there are financial risks associated with getting involved in this field.

**A. R.** : I think this is a very important point for developing countries: if the cost is excessive it will impede treatment of the HIV epidemic.

**P. D.** : Excellent cooperation between governments, academia, industry and philanthropies are needed to ensure a reliable vaccine supply. At present,
pharmaceutical companies are reluctant to enter or remain in the business of manufacturing vaccines because of the lack of a robust market in poor countries and the lack of financial incentives that make vaccine manufacture a risky business. Pharmaceutical companies require financial and economic incentives including fair pricing, guaranteed purchase of unsold supplies, tax incentives, a streamlining of the complex regulatory processes needed for vaccine licensure, liability protection, and awareness by government decision makers and the public to ensure a steady supply and demand for vaccines.
The symposium opened with a plenary presentation by Doctor Docteur Alice Croisier (Geneva, Switzerland) on bird flu.

This pathology particularly affects animals, although some cases of human infection have been recorded. The main proteins comprising the flu virus are hemagglutinin (H) and neuraminidase (N). Hemagglutinin incorporates 16 different subtypes; neuraminidase incorporates 9. They can undergo two types of structural change:

- drift: slight modification of the amino acid sequence: the subtype remains the same
- shift: major modification with emergence of a new subtype.

The avian influenza H5N1 virus was first observed over 10 years ago. It emerged in Asia (1996), carried by poultry and wild birds.

This highly pathogenic form of bird flu is capable of crossing species barriers, and contaminating man; on June 21, 2006, 130 deaths had been recorded on a worldwide level resulting from this emerging pathology. Interhuman transmission has probably already occurred in Thailand, although no formal proof exists.

The essential threat comes from the possible recombining of H5N1 with another flu virus from the A group, making it capable of infecting man directly.

The majority of current human cases are located in the Southeast Asia, i.e. Thailand, Vietnam and Cambodia. Cases have also been reported in Turkey, Egypt and Iraq. The disease affects all age groups, although the subjects are predominantly young. Slightly more women are affected then men, although this could be due to their lifestyle (child care, farm work involving contact with poultry).

This pathology is usually characterized by symptom emergence 2 to 6 days after contamination, with a fever above 38°C, often liquid diarrhea, and abnormal conjunctivitis. The acute respiratory syndrome may appear between the 4th and 13th day.

This pathology is continuing to expand its reach, and its future transmission is a genuine threat. No vaccine is available at the present time. The interval between various flu pandemics appears to vary from 10 to 30 years. Pandemics are systematically related to type A viruses.

The strategy for fighting avian influenza is currently being elaborated on a global level thanks to coordinated efforts by international organizations such as the FAO, WHO and UNICEF. It will require preparation of an immediate response, plus long-term action. Guidelines were drawn up and published in April 2006 (H5 Reference Health Structure).

A flu monitoring network (WHO Global Influenza Network) was set up as far back as 1947; it has now been supplemented with 3 H5N1 virus reference laboratories. It is unlikely that a vaccine would be available during the initial phase of a possible pandemic, as adapted vaccine development takes 6 months: 2 months for preparation, 2 months for efficacy testing and 2 months for the clinical development phase. Stocks of antiviral drugs are also limited. However, efficient intervention must take place within the first thirty days, after which it would already be too late. Limiting morbidity and mortality during the first wave of an epidemic would therefore depend on efficient action by nonmedical public authorities, including the following measures:

- isolation of diagnosed cases (quarantine at home);
- other more exceptional measures: are we ready for these?

Carers will be affected to the same degree as the general population; this is a major problem.

The H5N1 virus represents an immediate threat, which could persist for years. However, the fact that the WHO and FAO are giving priority to preparation on both a national and international level may make a big difference in terms of the human and socio-economic consequences of a flu pandemic.

The veterinary aspects of avian influenza were also tackled during a special session on emerging infectious diseases presented by Arjan Stegeman (Utrecht, Netherlands). Bird flu is a poultry disease; its highly pathogenic forms (High Pathogenicity Avian Influenza; HPAI) are represented by viruses H5N1 and H7N7. H7N7 was responsible for a major flu pandemic in the Netherlands, leading to the destruction of 30 million poultry and the death of one veterinary surgeon. This pathology affects the higher respiratory tract and can also cause conjunctivitis. However, some species such as ducks may remain asymptomatic, but continue to transmit the virus. The virus is mainly transmitted through the air from the higher respiratory tract – i.e. at short distances - but it also invades the digestive tract and may be transmitted by feces, leading to persistent long-distance transmission. From a veterinary viewpoint, preventive
action must focus on this aspect, as feces can contaminate objects, vehicles circulating from one farm to another, and farm workers. But controlling this mode of transmission is not as simple as it appears, as poultry farms have varying safety levels, or rather "biosafety" levels; they can range from industrial farms with high safety levels (FAO sector 1), to village backyards (sector 4; no protection), through FAO sector 2 farms - considered as having inadequate biosafety as they are in open contact with the environment (e.g. Thailand) -, or sector 3 farms, which are generally small producers.

The expansion of the pandemic between 1996 and 2003 means that the H5N1 virus now exists in an endemic state throughout most of the world, and may not disappear rapidly. The two foremost criteria for epidemic control are elimination and vaccination. The proper elimination of contaminated animals is very important and involves a genuine massacre... Transmission can be prevented in chicken and ducks fourteen days after vaccination. However, vaccine potency varies according to geographic zones: Hong Kong and Italy have reported good results, while Mexico and Asia have less favorable statistics.

The threat of an avian influenza pandemic subsequent to mutation or genetic recombination remains ongoing. Various measures are therefore recommended: contact between poultry farms and migrating or sedentary wild birds, live poultry markets and pet birds should be prevented. The circulation of poultry farm personnel and equipment must be regulated and hygiene measures strictly applied. Infected farms must be eliminated and neighboring farms depopulated. In areas with a high density of poultry farms, ponds or lakes, measures must be reinforced either by sacrificing birds throughout a zone, or vaccinating them. Farm personnel must be informed of the associated risks and trained in implementing hygiene measures.

James Sevjar (Atlanta, USA) reminded us that the West Nile virus was first isolated in 1937 in Uganda. This enveloped RNA Flavivirus, belonging to the Arbovirus family, is transmitted by Culex mosquitos. An upturn in West Nile virus infections has been observed in North and Central America. The epidemic spread rapidly from the East coast of the US between 2000 and 2005, affecting both humans and animal cases to an unprecedented extent. From 2005 onwards, the West Nile virus became endemic in the USA.

Limited epidemics have also broken out in Europe, including in France, in wet zones where the vector (culex modestus) and bird reserves (migratory birds) cohabit. Interestingly, 86% of human infections are silent. 20% of cases develop fever and headaches, with extreme fatigue and rash. Infection causes severe neuroinvasive encephalitis in 1% of cases (especially in ageing and immunodeficient people). The latter syndrome also incurs additional symptoms which initially led to confusion and mis-diagnosis, such as abnormal movements (shaking, myoclonias, cerebellous ataxia), or a Parkinson's syndrome provoked by virus neurotropism, in particular in thalamic centers.

Patients suffering from neuroinvasive encephalitis may also develop a poliomyelitic-like syndrome (absence of fever and meningitis, cephalgia in 20% of cases). The attack may be assymetric, often with monoplegia and possible respiratory problems, persistent fatigue, functional disorders and highly invalidating after-effects.

Lisa Jones-Engel (Seattle, USA) developed the theme of primate retrovirus transmission to man. She initially recapped already-known transmissions from primates to humans (mycobacteria, dengue), focusing on physiologic, morphologic, behavioral and genetic similarities. Humans and primates have been in close contact in Asia for centuries (temples, parks, hunting, pets, etc.), but the immunologic context has now evolved, particularly in terms of immunodepressed populations (HIV), hence offering new targets for pathogenic agents. Asia is an ideal territory for the study of virus transmission from non-human primates to man. Remarkably, a nonpathogenic retrovirus has been identified as a transmission marker (Simian Foamy Virus: SFV). Data collected on this virus show a seroprevalence of 3.4% in various populations (temple workers, owners of pet monkeys, hunters, trainers and people who have lived near temples). This seroprevalence was not explored in monkeys living in cities, or in the tourist population. However, several studies have revealed that 5% of foreign tourists have been bitten by monkeys versus just 1% of local tourists, with low figures for workers on the island of Bali. Using virologic techniques and additional approaches such as molecular biology, epidemiology, plus statistical and mathematical analysis, the author is currently researching emerging zoonoses in primates and evaluating the risk of their transmission to man. She concludes that it would be unwise to consider Africa as the only continent likely to foster new diseases; the infrastructures, populations and conditions found in Asia offer a favorable ground for the emergence of new infectious pathologies.
GLOSSARY

3TC: lamivudine
ABC: abacavir
ATC: apricitabine
APV: amprenavir
ATV: atazanavir
AUC: area under curve
AZT: azidothymidine
CBV: combivir (TM)
d4T: stavudine
ddC: dideoxycytidine
ddi: didanosine
EFV: efavirenz
FTC: emtricitabine
IDV: indinavir
PI: protease inhibitor
ITT: intent to treat
LPV: lopinavir
LPV/r: lopinavir boosted by ritonavir
MDR: multi-drug resistance
NFV: nelfinavir
NRTI: nucleoside analogue reverse transcriptase inhibitor
NNRTI: non-nucleoside analogue reverse transcriptase inhibitor
NVP: nevirapine
PR: Protease
RT: reverse transcriptase
SQV: saquinavir
TAM: thymidine analogue mutations
TDF: tenofovir
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