Nobel Lecture

25 years after HIV discovery: Prospects for cure and vaccine ☆

Luc Montagnier

World Foundation AIDS Research and Prevention, UNESCO, Paris, France

The impressive advances of our scientific knowledge in the last century allow us to have a much better vision of our origin on earth and our situation in the Universe than our ancestors. Life has probably started on Earth around 3 and half billion of years ago and a genetic memory has early emerged, based on an extraordinary stable molecule, the DNA double helix, bearing a genetic code identical for all living organisms, from bacteria to men. We are thus the heirs of myriads of molecular inventions which have accumulated over millions–sometime billions–of years. The environmental pressure has of course both maintained these inventions and also modulated them over the generations, through death of the individuals and sexual reproduction. For the last 30,000 years, our biological constitution has not changed: a hypertrophic cortical brain, a larynx to speak and a hand to manipulate. But for the last 10,000 years, another memory has emerged, which makes our species quite different from the others: this is the cultural memory which transmits knowledge and societal organization from generation to next generation, through the use of language, writing and more recently virtual communication means.

This revolution occurred in several sites on the earth almost simultaneously through sedentarization of human populations by agriculture leading to several civilizations. Each human being thus receives two luggages, the genetic memory at birth and cultural memory during all his life and he will become a real human only if he is benefiting of both. For the last 3 centuries, particularly in the 20th century, our scientific knowledge has increased exponentially and has diffused all over the world.

We have a tendency to consider ourselves as pure spirits, but the hard reality still reminds us of our biological nature: each of us is programmed to die and, during his life, is exposed to diseases. At the dawn of this new century, we are still facing two major health problems:

➢ New epidemics related to infectious agents (mostly bacteria and viruses)
➢ Chronic diseases (mostly cancers, cardiovascular, neurodegenerative, arthritic, auto-immune diseases, diabetes) linked to the increase of life expectancy and environmental changes related to human activities.

This presentation will be obviously focused to one new epidemic, AIDS, but we should not forget that there are other persisting and life endangering epidemics, especially in tropical countries such as malaria and tuberculosis.

Moreover, other new epidemics should not be excluded as human activities generate more favouring factors:

➢ Lack or loss of hygiene habits
➢ lack of water
➢ globalisation and acceleration of exchanges, travels
➢ atmospheric and chemical pollution leading to oxidative stress and immune depression
➢ malnutrition, drug abuse, aging, also leading to immune depression
➢ global warming leading to new ecological niches for insect vectors
➢ changes in sexual behaviours

This last factor and immune depression caused by malnutrition, drug abuse and increased co-infections, are probably the causes of emergence of AIDS as a global epidemic, affecting most if not all continents including recently Polynesia islands.

The causative agent existed in Africa before the emergence of the epidemics in Central Africa and North America in the 1970s. As there exists related viruses apparently well tolerated in non-human primates, it is tempting to consider AIDS as a zoonosis, resulting of the transmission to human of related viruses infecting primate species without causing disease.

But let us first recall the circumstances of HIV discovery in my laboratory at the Pasteur Institute (Fig. 1).

AIDS as a pathologic distinct entity was first identified in June 1981 by members of the CDC (particularly James Curran) after reports received from two medical doctors, Michael Gottlieb in Los Angeles and Alvin Friedman-Kien in New York, of clusters of opportunistic infections and Kaposi sarcoma occurring in young gay men which had related sexual intercourse.

Following publication of this report in the CDC Bulletin, similar cases were described in Western European countries and particularly in France by a group of young clinicians and immunologists led by Jacques Leibovitch and Willy Rozenbaum.

It was soon recognized that a similar disease, characterized at the biological level by a profound depression of cellular immunity and clinically by infections previously described in chemically or genetically immunodepressed patients, also existed in haemophiliacs and blood transfused patients.

The case of haemophiliacs was giving a clue as to the nature of the transmissible agent: these AIDS patients had received purified concentrates of factor 8 or 9, made from pools of blood donors which had been filtrated by bacteriological filters.

Fig. 1. The Pasteur team involved in the discovery of HIV in 1983–1984.

This purification process should have eliminated any soluble toxic compound and the filtration should have retained bacterial or fungal agents: only viruses could be present in the preparations given to patients. This is why I become interested in a search for viruses; but what kind of viruses? Many viruses have immunodepressing activity, in order to persist in their hosts. This is particularly the case of herpes viruses (cytomegalovirus) and retroviruses. A putative candidate was the Human T Leukemia virus (HTLV) described by R.C. Gallo and Japanese researchers.

Having more expertise on retroviruses (see biography Chapter I), we embarked on the search for an HTLV-like virus, at the suggestion of the French working group and also incited by the Institute Pasteur Production, an industrial subsidiary of the Institute, producing an hepatitis B vaccine from pool of plasmas from blood donors.

Knowing that retroviruses are usually expressed in activated cells, I have set up classical conditions to grow in culture activated lymphocytes, using first a bacterial activator of both T and B lymphocytes, Protein A, since I ignored in which subset of cells the virus was hiding out.

The reasoning at that stage was that we should look first in lymphocytes from swollen lymph nodes, supposedly the site where viruses accumulate in the early phase of infection.

I received in January 3 a biopsy of a patient with cervical adenopathy, a symptom already recognized as an early sign of AIDS. After dissection of the sample into small pieces and their dissociation into single cells, the lymphocytes were cultured in nutrient medium in the presence of Protein A and anti-interferon serum.

In fact, after addition of Interleukin 2, only T lymphocytes were multiplying well and produced a small amount of virus detected by its reverse transcriptase activity, measured by my associate Françoise Barre-Sinoussi. Only some 9 months later could I show also growth of the virus in B-lymphocytes transformed by Epstein–Barr virus (Montagnier et al., 1984).

The viral growth ceased as the cellular growth started declining, but we could propagate the virus in cultures of lymphocytes from adult blood donors as well as in lymphocytes from chord blood. This allowed characterization of the virus, and showed for the first time that it was different from HTLVs. A p24–25 protein could be immuno-precipitated by the serum of the patient and not by antibodies specific of the p24 gag protein of HTLV1, kindly provided by Dr R.C. Gallo.

Electron microscopy of sections of the original lymph node biopsy, as well as those from infected cultured lymphocytes, showed rare viral particles with a dense conical core, similar to the retro-lentiviruses of animals (infectious anaemia virus of horse, Visna virus of sheep, etc.), but different from HTLV. Unlike the case of HTLV, we could never see emergence of permanent transformed lines from the infected lymphocyte cultures (Fig. 2).
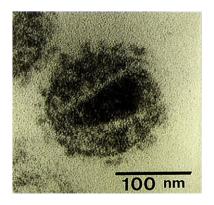

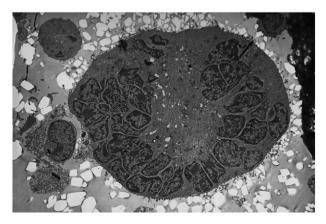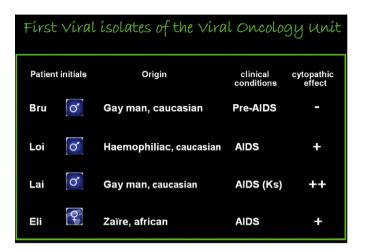**Fig. 2.** HIV at the electron microscope.

Fig. 3. Electron micrography picture of a giant cell (syncitium) resulting of the fusion of many lymphocytes expressing the HIV fusion protein (Montagnier et al., 1984).

These results were published in a Science paper in May 1983 (Barre-Sinoussi et al., 1983), together with two papers by Gallo and Essex groups in favour of HTLV being the cause of AIDS. During the following months, more data accumulated in my laboratory showing that this new virus was not a passenger virus, but was really the best candidate to be the cause of AIDS.

(1) The same type of virus was isolated from patients of different origins: gay men with multiple partners, haemophiliacs, drug abusers, Africans.

First Viral Isolates of the Viral Oncology Unit			
Patient initials	**Origin**	**clinical conditions**	**cytopathic effect**
Bru ♂	Gay man, caucasian	Pre-AIDS	−
Loi ♂	Haemophiliac, caucasian	AIDS	+
Lai ♂	Gay man, caucasian	AIDS (Ks)	++
Eli ♀	Zaïre, african	AIDS	+

(2) Besides immune-precipitation of viral proteins (p25, P18), serums from patients with lymphadenopathy syndrome and a fraction of the serums from patients with advanced AIDS, were positive in an ELISA test using proteins from partially purified virus (Brun-Vezinet et al., 1984).
(3) *In vitro*, the virus was shown to infect only CD4+ T lymphocytes and not the CD8+ subset (Klatzmann et al., 1984).
(4) A cytopathic effect was observed with isolates made from patients with late symptoms of AIDS. Particularly the third isolate made from a young gay man with Kaposi Sarcoma (Lai)

caused the formation of large syncitia, presumably due to the fusion of several infected cells (Fig. 3). Attempts to grow the first isolate Bru in T cell lines isolated from patients with leukaemia or lymphoma were unsuccessful. However, we discovered later (Wain-Hobson et al., 1991) that the Bru isolate was contaminated with the Lai isolate, which by contrast could be grown in T cell lines (CEM, HUT78) in laboratories which received our Bru isolate on their request.

In fact, a few laboratory isolates were shown to grow in mass quantities in T cell lines, facilitating analysis of the virus and its use for detection of antibodies by commercial blood tests.

Our data which I presented in September 1983 at a meeting on HTLV in Cold Spring Harbor (Montagnier et al., 1984) were met with skepticism and only in the Spring of 1984, the description of a quasi identical virus under the name of HTLV III by the group of R.C. Gallo (Popovicc et al., 1984) convinced the scientific community that this new retrolentivirus was the cause of AIDS. The group of Jay Levy in San Francisco also isolated the same kind of virus (Levy et al., 1984), followed by many other laboratories.

However, a few opponents led by P. Duesberg argued and are still arguing that there is no real demonstration that the virus does exist and is the cause of AIDS according to Koch's postulates.

In fact, the proviral DNA of the virus, renamed HIV (Human Immunodeficiency Virus) by an international nomenclature Committee, was cloned and sequenced (Alizon et al., 1984; Wain-Hobson et al., 1985; Ratner et al., 1985), showing the classical gene structure of animal retroviruses which Dr Duesberg helped himself to uncover at earlier times.

But in addition, new genes (Tat, Nef), important in regulation of the expression of the viral genetic information, were recognized from the DNA sequencing, making the viral genome probably the most complex known in the retrovirus family (Fig. 4). HIV and its Primate Cousins is therefore a well characterized entity only composed of DNA sequences none existing in the human genome.

A posteriori, two facts should have provided to the few remaining skeptics final conviction that HIV is the culprit in AIDS:

(1) Transmission of AIDS by blood transfusion has practically disappeared in countries where the detection of HIV antibodies in blood donors has been implemented.
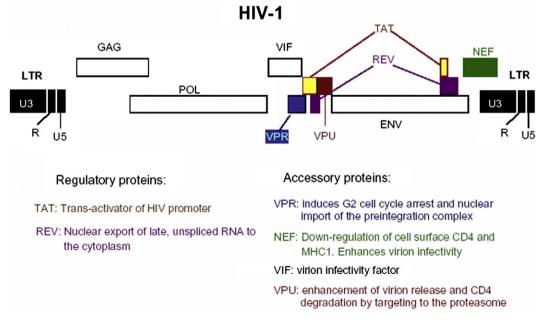

Fig. 4. Genome structure of HIV1: gag, pol, env are the genes coding for the structural proteins.

(2) The inhibition of virus multiplication by a combination of specific inhibitors of the viral enzymes (reverse transcriptase, protease), has greatly improved the clinical conditions of the patients. Mutations in the genome of HIV inducing resistance to these inhibitors has led to relapses and aggravation of the patients' condition.

In 1986, thanks to a collaboration with Portuguese colleagues, we isolated a second virus (which I named HIV2), from West African patients hospitalized in a Lisbon hospital (Clavel et al., 1986). They all had the signs of AIDS but had no antibodies against our first virus. In fact, they had only antibodies to the most variable protein of HIV, the surface glycoprotein. The patients had lost antibodies against the well conserved internal proteins of HIV2 which show common epitopes with their counterparts of HIV1, unlike the glycoprotein (Fig. 5).

The isolation of HIV1 (Montagnier et al., 1984) and HIV2 (Clavel et al., 1986) viruses from AIDS patients in Africa made us realize that we were dealing with a large epidemic of heterosexually transmitted viruses.

Evidence that HIV was not transmitted by casual contacts came from our study in a French boarding school where HIV infected haemophilic children were in close contact, day and night, with HIV negative non-haemophilic children: none of the latter was found HIV positive (Berthier et al., 1986).

The isolation of the virus causing AIDS allowed to implement rational prevention measures and also to start a search for efficient viral inhibitors.

The first candidate, azidothymidine, was an efficient inhibitor of HIV reverse transcriptase in *in vitro* experiments (Mitsuya). However, its use in AIDS patients was soon recognized as disappointing (Seligmann et al., 1994).

In fact, the treatment readily induced mutants of the virus resistant to AZT and did not extend the life span of the patients. The main obstacle of treatment with a single or two inhibitors was the capacity of the virus to mutate, which also impedes the design of an efficient vaccine and also explains the complexity of the pathophysiology of AIDS.

Only a combination of three inhibitors proved to be efficient on the clinical outcome. Since 1996, clinicians are using HAART (Highly Active Antiretroviral Therapy) to treat patients with high virus load and low CD4+ T cell number, preventing then most of the time from falling into lethal opportunistic infections (Zuniga et al., 2008).

The HIV variability

In fact, in order to escape to the immune reactions of their hosts, most viruses have a strategy to change their immunogenic epitopes. In the case of HIV, a conjunction of several factors put it to an unprecedented level.

I have listed below the factors which seem to be most responsible for this variability.

(1) Errors of reverse transcription,
(2) Genetic recombination,
(3) Incomplete neutralization by Vif of the activity of the APOBEC3G cellular gene,
(4) Oxidative stress.

The first is that the replicative enzyme, reverse transcriptase (RT), has no editing compensation, so that the transcription errors may reach $1/10^5$ nucleotides, far from $1/10^9$ of the cellular DNA polymerases.

However some other retroviruses, such as HTLV, do not show this variation rate, since once integrated, the proviral DNA remains replicated by the cellular DNA replicative machinery. The difference could be explained by the fact that the HIV infected cells die, so that the virus can maintain itself only by many cycles of new infections involving each time reverse transcription of its RNA into DNA. However, in *in vitro* infection of cell lines, also involving cytopathic effect and many cycles of re-infection, the virus seems to be stable, in the absence of immunoselective pressure.

Another factor of variation is genetic recombination. The immune responses (humoral and cellular) against the virus are unable to prevent a second virus infection of the host (because of virus variability induced by the previous factor and other causes), so that some cells could be co-infected by two viruses: this will also allow genetic recombination between the two viral RNAs existing each in two copies. The result is a "mosaic" virus in which many sequences from the two original viruses are entangled, starting from "hot spots" of recombination. This is particularly visible in Africa, probably because of repeated exposure to infection of many patients. The mosaic viruses, because of their selective advantage, then disseminate in the infected population. The original subtypes called A B C D E G… defined by the sequence of their envelope gene are thus replaced by A/G, B/C, etc… depending on the geographic location.

Moreover, two other factors have been more recently identified: In the lymphocytes are expressed a family of genes coding for enzymes

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Some Milestones in the Research of AIDS

1981	Identification of the disease in the USA
1983	First isolation of HIV
1984	Confirmation of HIV as the causal agent of AIDS – Biological and molecular characterization
1985	First blood test to eliminate transmission of HIV by blood transfusion
1986	Isolation of HIV-2

Some Milestones in the Research of AIDS[2]

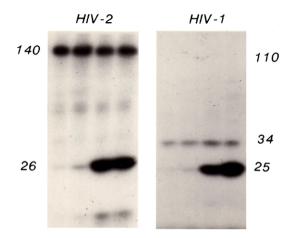|	
1987	First use of AZT as an antiretroviral drug
1991	Apoptosis as a mechanism of cell death in AIDS
1995	Decrease of HIV perinatal transmission with AZT
1995	Demonstration of high rate of HIV replication during the silent period of infection
1996	Identification of HIV main co-receptors
1996–1997	Generalization of HAART in developed countries

Fig. 5. Immunoprecipitation of radio actively-labelled proteins of HIV1 and HIV2 by a serum of an AIDS patient infected by HIV2; note in the HIV2 panel the precipitation of the envelope protein.

able to convert guanosine into adenosine in the viral DNA, fouling the viral genetic code (APOBEC3G). However, the virus has evolved a gene, Vif, which can more or less counteract this effect, rendering viable the viral DNA without completely avoiding mutations (Sheehy et al., 2002).

A last factor of variability, whose the importance has been probably overlooked, is oxidative stress (see below), a cause of RNA and DNA mutations (before integration of the proviral DNA): highly reactive molecules derived from oxygen can oxidize the bases, particularly guanine or deoxyguanine, thus modifying their coding capacity or inducing a wrong replacement in repair.

A combination of these factors could explain both the intrinsic variability of the virus in the host during the long evolution of infection, and also the increasing variability of the circulating strains as the epidemic is spreading in various populations.

We can at least act on this variability by decreasing the viral multiplication rate inside the host by antiretroviral treatment and also by neutralizing the oxidative stress.

The remaining problems:

How HIV infection results in the destruction of the immune system

In early years following the virus discovery, it was generally thought that the drop of CD4+ T cells was due to their direct infection by a cytopathic virus.

In fact, the viral isolates (like Bru) made in the early stage of the disease are not cytopathic, they use after binding to the CD4+ receptor of activated lymphocytes, a co-receptor (CCR5) which is the receptor for a chemokine.

Only viruses isolated from patients at late stage of the disease are cytopathic (like Lai) and their direct infection of the remaining T lymphocytes (by using another chemokine co-receptor CXC4) could account for the final drop of these cells.

In fact, the number of activated CD4+ T lymphocytes (the ones which only allow full replication of the virus), is probably a limiting factor of the initial infection, after the first contact with dendritic cells and monocytes of genital or rectal mucosa. It is obvious that inflammation and co-infections (bacterial, viral) could increase the number of activated T lymphocytes and therefore could increase the risk of HIV infection.

Recently, the virus has been found associated with the Peyer patches existing around the small intestine which constitutes a major source of activated T lymphocytes.

At the onset of infection, the virus replication is high in all the lymphatic tissues, taking advantage of the delay of reaction of the immune system (in time order, interferon, NK cells, CD8T cells,

antibody response) and then decreases while persisting in some lymph nodes (Fig. 6).

This is the beginning of the chronic phase which is generally asymptomatic, although the lymphadenopathy is often present. It has been shown that the virus replication continues in the lymph nodes, despite the immune response. This one starts declining, although there is a continuous renewal of T lymphocytes, both CD4+ and CD8+, which could last for years.

During this period, we have found two phenomena which could help explaining the indirect destruction of the immune system:
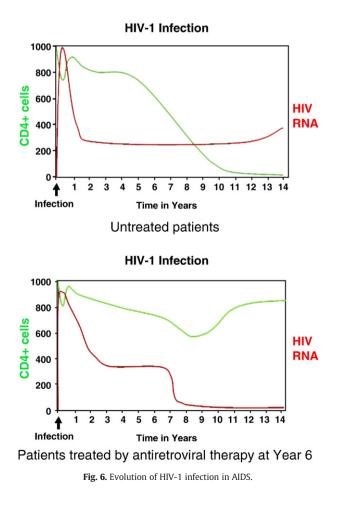
one biological: apoptosis
one biochemical: oxidative stress.

Apoptosis: my laboratory was the first to describe this program cell death in white blood cells cultured in medium deprived of interleukin 2 (Gougeon et al., 1991). All the subsets, not only the CD4+ T cells were affected when taken from the blood of asymptomatic HIV patients as well as in patients presenting with full blown AIDS: CD8+ T cells, NK cells, B lymphocytes, monocytes.

However, we found a good correlation between the drop of CD4+ T cells in patients and this in in vitro phenomenon (Gougeon et al., 1993) We surmised that in the in vivo situation, cells were still alive but in pre-apoptosis.

Indeed, we could detect in infected patients a general phenomenon of immune activation (Gougeon et al., 1996), which has been now well recognized as a major factor of AIDS pathogeny.

At the biochemical level, we also showed that the lymphocyte population of asymptomatic patients (CD4+, CD8+, NK) displayed the biochemical signs of oxidative stress (excess of free radicals derived from oxygen): namely fast degradation of oxidized protein,

Fig. 6. Evolution of HIV-1 infection in AIDS.

carbonylation of some of their amino acids (Piedimonte et al., 1997). In the patients' blood, we could detect similarly a hyper-oxidation of plasma lipids (Lopez et al., 1996) and oxidization of guanine.

What could be the origin of this strong oxidative stress? At least one HIV protein may contribute to it. It was shown by C. Flores, Mc Cord and their collaborators that the Tat protein, among many functions, inhibits the expression in lymphocytes of the Mn-dependent superoxide dismutase gene (Sevea et al., 1999). This enzyme is the key to transform the anion superoxide, highly oxidant into hydrogen peroxide. Tat has been shown to circulate in nanogram amounts in the blood of infected patients and to penetrate inside cytoplasm.

In addition, bacterial and viral co-infectors can also induce oxidative stress. We have been studying the possibility that a "cold" persisting bacterial infection could co-exist in HIV infected patients.

These studies were initiated because we observed that *in vitro* co-infection of lymphocytes with some mycoplasma species (*M. pirum*, *M. penetrans*, *M. fermentans*) and HIV could greatly reinforce the cytopathic effect of the latter.

Moreover, these small bacteria lack catalase, an enzyme able to convert hydrogen peroxide into water. Therefore they also generate oxidative stress and, furthermore, are activators of lymphocytes (Sasaki et al., 1995).

In summary, the pathophysiology of AIDS is complex. HIV is the main cause, but could also be helped by accomplices, and also have some indirect effects by wrongly activating the immune system through oxidative stress.

Prospects for the future

No cure. No vaccine, but maybe a cure by a vaccine

The advent of HAART has transformed AIDS in a tolerable infection, but whatever the length of the treatment, the inhibitors used have not reached the level of a cure! As soon as this treatment is interrupted, virus multiplication resumes within a few weeks and the immune system declines again.

This observation led researchers to think that there is a reservoir of virus, to which the drugs have no access (Richman et al., 2009), probably because the virus stays in a latent form in some tissues.

Our project is to design quantitative tests to evaluate the size of this reservoir and to prevent it from giving rise to actively multiplying virus by boosting the immune system against the most conserved parts of viral proteins.

A schematic protocol of this therapeutic immunization, aimed at achieving a functional eradication of HIV (Montagnier, 2007), could be the following:

(1) First, antiretroviral therapy (HAART) for 3–6 months to reduce viral load in the plasma to undetectable levels and maintain it until the protocol has been terminated.

(2) Then, treat by antioxidants and immunostimulants such as an orally absorbable form of glutathione to reduce the oxidative stress induced by viral proteins and by HAART. Reduced glutathione is known to induce a shift from Th_2 to TH_1 responses, therefore reinforcing cell-mediated immunity. Its effect can be enhanced by some synthetic immunostimulants, which are now close to approval for clinical use by regulatory authorities.

(3) After a 2-week treatment by the former products, start specific immunization against HIV proteins by a therapeutic vaccine. Trials with vaccine preparation made for a therapeutic use have already been carried out, with mixed results probably because the immune system of the patients was not sufficiently restored, or/and also due to the inadequacy of the immunogens. Our data by genetic engineering indicate that the native HIV glycoprotein must be modified in order to make immunogenic the most conserved parts of the protein, including the pocket involved in HIV binding. This will result in a neutralization capacity broad enough to cover potential escape mutants. I advise also to add in the vaccine preparation two other proteins involved in immuno-suppression, Tat and Nef, also modified to become non-functional while remaining immunogenic.

(4) After this vaccination, interrupt HAART. If the protocol has been successful, there will be no virus rebound, as evidenced by a low viral load and an increase of the CD4+ T cell component. Regular monitoring of these two parameters will assess the durability of the immunization. A strong cell-mediated immunity, in addition to the induction of neutralizing antibodies, will permit to interrupt a cycle of new cell infections by newly formed viral particles. This control already exists spontaneously in a small number of HIV infected patients, which show no immune depression even after many years.

Fig. 7. CIRBA—Centre intégré de Recherche Bioclinique d'Abidjan, créé en 1996.

This protocol is complex, but will be less expensive and for the patient much more tolerable than a life-long antiretroviral therapy.

The protocol can also be applied to patients in early stages of HIV infection, perhaps with a better chance of success, as their immune system will have a better ability to respond.

If, in this optimistic scenario, HIV infection becomes a curable disease, the impact on the epidemic itself will be considerable: in developing countries, HIV infection represents a stigma for the familial and professional life. Many infected individuals do not want to be tested and to learn their status, and as consequence, they keep transmitting the virus to new partners. The perspective of being treated for a cure immediately after the diagnostic of HIV infection will ease early testing and emergence of responsible behaviours.

Moreover, the success of a therapeutic vaccine will facilitate the design of an efficient preventive vaccine, based on the same viral components.

Meanwhile, it will be essential to make accessible the use of antiretroviral drugs to all patients who are eligible for it. This implies not only an international effort to lower the price of the drugs, which already has been partly achieved, but also a comparable effort to create adequate medical structures with trained doctors and research centres in developing countries. Our Foundation has chosen the mission to contribute filling these tasks in Africa (Fig. 7).[1]

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[1] The World Foundation AIDS Research and Prevention, in association with UNESCO and local governments, has created two Centers for AIDS Research and Prevention; The "Centre Intégré de Recherches Biocliniques d'Abidjan" – CIRBA (Fig. 7) in Abidjan (Ivory Coast) and the International "Chantal Biya" Reference and Research Centre for HIV-AIDS Prevention and care-taking" (CIRBC) in Yaoundé (Cameroun).