
ANRS
HIV vaccine research programme

December 2010

OVERALL GOALS

The overall goal of ANRS programme is to develop therapeutic and preventive HIV vaccines.

During the past 15 years ANRS has build a comprehensive programme dedicated to development of HIV vaccines. This programme includes: 1) laboratory research studies to define optimal immunogens; 2) pre-clinical in vivo studies in animal models; and 3) clinical trials evaluating the safety and efficacy of candidate vaccines. The ANRS has two major functions in this programme: 1) promotion and coordination of research activities, clinical, regulatory and legal aspects between various teams (ANRS HIV Vaccine Network); and 2) direct development of vaccine candidates.

In 2007, the ANRS HIV vaccine programme entered a new phase. The director of the ANRS, Prof. Jean-François Delfraissy appointed Prof. Yves Levy as the scientific director of the ANRS HIV Vaccine Programme

SPECIFIC PROGRAMMATIC THEMES

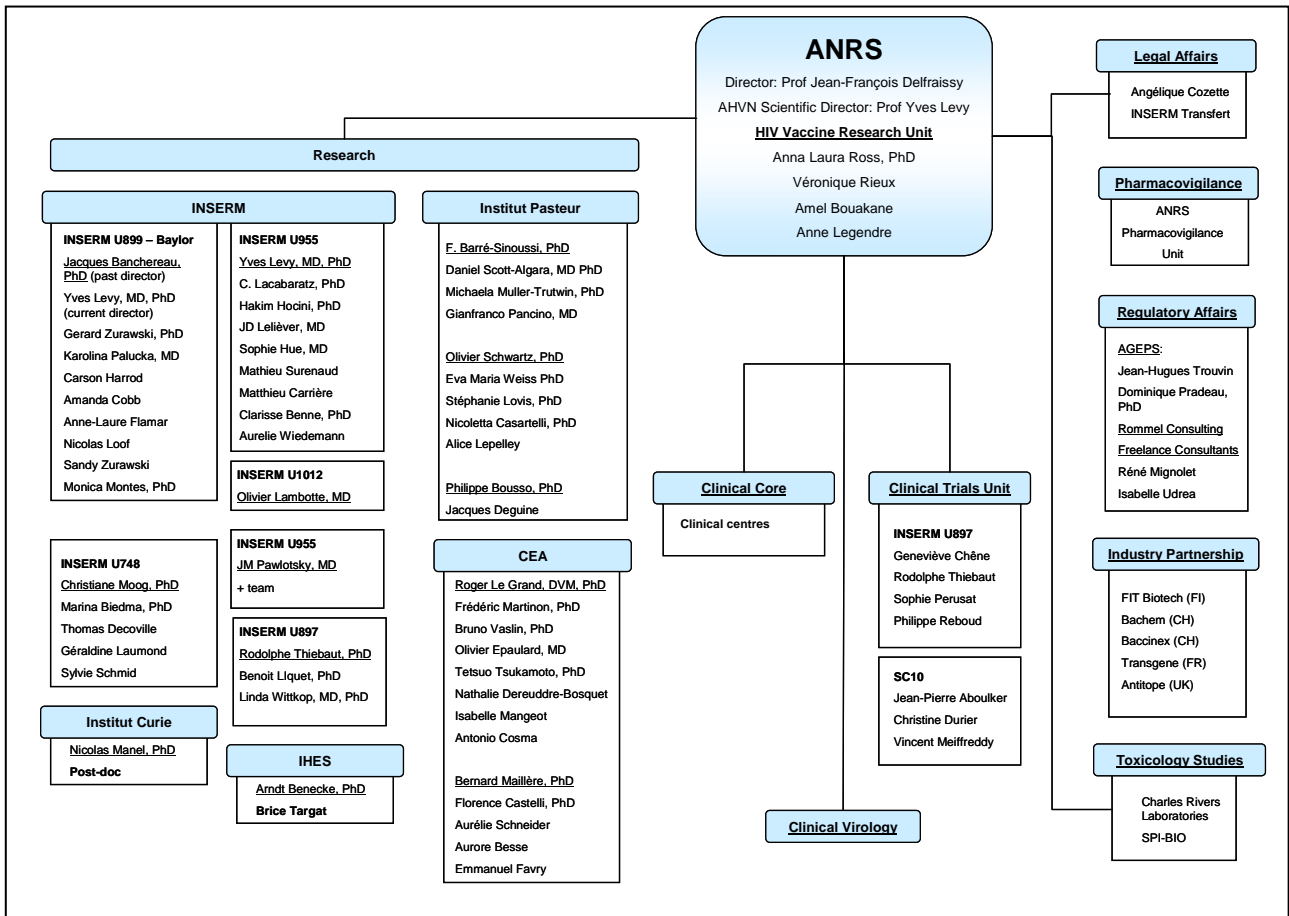
The next phase of the programme was proposed to tackle some of the many obstacles of the field that have been identified in the first phase. Indeed, despite recent encouraging data from the RV144 clinical trial, a number of critical aspects remain to be addressed. These form the objectives and goals for the next phase of the HIV Vaccine programme and include:

- Defining the immunological correlates of protection against HIV infection
- Establishing the breadth and the quality of immune responses elicited by vaccines
- Enhancing the immunogenicity of current vaccines
- Identification of the most effective antigen/adjuvant combinations to induce long-lived protective CD4 and CD8 T cell immune responses
- Understanding how HIV escapes the immune system

ANRS HIV VACCINE NETWORK (AHVN)

This research programme is carried out by a large network of some of the key experts of the field in France and the US (Figure 1). AHVN is a unique collaborative network, with a centralized strategic plan, involving scientists and clinicians from different background including: 1) immunologists skilled in dendritic cells, T, B and NK cells biology; 2) virologists; 3) cell and molecular biologists; 4) NHP model specialists; and 5) a network of clinical sites, investigators, biostatisticians and healthy human volunteers for preventive clinical trials. Global Network objectives are:

- Create a unique research and development programme
- Continue the development of ANRS candidate vaccines to be tested in clinical trials
- Diversify vaccine strategies by combining ANRS vaccines with other candidate vaccines
- Provide a strong background of expertise on regulatory, legal and ethical aspects
- Maintain the partnership between the ANRS and immunologists, virologists, cell biologists, molecular biologists, and primate model specialists
- Establish new partnerships with the clinical sites
- Improve the technology of immunological testing in vaccine trials



The ANRS vaccine research scientific programme is composed of 6 projects and built on common administrative, logistic and scientific cores (figure 2). The scientific objectives are:

ANRS’s candidate vaccine portfolio based on HIV Lipopeptides, recombinant Pox vectors and innovative novel vaccines based on the targeting of Dendritic Cells with recombinant anti-DC antibodies fused to selected HIV and HCV antigens.

Project 2) To analyze the immunogenicity of vaccines and their impact on innate and adaptive immunity. The aims are to increase the knowledge of basic mechanisms of HIV antigen presentation and DC biology relevant to vaccine research, and to perform preclinical analyses of candidate vaccines in cell culture systems. We will study the impact of antigen presentation on innate and adaptive immune effectors.

Project 3) To assess the immunogenicity of

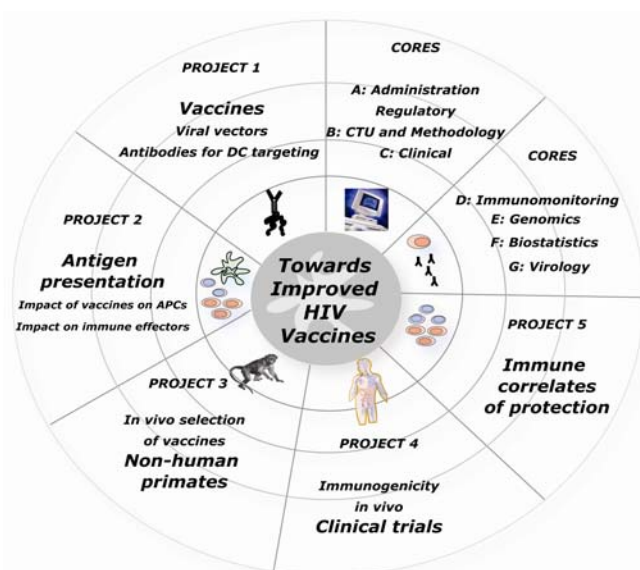


Figure 1 : ANRS HIV vaccine scientific programme

candidate vaccines in Non Human Primates (NHP). We will take the opportunity to assess different prime-boost strategies and the effects of dosing, adjuvant, route of administration on systemic and mucosal immune responses. The expertise of the teams on HIV/SIV physiopathogenesis should allow us to assess the effects of immunization on Host/Virus interactions and on mechanisms of pathogenesis control. Therapeutic effects of anti-DC/HCV vaccine will be evaluated in Chimpanzee models of HCV infection.

Project 4) To assess the safety and immunogenicity of candidate vaccines in healthy volunteers. The final goal is to identify the best prime-boost combination strategy and to integrate scientific inquiry into trial protocols from the beginning to maximize learning opportunities. This part of the programme is based on the development of several Phase I/II multicentric clinical trials. An important aspect of this project is the recruitment of volunteers participating to these trials and the interaction between basic scientists, clinical sites and patient communities. We will develop a specific programme of Social Sciences based on investigating the impact on the public of the communication surrounding HIV vaccinology.

Project 5) To establish the immune correlates of protection. Our strategy is to compare biological and molecular signatures elicited in vivo with different vaccine strategies (assessed in both prophylactic and therapeutic vaccination studies) to those of HIV-infected individuals who spontaneously control HIV replication (i.e. HIV Elite Controllers [HIC]). This part of the programme is developed through collaboration between the ANRS cohort of HIC patients and the AHVN. This project is built upon a strong effort of the immunomonitoring platform for the development and standardization of innovative tests developed in human trials.

Specific objectives and aims of the ANRS vaccine research programme

1. Project 1: To develop novel HIV and HCV candidate vaccines.

In this project, we will pursue the development of ANRS candidate vaccines (HIV LIPO5, ANRS-MVA (B) and anti-DC fusion proteins). This project will contribute to the overall vision and goals of the ANRS vaccine research programme since these candidate vaccines are currently being tested or expected to be tested in pre-clinical and clinical trials in a near future. Our hypothesis is that an epitope-based vaccine strategy will allow the induction of polypeptidic long-lasting T cell responses when combined in a prime-boost strategy.

Lipopeptides	Peptide Sequences	Phase I/II Studies (Humans)
LIPO-6	Gag (aa 183-214 ; aa 253-284)	ANRS-VAC04 ANRS-VAC09
	Nef (aa 66-97; aa 117-147; aa 182-205)	
	Env (V3, aa 303-335)	
LIPO-6T	Gag (aa 17-35 ; aa 253-284)	LIP01198 (ANRS-VAC10) ANRS 093, Vaccii2 (HPA04) ANRS 095, Primovac (HPA05)
	Pol (aa 325-355)	
	Nef (aa 66-97 ; aa 116-145)	
	Tetanus Toxin (TT 830-846)*	
LIPO-4	Gag (aa 77-85)	ANRS-VAC12 ANRS-VAC16
	Pol (aa 342-354, aa 476-484)	
	Nef (aa 68-82)	
	Tetanus Toxin (TT 830-843)**	
LIPO-5	Gag (aa 17-35; aa 253-284)	LIP01198 (ANRS-VAC10) HVTN042/ANRS-VAC19 (LIP04) ANRS-VAC18
	Pol (aa 325-355)	
	Nef (aa 66-97 ; aa 116-145)	

* A T-helper-stimulating tetanus toxin (TT) peptide is covalently linked to one lipopeptide

** The TT peptide is covalently linked to each lipopeptide

1.1 Peptide-based vaccines: Since 1994, the ANRS has developed a novel vaccine strategy based on the use of HIV lipopeptides which consists of large synthetic HIV peptides coupled to a lipid tail. The lipid moiety facilitates peptide entry into antigen-presenting dendritic cells, thereby allowing efficient processing, and expression of CD4+ as well as CD8+ T cell epitopes at the cell surface. The epitope-based approach is particularly interesting because the immune responses can be directed against highly

conserved and subdominant epitopes. ANRS has a large experience in the utilization of lipopeptides in vaccine. Four formulations of lipopeptide vaccines have already been tested (LIPO-6, LIPO-6T, LIPO-4 and LIPO-5) in Phase I/II clinical trials (table). HIV LIPO 5 vaccine has been tested in phase II studies eliciting CD4+ and CD8+ T cell responses in both healthy and HIV-infected volunteers. We will pursue its development in several phase II studies in combination with recombinant DNA or Poxvirus HIV candidate vaccines (project 2).

1.2 DNA/viral vectors-based vaccines: In the past three years, ANRS has also developed or tested, in collaboration with other industrial partners, HIV DNAs (Eurovacc/Cobra; Fit Biotech) and recombinant Pox Virus vectors [ANRS-MVA (Gag/Pol/Nef) and Eurovac/Sanofi NYVAC (C)]. The goal is to develop a prime-boost strategy combining two or more of the candidate vaccines developed by the ANRS. We have developed a recombinant MVA vector encoding the homologous sequences contained in the HIV LIPO5 in collaboration with Transgene. Transgene MVA technology is based on a specific MVA isolate, MVATGN33. Recombinant HIV antigens include the full-length codon-optimized sequence of gag (amino acid (AA) sequence 1-512) fused with fragments from pol (AA: 172-219; 325-383; 461-519) and nef (AA: 66-147; 182-206) from Bru/Lai isolate (Los Alamos accession number K02013). To enhance further Gag expression, within gag poly-C, poly-G (longer than 3 nucleotides) and poly-GC (longer than 8 nucleotides) motifs were replaced. The ANRS-MVA (Gag/Pol/Nef) vaccine candidate has recently completed toxicology evaluation (Charles River, Scotland).

1.3 Dendritic cell-based vaccines: The hypothesis is that vaccines targeting antigens to DCs offer a potent and novel means of improving vaccine delivery that allows dose sparing and precise control of the type of immune response. The ANRS and the Baylor Institute for Immunology Research (BIIR)/Inserm U899 have jointly developed prototype HIV and HCV vaccines based on DC-targeting using recombinant anti-DC antibodies fused to antigens.

B and CD8+T lymphocytes are the effectors of the adaptive immune system while CD4+T lymphocytes regulate their functions. Each of these cell types is composed of subsets with specialized functions. The CD4+T cell compartment is particularly complex as it includes Th1, Th2, Th17, Tfh, and regulatory T cells. Upon antigen encounter, naive T cells differentiate into effector or memory cells. Many of the studies relating to T cell polarization have focused on T cells themselves. Yet, T cells are under the control of dendritic cells through both the presentation of antigen-derived peptides in the context of MHC molecules and the delivery of polarization signals. DCs are unique in their ability to prime naïve T cells, though their effects on B cells and other immune effectors, such as NK, NKT, and $\gamma\delta$ T cells, are being established. While DCs are viewed as stimulators of immunity, more recent studies highlighted their critical role in the induction of peripheral tolerance. The distinct functions of DCs depend on the type of maturation signals they receive as well as the existence of distinct subsets (figure 3).

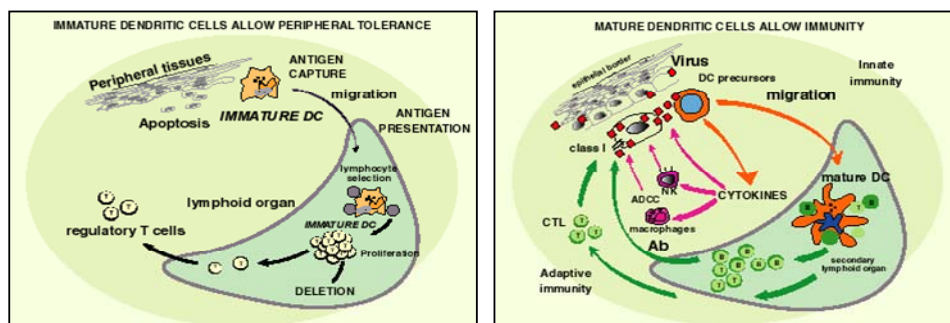


Figure 1 : Immature DCs allow tolerance: Tissue DCs constantly sample their environment, capture antigens and migrate to draining lymph nodes. However, in the absence of inflammation, DCs are immature and antigens are presented without costimulation leading to either T cell deletion or generation of regulatory T cells. **Mature DCs allow immunity:** Tissue inflammation allows DCs maturation and migration, in large numbers, to draining lymph nodes. Translocation of MHC/peptide complexes to cell surface and expression of appropriate costimulatory molecules allow T cell priming, B cell activation and the initiation of adaptive immune response.

Two main DC differentiation pathways have been characterized: a myeloid pathway that generates both Langerhans cells (LCs), which are found in stratified epithelia such as the skin, and interstitial DCs (intDCs), which are found in all other tissues. The second pathway generates plasmacytoid DCs (pDCs), which secrete large amounts of IFN α after viral infection (Figure 4). Each of these DC subsets has common as well as unique biological functions determined by a unique combination of cell-surface molecules and cytokines. For example, intDCs induce the differentiation of naïve B cells into immunoglobulin-secreting plasma cells, whereas LCs are particularly efficient activators of antigen-specific CD8 $^+$ T cells. Both LCs and intDCs activate memory T cells.

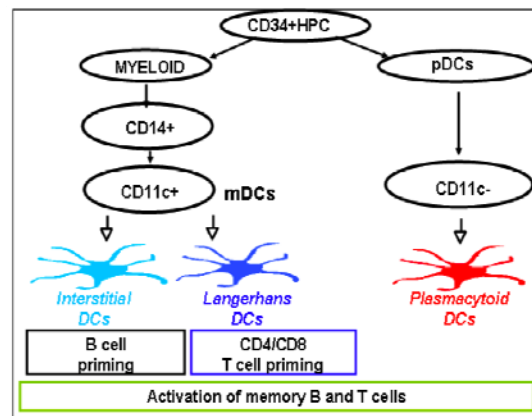


Figure 2 : DC Subset

DCs have diverse cell surface receptors known, or suspected, to be involved in antigen capture. These include DEC-205, LOX-1, DC-SIGN, DECTIN-1, DC-ASGPR, DCIR, CLEC-6, Langerin and

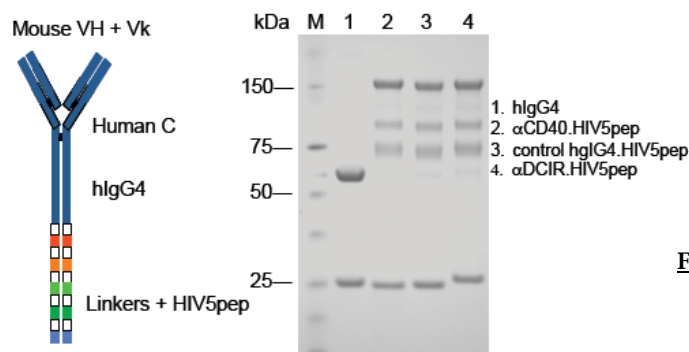


Figure 3 : Construct of anti-DC HIV or HCV vaccine

Mannose receptor - all of which are C-type lectin receptors and have lectin-like domains thought to mediate recognition of pathogen-derived carbohydrate-bearing antigens. Other DC receptor classes can also internalize antigen with concomitant activation signals, e.g., CD40, CD1d, and MARCO. One strategy to improve vaccine efficacy is the specific targeting to DC of antigens conjugated to monoclonal antibodies that bind internalizing DC-specific receptors (DCIR). The potential of DC-targeting for vaccination was well established in key mouse studies showing significant dose-sparing and broader responses than normally seen with other types of immunization. The targeting of HIV Gag antigen to DCs via DEC-205 extended these concepts to a clinically relevant antigen and confirmed the benefits of DC-targeting – i.e. significant dose-sparing, protective responses from a single vaccination, and expansion of both antigen-specific CD4 $^+$ and CD8 $^+$ T cells (Bozzacco *et al.*, *PNAS*, 2007; Cheong *et al.*, *Blood* 2010).

The project is to design vaccines that are able to target HIV and HCV antigens to different DC subsets while varying both co-activation signals and their exposure to different antigen-processing pathways. In this purpose, we use bifunctional antibodies directed against DC-specific receptors and bearing

target antigens fused to the C-terminus of their heavy chain. This strategy allows the unique targeting of antigen to different DC subsets, or to the same DC via different receptors, enabling antigen delivery concomitant with unique DC activation signals, thereby permitting efficient antigen processing and presentation, with a fine control of antigen-specific B cell and T cell expansion.

1.4 Ex vivo DC platform: we are currently testing in a Phase I study among HIV-infected patients ex vivo-generated DC vaccines (interferon- α /LPS) pulsed with HIV peptides (HIV LIPO-5). The aim of this study is to improve the immune responses in HIV-infected patients before stopping antiviral drugs. Expected results from these studies should provide insights into the correlates of immune control of HIV replication.

2. Project 2: Immunogenicity of vaccines and their impact on innate and adaptive immunity.

Our aim is to provide an innovative view of the activation of the immune system when it encounters candidate vaccines. We will compare immune responses induced by HIV particles, HIV-infected cells, and vaccine candidates. It is of great importance to understand the immediate interactions between candidate vaccines and the innate immune system, since these early crosstalks will shape the nature of adaptive responses. In this project, we will address the questions related to the impact of vaccine vectors, antigen design and type of antigen-presenting cells on the immunogenicity of vaccines with a particular focus on innate immunity (plasmacytoid DC and NK cells). We will also study the repertoire and function of vaccine-specific T cells in *in vitro* systems. The role of vaccines in modulating the microenvironment of secondary lymphoid organs is largely unappreciated. Most importantly, we will investigate how vaccines impact on lymphocyte behaviours *in vivo*.

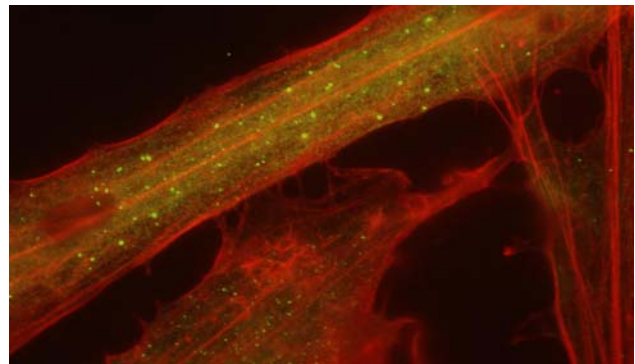


Figure 4 : ANRS-HIV-MVA infected primary myotubes
Gag is green (from Brandler et al, J Virol 2010)

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2.1 Immunological and virological characterization of HIV vaccine candidates: The immunogenicity of recombinant vectors such as Poxvirus is determined by their capacity to induce apoptotic or inflammatory responses after delivery. We have shown that ANRS MVA-HIV infects and drives Gag expression in primary macrophages, DCs, epithelial and muscle cells (figure 6). MVA-HIV infected DCs matured, efficiently presented Gag, Pol and Nef antigens and activated HIV-specific CTLs (Brandler, Lepelley et al. 2010).

Our aim is to provide an innovative view of the activation of the immune system when it encounters candidate vaccines (peptides, recombinant vectors, anti-DC fusion proteins). We will compare immune responses induced by HIV particles, HIV-infected cells, and vaccine candidates. In experimental *in vitro* culture experiments, we will assess how HIV-infected cells or vaccine-exposed cells are sensed by the innate immune system, with a focus on plasmacytoid DCs (pDCs). We have reported that cellular factors such as Apobec3G acts not only as an intra-cellular HIV barrier but also as a factor modulating the immune adaptive response facilitating antigen presentation. Our hypothesis is that cellular factors may influence the generation of epitopes and the establishment of adaptive immune responses. We will examine whether tetherin or Apobec-3G, by altering the routing of HIV

particles, modifies the ability of infected cells with HIV or ANRS-MVA-HIV vaccine to present viral antigens by MHC molecules.

2.2 Characterization of Natural Killer cell response to Dendritic cells loaded with HIV antigens:

Reciprocal activation of NK cells and DCs through NK/DC interactions is now recognized to be an important dynamic connecting the innate and adaptive arms of the immune system. Our hypothesis is that a deeper understanding of the molecules and pathways involved in NK/DC interactions and their role in orchestrating the innate and adaptive immune response is crucial to finding new correlates of protection, and to the development of new vaccine strategies.

We have already established an *in vitro* model designed and validated to study the impact of HIV antigen-loaded DCs on their interaction with NK cells (Cummings 2009). In this aim, we will characterize NK cell subsets implicated in vaccine responses phenotypically and using transcriptomic approaches (immunology core). The capacity of NK cells activated by vaccine-loaded DCs to control HIV replication and to shape CD4 and CD8 vaccine-specific T cell responses will be studied using *in vitro* culture systems.

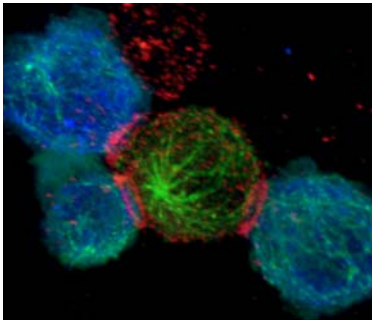


Figure 5 : A polysynapse formed between one infected cells and three target cells (in blue). Gag is red, tubulin is green (Rudnicka et al)

2.3 Impact of CTLs and NK cells on HIV replication and cell-to-cell transmission:

We aim to establish an *in vitro* system designed to study the effects of CTL and NK cells in cell-to-cell HIV transmission. There are multiple ways to assess the activity of CTLs. For instance, it is possible to cocultivate CTLs with peptide-loaded APCs or with HIV-infected cells, and then follow their ability to produce cytokines using an Elispot assay or flow cytometry. Other assays enable to evaluate the ability of CTLs to kill HIV-infected cells, or to suppress HIV replication, by measuring the capacity of *ex vivo* HIV specific CD8 T cells to kill autologous infected CD4 T cells by measuring the disappearance of Gag⁺ cells in culture. Much less is known on how CTLs interfere with cell-to-cell HIV transmission. Cell-to-cell spread accelerates viral dissemination, and likely influences pathogenesis and immune evasion.

O. Schwartz's team has developed, in collaboration with the Pasteur Imaging facility, a real-time microscopy analysis, to visualize viral intercellular spread. HIV readily forms virological synapses at the interface between infected cells and target cells. Other modes of retroviral cell-to-cell spread include polysynapses, which allow simultaneous transfer to multiple targets, filopodial bridges or thinner nanotube-like structures formed between infected cells and more distant targets (Figure 7).

It is thus of interest to determine and to visualize how CTLs and NKs may inhibit this important mode of viral replication. Various CTL types (CTL clones, uncloned CD8 T cells from HIV-infected individuals) will be analyzed. We will similarly analyze the behaviour of various NK subsets. In the future, CTLs and NK cells from individuals vaccinated during prophylactic and therapeutic vaccine studies could be also tested in this system. Hopefully, this part of the project should help us understand how vaccine-elicited cellular immunity may antagonize HIV cell-to-cell spread.

2.4 Repertoire and function of vaccine-specific T cells generated *in vitro* and *in vivo*:

We hypothesize that Regulatory T cells (Treg) participate to T cell responses elicited by HIV vaccination and that those Treg may dampen HIV-specific immune responses. The role of Treg in the modulation of vaccine-elicited immune responses in humans is largely unknown. We will combine *in vitro* and *in*

vivo studies to evaluate the generation, phenotype and function of T cell repertoire generated in response to various vaccine candidates.

The phenotype, function and specificity of Treg in prophylactic and therapeutic HIV vaccine trials developed in the programme (project 4) will be studied. A similar approach will be developed in vaccine trials performed in NHP allowing the characterization of Treg and effector T cell responses in secondary lymph nodes and mucosa (project 3).

2.5 Epitope characterization and predictive immunogenicity: Recent data including ours have shown that the magnitude of T cell responses is largely dependent on the size of the epitope-specific naïve T cell repertoire. This repertoire is shaped during T cell ontogeny by the stochastic rearrangement of Tcr genes and the selection of clonotypes by self-peptides. Thus, our hypothesis is that the evaluation of the frequency of pre-existing T cells in naïve donors will provide relevant insights on the immunogenic potential of vaccine candidates but few data are available either for defined epitopes (Wang, Cohen et al. 2007) or proteins (Geiger, Duhon et al. 2009) our own data concerning LIPO-5 vaccine). Finally, the T cell response induced by vaccine candidates is expected to be adapted to natural sequence variations of circulating viruses, to be recalled by infected cells and to limit virus infection.

We will evaluate the size of naïve T cell repertoire recognizing DC transfected with plasmids encoding the whole HIV proteome with the final attempt to compare sequences already inserted in ANRS HIV vaccines with sequences generally targeted by the T cells in seropositive donors.

2.6 Lymphocyte dynamics in lymph nodes following administration of HIV candidate vaccines viral protein in mice models: Immune responses are highly orchestrated and dynamic events that largely rely on the ability of immune cells to migrate in organs and tissues, to interact with each other in order to exchange information and to disseminate in the body to fulfill effector functions. Our team as well as other workers have shown that two-photon imaging is the technique of choice to decipher immune cell migration and interactions in live mice (Celli, Lemaitre et al. 2007; Azar, Lemaitre et al. 2010) (Figure 9). We suggest to use two-photon imaging to address how MVA-HIV vaccine candidate affects lymphocyte distribution and behaviour in draining lymph node in adoptive transfer experiments. The effects of MVA on kinetics, accumulation and function of NK cells and on the stability of DC/T cell interactions will be studied. In addition, we will also test the possibility that HIV viral proteins (in particular Nef) may interfere with steady-state T cell migration to lymph nodes.

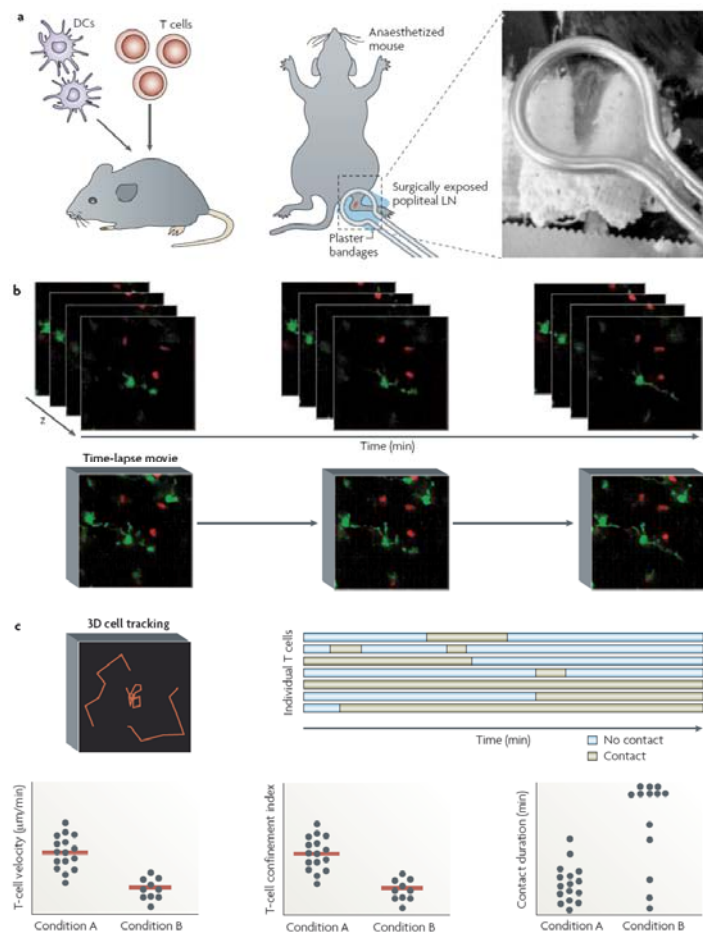


Figure 6 : Two-photon intravital imaging of immune cell interactions. The figure shows the basic steps for data collection and the new set of biological information delivered (from Bousoo, Nat Rev Immunol, 2008)

3. Project 3: Assessment of candidate HIV vaccines in non-human primates

Animal models can accelerate the development of new vaccines through the discovery new strategies and the translation of innovation into the clinic. Nonhuman primates (NHP) are the only animal models that reproduce the physiopathology of AIDS. In this project, we will thus use NHP models for the preclinical evaluation of immunogenicity and efficacy of ANRS vaccine candidates. We will also use these models as a discovery pathway, by studying both the nature of the protective immune responses (correlates of protection) and the trigger mechanisms of these protective responses. For the vaccine studies, we will use a well-defined macaque model (*cynomolgus macaques*). For the research of correlates of protection, we will use the same vaccinated cynomolgous macaques after challenge with relevant SIV or SHIV, as well as a model of strong natural protection against AIDS (African green monkeys, AGM). Studies in animal models will be strongly coordinated with programs on in vitro characterization of vaccines, clinical trials in humans and search of correlates of protection in long-term non progressor patients. The programme will be organized into three different aims.

3.1 Immunogenicity of the different vaccine candidates developed by the ANRS. The rationale is that different combinations of adjuvant, delivery methods and antigens, in different prime boost strategies can be used to improve vaccine efficacy. Appropriate number of animals will be used for statistically powered comparisons using a variety of state-of-the-art assays for assessing antigen specific T and B cell responses. In addition, we will improve and innovate in the technology for monitoring vaccine induced immune response. Indeed, the identification mechanisms associated with breadth and durability of the vaccine induced response (including the role of T regulatory cells) and immune-correlates of protection we will explore in challenge models in Aim 4, will need in deep characterization during vaccination process of cells with complex phenotype and functions that can be explored with new powerful tools (multiparametric flow cytometry, mass cytometry, micro-arrays) and which interactions in the process of vaccination can be modelled in a system biology approach. We expect here to optimize our vaccine strategies and define the best strategies to be tested in humans. The best candidates will be tested for efficacy in aim 4

3.2 Evaluation of vaccine-elicited mucosal responses. The consortium will generate new vaccine approaches for blocking HIV at the rectal and cervico-vaginal portal of entry. Different tools will be developed to characterize local specific cells and antibodies that may contribute to protection. Animal models are particularly relevant here because the need to access to tissues that are difficult or impossible to obtain from healthy volunteers. In addition, we expect to identify blood biomarkers that correlate with induction of mucosal immune responses in macaques and that can be translated into human clinical trials to predict mucosal immunity.

3.3 Identification of correlates of protection in vaccinated and non-pathogenic NHP models of SIV infection. We will compare the innate and adaptive immune responses in three groups of protected and non-protected animals. In the first group we will extensively compare anamnestic responses in vaccinated macaques (those with controller genetic background will be excluded) protected or not from experimental challenge. In the second group, vaccine related protective responses will be compared to responses in non-vaccinated animals with natural control of disease progression which we know in cynomolgus macaques to be strongly associated with genetic background (H6 MHC haplotype in cynomolgus macaques). In the third group we will compare the immune responses that have been identified in groups 1 and 2 to be related to the efficient control of virus replication and/or viral reservoirs to the immune responses that do not protect against viral replication but do protect against the development of AIDS (AGM model). This is an important issue as it has become clear that humans with undetectable viremia still can progress, albeit very slowly, to AIDS. This progression to AIDS in humans and NHP is associated with chronic immune activation. It will be crucial to compare in NHP (vaccinated macaques, natural controller macaques and AGM) the early immune activation

pattern that lead to the induction of efficient anti-viral responses to the one that lead to efficient control of chronic immune activation. For the identification of the mechanisms responsible for the induction of the protective immune responses, we will focus the study on the early events in tissues (mucosae, lymph nodes) by analyzing key factors of innate and adaptive immunity during primary infection in vivo. The protective natural immunity could then be reinforced by vaccination, while early disorders shall need to be corrected by vaccines.

4. **Project 4: Assessment of candidate vaccines human**

The ANRS' scientific programme recognizes that clinical trials represent an unequalled opportunity to analyze the human immune response to vaccination. Our goal is to extend the portfolio of the candidate vaccines that could be tested in prime boost combinations and to develop original approaches to deliver immunogens (see also Project 1). Several questions need to be addressed in phase I/II trials including safety, immunogenicity, dose, delivery, adjuvants, quality and sustainability of immune responses. Some conclusions were provided by previous trials: safety of lipopeptide vaccination (Durier, Launay et al. 2006); immunogenicity of different doses of LIPO5 vaccine administration (Salmon-Ceron, Durier et al. 2010); immunogenicity of ID versus IM administration of HIV lipopeptides (Launay, Durier et al. 2007). We have also tested the hypothesis that the prime vaccination using recombinant DNA may impact the magnitude and the breadth of the response to a heterologous boost in the EV03/ANRS VAC20 trial (Levy 2010).

In this project, we will extend the number of phase I/II clinical trials combining recombinant/protein boost vectors. Although there are no clear go/no go criteria for moving from phase II to phase IIb/III, dissecting vaccine-elicited immune responses is essential for defining the ability of vaccines to prevent infection or disease and for the subsequent discovery of vaccine-induced correlates/signatures of protection. The goal is to identify the "best-in-class" combination that we will test in phase IIb/III efficacy trial in healthy volunteers at medium/high risks of infection.

In addition, the ANRS will develop a programme aimed to demonstrate the proof of concept of therapeutic immunization. Several lines of evidence suggest that the immune system contributes to the long-term control of HIV replication. This is well exemplified by a subgroup of patients called "Elite Controllers" (project 5) that provides evidence that durable containment of HIV replication and/or prevention of disease

progression without antivirals (ARV) is possible. Therefore, the hope is that therapeutic immunization might mobilize some of the mechanisms that mediate control of viral replication in these patients.

The clinical vaccine programme of the ANRS HIV vaccine programme will be linked to and build upon the tools allowing an extended analysis of immune responses including molecular biology,

DNA GTU/LIPO Prime Boost Phase I/II: outline

Randomized phase I/II study of the safety and immunogenicity of a combination of DNA GTU-MultiHIV B followed a LIPO 5 boost in healthy volunteers

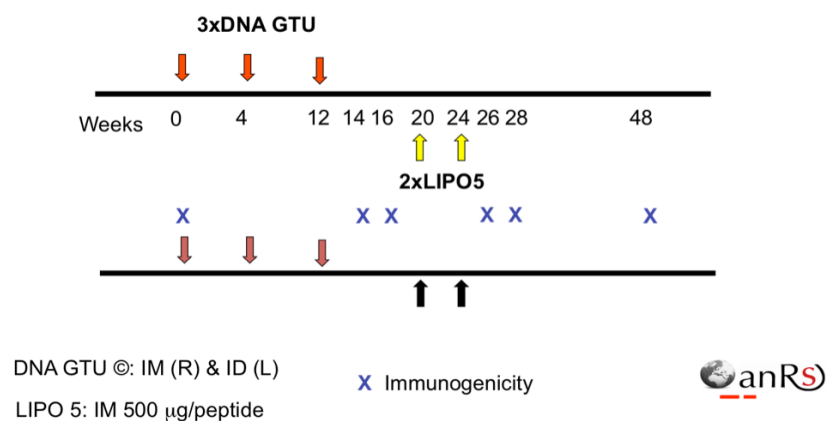


Figure 7 : Design of the prophylactic trial ANRS/VRI Vac 21

immunology, virology, genomics, bioinformatics approaches, to optimize both vaccine design and information on candidate vaccine biology. Several vaccine platforms will be tested

4.1 prime/boost strategies in healthy volunteers:

These randomized, double blind, controlled studies will be performed in healthy volunteers recruited through the ANRS volunteers network and in the network of clinical centres of the AHVN (clinical core). Primary end points include safety and immunogenicity. Immunological tests will be performed in a central laboratory (Immunology Core: Mondor Immunological Center) according to SOP and internationally agreed criteria. Trials will be designed as superiority trials when two different combinations of candidate vaccines will be compared and conducted in intent-to-treat analyses (Biostatistics core and Clinical trial Unit, Inserm Bordeaux).

4.2 Therapeutic immunization clinical studies in HIV-infected individuals:

Expected results include identification of correlates of immune protection/control of HIV replication (Immunology core) and HIV escape mutations using a "deep sequencing" 454 analysis of HIV variants following viral rebound (Virology Core Inserm U955).

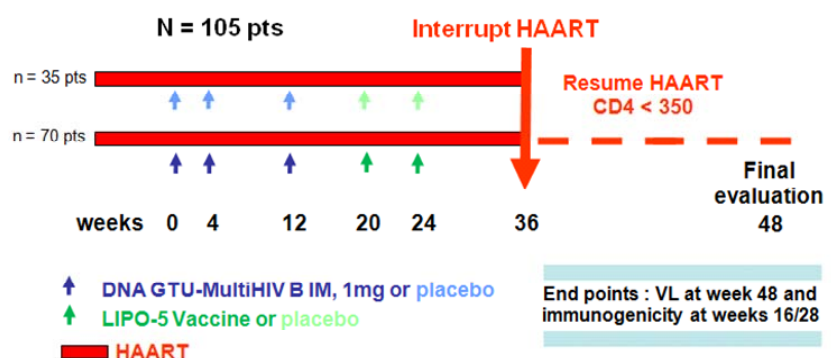


Figure 10: Design of the therapeutic vaccine trial LIGHT

5. Project 5: Identification of immune correlates of protection

The aim of this project is to identify functional and gene expression signatures associated with the control of the HIV infection in: i) patients who spontaneously control HIV replication without treatment (HIV controller [HIC]); ii) in patients enrolled in therapeutic immunization studies or chronic HIV-infected patients treated or not with antiviral drugs. The hypothesis is that an immune response associated with the spontaneous control of HIV in natural infection may prove similarly efficient at controlling early HIV replication in vaccinees. The aim is to establish a large data set allowing the comparison of these signatures at the population and at cellular levels following in vitro stimulation of immune cells with HIV candidate vaccines.

We surmise that these analyses, in comparison with those from viremic patients, ARV treated patients, and those enrolled in therapeutic immunization studies (project 4) would help to define a robust "controller signature". By its implication in the AHVN, the ANRS Elite Controller cohort provides an impressive infrastructure for rapid progress towards identification of correlates of protection. We will perform: (i) an extensive blood transcriptome analysis of a large population of HIC (n=86), well described in terms of clinical, immunological and virological characteristics; (ii) Gene expression profile of purified T cell populations from HIC patients with strong or weak immune responses against HIV antigens would help to define markers of HIV control; (iii) we will compare functional and molecular responses to in vitro stimulation of immune cells from HIC and vaccinated patients with HIV candidate vaccines. The transcriptomic analysis will be performed at the immunology Core of the ANRS vaccine research programme following SOP of the laboratory for transcriptome analysis on Illumina Technology. A large set of genes will be analyzed and a relevant gene signature will be used for comparing the different groups (Biostatistics Core).

D. ANRS HIV vaccine research programme core facilities

In addition to the scientific programme, the efforts of the AHVN research scientists are facilitated by core laboratories in immunology, virology, biostatistics, bioinformatics, clinical trial units and a network of clinical sites. A detailed description of the ANRS HIV vaccine research programme core facilities is provided in Annexe 5.

The ANRS legal and regulatory Core. ANRS is the principal research stakeholder in the fight against HIV/AIDS and viral hepatitis in France. This core will be in charge of all legal and regulatory aspects pertaining to the clinical programme. The ANRS HIV vaccine research unit has a strong expertise in regulatory affairs and is in charge of overseeing the production and validation of clinical batches of ANRS vaccine candidates. The core will be in charge with the Clinical Core of the organization of the recruitment of volunteers for vaccine clinical trials and for preparing the necessary regulatory aspects for the competing authorities in relation to ANRS vaccine clinical trials.

Key Personnel: JF Delfraissy, MD (ANRS Director), AL Ross, PhD (Head of the HIV Vaccine Research Unit), V Rieux (Project Manager), A. Bouakane, PhD (Project Manager), A Cozette (Head of legal affairs), T Menvielle (Head of financial department).

The Mondor Immunology Centre (MIC) facility fills an urgent need for immunology laboratory services to provide support for the ANRS sponsored clinical programme. The need for standardization is critical to ensure compliance with GLP/GMP guidelines regarding the handling, processing, storage and traceability of clinical trial samples, development of new assays, data management, and documentation required for submissions to regulatory agencies and product licensure. The MIC has developed novel tools to assess induced immune responses in individuals involved in vaccine and immunotherapeutic trials. The missions are: i) to develop up-to-date and standardized technologies to monitor the immune status of patients at various stages of their disease using standardized assays according to GLP/GMP, ii) to provide scientific, bioanalytical and technical support in national and international clinical trials by performing analysis of immune responses to novel vaccines and immunotherapies against viral and malignant diseases, and iii) to identify correlates of immune protection with the aim of generating novel immunotherapies and better vaccines.

In the last two years, the MIC has developed standardized tests for flow cytometry analyses of vaccine trials in collaboration with Eurovacc (CHUV Lausanne, Pr Pantaleo) within the CAVD programme funded by the Gates Foundation. Our objective is develop standardized assays and common SOP with the Baylor/ANRS/Inserm U899 Institute of Immunology, Dallas through sharing samples and developing common clinical trials, assays and technology transfer in the next two years. The cellular platform, based on multiparametric flow cytometry and cytokine multiplex technology, as well as the genomic platform based on the BeadChip Illumina platform work in tight collaboration with the biostatistics and bioinformatics platforms.

Key personnel: C Lacabaratz, PhD (Flow cytometry manager); JD Lelièvre, MD, PhD (interaction with the Clinical Core); S Hüe, PhD (Multiplex manager); A Wiedemann, PhD (Engineer); M Surenaud (Engineer); C Manier (Engineer); C Krief (Technician); H Hocini, PhD (Genomic platform, manager); P Tisserand (Technician).

The Clinical Core is in charge of the recruitment of healthy volunteers and HIV-infected patients enrolled in vaccine trials and to develop phase I/II trials. The clinical core is in charge of the recruitment of healthy volunteers and HIV-infected patients enrolled in vaccine trials and to develop the ANRS HIV vaccine clinical programme, the coordination between clinical and immunomonitoring platforms and with CTU. The Clinical core includes four clinical sites across France. The limited number of centres allows the specialisation in the field of preventive HIV vaccines of the medical staff involved. It coordinates the recruitment of healthy volunteers ahead of each preventive vaccine trial together with a specialised communication agency. The clinical core also coordinates the prospective

multicentre cohort ANRS COV1-COHVAC of participants in preventive vaccine trials. The main objective of this cohort is to examine the incidence and the severity of clinical events occurring since the first injection of a vaccine candidate till the end of follow up (7 years).

Key personnel: JD Lelievre, MD (Henri Mondor Hospital, Créteil), O Launay, MD (Cochin Hospital, Paris), I Poizot-Martin, MD (Marseille Hospital), F Lucht, MD (St Etienne Hospital), C Desaint (COHVAC Project Manager), AL Ross, PhD (Head of the ANRS HIV Vaccine Research Unit), V Rieux (Project Manager) A. Bouakane (Project Manager).

The Clinical Trials Unit Core is responsible for providing the methodological and logistical resources to adequately assess the safety and immunogenicity of HIV vaccine candidates tested in clinical trials. HIV vaccine trials require the involvement of a highly experienced platform able to coordinate the conduct and analysis of the trials and observational cohorts at the international level with available experienced staff, adequate space, and facilities. The establishment of a specific CTU dedicated to HIV vaccine trials will benefit from the experience of the Clinical Trials Unit (CTU) at ISPED and Inserm Unit 897, as a platform of excellence for clinical trials and epidemiological studies in HIV/AIDS sponsored by the ANRS since 1996. A team of around 30 experienced persons comprising researchers, study coordinators, clinical monitors, statisticians, data managers, clinical research assistants, administrative assistant and secretaries provides a highly skilled staff in HIV trials. The group is part of the Inserm “Epidemiology and Biostatistics” research centre. In particular, we are related to the HIV team and the ANRS CTU (head: G Chêne) and to the Biostatistics team (10 permanent researchers, head: D Commenges) located at the Institute of Public Health, Epidemiology and Development (ISPED).

The CTU and the Biostatistics team at the Inserm U897 take the advantage of a platform: The Centre of Research and Development in Medical Informatics (CREDIM). Technical resources available include software tools for clinical data (SAS, STATA) and study management, monitoring tools, and centralized and secure databases (SQL server) and Internet servers, all within a protected environment. In addition to the management servers of the research team’s environment (domain controllers, firewall (CheckPoint Software) and files server), the CREDIM also holds a server dedicated to the databases (Microsoft SQL/Server), a server for backups (ArcServe), a server for the developments (Visual Basic and Visual Basic.NET, Microsoft ASP and ASP.NET) and two Internet servers (Microsoft IIS).

Key Personnel: G Chêne, MD, PhD (Head of CTU); C Fagard, MD (Deputy Head of CTU); R Thiébaud (researcher), L Richert, MD; 3 project coordinators; 6 clinical research assistants; 3 data managers; 1 statistician; 2 information technology specialists; 1 secretary.

The biostatistics core is part of the INSERM U897 Epidemiology and Biostatistics research centre. The group will be devoted to the development and analysis of trials evaluating immune-based interventions against HIV such as immunotherapy and vaccine. The objective of this group is to give a high-skilled support for the analysis of clinical trials (performed by the CTU) and for the integration of all data generated in the network and to develop/adapt statistical methods for this specific context. From a biostatistical point of view, several challenges are arising in the field of HIV vaccine trials, in particular the definition and the analysis of the endpoints, the optimization of trial designs as well as the integration of various data. The core will take the advantage of its experience in complex analyses of immunological data, development of new biostatistical methods for experimental and clinical research data (in Fortran with implementation in standard software such as SAS® or R). Also, it will take advantage of a Linux network environment including 15 bi-processors for calculation.

Key Personnel: G Chêne, MD (Head of CTU U897), D Commenges (Head of the biostatistics team), R Thiébaud, PhD (researcher), L Wittkop, MD, PhD, B Liquet, PhD (assistant professor), Marta Avalos, PhD (assistant professor), L Richert, MD.

The core virology laboratory is an expert centre in clinical virology which has been involved in the virological monitoring and management of a very large number of clinical trials in HIV and/or HCV infected patients, in collaboration with ANRS, individual academic investigators and private drug companies. The missions of the core virology laboratory are: i) to use and develop up-to-date and standardized technologies to monitor viral replication and genotypic changes of viruses in clinical trials with antiviral drugs or vaccines; (ii) to provide virological expertise in national and international clinical trials. In the past few months, the core virology laboratory has developed expertise in novel, ultra-sensitive technologies aimed at characterizing viral genome sequences and their changes over time on antiviral or immunological therapies. In particular, the virology core laboratory has developed the novel technology Ultra-deep sequencing by means of pyrosequencing (UDPS) allowing characterization of viral sequences and identification of minor viral populations down to 0.5%-1% within viral quasispecies mixtures.

Key personnel: JM Pawlotsky MD, PhD (Head of the team 18, Inserm U955); S Chevaliez, (PharmD, PhD); M Bouvier-Alias, (PharmD); C Rodriguez, (PharmD); A Soulier; F Darthuy, (Technicians).

The humoral core, part of the unit 748 in Strasbourg participates in the development and standardization of antibody inhibitory assays since many years. They are part of collaborative programs to improve inhibitory assays to best reflect in vivo protection and to define the correlate with in vivo protection. The most accurate assays will be used to follow up the humoral immune response induced by in the systemic and mucosal compartments by various vaccine protocols, in NHP and in humans.

Key personnel: C Moog PhD (Head of the platform); M. Biedma PhD (development of ADCC assays); B. Su PhD (Fc mediated inhibitory assays) T. Decoville (Engineer), S. Schmidt (Engineer); G. Laumond (Engineer); J. Penichon (technician)

The Bioinformatics and medical systems biology core will participate to the global efforts to develop an integrated approach to analyze immune responses in human and NHP vaccine trials. The "systems epigenomics group" is dedicated to the integrated analysis of genomic and epigenomic activity in human physiopathology using tools from experimental whole-genome biology to bioinformatics and mathematics. The medical systems biology core activity concerns the identification and quantification of nucleic acids in patient and model system samples. The activity is split into two complementary and highly integrated aspects: (i) experimental functional genomics, and (ii) systems biology and bioinformatics. The experimental facility with a current capacity of 2000 samples / year includes automated sample preparation (Zephyr Caliper SPE workstation; Qiacub), automated biochemical samples analytics (NanoDrop8k, LabChipGX, Bioanalyzer), automated qPCR analysis (Agilent MX3005, Applied Biosystems AB7900), two array platforms (Illumina iSCAN, Applied Biosystems AB1700), and a high-throughput sequencing machine (Ion Torrent 316). This core will work in close relationship with the biostatistics core and the immunology core (M.I.C).

Key personnel: A. Benecke (CR1, CNRS; Head of the team); C. Lavelle (CR1, CNRS); B. Targat (Engineer); 2 Post-Docs