RESINFIT - Anti-infectieux : supports moléculaires des résistances et innovations thérapeutiques

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

AERES report on the research unit

Support moléculaire des résistances et innovations thérapeutiques

From the

University of Limoges

February 2011

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

AERES report on the research unit

Support moléculaire des résistances et innovations thérapeutiques

From the

University of Limoges

Le Président de l'AERES

Didier Houssin

Section des unités de recherche

Le Directeur

Pierre Glorieux

February 2011

Research Unit

Name of the research unit: Support moléculaire des résistances et innovations thérapeutiques

Requested label : UMR_S INSERM

N° in the case of renewal : EA

Name of the director : Ms. Marie-Cécile PLOY

Members of the review committee

Committee chairman

M. Laurent GUTMANN, Hôpital Européen Georges Pompidou, France

Other committee members

M. Thierry DE FRANCE, University Lyon 1, France

M. Joost VAN MEERWIJK, University Toulouse 3, France

M. Thierry NAAS, CHU Bicêtre, Le Kremlin Bicêtre, France, CSS representative

M. Ulrich BLANK, University Paris 7, Paris, France

M. Eric THERVET, Université Paris 5, France

Observers

AERES scientific advisor :

M. Nicolas GLAICHENHAUS

University, School and Research Organization representatives

Ms. Catherine LABBE-JULLIE, INSERM

M. Serge VERDEN, Université de Limoges

Ms. Evelyne JOUVIN-MARCHE, CNRS

M. Ahmid SIAHMED, CHU

Ms. Marie SENGELEN, CHU

Le membre du CNU n'a pas pu se déplacer

Report

1 • Introduction

- **Date and execution of the visit**

The visit started on February 16, 2011 at 8:30 am and ended the same day at 2:15 pm. The scientific program included an overall presentation of the Unit by its Director followed by two scientific presentations of the past activity and projects by each one of the two team leaders. Meetings with PhD students, engineers, technicians and administrative staff, researchers with permanent positions were also organized.

- **History and geographical localization of the research unit, and brief presentation of its field and scientific activities**

This unit was created in 2000 as an "equipe d'accueil". It became an AVENIR team in 2007. The unit is Centre de Référence for the Cytomegalovirus. The unit interacts with the CIC-P. It is part of the GEIST (SFR) superstructure. The unit will move to a new building in 2013.This building will house common platforms with the other units and the hospital laboratories which is at one end of the building.

- **Management team**

The head of the lab in Ms. Marie-Cécile PLOY.

- **Staff members**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	9	8
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	0	1
N3: Number of other researchers including postdoctoral fellows (Forms 2.2, 2.4 and 2.7 of the application file)	13	8
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	1	1
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	1	
N6: Number of Ph.D. students (Form 2.8 of the application file)	5	
N7: Number of staff members with a HDR or a similar grade	7	6

2 • Overall appreciation on the research unit

- **Summary**

This emerging team has obtained an Avenir (2007). Taking the opportunity of their strategic situation in the microbiological laboratory of the CHU of Limoges they have now expanded their research on antibiotics resistance and integron towards resistance to antiviral drugs of CMV. The recent important discoveries of their Avenir team are illustrared by very good publications in Science, Embo, and Plos. The project is very clear, convincing and well balanced between the integron and the CMV parts. They have built up the tools to achieve their goals and get nice insights. The director of the team (MC Ploy) and its new co-leader (S Alain) are very efficient, dynamic and interactive.The presence of a full time Inserm researcher recruited in 2008 will reinforce the team which is very attractive for the formation of forthcoming medical and scientific PhD.

Their capacity to raise fundings (PHRC; ANR) testify of their capacity to build an efficient cooperative research.

Overall this emerging team is very good.

- **Strengths and opportunities**

- Ability to build a research unit focusing on both viruses and bacteria in which the two sub-teams are working together thus highlighting the quality of the management;

- Strong links between fundamental and clinical research ;

- The research unit is also Centre National de Reference (CNR) for the Cytomegalovirus (CMV) ;

- A few excellent publications during the past 5 years despite the fact that this is an emerging team;

- Ability to recruit and attract young researchers ;

- Ability to develop fruitful and pertinent collaborations and partnership ;

- Ability to raise funding ;

- Opportunity : antibiotic resistance is a major issue in Public Health ;

- Opportunity : the research unit will move to a new building in a few years.

- **Weaknesses and threats**

The team only includes one full-time researcher, i.e. without clinical or teaching duties who is also assuming some administrative or routine duties.

- **Recommendations**

- Clarify how the multiplex analysis of integrons will be useful in the clinical practice in Intensive Care Units (ICU) ;

- The small project on the longitudinal Evaluation and response by a Quantiferon CMV kit technique in renal transplant recipients do not seem to be relevant in this project;

- Keep focusing on the limited number of projects described in the written document.

- **Production results**

A1: Number of permanent researchers with teaching duties (recorded in N1) who are active in research	7
A2: Number of permanent researchers without teaching duties (recorded in N2) who are active in research	1
A3: Ratio of members who are active in research among staff members [(A1 + A2)/(N1 + N2)]	88%
A4: Number of HDR granted during the past 4 years (Form 2.10 of the application file)	0
A5: Number of PhD granted during the past 4 years (Form 2.9 of the application file)	4

3 • Specific comments

The scientific strategy includes :

- Strains (bacteria and virus) isolated from patients infected with resistant microorganisms

- Epidemiology studies : prevalence and polymorphism of the resistance

- Generic vehicle of resistance (*in silico* studies)

- *In vivo* and in vivo functional studies

- Clinical studies

- **Appreciation on the results**

  — The relevance and the originality of the research, the quality and the impact of the result

The research unit includes two sub-teams that are working respectively on the development of antibiotic resistance in bacteria and the development of resistance to anti-viral drugs in CMV.

1. Antibiotic resistance in bacteria

Several members of the unit are working on integrons. Integrons constitute a molecular plateform that allows the capture and expression of genes embedded within gene cassettes. The capture is non-random and occurs through on a site-specific recombination integrase-mediated mechanism. In addtion, there is a high correlation between integron carriage and antibiotic resistance. Main discoveries during the past 5 years include :

- The demonstration that a protein involved in the SOS response, lexA, regulates integrase expression when bacteria are exposed to antibiotics (published as a Breva in Science) ;

- The demonstration that the SOS response promotes qnrB quinolone-resistance determinent expression (EMBO Rep. 2009) ;

- The development of a quantitative multiplex real-time PCR for detection class 1, 2 and 3 integrons (J. Antimicrob. Chemother. 2010) ;

- The identification of numerous promoter variants which correlate with variant of the integrase resulting in a balance for the regulation between antibacterial activity (gene cassettes expression) and the excision of these gene cassettes (Plos Genet. 2010).

2. Cytomegalovirus

Other members of the unit are working on Cytomegalovirus (CMV). The CMV persist lifelong in a latent stage, usually harmless. It is a leading cause of birth defect (1%) and results in severe infection in immunodeficient patients. There is no vaccine so far. Antiviral coumpounds (gancyclovir, cidofavir, foscarnet, benzimidazoles, maribavir) do exist but they are toxic and cannot be used in pregnant women. Gancyclovir, cidofavir, foscarnet target the U54 polymarase. Benzimidazoles targets the encapsidation terminases complex UL56 /UL89. Maribavir targets UL97 (Serine -thréonine-kinase). During the past 5 years, the unit members have identified CMV variants that were resistant to anti-viral drugs and mapped these genes. They worked on the structure of the above proteins and other proteins which are in the same region and are targets, or potential targets, of antiviral drugs. Main discoveries during the past 5 years include :

- The demonstration that 13% of patients were either clinically or virologically resistant. Data showed that virological resistance was associated with unfavorable outcome (J. Antimicrob. Chemother. 2010) ;

- The demonstration that many mutations in the UL54, UL97, were responsible for antiviral-resistance in patients (Antivir. Therapy, 2006, 2009) ;

- Structure-function analysis of UL27 (potential regulator of UL97), UL97, UL56 and UL89 and their protein-protein interaction (ul27/UL97;UL56/UL89) (Antivir. Therapy, 2007,2008, 2009, Proteins, 2010);

- The development of several assays to monitor UL54 and other UL activity (J. Virol. Methods, 2007) ;

- The development of high throughput genotyping (2009).

— The quality and the number of the publications, scientific communications, thesis and other outputs

From 2006 to 2010, the members of unit have published 35 publications in peer-review Journals, among which 20 as first and/or last author. Most significant papers from the « bacteria team » were published in Science (2009), Plos Genet. (2010), EMBO Rep. (2009), Antimicrob. Agents Chemother. (2006), Emerg. Infect. Disease (2006), J. Clinical Microbiol. (2007), J. Antimicrob. Chemother. (2006, 2010), J. Med. Microbiol. (2009). Most significant papers from the « virus team » were published J Clin Virol (2009), Antivir Ther (2009a, 2009b, 2007), J Antimicrob Chemother (2010), J Virol Methods (2006).

— The quality and the stability of partnerships

They have excellent collaborations with Didier Mazel (Institut Pasteur), a team working on other types of integrons, and Serge Bouaziz (UMR8151, Paris) a specialist of protein structural studies.

- **Appreciation on the impact, the attractiveness of the research unit and of the quality of its links with international, national and local partners**

— The number and the reputation of the awards obtained by staff members, including invitations to international conferences and symposia

They have received different regional prizes for their studies, in particular those which associate fundamental and clinical studies. MC Ploy has been invited to the NIH to do a conference on integrons.

— The ability to recruit high levels scientists, post-docs and students, and more particularly from abroad

During the past 5 years, a young researcher was granted a CR1 INSERM position in the team.

Four PhD have defended their thesis since 2006 and five are in process.

— The ability to raise funds, to successfully apply for competitive funding, and to participate to scientific and industrial clusters

The unit members have been able to raise significant funding since 2006. Funding sources include 2 PHRC, 1 grant from the FRM, 2 ANR contracts as participant, EU as a participant (project PILLS). Industrial contracts were also obtained (Biomerieux).

— The participation to international or national scientific networks, existence of stable collaborations with foreign partners

The unit members have developed long term collaborations with international (Germany and Spain), national (Grenoble, Paris) and local teams. In addition, the unit participated to the EU PILLS project.

- **Appreciation on the management and life of the research unit**

— The relevance of the research unit organization, quality of the management and of the communication policy

The unit includes 1 technician with a permanent position (who is part time in the unit), 1 technician without a tenured position and 2 Attachés de Recherche Clinique (ARC) with permanent position. All are very happy to work in the unit. They have the opportunity to follow scientific courses and training, to sign papers when they have significantly contiruted to the work and to attend weekly lab meetings.

— The relevance of the initiatives aiming at the scientific animation and at the emergence of cutting edge projects

Lab members attend regular scientific meetings weekly. The scientific exchanges between the bacterial part and the viral part of the unit is obvious. Most of their lab meetings are common and the students share the same spaces.

— The contribution of the research unit staff members to teaching and to the structuration of the research at the local level

Lab members are involved in teaching and many of them have important clinical duties.

- **Appreciation on the scientific strategy and the project**

— The existence, relevance and feasability of a long term (4 years) scientific project

During the next 5 years, the members of the integron team are planning to :

- Expand the knowledge of the dynamic of integron resistance cassettes and expression of the SOS system;

- Perform studies on transmission of integrons and exchanges of cassettes under different stress conditions (antibiotics and modifications of the environment) in simple conditions, in biofilms and in murine models in which axenic animals are reconstituted with human flora ;

- Investigate the relevance of integron detection in effluent samples (PILLS european project) and in biological samples (Evaluation of rapid methods to detect Resistance Integrons in Sepsis (IRIS project). Theywill also evaluate the presence of Integrons in the context of cattle (DEFI-VIANDE) for which they have received an ANR grant.

For the next 5 years, the members of the CMV team are planning to :

- Elucidate the structure/function and activity of the different domains of the terminases complex within wild type and mutant enzymes and the existing inhibitors to better understand the mechanisms of action of new antiviral drugs including AIC 246 and Maribavir;

- Decipher the 3D structure of UL97/UL27 for which no full structure exists in order to better understand how the present inhibitors could be refined;

- Search for new mutations and the consequences on the replicative activity using a new model of in vitro recombinant virus and compare it with kinetics of the isolated targets enzyme;

- Elucidate the relation between the polymorphism of the virus and the kinetic of emergence of resistant variants taking advantage of their cohort studies;

- Develop in cooperation with their industrial partner (Luminex) a rapid technology to identify in the different antiviral targets (polymerase, threonine kinase, terminases) all kown mutations and to add in an easy way new mutations in the kit for detection.

All these projects are well in line with their previous projects. They have been able to develop the tools to achieve their goals (genetic, enzymology, biochemistry and animal models). They are still improving the methodology to get further insights into their projects and have found the good partners to complete the structural part of their project. Thier cooperation with the clinicians and their involvment as leader of a CNR (CMV team) allow to address fundamental questions in partnership with the microbiological reality.

— The originality and existence of cutting edge projects

These projects are timely since the resistance to antibiotics and antiviral drugs is becoming a real problem. They will help to understand how the resistance develops and disseminates and to get strong insights into the mechanism of these resistances. This is a first step to improve drugs or find tools to get new drugs.

Intitulé UR / équipe	C1	C2	C3	C4	Note globale
ANTI-INFECTIEUX : SUPPORTS MOLÉCULAIRES DES RÉSISTANCES ET INNOVATIONS THÉRAPEUTIQUES	**A+**	**A**	**A**	**A**	**A**

C1 Qualité scientifique et production
C2 Rayonnement et attractivité, intégration dans l'environnement
C3 Gouvernance et vie du laboratoire
C4 Stratégie et projet scientifique

Statistiques de notes globales par domaines scientifiques

(État au 06/05/2011)

Sciences du Vivant et Environnement

Note globale	SVE1_LS1_LS2	SVE1_LS3	SVE1_LS4	SVE1_LS5	SVE1_LS6	SVE1_LS7	SVE2 _LS3 *	SVE2_LS8 *	SVE2_LS9 *	Total
A+	7	3	1	4	7	6		2		30
A	27	1	13	20	21	26	2	12	23	145
B	6	1	6	2	8	23	3	3	6	58
C	1					4				5
Non noté	1									1
Total	**42**	**5**	**20**	**26**	**36**	**59**	**5**	**17**	**29**	**239**
A+	16,7%	60,0%	5,0%	15,4%	19,4%	10,2%		11,8%		12,6%
A	64,3%	20,0%	65,0%	76,9%	58,3%	44,1%	40,0%	70,6%	79,3%	60,7%
B	14,3%	20,0%	30,0%	7,7%	22,2%	39,0%	60,0%	17,6%	20,7%	24,3%
C	2,4%					6,8%				2,1%
Non noté	2,4%									0,4%
Total	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

*** les résultats SVE2 ne sont pas définitifs au 06/05/2011.**

Intitulés des domaines scientifiques

Sciences du Vivant et Environnement

- **SVE1 Biologie, santé**
  - **SVE1_LS1 Biologie moléculaire, Biologie structurale, Biochimie**
  - **SVE1_LS2 Génétique, Génomique, Bioinformatique, Biologie des systèmes**
  - **SVE1_LS3 Biologie cellulaire, Biologie du développement animal**
  - **SVE1_LS4 Physiologie, Physiopathologie, Endocrinologie**
  - **SVE1_LS5 Neurosciences**
  - **SVE1_LS6 Immunologie, Infectiologie**
  - **SVE1_LS7 Recherche clinique, Santé publique**
- **SVE2 Ecologie, environnement**
  - **SVE2_LS8 Evolution, Ecologie, Biologie de l'environnement**
  - **SVE2_LS9 Sciences et technologies du vivant, Biotechnologie**
  - **SVE2_LS3 Biologie cellulaire, Biologie du développement végétal**

Hôtel de l'Université
33 rue François Mitterrand
BP 23204 - 87032 Limoges cedex 01
Tél. 05 55 14 91 00
Fax 05 55 14 91 01
www.unilim.fr

Université de Limoges

Limoges, le 12 avril 2011

Le Président

à

Monsieur le Président
AERES
20 rue Vivienne
75 002 PARIS

Service Recherche

Affaire suivie par V. REYTIER

Tél. 05 55 14 91 49

recherche@unilim.fr

Nos réf : Rech&VR n°676

OBJET

General comment for the Unit EA 3175 « S2UR120001488 – Anti-infectieux : supports moléculaires des résistances et innovations thérapeutiques – 0870669^{E} »

Dear Mr President,

We thank the committee for the positive comments on the team structure, as well as for the constructive evaluation and scientific suggestions.

The AERES committee highlighted three points:

- Clarify how the multiplex analysis of integrons will be useful in the clinical practice in Intensive Care Units. We redefined the objectives of the study on the InteRest of Integrons detection in Sepsis (IRIS project). The primary aim is not to evaluate the usefullness of an integron detection method in clinical practice. Firstly, we wish to establish the predictive value of the presence of integrons as a marker of overall resistance to antibiotics in Sepsis due to Gram-negative bacteria, as previous studies suggested a relationship between integrons and multidrug resistance. We will study that directly from biological samples as we developed a multiplex PCR able to detect class 1, 2 and 3 integrons from strains but also from biological samples.

We agree this is too early to transfer the technology to a clinical application without-t having shown first that such an integron detection correlates with antibiotic resistance. We thus have undertaken a preliminary work in this regard during 2010. In an epidemiological study conducted on 235 pathogenic GNB isolated from blood cultures (protocol "Hemotion", data under publication), among which 24.3% had a RI, we showed that 78.6% of enterobacteria expressing acquired resistance to at least 2 families of ATB (among β-lactams, aminoglycosides, fluoroquinolones and cotrimoxazole) had a RI (class 1 and / or 2). The link was particularly important with resistance to cotrimoxazole (93.6% resistant strains had an integron), aminoglycosides (71.4%), ciprofloxacin (70.7%) and extended spectrum β-lactamase (ESBL) phenotype (88.9%). Moreover, among the strains that harbored no integron (intI-), only 8.7% presented acquired resistance to 2 families of ATB versus 81.5% for strains that harbored an integron

(intI+). Thus, 8% of intI- strains were resistant to cefotaxime, and only 3% if we considered E. coli. The ESBL phenotype concerned 0.7% of intI- enterobacteria and 1.5%. of intI- E. coli. Similarly, for amikacin, 2.9% of intI- enterobacteria were resistant. These data suggest that the absence of integrons has a potential negative predictive value of acquired resistance to ATB. In addition, to better apprehend the organizational aspects of the IRIS project, a feasibility study, F-IRIS, conducted on 50 patients, began in January 2011 in a single center (CHU Limoges), in cooperation with the CIC-P 0801. Patients were recruited during their admission to the intensive care unit with SIRS of urinary or gastrointestinal origin. This feasibility study for which all the regulatory and organizational aspects have already been satisfied (ethic committee notification, CCTIRS and CNIL declaration, biological collection duly constituted and reported, CRF, management of biological samples) showed potential recruitment of 5 to 10 patients per month. Thus, these preliminary results showed both the feasibility of a study to detect integrons from biological samples and the relationship between integrons and resistance (to at least 2 families of antibiotics). We now have the scientific and organizational base necessary to perform a large multicenter trial to study the predictive value of detection of integrons in biological fluids as a marker of acquired resistance to antibiotics. We thus rewrote the project IRIS with all these new data and focused the objective on the study of the predictive value, which is the necessary first step to evaluate the potential interest of the integron detection.

-**The small project on the longitudinal evaluation and response by a Quantiferon CMV kit technique in renal transplant recipients do not seem to be relevant in this project.** Antiviral resistance is a major issue in the management of Cytomegalovirus (CMV) infection, given the growing number of patients treated with immunosuppressive drugs, which entails more extensive use of antiviral drugs, especially for long-term prophylaxis. Antiviral treatment failure is observed in more than 10% of patients. As mentionned by the committee, though immune response is also a factor of antiviral failure, it is not strictly related to our project which focused on virology, ie (i) what is the impact of resistance mutations on the function of antiviral targets and on viral replication (ii) is there a specific mode of selection of viral resistant strains, (iii) are there specific patterns of mutations within UL97 and UL54, (iv) is there a role for co-infection?

-Keep focusing on the limited number of projects described in the written document. We totally agree with the committee on the need to focus our efforts on the projects described in our document.

We hope the committee will agree with these comments.

Sincerely yours.

J. FONTANILLE

UNIVERSITÉ DE LIMOGES