BMGIC - Biothérapies des maladies génétiques et du cancer

Rapport Hcéres

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

Section des Unités de recherche

AERES report on the research unit

Biotherapies of genetic diseases and cancer

From the

Université Bordeaux 2 Victor Segalen

INSERM

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Le Président
de l'AERES

Jean-François Dhainaut

Section des unités
de recherche

Le Directeur

Pierre Glorieux

Mai 2010

Research Unit

Name of the research unit: Biotherapies of genetic diseases and cancer

Requested label: UMR_S INSERM

N° in the case of renewal: U876

Name of the director: M. Hubert De VERNEUIL

Members of the review committee

Committee chairperson

Mrs Anne GALY, Genethon, INSERM, Evry

Other committee members

Mrs Nathalie CARTIER-LACAVE, INSERM, Paris

M. Marcel DECKERT, Université de Nice

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Committee members suggested by CNU, CoNRS, CSS INSERM, CSS INRA, INRIA, IRD...

Mrs Mireille CLAUSTRES, CNU member

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Observers

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M. Alain BLANCHARD (Université de Bordeaux 2)

Report

1 • Introduction

- **Date and execution of the visit:**

The site visit took place on November 5th 2009 at the U876 laboratory and the reception was well-organized. After a general introduction from the Director, the committee heard and questioned the scientific projects presented by each team, talked about the life and organization of the unit with the different staffs and discussed with a representative from Bordeaux 2 of the University strategy and implication in the unit. The visit ended with a closed door Committee session to share appreciations.

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

The Unit is a 800-900 m2 laboratory located on the campus of Bordeaux 2 in close proximity to the Pellegrin Hospital. The project proposed for 2011-2015 is the evolution of a project that was initiated in 2002 as a single team Unit working on gene therapy in stem cells as applied to porphyria and skin. In 2004, this small Unit incorporated a team of Hematologists working on leukemia and the group was renewed as an Inserm Unit in 2007-2010 with such 2 team-structure entitled "Gene Transfer into Stem Cells". The new project for 2011-2015 is constituted of the same 2 teams working in parallel: Team 1 "Gene Transfer into Hematopoietic Cells and Epidermal Cells" and Team 2 " Leukemic Hematopoiesis and Therapeutic Targets". The teams are independent but share a common expertise in hematopoiesis, stem cells and cellular signaling. Practically, they also share common equipment and general resources. The projects of the 2 Teams are grouped under the title "Biotherapies of Genetic Diseases and Cancer", which is a theme in line with the strategic orientation of the University. The Unit is part of the IFR 66 Federative Research Institute "Infectious Diseases and Cancer" and actively contributes to the "Ecole Doctorale E154 "Sciences de la vie et de la Santé" at Bordeaux 2.

- **Management team:**

The Unit is directed by Dr. Hubert de Verneuil who is also responsible for Team 1. Dr. François-Xavier Mahon leads Team 2.

- **Staff members (on the basis of the application file submitted to the AERES):**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	12	15
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	3	3
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)	8	7
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	8	9
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)	1	1
N6: Number of Ph.D. students (Form 2.7 of the application file)	7	7
N7: Number of staff members with a HDR or a similar grade	11	12

2 • Overall appreciation on the research unit

- **Summary:**

This Unit is conducting applied research in hematology and dermatology and is already well-established both in terms of staff, infrastructure and expertise. Under the leadership of the current applicant Director, the Unit has demonstrated a constant scientific productivity of good quality including a contribution of high impact in dermatology. Consequently, the scientific projects for 2011-2015 remain essentially in the continuity of current work but vary in quality and originality. In gene therapy, the interest on porphyria continues and evolves towards a gene and cell therapy approach through iPS both in CEP mice and humans. Such project is technically ambitious and has certain merits, however certain aspects are ill-conceived. A new gene therapy application is proposed in pancreatic adenoarcinoma but this is a high-risk and disconnected project. In dermatology, the group continues to study XPC as an original model to understand metabolic modifications and cancer initiation. In addition, spectacular clinical results in the treatment of infantile hemangiomas (IH) have prompted a novel project of high potential to study beta-adrenergic signaling in hemangiomas and tumor angiogenesis. The second team of the Unit remains largely focused on CML as a model of leukemic hematopoiesis to discover new therapeutic targets and to optimize treatment through a coherent and solid program on the different pathways to cell death, identification of specific microRNAs and the study of ROS in genetic instability. As a whole, the Unit is performing well and is recognized in the field of rare diseases. However, it needs to address certain weaknesses to increase its impact in the field. It would benefit from a synergy between projects and reinforcement of the strong underlying scientific theme around hypoxia and genetic instability. For a Unit called "Biotherapies of Genetic Diseases and Cancer" there needs to be a more clear clinical perspective for biotherapies and in particular a clear plan for the gene therapy of porphyria is needed.

- **Strenghts and opportunities:**

The main strength of this Unit lies in its ability to perform applied medical research through proximity to, and involvement with, the clinic. Such strong link should be an asset for the recruitment of dedicated high-performing researchers by providing a relevant context for research.

- **Weaknesses and threats:**

Several problems will hamper the pursuit of excellence and dilute ressources:

• The scientific projects are of uneven quality: The project on adenocarcinoma is disconnected from others, difficult, immature and high risk for the investigator;

• Considering the unit as a whole, there is a dispersion in the topics and disconnection between projects that do not tend towards a homogenous scientific goal. The project on porphyria has long been used as a common denominator but a new connection linking hematology, dermatology and biotherapy must now be found;

• A clear clinical plan for biotherapies is lacking particularly for the gene and cell therapy of porphyria. This will require dedicated resources or should be revised.

- **Recommendations to the head of the research unit:**

The head of the research Unit has the difficult task of managing a global project that reconciles the investigators divergent interests into a productive collegial and meaningful scientific goal. This is complicated by a natural and uneven evolution of projects. The former flagship project on porphyria provided the initial directions of work and led to the existence of the viral vector platform but now seems to be eclipsed by emerging themes in cell signaling and cancer that are more strongly anchored in clinical practice and provide a more cohesive theme of study than porphyria. In such context, the Head of the research Unit should be commended for a good and harmonious management, for consistency in the quality of the resarch as well as for

promoting young investigators to develop their own projects. The committee would like to encourage the Director to place further efforts to achieve higher levels of scientific impact. This can be done by developing a more pro-active strategy in terms of project orientation and recruitments that will strengthen the scientific expertise of the Unit. It would be wise a) to reinforce projects with highest potential through novel recruitments and through reorientation of internal resources b) to create scientific synergies within the Unit particularly around the theme of respiration/death and genetic instability/cancer which can be extended to all subjects. A clear and practical plan for the clinical gene and cell therapy strategy of the Unit should be developed. The committee would also like to encourage the Director to seek higher levels of funding and additional European notoriety through submission of larger grants and European project coordination, rather than constantly entertaining multiple small contracts.

- **Production results:**

A1: Number of permanent researchers with or without teaching duties (recorded in N1 and N2) who are active in research	15
A2: Number of other researchers (recorded in N3, N4 and N5) who are active in research	17
A3: Ratio of members who are active in research among permanent researchers [(A1)/(N1 + N2)]	1
A4: Number of HDR granted during the past 4 years	1
A5: Number of PhD granted during the past 4 years	7

The two teams have been actively publishing for the past 4 yeats signing a substantial number of papers as leaders. These papers include some publications in good journals including top-tier journals. Yet, the majority of the publications has been in journals with average to low impact factor. This is particulary true of most of the clinical studies published by team 1. It is also remarkable that besides their original work, the researchers of this Unit have engaged and published a large number of collaborations which provides another incidator of the impact of their work.

	Number of Publications since 2005 and constributions	
	As leader	As collaborator
Team 1	58 including 25 scientific studies znd 33 clinical studies	58 Including 30 scientific studies and 28 clinical studies
Team 2	24	43
Total	82	101

3 • Specific comments on the research unit

- **Appreciation on the results:**

The results of research are of good quality both in the field of fundamental scientific and clinical data. Among the results obtained it is worth mentioning highy original observations in the treatment of IH with beta2 blockers, which have had a widespread impact in the international literature and clinical practice.

The productivity is very good. Team 1 has a steady stream of 8-15 peer reviewed publications per year since 2005 in relation with the scientific activities and about 10 peer-reviewed papers per year concerning the clinical activities. Team 2 has about 10-15 peer-reviewed papers per year. Overall, the quality and impact is

very good as several papers have been published in high impact journals such as New England Journal of Medicine, Blood, Cancer Research and J. Biol Chem.

Partnerships have been established with pharmaceutical companies such as Abbot for testing novel molecules for CML and with Pierre Fabre for the development of a topical formulation of propanol for hemangiomas.

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

Three investigators are internationally-recognized experts who are regularly invited to present their research at international meetings but plenary talks at large international meetings are not mentioned. One member is an associate editor of several journals in dermatology. Another is a member of the scientific advisory counsels of several charities. All contribute to University, Hospital or National Institutions committees.

The overall number of PhD students and postdocs is very good (7 and 5, respectively) but there is a relative weakness in the ability of the Unit to attract high level full-time researchers.

Most post-docs and students are recruited at the local or national level, though through competitive calls. There is no shortage of resources to conduct good science. Many of them want to stay in science. Regarding the continuous scientific output of the Verneuil department, many researchers are successful in publishing quality papers. Of course, it is not always easy to attract high quality post-docs at the international level. Most labs that succeed in doing so are very well established at the international scene by a continous output of top level papers (IF>10), which is not the case for this research team. Improvement of the impact of their fundamental research and integration of the department at the international level may open new opportunities.

Research funds have been secured from several contracts with industry, patients associations and ANR. Funding from the European Comission is not entirely clear as 2 networks are mentioned (GeneSkin and LeukemiaNet but no funding is specified).

The participation to collaborative and international activities is adequate. In dermatology, the networking is well developed. A Unit member is the coordinator of a special interest group on vitiligo in the International federation of pigment cell society. At the national level, this investigator also coordinates a national reference center for rare skin diseases. In hematology, an important national multicenter clinical study “STIM” has been coordinated. Several international collaborations have been established within the various projects with reputed investigators. A long-standing collaboration with Punan Malik concerns the genotoxicity of rHIV vectors in erythroid cells and has generated 1 common article in the past 4 years. A collaboration with University of Columbia has led to a common patent application on NOX inhibition as a target for prevention and treatment of cancer. Several collaborations with other national teams have generated joint publications on the mechanisms of leukemogenesis in myeloproliferative diseases.

Research contracts that have been established with industrial partners contribute to support the costs of research in the laboratory.

- **Appreciation on the strategy, management and life of the research unit:**

There seems to be an efficient and friendly organization of the work in the Unit. The laboratory management seems reasonable and collegial. The laboratory is spacious and clean. Common consumables are shared to alleviate costs. Various researchers share the regulatory responsibilities concerning animals, radioactivity, GMOs, human cells etc… for the whole group. Weekly laboratory meetings are held on a regular basis and students encouraged to present. No particular comment was raised concerning the communication policy in the Unit. A good atmosphere was evident among individuals. However, the committee must relay that there are problems with the restricted access to the laboratory that should be resolved to facilitate the work of the investigators and staff while protecting their equipment and belongings.

The Unit is actively involved in teaching and training. It contributes to scientific animation in particular with the organisation of the "Cours annuel de dermatologie Pediatrique" and will organize the next international conference of Pigment Cell in 2011.

The investigators contribute to teaching and training according to their University and Hospital duties. For some staff members the latter take a significant part of their time.

- **Appreciation on the project:**

All of the projects proposed have a strong clinical relevance to the fields of hematology, dermatology or cancer by studying treatments or mechanisms of pathophysiology of these conditions. The projects are developed and clinically-relevant but of uneven quality. The most exciting and original project is proposed by the dermatology group (Team 1-Projects 3 and 4), which shows a great example of how clinical observations can inspire a novel line of research. Two separate models test the hypothesis that hypoxia-regulated pathways may play a significant role in the initiation of tumorigenesis. The model using XPC-deficient cells is a pertinent precancerous model to examine the relationship between metabolism, NAPDH activity, NOX-1, the generation of ROS and the induction of carcinogenesis. The study of hemangiomas examines the role of beta blockers and hypoxia in the angio/vasculogenic cascade and tumor angiogenesis. The proposed studies are feasible, articulate, well developed and have a strong potential for innovation. There should be more overlap between this expertise and the other projects. For its innovation, structure and impact, the committee rates this projet "excellent".

The study of CML as a model for leukemic hematopoiesis and therapeutic targets (Team 2) presents a solid and coherent program. Various pathways of cell death, are studied through cell signaling, pharmacology and molecular approaches. This is not a very original topic but the Team has succesfully studied the clinical, cytogenetics and pharmacology aspects of CML in the past. More innovation is proposed for instance by identifying microRNAs that could be involved in the physiopathology of CML, by studying the role of ROS in genetic instability and by examining the mobilisation of LINE retrotransposon are markers or consequences of chromosomal instability in CML. The studies are feasible but the lack of sufficient force in bioinformatics and high-throuput molecular biology will probably be a limitation in this highly competitive field. Yet, results are likely to bring clinically-relevant advances with the possibility to bring novel compounds to the clinic and to extend to other diseases. The expertise in cell signaling is also coherent with that of Team 1-dermatology. The committee rates this project "very good".

The therapeutic projects on porphyria consist of a) pursuing a gene transfer approach and b) developing a method of corrrection using genetically-modified iPS. The feasbility of these projects will be supported by the undeniable expertise of the group in congenital erythropoietic porphyria, hematopoiesis as well as in the use of HIV-derived lentiviral vectors for which they have established a local platform for production of research-grade batches. The iPS technology is completely new to the laboratory but the principal investigator has followed a training on the topic and should rapidly become proficient. On the other hand the committee has some reservation on certain aspects. In spite of funding, the clinical gene therapy project fails to progress convincingly due to lack of a clear plan taking into account the rarity of patients, GMP availibility and regulatory requirements for product biosafety. The iPS project is essentially an application of a trendy technique, it is not hypothesis-driven and appears to be also not clinically-feasible for the near future. The strategy of including a suicide gene to reduce tumor formation by iPS-derived cells, will have to be thoroughly validated . The committee is surprised to see so little contribution from internal collaborators on the problem of genotoxicity of integrative vectors, or tumor formation by iPS. Why restrict iPS to porphyria and not envision this project as an opportunity to gain knownledge in cell differentiation and cancer ? Why are lentiviral vectors not used more abundantly in other projects of the Unit ? Will the unit be sufficiently competitive in the field of iPS, considering that major research institutes in the world investing significant resources? In spite of these reservations, given the notoriety of the investigators in porphyria, the committee rates this project "very good".

The project on molecular targets and gene therapy of pancreatic adenocarcinoma is clearly apart from the others. It is supported by a young investigator with a solid expertise of FGFR signaling and the desire to develop a novel application for the gene therapy platform. However, the project is not clearly hypothesis-driven and the project is risky. The in vivo targeting of pancreatic tumor cells by gene therapy is a highly-difficult goal, not mastered by the investigator and combining technical difficulties with the complexities of the mechanisms of cancer escape that will compromise the focus of the project. On the other hand, it seems that there are

obvious links between the skills developed by the investigator on FGFR expertise or 3D in vivo models that could be useful to other projects, notably to understand or to model vascular formation. As it is, this risky project is rated “good” by the committee who strongly recommends a revision to find cohesion with the other projects while maintaining an opportunity for this young investigator to develop professionally. The project on skin and hemangiomas is the most cutting edge project of the Unit.

The Unit is sharing certain common resources and equiment but the 2 Teams have an independent budget.

4 • Appreciation team by team

Team 1 : Gene Transfer in hematopoietic and epidermal cells

Team leader : M. Hubert De Verneuil

Team 1 comprises 2 groups with distinct research themes:

- Group A : Biotherapies with two separate projects
  - Project 1: Gene therapy of porphyria;
  - Project 2: Pancreatic cancer.
- Group B : Dermatology also separated In 2 projects, but this group is more focused than group A.
  - Project 3: Cutaneous photoprotection;
  - Project 4: Hemangiomas.

As a result, the committee decided that the diverse topics and qualities of the projects in Team 1, particularly in Group A required separate evaluations to provide the Unit Director with very clear perspectives on each of these projects. Therefore, 3 separate evaluations for Team 1 were performed.

Team 1 – Group A

Project 1 : Gene Therapy of Porphyria using genetically-modified iPS cells

Project leader : M. François MOREAU-GAUDRY

- **Staff members (on the basis of the application file submitted to the AERES):**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)		4
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)		1
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)		0
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)		2
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)		0
N6: Number of Ph.D. students (Form 2.7 of the application file)		2
N7: Number of staff members with a HDR or a similar grade		3

- **Appreciation on the results:**

The treatment of porphyrias has long been a major focus of interest for Team 1 and led to elegant preclinical studies and to the development of an RNAi and knock-in mouse model of this pathology. Since 2005, only about 6 papers have been published on porphyria but one is currently in revision in a good journal. One PhD student has been trained on the project since 2005.Two new PhD students have been recruited for the new project.

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

One of the team leaders has been invited to several international symposia dedicated to the subject of porphyrias or erythropoiesis.

There has not been any recruitment of high-level scientists from abroad.

In the past this project has been successfully funded by AFM (2005-2006) for preclinical gene therapy studies in models of porphyria, by ANR (2006-2008) for the gene therapy of porphyria, by AFM (2007) for the production of clinical vector batch and by AFM for a liver targeted gene therapy (2008-2009). For the future project and the next 3 years, funding has been secured from ANR with the Genopat project: iPSGENETHER (2009-2011) for the treatment of poprphyria with gene-modified iPS.

This project involves a collaboration with a laboratory in Cincinatti.

This project concerns a rare disease which represents a valuable and interesting model both in scientific terms but also for patients afflicted with this condition. However, a weakness of this project is the lack of a clearly developed clinical plan, possibly due to a lack of patients owing to the low frequency of this disease, to obtain concrete results matching the scientific investment.

- **Appreciation on the strategy, management and life of the team:**

The Team is actively contributing to ED 154.

- **Appreciation on the project:**

This group develops new therapeutic approaches for erythroid porphyria, a very rare disease. A gene transfer approach has been developed in the perspective of a clinical application. The clinical project is currently held back by the lack of clinical-grade vector, pending demonstration of the safety of the vector with respect to genotoxicity and the lack of patient recruitment. A clear plan must be developed to overcome all of these difficulties and to provide a clear concrete perspective for clinical trial and access of treatment to patients.

A new research project has been developed to address the lack of patients cells and the concerns related to insertional mutagenesis with the current integrative vectors. The project consists in generating iPS and reprogramming them into hematopoietic cells before genetic correction. For the murine project, iPS will be derived from fibroblasts of CEP mice using minimal combinations of reprogramming genes and optimizing the use of small molecules such as valproic acid in the culture conditions. The investigators propose to use non-integrating lentiviral vectors to avoid the persistence of reprogramming genes in the cells and to facilitate the induction of differentiation. The approach has already been used by others but is not easy. Furthermore, a certain level of genomic integration will occur anyway. To eliminate transformed clones that may result from the stable integration of Oct4 or Sox2 genes, the investigators propose to insert a suicide TK gene in a bicistronic construct to eliminate the cells with ganciclovir. It is not certain if this approach will be efficient with such highly tumorigenic cells, considering evidence for epigenetic silencing and secondary mutations in these cells. An alternative strategy should be envisioned. Some groups have achieved reprogramming with proteins instead of genes, for instance. Once reprogrammed cells are obtained, the iPS will be induced to differentiate into HSC on OP9 stroma. The iPS-derived HSC will be gene-corrected by lentiviral mediated gene transfer or homologous recombination. Both the differentiation and correction projects are vaguely described but will probably utilise existing technologies from other groups. Then, the iPS-derived HSC will be transplanted into CEP mice to test their biological activity, hematopoietic and therapeutic potentiaL In parallel, the group proposes a project to derive human iPS from fibroblasts and keratinocytes in collaboration with a team in Montpellier . Similarly the iPS will be derived with NIL and induced to differentiate into HSC using conditions published by others. The hematopoietic potential of iPS-derived HSC will be tested in the xenograft models using immunodeficient NOG mice. Particular focus is placed on erythroid production. Both projects are therefore applied research and are technology oriented. These new techniques are extremely complex and hold great potential for regenerative medicine. However, it is unlikely that major progress will occur if there is no effort to understand fundamental questions related to cell reprogramming and differentiation, notably the tumorogenicity, genetic instability of the cells. It is therefore a pity that no more effort is being placed in the group to develop hypothesis-driven projects which could bring major contributions in the field considering the expertise in hematopoiesis and cell signaling already in place in the group.

Overall, this is an ambitious and technologically-innovative project. It seems that it will be feasible in 4 years to obtain the technical proof of principle for the approach. It is however unlikely that this will lead to therapeutic application during this period of time. Again, a clear plan for the eventual use of iPS in man and particularly in porphyria patients should be developed.

The iPS technology has high potential.

- **Conclusion:**

  - Summary:

The aim to treat porphyria by cell and gene therapy has not reached the clinical stage but has been largely revised through a new approach with iPS. The project for the next 4 years will be a technology-oriented research effort on iPS generation and hematopoietic reprogramming. It is a difficult project which appears to have little likelihood to be in the clinic soon. Nevertheless, this effort has merit on a scientific and strategic front because this techology has clearly made an important change in the field of regenerative medicine and it is worthy for this Team and for the Bordeaux university to invest in this complex field.

— Strengths and opportunities:

This project will strengthen the already strong expertise in the group on cell culture and hematopoiesis, which in turn is hoped to facilitate the production of iPS and the study of their differentiation in various models. The "niche" of expertise with porphyria that is rather unique in the world should ensure some level of originality to the project. The iPS technology in the Unit could serve as a platform for the study of cancer mechanisms and genetic instability in other models such as skin.

— Weaknesses and threats:

This is a highly-competitive field. The NIL approach may not be sufficiently efficient. The project may be successful but if it remains purely a technology application it will only confirm other studies. It may be difficult to overcome certain difficulties associated with reprogramming and differentiation such as tumorigenesis or genetic instability without more scientific investment on fundamental questions in the project.

— Recommendations:

Invest more effort in fundamental questions and try to create synergy with other projects of the Unit.

Project 2. Molecular Targets and gene therapy of pancreatic adenocarcinoma

Project leader: Mrs Sandrine DABERNAT

- **Staff members (on the basis of the application file submitted to the AERES):**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)		4
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)		0
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)		0
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)		1
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)		0
N6: Number of Ph.D. students (Form 2.7 of the application file)		1
N7: Number of staff members with a HDR or a similar grade		0*

0* : The PI does not have an HDR. The PhD Student is probably trained by the leader of this group under the Direction of the leader of Team1/Project1.

- **Appreciation on the results:**

This new project is proposed by a young MCU-PH who was previously involved in growth and expansion of pancreatic epithelium and particularly in FGFR3 signaling in cancer cells. Based on that, the leader has chosen to drive her project into a cancer gene therapy project using sindbis pseudotyped non integrative lentiviral vectors carrying suicide gene and/or apoptosis inducing factors. The question addressed is relevant but lacked of some originality since very competitive teams are already involved in cancer gene therapy using suicide gene vectors and have obtained very modest success. Although the therapy of pancreatic adenocarcinoma is of evident medical interest, this project is immature and too risky. The researcher who is in charge of this project

has 14 publications from 2005 in relevant Journals. Some collaborations have been indicated in the oral presentation of the project, mostly local for animal facility or vectorology.

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

This group is new and most of the following criteria cannot be applied. Some grants have been obtained from INCA, Ligue Régionale contre le Cancer, and IFR66.

- **Appreciation on the strategy, management and life of the team:**

This young group is really motivated by the subject; however, there are too many sub-projects, and it does not seem to exist a "general vision" about the direction towards the group is going. It is difficult to evaluate the exact composition, as several collaborators are also involved in other projects of Team1; the group involves one technician and one PhD student. This group is really involved in teaching (PCEM1 Bordeaux and Reunion Island) and, since 2009, is RESPONSIBLE for Bio Cell teaching in PCEM1 and participates to other courses.

- **Appreciation on the project:**

The project on gene therapy does not seem feasable in 4 years.

- **Conclusion :**

— Summary:

The project on molecular targets and gene therapy of pancreatic adenocarcinoma is too vast, complex, and risky.

— Strengths and opportunities:

This young group is enthusiastic, with strong motivation for research, and must be left an opportunity to develop. Strong motivation of the group leader.

— Weaknesses and threats:

The committee found that this new project was highly risky, too vast and dispersed. For specialists, the "gene therapy part" is not competitive. In addition, this project appears relativly isolated from the others projects.

— Recommendations:

The "gene therapy" part should be discouraged (as it is), and the group should be rather encouraged to focus efforts on FGFR signalling basic research (and even reinforce this theme) as they have already a strong expertise in this field and can collaborate with leaders in USA. This group should be also encouraged to establish links with Team1/Project4 (infantile haemangiomas and tumor angiogenesis) as expertise and skills could be shared with this team.

Team 1 – Group B

Project 3: Cutaneous photoprotection: Study of metabolic modifications and cancer initiation

Project leader: F. MAZURIER

And

Project 4: Beta adrenergic signalling in infantile hemangiomas: application to tumor angiogenesis

Project leader: A. TAIEB

- **Staff members (on the basis of the application file submitted to the AERES):**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)		6
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)		1
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)		4
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)		2
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)		
N6: Number of Ph.D. students (Form 2.7 of the application file)		1
N7: Number of staff members with a HDR or a similar grade		3

- **Appreciation on the results:**

The continuous high quality studies on the rare genetic cutaneous disease XPC opened original avenues to understand how oxidative and metabolic stresses affect skin cancer initiation. In addition, original clinical observations in infants regarding the treatment of the benign vascular tumour hemangioma led to an outstanding research project on beta-adrenergic signaling on tumor angiogenesis that will impact both clinical practice and fundamental cancer research. The proposed project has high relevance for the cancer field. The team extends earlier work in the field making use of the appropriate in vitro and in vivo models, some of them being developed in their own lab.

In view of the recent development of these projects the output is good. Since the last 3 years, about 6 papers high impact research papers (JID, JBC, N.Engl.J.Med) and a similar number of clinical papers have been published on these research topics. The more senior PI working in IH is an internationally recognized expert in the field. The more junior PI working in XPC has until now been mainly active in the field of heamatopietic stem cells (about 20 high quality papers since 2000), will also succeed in hitting the international scene in the field of cancer because of the relevance of his projects.

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

In 2008 and 2009, the PI of project 4 gave about 13 invited lectures.

Since 2005, there has been no recruitment of high-level scientists from abroad. One full-time Postdoc and one PhD student work on the XPC project. One full-time Postdoc works on the IH project. One PhD student has been trained since 2005. Two new PhD students have been recruited for the XPC project. The ability to recruit foreign post-docs is a continuous challenge. By increasing the visibility in the field of fundamental cancer research, combined with the clear clinical links they established, they should try to attract good quality postdocs from abroad. In this respect, increasing the international networking on the basic cancer research scene would be benefical.

The team succeeds in raising sufficient competitive funding, also from industry. Most of the funding comes from national funding (Preclinical studies in scleroderma with Novartis (2006), preclinical gene therapy studies in XPC with Association Enfants de la Lune (2008), skin substitutes witn Integra Corp. (2008-2009). The XPC project has been funded by ARC-INCA 2007-2009. The IH project is funded Pierre Fabre (2008-2009)) . Over time the team should try to get more access to international funding, e.g. EU funding. The committee realizes that this is not always an easy task.

The dermatology group as a whole is part of NRCRSD (National Reference Centre for Rare Skin Diseases), the burn unit, and the Vitiligo European Task Force. The XPC project : Collaboration with Columba University.

The research activity of the Dermatology group has concrete impacts on clinical practice (beta-adrenergic signalling in IH), and translational research with industrial development of skin substitutes (Integra Corp), and of a topical formulation of propanolol (Pierre Fabre), and preclinical studies (scleroderma and XPC).

- **Appreciation on the strategy, management and life of the team:**

The Team is actively contributing to ED 154 and teaching and training at University Bordeaux 2.

- **Appreciation on the project:**

Project 3 (XPC) and Project 4 (IH) are both excellent, highly feasible, original with a high impact and relevance in several fields including dermatology, angiogenesis and cancer research. This team is trying to unravel the relationship between metabolic modifications and cancer initiation using XPC-deficient cells and mice (Project 3), and studies the role of ß-adrenergic signalling in hemangiomas (Project 4). In preliminary experiments, they found that XPC knock-down in keratinocytes results in metabolic changes and increased proliferation. Within the field of cancer research there is a revival of the hypothesis that metabolic changes contribute significantly to tumoregenesis. One of the critical steps in this metabolic change was the overactivation of NOX1, leading to increased ROS production. Using in vitro cellular XPC knock-down they will try to identify the initial cellular changes leading to the altered behaviour of these cells by making use of biochemical approaches and RNA profiling experiments. In additon, the research team has the availability of Nox1-/- and tissue-specific XPC-/- in vivo models that will be instrumental in the proposed research. In view of the availability of the required tools and the expertise of the team in the field the feasability of the project scores high. The second dermatology project originates from their discovery of the use of the beta-blocker propranolol to treat successfully hemangioma vascular tumors in children. This lead to the development of a highly relevant project to study the contribution of the beta-adrenergic pathway in angiogenesis in tumor development. They will use both cellular and in vivo approaches to identify these signaling pathways. The relatively easy access to clinical samples is an asset for this project. In addition, they will analyze whether genetic variation in an endogenous beta-adrenergic inhibitor, GRK5, is affected in hemangiomas. To conclude, this team had clearly the best overall developed strategy.

In view of the well-developed future strategy, this project would benefit from allocating more resources to it. The recruitment of at least one additional post-doc and predoc would be instrumental for the further development of this project.

The proposed projects are original and highly relevant to the cancer field. This is a trendy research avenue and the program is highly feasible and well-conceived.

- **Conclusion:**

  - Summary:

This project proposal is of high quality and has a well-developed strategy. Therefore it was very much appreciated by the Committee.

  - Strengths and opportunities:

The required expertise, tools and access to clinical samples are present within the research team. There is currently a COST program on hypoxia sensing, which seems still open to additional partners. It would be benificial for networking to try participating in this COST action.

  - Weaknesses and threats:

One concern is the lack of high levels full time scientists (with the exception of the PI for project 3).

  - Recommendations:

Consider attracting or allocating additional resources (personnel) to this project.

Team 2 : Leukemic hematopoiesis and therapeutic targets: model of chronic myeloid leukemia and other myeloid disorders to optimize treatment

Team leader : M. François-Xavier Mahon

- **Staff members (on the basis of the application file submitted to the AERES):**

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)		3
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)		1
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)		3
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)		1
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)		1
N6: Number of Ph.D. students (Form 2.7 of the application file)		4
N7: Number of staff members with a HDR or a similar grade		3

- **Appreciation on the results:**

Team 2 is structured around the study and treatment of leukemia. This team proposes 3 projects. The first project is to study the mechanisms of tyrosine kinase inhibition and resistance in CML. The second project is to study the leukemogenesis of CML and other myeloid disorders. The third project is translational and clinical research.

The overall scientific originality of the research is not very high (CML cell lines, cell signaling). However, the research program of Team 2 has high quality values in oncology and molecular or cellular hematology and is

very clinically-relevant, as it addresses novel mechanisms of resistance to tyrosine kinase inhibitor and novel markers for predicting relapse.

Since 2005, this team has published more than 65 peer-reviewed papers in the field of hematology, oncology and immunology including high impact journals such as Blood, Leukemia, Cancer Research, Journal of Clinical Oncology. 4 PhD students have been trained since 2005.

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

Since 2007, the team members gave a total of 19 invited conferences in the fields of hematology and chronic myelogenous leukemia.

Since 2005, there has been no recruitment of high-level scientists from abroad.

Fundings from Charity Associations (Fondation de France, ARC, Ligue contre le Cancer, Association Laurette Fugain) on molecular mechanisms of CML resistance to tyrosine kinases inhibitors and studies on other myeloid disorders. Funding from INCA (Team leader:, Tyrosine kinases in haematological malignancies).

The team leader is the lead investigator of a national PHRC for the multicentric study STIM (Stop Imatinib) that started in 2007, and the coordinator of an INCA network (Tyrosine kinases in haematological malignancies, 5 research teams) (2008-2010). Team 2 is a partner of the European research network LEUKEMIA NET.

One of the reaserchers of the team has a collaboration with the Ipsogen company as part of a CIFRE PhD scholarship (LINE retrotransposon expression in CML genetic instability).

The research activity of the Hematology group has concrete impacts on the understanding of the molecular mechanisms of resistance to tyrosine kinase inhibitors in CML patients and on the identification of clinically-relevant novel markers for predicting relapse (international patent IB2009/006622 with CNRS).

- **Appreciation on the strategy, management and life of the team:**

The members of the team are actively contributing to teaching and training at University Bordeaux 2 and ED154.

- **Appreciation on the project:**

The investigators study CML as a model for leukemic hematopoiesis and therapeutic targets. To understand and to overcome the mechanisms of resistance to tyrosine kinase inhibitors in patients, the investigators conduct biochemical studies in cell lines to study novel inibitors of ABL such as nilotinib and examine the various pathways of cell death notably bcl2 and the intrinsic mitochondrial apoptotic pathway. The effects of pharmacological agents such as ABT737, which is a BH3 mimetic acting on Bcl2, BclXL and Bcl-W, is being studied in detail. Besides this classical approach, the investigators have also developed more novel orientations to identify microRNAs that could be involved in the physiopathology of CML or other leukemias. They also propose to study the role of ROS in genetic instability notably by examining the mobilisation of LINE retrotransposon to determine if they are markers or consequences of chromosomal instability in CML. The studies are feasible but the lack of sufficient force in bioinformatics and high-throuput molecular biology will probably be a limitation in this competitive field. Yet, results are likely to bring clinically-relevant advances with the possibility to bring novel compounds to the clinic and to extend to other diseases. It would be worth while considering to try raising additional funding, e.g in joint projects at the national or international level, to develop non-biased screening strategies to identify novel genes involved in therapy resistance in the different myeloid disorder models studied within the research team. The expertise in cell signaling is also coherent with that of Team 1-dermatology.

There are no cutting edge projects. However, there are specific topics including miRNAs profilling and characterization of novel cell death pathways in CML pathophysiology and studies of the involvement of oxydative stress and LINE retrotransposon expression in CML genetic instability that show innovative prospects.

An effort to extend the expertise of the team in molecular and cell signaling on studying other myelogenous and haematological diseases was noted.

- **Conclusion:**

  - Summary:

This Team is performing very good applied research but needs to remain competitive. The committee encourages the new directions of research that are taken to extend the previous work on CML and to broaden horizons.

  - Strengths and opportunities:

The team has a long-term implication and expertise in onco-hematology and CML studies – The coherence of the research project and deep interaction with the clinic and hematologists (clinical networks) has been noted by the committee – A MCU who recently joined Team 2 has an expertise in bioinformatics and gene expression analysis.

  - Weaknesses and threats:

The Team needs to cultivate a competitive edge to measure against the strong international competition in the field of oncology, advanced technologies in the field of leukemia.

  - Recommendations:

The expertise of Team 2 should be extended on other myelogenous and haematological diseases.

Note de l'unité	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	**A**	**A**	**A**	**A**

Nom de l'équipe : *GENE TRANSFER IN HEMATOPOIETIC AND EPIDERMAL CELLS*

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	*A+*	*A*	*A*	*B*

Nom de l'équipe : *LEUKEMIC HEMATOPOIESIS AND THERAPEUTIC TARGETS*

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	*A*	*A*	*A*	*A*

Université Victor Segalen Bordeaux 2

UNIVERSITÉ DE BORDEAUX

Monsieur Pierre GLORIEUX
Directeur de la section Unités de recherche
AERES

Bordeaux, le 22 février 2010

Monsieur le Directeur,

Je vous transmets les observations de Monsieur Hubert de VERNEUIL, Directeur de l'Unité « Transfert de gènes dans les cellules souches à visée thérapeutique », faisant suite au rapport du Comité de visite de l'AERES.

Je vous prie de croire, Monsieur le Directeur, à l'assurance de mes sincères salutations.

Le Vice-Président du Conseil Scientifique,

Alain BLANCHARD

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Transfert de gènes dans les cellules souches à visée thérapeutique
INSERM U876

Professeur Hubert de VERNEUIL
Case 10 - 146, rue Léo-Saignat - 33076-BORDEAUX Cedex

<u>Objet :</u> Observations de portée générale sur le rapport d'évaluation de l'unité INSERM U876

Project 1 :

1-A Current status for the preclinical and clinical trial for Congenital Erythropoietic Porphyria:

Following the conclusive results in the murine model of the disease (Am J Hum Genet 2008), a preclinical and clinical program started in 2008. The original vector HAEW was improved i) by the replacement of the wild-type WPRE sequence with a mutated PRE and ii) by the adjunction of insulators. Several tests on $CD34^+$ cells demonstrated a similar efficiency of this modified vector HAUPIns compared to the previous one.
Research contract was accepted by AFM (Association Française contre les Myopathies) to start pre-clinical, toxicological studies and eventually a clinical trial if the results are satisfactory. A PHRC (Projet Hospitalier de Recherche Clinique) was obtained for the clinical part. A meeting with AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé) was organised in april 2008: the agency required *in vivo* experiments with the new vector pre-clinical lot. A feasibility study of a vector production lot, proposed by a company, was accepted by the main investigator in october 2008. Several assays were performed during the 2009 year without any success (low titer and only 10% of transduction on $CD34^+$ cells).
Moreover, several companies or certified laboratories were contacted for the toxicological protocol with the murine model without any clear answers on the conditions (particularly no quotation).
In the mean time, in vitro genotoxic tests were initiated on murine stem cells in collaboration with P Malik (Cincinnati, Ohio) and C Baum (Hanover, Germany). A new company has to be identified for providing preclinical and clinical lots. The research contract with AFM has to be revised.
Concerning the recruitment of the patients: CEP is a very rare disease. It is estimated that 1 in every 2 million people is affected by the disease. The protocol is planned at the European level, with the help of EPNET (European Porphyria Network; coordinator: Pr JC Deybach, Centre Français des Porphyries, Colombes, France). There are no patients in France fitting with the inclusion criteria, since most of them are treated by bone marrow transplant (BMT) during the first two years of life. As a starting point, the protocol is scheduled for three patients, which requires one or two batches of vector.

1-B iPS project

We agree that the iPS technology is very complex and will require many efforts of the team 1 to get a good expertise in this field of research. We are aware that integrase-deficient lentiviral vectors preventing genomic alteration could be possibly not efficient for reprogramming. For this reason, we have also included lox sequences in each vector to easily switch the protocol towards the use of integrative vectors, followed by excision of reprogramming sequences by transient expression of Cre recombinase (resulting in a very small genomic scar). To improve the efficiency of our vectors, we are also developing a new vector containing 3 reprogramming factors plus an anti-p53 shRNA flanked by lox sequences to easily reprogram somatic cells with a single vector. The vector driving EGFP and HS-TK under the control of the nanog promoter described in the project is already functional. This approach allows to easily screen nanog positive cells at the early stage of reprogramming and later will permit us to prevent teratoma formation after reinjection of unwanted residual iPS cells after hematopoietic differentiation. The development of iPS technology is not the final goal but a prerequisite to understand more fundamental mechanisms in the field of the biology of dedifferentiation. First, this technology will be applied to reprogram CD34 positive cells from patients with chronic myeloid leukemia (CML; collaboration with team 2), for a better understanding of pathophysiology of this cancer and the resistance to tyrosine kinase inhibitors. The second approach will be to investigate the effect of hypoxia (role of HIF factors) in the generation of iPS in a collaboration with the dermatology group (project 3). Third, this iPS project is a new promising approach in the future of gene therapy in general. Our laboratory will use CEP and xeroderma pigmentosum (XP) diseases as models for these applications.
In conclusion, iPS technology expertise will give rise to many novel transversal projects in our laboratory and could be offered later as a service of the vectorology platform of our future SFR "Transbiomed".

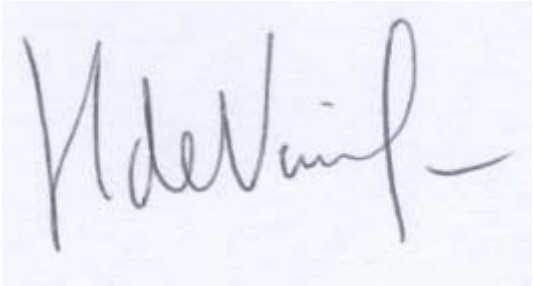
Project 2 :

The cancer gene therapy project presented to the comity started mid-2009. Therefore it is immature since very new. We are aware that it is also risky, as are most innovative projects involving new technological approaches.

The main goal of the project is to find molecular targets with anti-tumour properties against pancreatic adenocarcinoma and to develop new methods to use them in vivo. To date, the use of targeting small inhibitory molecules showing therapeutic advances in other types of cancers have failed when applied to pancreatic cancer. In the meantime, a growing set of data is now available on factors involved in pancreatic adenocarcinoma development and aggressiveness. Their potential therapeutic qualities have first been demonstrated in vitro. However, modulating gene expression in vitro is not sufficient to efficiently design therapeutic strategies. According to many sources, including the National Cancer Institute, cancer gene therapy is a relevant option for chemo- and radio-resistant tumours. Until recently, cancer gene therapy used non viral vectors to deliver genes in tumours with low efficiency or viral vectors with major adverse side effects. As to now, recent publications confirm that lentiviral vectors represent a good tool to transfer genes in cancer gene therapy approaches. Our lab having notorious expertise in gene therapy and in gene delivery using lentiviral vectors, we think that developing a cancer gene therapy program is fully into the general aim of the lab, which is called "Biotherapies of genetic diseases and cancer". Project 1 PI, who is expert in gene therapy and delivery is of course intellectually closely involved in the project. We indicated that FGFR3 is

included in the project since we test the hypothesis that FGFR3 regulates the pancreatic tumour growth. In parallel, we develop a targeted secured method to efficiently deliver toxic gene in pancreatic tumour cells. According to our preliminary results, this gene delivery system could be used in future to deliver an inhibitory isoform of FGFR3 in pancreatic tumour cells. To fully take advantage of the scientific options of our lab, we are currently developing a collaboration with the team 2, to test the toxic effect on pancreatic tumor cells of the inhibitory molecules active on LMC such as tyrosine kinases inhibitors that act on apoptosis.

Finally, we don't pretend that we can find a way to cure pancreatic adenocarcinoma in 4 years. However, we believe that our approach might provide important options for designing therapies. Nowadays, cancer treatment necessitates targeted programs and we think that the present project is fully relevant to the field.



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