Small Scale Collaborative Project

RAPSODI

PRe-clinicAl studies of a PSA-based human vaccine candidate targeting visceral, cutaneOus and mucocutaneous Leishmaniasis and Development of the associated procedures for further clinIcal trials

FP7 Contract Number: 223341

PUBLISHABLE SUMMARY

Period: M1 to M18

Publishable summary

1.1 Context

Leishmaniasis has been categorized by the World Health Organization as a “re-emerging and uncontrolled disease”, having detrimental effects on the development of endemic countries.
Various drugs (usually quite expensive) are currently being employed, which raises the problem of the development of resistance. On the other hand, vaccination is expected to be more cost-effective mean and can reach the goal of global control and/or eradication of the parasite infection. But, no such vaccine is available today.
Furthermore, tools enabling the precise categorization of the target population are also missing. As a consequence, it is still impossible to detect the individuals who should be vaccinated, and those who should not because of natural immunisation (innate or acquired immunity).

1.2 Objectives of the project

RAPSODI will first develop a human vaccine candidate based on a crude excreted-secreted antigen obtained from promastigote culture supernatant of *Leishmania infantum,* which gave highly promising results on dogs, a natural reservoir of visceral leishmaniasis (the more severe form). RAPSODI will also investigate the possibility of extending the action of the vaccine candidate to cutaneous and mucocutaneous leishmaniasis. At the end of the project, the vaccine candidate should be ready for human clinical trials.
RAPSODI will also develop assays for population categorization, together with a marker signature for genetic susceptibility assessment. Finally, RAPSODI will provide the immunoassay associated to the efficiency follow-up of vaccination.

1.3 Work performed and results obtained

The project workplan is globally divided in three axes: i) the vaccine candidate definition and production; ii) testing the antigens on human cells and the vaccine candidates on animals and iii) the setting-up of a toolbox for unraveling the specific features (parasitological, immunological and genetic) of resistance. Transversally, the recruitment of individuals from various conditions is needed to feed the project with appropriate and documented samples. The overall results should lead to an operable vaccine candidate and the related protocols for the qualification and follow-up of patients to vaccination.

Vaccine candidate antigens were produced under GMP standards. These antigens were distributed among partners for the toolbox setting-up on patient samples. In parallel, the formulation into vaccine candidates and their related analysis are in progress.

As the vaccine antigens are not strictly defined once and for all yet, all preliminary testing was possible, but the results are promising.

Regarding the definition of parasitological features of resistance, the goal of applying the same PCR approach to detect and quantify *Leishmania* infection within RAPSODI project, and make this comparable among partners, was achieved; more work aiming at identifying the parasitic load threshold for asymptomatic and symptomatic individuals and identifying the causative species of leishmaniasis is on-going.
Concerning the definition of immunological markers of resistance, an ELISA assay to determine plasma levels of different human immunoglobulin isotypes specific for Leishmania

antigens is about to be finalized. This test will be to evaluate efficient vaccination and to discriminate vaccinated from infected individuals. In parallel, studies on the cell mediated immunity to Leishmania infection are on-going.
As far as genetic features are concerned, contrasting macrophage infection phenotypes were evidenced on limited human samples, validating the proposed experimental approach. A robust and useful macrophage infection micro assay adapted to small field blood samples is now ready to use.

Apart from legal requirements (namely ethical clearance), the patient recruitment procedure needed to be standardized among the various population and phenotypes. An important work aiming at defining precise and common inclusion/exclusion criteria was performed; also, VL, CL and ML diagnostic methods and epidemiological, clinical, parasitological and immunological criteria were clearly established and standardized. The resulting protocols should be soon made available to the scientific community. A common procedure of sample handling was also designed and training sessions were organized within the consortium in order to avoid discrepancies related to manipulation bias.

The next steps will seek for gathering more comprehensive data on the efficiency of vaccine antigens and candidates on the relevant in vivo models, and finalize the conclusion of the definition of features of resistance.

1.4 Potential impact and use

Leishmaniasis is affecting 88 endemic countries with a potential of 360 Mo individuals at risk; its annual incidence is close to 2 Mo individuals, while its prevalence accounts for 14 Mo individuals; approximately 59 000 patients are dying each year. By developing solutions to circumvent such a “re-emerging and uncontrolled disease”, the vaccine candidate issued from RAPSODI shall have a great impact on the development of endemic countries, and improve the quality of life of their inhabitants by limiting social and health burdens. In addition, and quite independently from the vaccine development itself, the categorization tests will be very useful to enhance epidemiological data from the endemic areas, and could be useful to whatever clinical trials. Finally, the standardization and training activities will help enhancing a common view of dealing with leishmaniasis-related issues among the scientific community.

In order to promote the project results among the scientific community but also among the population whose life is at stake, a website has been set up (www.fp7-rapsodi.eu). A logo was also created, together with an introducing booklet and a general graphical charter, in order to provide a distinguishable identity.

Section 2 - Project objectives and major achievements during the reporting period

2.1 General project objectives

The global aim of RAPSODI is to develop a human vaccine candidate against most or all *Leishmania* species that cause the most severe forms of leishmaniasis in the world. A unique vaccinal solution will thus be provided to protect the population against the various clinical phenotypes (namely visceral, cutaneous and mucocutaneaous leishmaniasis, VL, CL and ML respectively).
RAPSODI will also develop assays for population categorization, together with a marker signature for genetic susceptibility assessment. Finally, RAPSODI will provide the immunoassay associated to the efficiency follow-up of vaccination.

2.2 Objectives for the reporting period, work performed and main achievements

The following table lists the objectives of the project for M1-M18 period.

WP	Objectives	Achievement status	Comments
1	Choice between PSA and peptides	**Complete**	
1	Availability of antigens to partners	**Partially**	Logistics issues more difficult than expected + new peptides to distribute
1	Antigen formulation	**Partially**	Formulation of new peptides missing
2	Definition of recruitment framework (criteria + data management)	**Complete**	
2	Recruitment of half of the patient	**Partially**	IPT started with delay; IOP may have a problem of frequency and/or season dependence
3	A common PCR tool for low level parasitemia	**Complete**	
3	A common ELISA tool for humoral response	**Partially**	Best concentration still needs to be defined + cut-off
3	Common tools for measuring specific cellular mediated immunity to antigens	**Partially**	Comparison checks needed
4	Proof of concept of the approach	**Complete**	
4	Proof of feasibility in the field	**Complete**	
5	Feasibility of universal vaccine	**Partially**	Possible only if conditions on epitopes and functionality are met
5	Test for discriminating asymptomatic from non infected individuals	**Partially**	Data missing for Old World Leishmania
6	Operational sharing of standardized protocols	**Partially**	Some protocols are still missing

*Li*ESAp was produced and analysed by VIRBAC as planned. The very low productivity of *La*PSA would result in the production of a very expensive vaccine (hundreds of euros per dose), that make unrealistic any development for human mass vaccination. As peptide production is far less expensive, the decision was finally taken to select only the peptides as active ingredients for the vaccine. Virbac subcontracted the *sp*PSA production and analysis

as intended. The 3 peptides A17E, A17G and E34Pc that was found to be efficient in combination in dogs in the frame of the Vaxileish project have been produced and controlled. However, preliminary results on human cells revealed that these 3 peptides alone may not be sufficient to cover both all species responsible for human leishmaniasis and the diverse HLA classes groups. More work, unforeseen in the initial plan, was thus needed to define additional peptides. A full investigation of peptides was performed by IRD and IMTAvH, in narrow collaboration with the other partners, with specific focus on both the genetic diversity of PSA, and the need to take into account the high polymorphism of HLA Class I and II molecules. Five peptides designed by IRD (3 HLA class I multi-epitope peptides and 2 HLA class II multi-epitope peptides) providing a good coverage of the world human population and maximized the number of PSA epitopes will be tested by IOP, IPT and IMTAvH in endemic areas. In addition, 6 peptides designed by IMTAvH that evidenced reactivities in a pilot experiment may be tested by the IMTAvH in endemic areas of american tegumentary leishmaniasis.
Common protocols for clinical evaluation (inclusion/exclusion criteria), sample collection and preservation and diagnostic methods were defined for recruitment of human groups (symptomatic, asymptomatic and healed) from target populations in endemic areas for VL, CL and ML.
Common protocols of cellular biology and for immunological assays were provided by IRD in order to establish the immunological profile (humoral and cellular) associated with resistance and susceptibility to the parasite in the different human groups.
The procedure for the evaluation of the performance of the PCR methods developed by each partner laboratory was also defined. A PCR workshop was organized in order to evaluate the PCR methods available. The goal of applying the same approach to detect and quantify *Leishmania* infection within RAPSODI project, and make this comparable among partners, was achieved.
Concerning the definition of genetic features of resistance, contrasting macrophage infection phenotypes were evidenced on limited human samples as encouraging results. It contributes to validate the proposed experimental approach to analyze the impact of human genetic diversity on the macrophage response upon infection. We evidenced the necessity to improve and standardize a micro-assay of macrophage infection applicable to limited volume of field blood samples and to large scale study. This *in vitro* cell model certainly offers a good opportunity to identify genes with differential expression between "permissive" and "less permissive" human macrophages. SAGE libraries representative of the contrasting phenotypes (high versus low permissiveness) and of the different culture conditions (infected versus uninfected condition) were constructed and transcriptomic databases were established; it definitively validated the interest of the study.
Concerning immunological and genetic assays, different training activities and methodology transfer have been carried out during the M1-M18 period. A summary of the project is now available on the EC website and a booklet for presentation of the project was produced. Also, Rapsodi project was presented in various congresses.

2.3 Most important problems during the period including the corrective actions undertaken

Preliminary results on human cells revealed that the initial combination of 3 peptides (A17E,A17G and E34Pc) is not sufficient to cover both all species of human leishmaniasis and the diverse HLA classes groups. More work, unforeseen in the initial plan, was thus needed to define additional peptides. As a consequence, the initial plan had to be modified:

- The recruitment campaigns (WP2) and diagnostic testing (WP3, WP5) had to be adapted to involve the maximum of individuals and samples with the final set of peptides

- Some additional assays on dogs (WP1) turned out to be necessary to determine the efficiency and dose effect of these new peptides. In the end, two studies are planned: the first one will determine the efficiency of the diverse new peptides (from October 2010 until April 2011) and the 2nd study, focusing on dose effect, is forecasted from June 2010 until February 2012.

This delay is also profitable to WP3 and WP4 that have experienced some difficulties and unforeseen amount of work, respectively.

Overall, we believe necessary to revise the original end date of the programme to mid-2012 in order to be able to achieve RAPSODI's goals.