



Project number: **LSH-2002-1.2.5-2**

Project acronym: **PHARMA-PLANTA**

Project title: **RECOMBINANT PHARMACEUTICALS FROM PLANTS FOR HUMAN HEALTH**

Instrument: Integrated Project

Thematic Priority: Life Sciences 1

SECOND PERIODIC ACTIVITY REPORT OF THE PHARMA-PLANTA CONSORTIUM

Publishable Executive Summary

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PUBLISHABLE EXECUTIVE SUMMARY



Pharma-Planta is an EU Sixth Framework Integrated Project whose primary goal is to develop an approved production pipeline for plant-derived pharmaceutical proteins (PDPs). Although previous research has provided proof of the PDP concept, Pharma-Planta aims to develop an entire production chain by taking candidate pharmaceutical molecules from the expression platform through all stages of production and processing, ultimately to initiate phase I human trials in Europe. The Pharma-Planta Consortium currently comprises 40 interacting groups representing 33 public institutes and SMEs from 11 European Member States and South Africa.

The objectives of the program can be summarized as follows:

- To produce recombinant pharmaceutical molecules in transgenic plants. These will be developed through all regulatory requirements, GMP standards and pre-clinical toxicity testing and will be evaluated in Phase I human clinical trials.
- To develop robust risk assessment practices for recombinant pharmaceutical molecules produced in plants, based on health and environmental impact, working with regulatory authorities within the EU as well as public groups to ensure that the production systems are as safe and as acceptable as possible, and that they comply with all biosafety regulations.
- To define and carry out a coordinated program for securing and managing intellectual property that will facilitate the availability of high priority plant-derived recombinant pharmaceuticals to the poor in developing countries while simultaneously allowing the products to be developed commercially in Europe and North America.
- To develop and refine new strategies for the expression of recombinant pharmaceuticals in plants, which can be used on a generic basis for molecules that are normally expressed poorly.

- To develop and generate transgenic plants expressing a second generation of recombinant molecules that will be used in future clinical trials.

At the beginning of the project, eight target molecules were chosen representing four key indication areas – HIV, tuberculosis, rabies and diabetes. These molecules comprised two HIV antibodies, two HIV antigens, two rabies antibodies, a TB antigen and a diabetes autoantigen. Early in the first project year, the two HIV antibodies were selected for fast-track production, meaning that these molecules were to be taken through the production pipeline as pioneers, progressing through the key areas of risk assessment, plant production, scale-up and regulatory development, with the aim of submitting at least one of them for clinical trials within the five years of the program. All eight targets were sent through a development loop of enabling technologies for improved PDP expression.

To facilitate this, the project was divided into six interacting work packages, as shown in **Figure 1.1** below. WP1 provides the target molecules and the assays for their detection in transgenic plant material. WP2 considers the potential environmental impact of different strategies for PDP production, and leads interactions with appropriate regulatory agencies and other stakeholders. WP3 provides expression platforms for the fast-track molecules and generates the bulk material for their production. WP4 is the development loop, and involves a diverse range of expression platforms and technologies for improving protein yields. WP5 oversees the scaling, processing, quality assurance and quality control of the fast-track material, and also leads interactions with regulatory agencies involved in GMP for pharmaceutical production. Finally, WP6 is charged with organizing and performing the clinical trials.

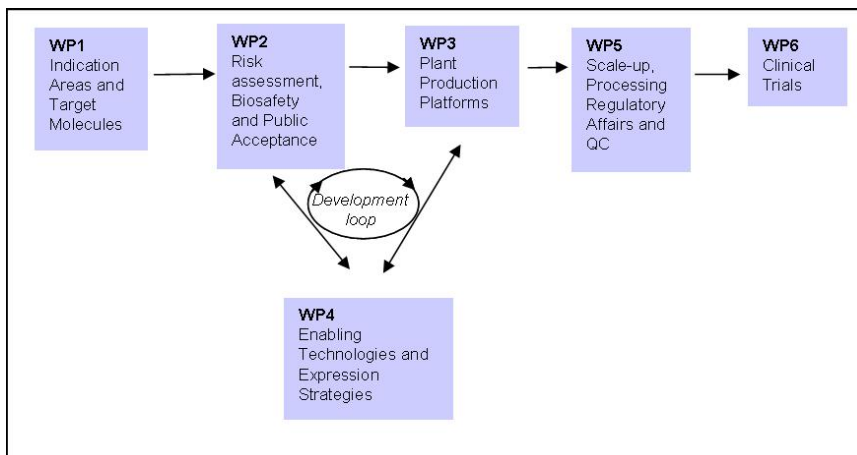


Figure 1.1
Organization of the
Pharma-Planta
program

The program is coordinated by the Fraunhofer Gesellschaft, which provides professional project management, administrative and financial coordination, and IT support. Scientific aspects of the project are coordinated by Professor Julian Ma of St George's Hospital Medical School, where the clinical trials will be performed. The management of the program also includes components to carry out biosafety risk evaluation (partner 5a), management of intellectual property (handled by MIHR, partner 32) and a comprehensive training program (chaired by Paul Christou, partner 35). Two rounds of competitive applications for PhD studentships were invited in year 1 of the program, and the projects approved in the second round of applications are now being advertised to prospective candidates. These studentships complement and extend the work envisaged within the Technical Annex thus giving the project added value.

During the second year of the project, the consortium has continued to make significant progress in all areas. WP1 scientists, having supplied gene constructs, reagents and assays for the majority of the target molecules in year 1, have focused on the molecules that were delayed in the first project year (primarily the rabies antibodies) and on a new HIV antibody to replace one of the fast-track antibodies that gave disappointing expression levels in transgenic plants. In the tuberculosis subproject, WP1 scientists have carried out further work on the development of immunological assays and associated reagents.

While WP1 has focused on expression constructs and assay development, the major activity of WP2 has been consultations with European regulatory authorities. As the regulations are evolving in this area, Pharma-Planta scientists have made a significant contribution to the discussions on PDPs. This activity includes participation in the EFSA (European Food Safety Agency) Self Tasking Working Group on non-food crops. Discussions are also in progress with EMEA (The European Agency for the Evaluation of Medicinal Products) to help to tune the regulatory procedures currently used for cell culture methods, and interpret them for the production of PDPs. These activities are being used to prepare pilot proposals for the production of HIV antibodies in tobacco and maize in preparation for phase I clinical trials. These regulatory discussions are also important for informing our deliberations within Pharma-Planta on what standard operating practices would be appropriate for these crops in a large scale production

schemes in both the EU and South Africa. As part of the generation of data for the risk assessment studies, research on root exudates has shown that although some recombinant proteins are rhizosecreted by transgenic plants, these proteins are almost immediately sequestered, and there is no bioavailability of active protein in the soil, as measured by highly sensitive ELISA.

In WP3, progress has been made in the production of transgenic plants expressing all of the new target molecules, as well of continued analysis of the existing transgenic lines. An important aspect of the work this year was the decision by WP2 to reintroduce tobacco into the fast track. Therefore, the work has been coordinated between two partners in Europe plus the CSIR in South Africa to introduce elite maize breeding lines. Stable transgenic tobacco and maize lines of have been obtained with various constructs containing the components of the HIV antibodies. An extensive screening program including more than 800 primary transgenics and offspring from selected tobacco lines has been carried out, and this has produced two transgenic lines producing the secreted form of antibody 2G12. These lines have been advanced to the T3 and T4 generation. The antigen-binding properties of the antibody have been confirmed by ELISA and Biacore assays. Laboratory-scale quantities of the antibody have been purified from tobacco cells and used for virus neutralization assays, which confirmed the efficacy of the plant-derived preparation and indicated that the plant-produced antibody is comparable to the CHO-cell derived antibody. The maize work initially focused on the model variety HiII, but has in the latter part of the year been directed towards the transformation of the elite South African genotype M37W. This is important because in the long term the consortium can save up to 2 years in breeding efforts to achieve germplasm that can be used for production in the field. Over the last 12 months, more than 100 primary transformants (variety HiII) containing HIV antibody transgenes have been generated and analyzed, and over 150 lines from the M37W elite genotype were generated. These lines represent diverse germplasm containing versions of the light and heavy chains of both antibody genes introduced as either clean DNA fragments or whole plasmids.

WP3 also encompasses plastid transformation and expression. An additional series of constructs for improved expression of the HIV antigens in chloroplasts has been

produced. These include codon optimized versions of the genes, as well as dicistronic (p24-nef) versions, and versions with modified 5' UTRs. Constructs for expression of the diabetes and tuberculosis antigens have also been developed. Efficient plastid transformation procedures have been achieved with the Maryland Mammoth variety of tobacco, and homoplasmic transplastomic tobacco plants of the Petit Havana variety, containing several cassettes with HIV and diabetes antigens have been obtained. Maryland Mammoth plants expressing the HIV antigens have also been produced. Significant levels of expression of these antigens have been demonstrated in plastids of both tobacco cultivars.

Vectors for lettuce plastid transformation are now available, and the tissue culture constraints on plastid transformation of this species have been overcome. The production of transplastomic tomato and lettuce plants expressing HIV antigens is underway and the first putative transplastomic shoots and calli have been obtained. Nuclear transformants with plastid targeted Cre recombinase have been produced for all species in the program and the efficacy of the marker excision system has been demonstrated in tobacco.

WP4 considers a diverse array of processes to improve recombinant protein yields, including protein folding, assembly, targeting, modification and proteolytic digestion. WP4 is well within schedule for the completion of all deliverables set in the previous implementation plan, and no major problems were encountered this year. Several new collaborations within WP4 and between WPs have been initiated. Highlights of the year include new approaches for the expression of HIV Nef (which has proved to be the most problematic target molecule), the generation of plant lines overexpressing individual chaperones, the generation of fusions between elastin-like peptides and HIV neutralizing antibody chains that have resulted in increased accumulation of functional antibody in transgenic plants, the identification of proteases secreted in the tobacco leaf apoplast and in the medium of cultured tobacco cells (and some of their genes have been cloned), constructs for the RNAi-induced silencing of selected proteases are now available, the production of transgenic *Arabidopsis* plants expressing high levels of HIV antibodies and derivative single chain fragments in their seeds, and the achievement of high level hGAD-65 expression using the cowpea mosaic virus expression system in *N. benthamiana*.

Although sufficient transgenic plant material expressing the fast-track antibodies remains unavailable, the WP5 groups have continued to develop and refine procedures for the recovery and purification of antibodies from tobacco leaf extracts. In particular, the influence of buffer composition, pH and salt content on extraction efficiency was investigated in detail and an optimized buffer system was created for further upscaling and process development work. Additionally, WP5 scientists initiated the evaluation of advanced filtration technologies for crude extract clarification, provided by one of the new consortium partners (Sartorius AG, Göttingen, Germany). Also in WP5, consultation exercises were intensified with the aim of obtaining useful regulatory guidance for the production of a clinical batch of antibody from plant material. A meeting with representatives of EMEA in London provided valuable information about the major concerns of the regulators. In part because of the regulatory burden, a major change in the clinical trial design was undertaken by WP6. Instead of a trial involving an injectable antibody, the consortium now intends to develop a topical agent for application in the vagina, which may comprise one or more of the HIV neutralizing antibodies.