



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

FIRST PERIODIC ACTIVITY REPORT OF THE PHARMA-PLANTA CONSORTIUM

Publishable Executive Summary

PUBLISHABLE EXECUTIVE SUMMARY



THE PHARMA-PLANTA CONSORTIUM brings together more than 30 academic research laboratories and companies representing 11 countries within the EU as well as South Africa. These participants have united within the context of an EU Sixth Framework Integrated Project to develop technologies for the production of plant-made pharmaceuticals (PMPs). While previous research has provided proof of the PMP concept, the aim of the Pharma-Planta program is to develop an entire production chain by taking candidate pharmaceutical molecules from the expression platform through all stages of the production chain ultimately to initiate phase I human trials in Europe. Eight target molecules have been identified by the consortium representing four key indication areas – HIV, rabies, tuberculosis and diabetes – two of which, HIV and tuberculosis, fall within the Grand Challenge Millennium Goals. Four of the target molecules are antibodies and four are antigens that could be developed into vaccines. The aim is to fast-track these molecules through the key elements of risk assessment, plant production, scale-up and regulatory affairs, with the aim of submitting one of them for clinical trials within the five years of the program. Two anti-HIV antibodies have been chosen for this purpose.

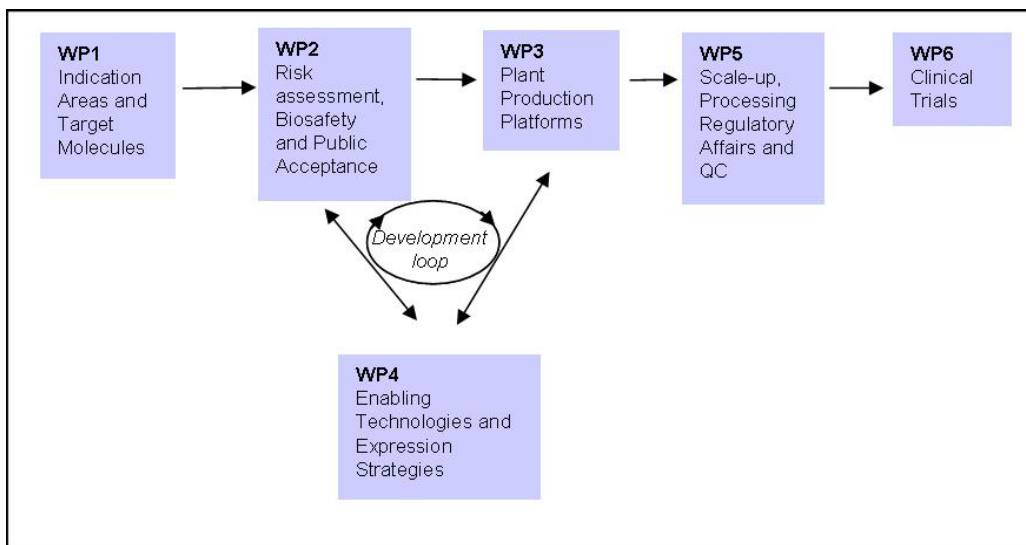
The program is coordinated by is the Fraunhofer Gesellschaft, which provides professional project management, administrative and financial coordination, and IT support. Scientific aspects of the project are coordinated by Professor Julian Ma of St George's Hospital Medical School, London. Professor Ma is also chair of the Executive Committee, the major decision making body of the consortium, and a permanent member of the Science & Technology Committee, which oversees scientific progress and makes key decisions concerning the direction of the scientific work. 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. In this context, we invited two rounds of competitive applications for PhD studentships as envisaged in the Technical Annex. We have funded

five studentships in the first year, and we can fund three more. The Training Committee solicited external peer reviews to assure the highest possible standards in selecting the successful candidates. These studentships complement and extend the work envisaged within the Technical Annex by giving the project added value. We are in the process of formulating a mechanism to utilize the remaining studentships as a mechanism to allow external collaborators to enter the consortium as part of our policy to encourage other scientists to link up with the project.

The objectives of the program can be summarized as follows:

- To produce a recombinant pharmaceutical molecule in transgenic plants, which will be developed through all regulatory requirements, GMP standards and pre-clinical toxicity testing. This will then 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.

In order to achieve these objectives, the Pharma-Planta program has been divided into six Workpackages, shown in the figure below. WP1 is charged with the provision of target molecules and the development of assays for their detection in transgenic plant material. The purpose of WP2 is to carry out a detailed and challenging study of the potential environmental impacts of different models for the production of pharmaceutical molecules within the Integrated Project and to determine the choice of production system(s) for the fast-track targets. The role of WP3 is to develop the plant expression platforms for the fast-track molecules, while WP4 seeks new expression technologies to improve recombinant protein expression and accumulation. WP5 oversees the scaling, downstream processing, quality assurance and quality control, feeding directly into WP6 which handles the clinical trials. In the first year of the project, most of the work has been carried out in WPs 1-4.



Over the last 12 months, the consortium has made good progress towards its initial goals. WP1 scientists have supplied gene constructs, reagents and assays for the majority of the target molecules, and in the case of the fast-track HIV antibodies these assays have been validated using standard operating procedures. While WP1 has focused on expression constructs and assay development, WP2 has carried out an extensive consultation on the production platforms that will be used for the fast track molecules identified in the program. This has involved visits to the regulatory authorities and stakeholders in the EU, North America and South Africa. The principal publication is a consolidated report

(see **Appendix II**) detailing the activities, observations and reasoning associated with some key decisions made during the first year of the program. Based on this consultation exercise, maize and tobacco were chosen as the major production crops, although others are still being pursued as part of the strategy development program. Both crop systems have their merits, and our opinion is that the inclusion of both of these crops for fast-track production within the program will provide valuable experience of the two contrasting systems. WP2 was also involved in some of the key dissemination activities of the consortium. For example, press briefings were held in July 2004 to publicize the program in the UK, Germany and Italy, and a press release was published in South Africa. At the same time, the Pharma-Planta public access web site was launched to describe the objectives and participants in the program and this includes a section on frequently asked questions (see <http://www.pharma-planta.org>). There have been many requests for Pharma-Planta representatives to participate in meetings on PMPs. For example, several program participants organized sessions and gave presentations at the cPMP meeting in Montreal at the end of January 2005.

The role of WP3 is the development of plant production platforms, and over the past year progress has been made in the production of transgenic plants expressing nearly all of the chosen target molecules. Appropriate constructs have been designed for the generation of nuclear transgenic tobacco lines expressing the two anti-HIV antibodies, and both the heavy and light chains of both antibodies have been detected in leaf extracts by immunoblot analysis. Other target molecules have also been expressed successfully in tobacco plants or BY-2 cells. Similar progress has been made by the maize group, where the aim is to generate transgenic maize lines that express the two anti-HIV antibodies specifically in seed. Over 150 primary transformants have been generated and many of these have been shown to carry the heavy and light chain transgenes for the relevant antibody. A further component of WP3 is the plastid transformation platform. Gene constructs for the expression of GAD67/65, HIV p24 and Nef in tobacco chloroplasts have been produced and introduced into tobacco (cv. Petit Havana) by microprojectile bombardment. Transplastomic tobacco plants containing genes encoding GAD67/65 and HIV p24 have been produced and are being analyzed for expression. Efforts are underway to develop Maryland Mammoth as an alternative tobacco cultivar for the

plastid-based expression of vaccine genes, on account of its greatly superior potential for leaf biomass production. As has also been found with other tobacco cultivars it does not respond as readily as Petit Havana to plastid transformation protocols, and to date only a single transformant has been obtained. Further work in WP3 includes the transformation of tomato and lettuce plastids and the development of systems for marker excision from the plastid genome.

WP4 considers a diverse array of processes to improve recombinant protein yields, including protein folding, assembly, targeting, modification and proteolytic digestion. Overall, WP4 is well within schedule for the completion of each short-term deliverable. No major problems have been encountered by the partners and several collaborations within WP4 and between WPs have been initiated. The principal achievements of WP4 this year include extending the palette of target proteins to include HIV Nef for localization and expression studies, the development of plants overexpressing the chaperones endoplasmic reticulum chaperonin and BiP, the production of plants expressing antibody chains fused to elastin-like peptides, the isolation of two maize glycosyltransferase cDNAs and the detection of a large number of apoplastic proteases which are now being characterized using proteomic technology.

Although sufficient transgenic plant material expressing the fast-track antibodies is not yet available, the WP5 groups have already started working on the early steps of processing, extraction and purification using non-transgenic tobacco leaves (either untreated or spiked with purified antibodies from CHO cells) and transgenic leaves expressing an antibody unrelated to the Pharma-Planta program. Various combinations of equipment and protocols for cell disruption have been compared, and the stability of the antibodies under each set of conditions has been evaluated. Progress has also been made in the development of aqueous two-phase partitioning and synthetic affinity ligands. To identify urgent issues to address in the context of production under GMP requirements, the draft document “Points to consider on quality aspects of medicinal products containing active substances by stable transgene expression in higher plants” (CPMP/BWP/764/02) was discussed extensively within the consortium, and communication with European regulatory authorities was initiated.

Groups involved in WP6, along with colleagues in WP5, have been involved in preliminary discussions with regulatory advisors in preparation for the regulatory submission that will need to be made to the MHRA prior to the clinical trial. They have attended all management meetings and general meetings to provide input and advice on the activities leading up to the clinical trial.