PUBLISHABLE EXECUTIVE SUMMARY

GIANT

Gene Therapy: an Integrated Approach for Neoplastic Treatment

Introduction

Translation of genetic knowledge from the Human Genome into disease-specific therapy for untreatable congenital disorders and acquired diseases is now reality. However, the gene therapy vectors currently used in experimental settings can be developed for safe clinical application only if fundamental problems are solved: i.e. the limitation of vector dose by attachment targeting and expression control, and a decrease of non-specific toxicity. Minimisation of vector immunogenicity (stealthing) is necessary to reduce bloodstream and immune-mediated reduction of effective vector concentration. To be a successful vehicle for gene therapy, each vector type (both viral or non-viral) has its own particular qualities and limitations, and its degree of development also depends on the intended medical application. The choice of the target, the transgene, and duration and stability of its expression all influence final vector choice and design. With ongoing research and discoveries in the field of molecular genetics, it is expected that new generations of genetic vectors will be essential for the translation of molecular research into clinical practice.

GIANT project outline

The GIANT project will provide an international resource to apply innovative technologies for modification of existing gene therapy vectors, focusing on increased targeting and decreased vector immunogenicity by “stealthing”. During a period of 5 years, this Integrated Project will address the current shortcomings in gene therapy for human prostate cancer by developing and testing vectors both in a preclinical and clinical setting. This translational action will serve as a template for the development of innovative treatments based on gene therapy, which can be applied in generic terms to other tumour types, using appropriate, tumour-specific targeting.
The overall objective of GIANT is the production of an optimally retargeted gene transfer vector with minimal immunogenicity that will be both clinically tested and ethically approved for prostate cancer therapy. The project is broken down into 6 subprojects, called workpackages (WP), which are described in more detail below.

WP1 Genetic retargeting

In this WP new human adenovirus-derived vectors will be developed, generated and produced to be used as therapeutic agents in prostate cancer. The necessary selectivity will be incrementally increased at two levels: (1) by modifying the tropism of the viral vector by including protein ligands in the viral capsid, and (2) by employing prostate and/or tumour-specific promoters to drive expression of the therapeutic transgene.

WP2 Chemical detargeting and retargeting

WP2 has been designed to specifically address the problems associated with immunological reactivity, and adhesion to multiple cell types in the bloodstream which genetic retargeting in WP1 alone can not control. Chemical modification will be used to (i) make the vector particles invisible to cellular and non-cellular components ("outside stealthing"); (ii) retarget the vector particles to specific surface molecules and/or detarget the vector; (iii) to combine chemical "outside stealthing" strategies with "gutless" vector technology ("inside stealthing").

WP3 Target exploration

This WP seeks to expand the range of targeting molecules present on the surface of prostate cancer cells, which can be used to expand the potential targets used for gene therapy against the tumour.

WP4 Non-viral vectors

This WP will explore non-viral technology for delivery of therapeutic DNA, but will also combine the best of viral and non-viral technologies by including the genomes of therapeutic adenoviruses within state of art synthetic delivery vectors. By coupling the superior pharmacokinetic properties of

non-viral vectors with the powerful and controlled anticancer activity of adenoviruses, including conditionally replicating adenoviruses, intravenous delivery of gene therapy for targeted treatment of prostate cancer should become feasible.

WP5 Preclinical testing of vectors

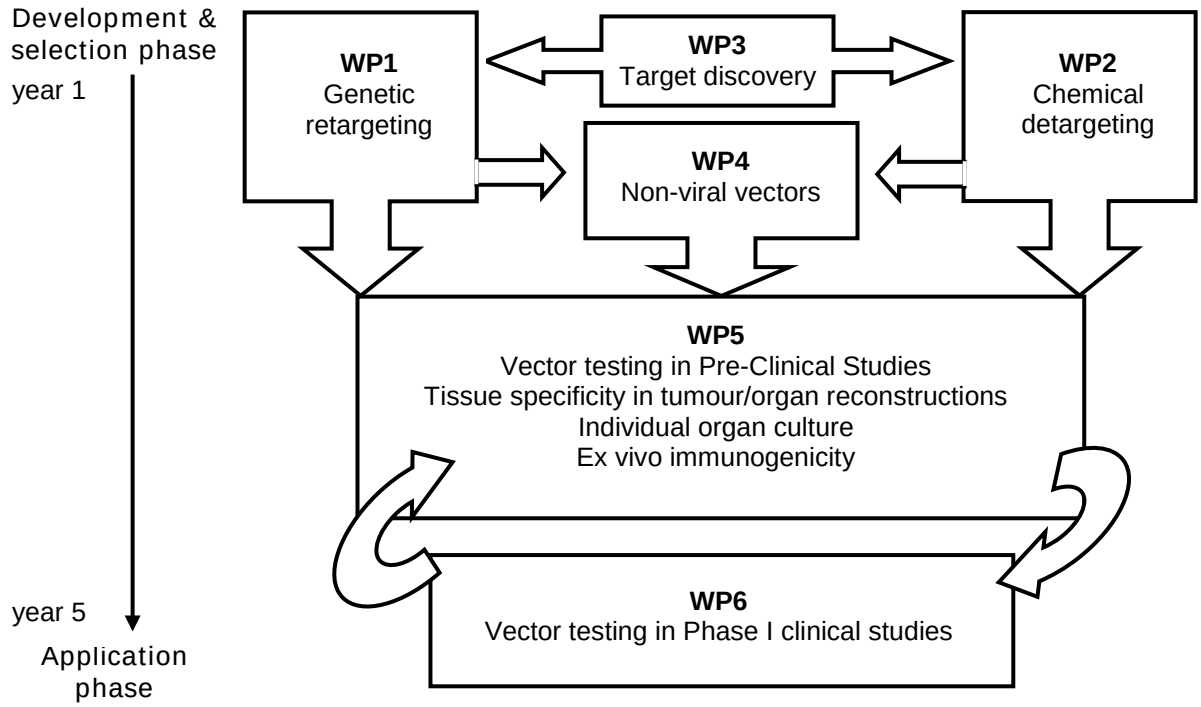
The objective of WP5 is to develop and validate physiologically and immunologically relevant pre-clinical standardized testing of modified gene transfer vectors. This testing package includes *in vitro* assays, immunogenicity assays and an orthotopic xenograft mouse model. Based on the results of WP5, it will be decided if a vector is suitable for clinical testing.

WP6 Clinical testing of vectors

Within the time-frame of GIANT, one vector developed in this programme can be clinically tested in a Phase I trial as a therapy for prostate cancer. An adenovirus that only replicates in prostate cells, called Ad[I/PPT-E1A], has now been selected for the GIANT clinical trial. This trial will be performed in a multi-center setting using a standardized protocol.

A schematic overview of the GIANT project design is presented in Figure 1.

Figure 1. GIANT project design



Organisational structure of GIANT

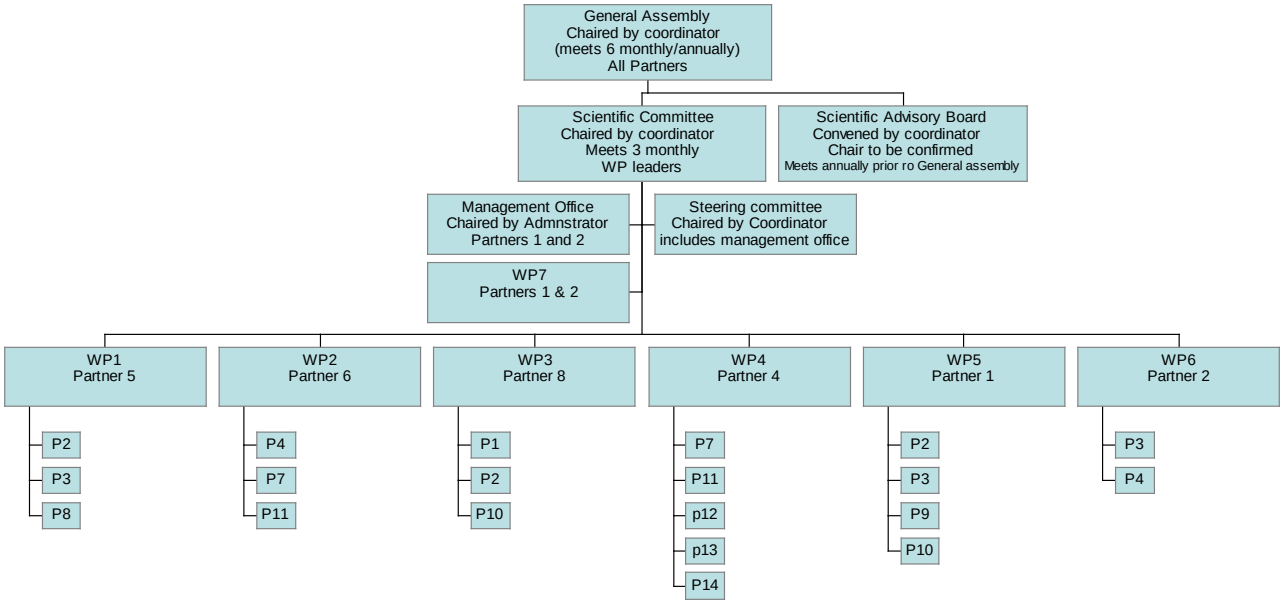
The GIANT consortium is composed of 14 participants, involving 9 academic institutes and 5 SMEs. The GIANT participants have a long record of EU-based scientific collaboration and expertise in ethically approved clinical vector generation. The SMEs own international patents on retargeting vectors and target discovery methods, providing a technology platform for further exploration of promising targets and innovative approaches to facilitate treatment of neoplastic diseases. The following partners (principal investigator in brackets) contribute to GIANT:

1. University of York, United Kingdom (Prof. dr. Norman Maitland)
2. Erasmus MC, Rotterdam, The Netherlands (Prof. dr. Chris Bangma)
3. Uppsala University, Sweden (Dr. Magnus Essand)
4. University of Oxford, United Kingdom (Prof. dr. Leonard Seymour)
5. Leiden University Medical Center, The Netherlands (Prof. dr. Rob Hoeben)
6. University of Ulm, Germany (Prof. dr. Stefan Kochanek)
7. Academy of Sciences of Czech Republic, Prague, Czech Republic (Prof. dr. Karel Ulbrich)

8. Got-a-Gene AB, Göteborg, Sweden (Dr. Leif Lindholm)
9. Scuron, Rotterdam, The Netherlands (Dr. Wytske van Weerden)
10. Pro-Cure Therapeutics Ltd, York, United Kingdom (Dr. April Frazier)
11. Ludwig-Maximilians-Universitaet, Muenchen, Germany (Prof. dr. Ernst Wagner)
12. Université de Strasbourg, France (Dr. Jean-Paul Behr)
13. Polyplus Transfection SAS, Illkirch, France (Dr. Patrick Erbacher)
14. Hybrid Systems Ltd, Oxford, United Kingdom (Dr. Kerry Fisher)

GIANT is coordinated by Prof. dr. Norman Maitland, chief of the Department of Biology of the YCR Cancer Research Unit, University of York, United Kingdom. The Steering Committee, composed of the project coordinator, the head of the management office located in Rotterdam, The Netherlands, and a technical coordinator for material exchange takes care of the day-to-day management of GIANT. All major project decisions are taken by the General Assembly, which is composed of one representative for each partner. Important scientific decisions during the project are taken by a Scientific Committee, composed of the project coordinator and the WP coordinators. A Scientific Advisory Board consisting of experts covering the fields of virology, oncology, ethics and regulatory affairs advise the General Assembly and the Scientific Committee. An overview of the organisational structure of GIANT is presented in Figure 2.

Figure 2. Organisational structure of GIANT



Results of GIANT year 5

The GIANT project has been extended with six additional months and has ended on 30 June 2010. The extension has enabled the consortium to complete the preclinical development and preclinical testing of a number of targeted vectors, and to proceed with the preparations for the clinical introduction of Ad[I/PPT-E1A] through a Phase I trial.

In the final 18 months, the technology for genetic targeting of adenovirus vectors to prostate cancer cells was used to generate several vectors for preclinical evaluation. New tumor-specific vectors with enhanced potency or specificity were generated and evaluated. In parallel, approaches have been taken for developing new test systems for functional characterization of these viruses. This yielded the development and implementation of new technologies that may reduce and replace animal experimentation, e.g. a system for growth of human tumors in chicken eggs, and the extraction of antigen–specific immunoglobulins from commercially available human immunoglobulin preparations. The development of chemically de- and retargeted adenoviral vectors has focused on overcoming the barriers for vector delivery with the ultimate goal to achieve efficient *in vivo* vector delivery. Strategies for polymer coating of adenovirus in combination with ligand-mediated targeting were further developed optimised. An example is the Bungarotoxin system that demonstrated to be a flexible way of targeting and retargeting of vectors. For the

development of binders against novel prostate cancer-specific antigens, several strategies were developed for the incorporation of scFv and scTCR, including camelized antibodies. Targeting of HLA/multiMAGE complexes was identified as a promising and safe targeting strategy due to the very high specificity of this particular receptor. For the development of non-viral vectors, further important advances have been made in overcoming many of the limitations to non-viral delivery. Vectors were made bio-responsive via coating with endosomal pH responsive or thermoresponsive polymers. Specificity was added to these vectors by introducing prostate cancer specific binders to the coating. Proof of concept for these technologies was obtained in a mouse model using Sindbis RNA genomes. The latter stages of the GIANT project placed a heavier workload on the partners involved in WP5, whose main task was to test vectors generated in the project in clinically relevant models. Comparisons of the ability of GIANT vectors to transduce 2D and 3D cellular models of prostate cancer revealed a number of significant differences between different cell types, but most significantly, confirmed the probelems encountered in the mouse models with PC346C (and in early human trials elsewhere) of the ability of various vector types to diffuse attach and penetrate complex structures. Such difficulties were not experienced with 2D monolayer cultures and this phenomenon was not related directly to receptor expression levels on complex tissues. Rather, the bioavailability of receptors was critical, in relation to injection points and adhesion contacts (and tight junctions) between cells. For oncolytic vectors these restrictions would be of less importance, as there is progressive tissue destruction, but as we observed in the xenograft models, even oncolytic effects also seem to be restricted. The second main testing task was to produce critical pre-clinical data for the clinical trial safety dossier. As our oncolytics are human-specific, there was little point in conducting toxicology trials in animal models, beyond those required by law. This is even true in non-human primates where the infections are at best semi-permissive. Accordingly, the consortium decided to carry out pre-clinical testing in a range of relevant primary human cell cultures, grown from tissues, which were likely to be infected at detectable levels in a clinical setting. The results of this dossier are now collated for presentation and reveal the exquisite specificity of the GIANT virus (Ad[I/PPT-E1A]). Even in human hepatocytes, which are extremely sensitive to infection by AdWT, there was no evidence of viral replication and toxicity at viral multiplicities of infection <500 viral particles/cell, a multiplicity which would only rarely be achieved in patients' livers.
Significant progress has been made in the preparations for the GIANT Phase I clinical trial. The production of the clinical batch of Ad[I/PPT-E1A] was completed. The batch was tested for toxicity in human primary cells and mice. Quality control testing will be finished in the summer of 2010. Submission of the dossier to the regulatory authorities in The Netherlands for approval is foreseen in the last quarter of 2010. It is anticipated that recruitment of patients can be initiated mid 2011.

GIANT public relations

Results from GIANT have been published in international scientific journals (selected publications are listed below) and have been presented at various international scientific congresses. A highlight is the publication of a series of 3 review articles from the GIANT consortium in the July 2010 issue of Human Gene Therapy, a peer-reviewed journal that is the official journal of the European Society of Gene and Cell Therapy and many national gene therapy societies. Furthermore, members of the consortium have been active in informing the general public on gene therapy and GIANT in numerous ways. Three doctoral and two Master of Science theses on GIANT studies have been defended by students from UU and LMU.

Selection of GIANT publications in year 5

1. De Vrij J, Willemsen RA, Lindholm L, Hoeben RC and the GIANT FP6 Consortium. Adenovirus-derived vectors for prostate cancer gene therapy. Human Gene Ther 2010;21:795-805.
2. Schenk E, Essand M, Bangma CH and the GIANT FP6 Consortium. Clinical adenoviral gene therapy for prostate cancer. Hum Gene Ther 2010;21:807-13.
3. Maitland N, Chambers K, Georgopoulos L, Simpson-Holley M, Leadley R, Evans H, Essand M, Danielsson A, van Weerden W, de Ridder C, Kraaij R, Bangma CH and the GIANT FP6 Consortium. Gene transfer vectors targeted to human prostate cancer: do we need better preclinical testing systems? Hum Gene Ther 2010;21:815-27.

4. Danielsson A, Elgue G, Nilsson B, Nilsson B, Lambris JD, Tötterman TH, Kochanek S, Kreppel F, Essand M. An ex vivo loop system models the toxicity and efficacy of PEGylated and unmodified adenovirus serotype 5 in whole human blood. Gene Therapy 2010;17:752-62.
5. Espenlaub S, Corjon S, Engler T, Wagner E, Ogris M, Fella C, Kochanek S, Kreppel F. Capsomere-specific fluorescent labeling of adenovirus vector particles allows for detailed analysis of intracellular particle trafficking and the performance of bioresponsive bonds for vector capsid modifications. Hum Gene Ther 2010, in press.
6. Willemsen RA, Pechar M, Carlisle RC, Schooten E, Pola R, Thompson AJ, Seymour LW, Ulbrich K. Multi-component polymeric system for tumour cell-specific gene delivery using a universal Bungarotoxin linker. Pharm Res 2010, in press.

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