THE JOURNAL OF GENE MEDICINE
J Gene Med 2002; **4**: 105–108.
DOI: 10.1002/jgm.250

First Meeting of the Gene Vector Production Network, 4–5 October 2001, Evry, France

Introduction

The first meeting of the Gene Vector Production Network (GVPN), convened by the AFM (Association Française contre les Myopathies), was held at the recently built Genocentre, located next to the Genethon Laboratories in Evry, France. The meeting was attended by over 250 participants, many of whom are current or potential users of the gene transfer reagents (viral vectors, plasmids, cell lines) produced by the GVPN. The conference was supported by the European Community (High Level Scientific Conferences) and by numerous biotechnology companies, whose presence clearly showed their interest for a network whose goal is to develop vectors for gene therapy applications. The scientific committee consisted of M. Mezzina (GVPN coordinator), C. Bagnis (Marseille), O. Danos (Evry), H. de Verneuil (Bordeaux), P. Lehn (Paris), P. Moullier (Nantes), J. C. Pagès (Tours) and J. Salama (AFM).

In their introductory talks, E. Molinié (President of the AFM) and O. Danos (Scientific Director of Genethon) reviewed the scientific context in which the GVPN was launched and outlined its initial aims, as well as the goals of the meeting. The GVPN was founded in 1997 by an initiative of the AFM to promote gene therapy in the French scientific community by providing researchers with suitable vectors and reagents. It was funded entirely by the AFM as part of a 5-year program aimed at providing a major impetus for bringing gene therapy research from the bench to the clinical setting. The goal of this meeting was to make a first assessment of the GVPN, to allow the numerous GVPN users to meet and exchange their experience, and finally to envisage future prospects.

The GVPN custom services: background and present status

In the first session, M. Mezzina (GVPN coordinator) provided an overview of the different activities of the GVPN. P. Noguiez-Hellin (Genethon, Evry), C. Bagnis (Marseille), and P. Moullier (Gene Therapy Laboratory, Nantes) reported on the activities of the three different vector production sites participating in the network.

The network started in Nantes with the manufacturing of adenovirus (Ad) and adeno-associated virus (AAV) vectors for a limited number of investigators. When the teams in Evry and Marseille joined the network (in November 1997 and April 1998, respectively), the GVPN developed rapidly. The network is currently

Figure 1. The new Genocentre, Evry, France

custom-manufacturing four types of viral vectors: murine leukemia virus (MLV), Ad, AAV, and HIV lentiviral vectors. It also provides custom services for the construction of plasmids and the generation of producer cell lines. Investigators can download a request form from the GVPN website (www.genethon.fr/gvpn), which also features a database. Requests are reviewed by a panel of experts in order to establish a priority ranking based on scientific merit. To date, the GVPN has assisted over 300 investigators worldwide by providing reagents and expertise in vector know-how. The sites in Nantes and Marseille focus mainly on Ad/AAV and MLV/HIV vectors, respectively, whereas Genethon produces all types of viral vectors. Over 2000 batches of reagents (mostly viral vectors) have been produced to date. Most of these batches (60%) were requested by academic and non-profit institutions, such as INSERM and CNRS, for whom the services have so far been free of charge. More recently, biotechnology companies have shown an interest in GVPN and they may become users or even partners. The experience so far clearly indicates that the GVPN launch was successful.

However, many critical issues still need to be addressed, in particular the standardization of the vector batches delivered by the three production sites, including validated production, purification, and quality control procedures. E. Aguilar-Cordova (Boston, USA) pointed out that because of the difficulty in agreeing on harmonized production procedures (such harmonization may, in fact, even slow down research in the area), it will be very important to define reference standards allowing comparison of the various vector batches produced by the different GVPN sites (titers, purity, etc.). Such standardization should allow the GVPN users to obtain more reproducible results, and hence better comparability between different gene therapy approaches. In addition, standardization is important for clinical trials; approval by regulatory bodies will require preclinical determination of safety and efficacy profiles of the vector to be used (X. Chenevisse, AFSSAPS, Vendargues).

Up-scaling of the vector batches for experiments involving large animals or even for clinical trials represents another major challenge for the GVPN in the near future. Because the vector batches currently produced by the GVPN are rather small, they permit studies in only small or medium-sized animals, such as mice, rats or rabbits. Thus, Genethon is at present developing new processes for up-scaling its MLV, HIV, and AAV vector production and evaluating novel procedures for quality control. Genethon is also coordinating a platform involving industrial partners in order to integrate the broad technological expertise required for the production of large amounts of vectors (for work with large animals) and of GMP-grade reagents (for clinical trials). Henogen, a biotechnology company based in Charleroi (Belgium), has recently become a pilot partner. Genethon and Henogen plan to co-produce GMP-grade batches of MLV vectors for clinical trials, in the near future.

Scientific presentations

The scientific presentations highlighted the efficiency and the limitations of the different types of vectors produced by the network. Abstracts can be found at www.genethon.fr/gvpn/conference.html.

MLV vectors versus lentiviral vectors

Several presentations focused on the use of MLV vectors and HIV-derived lentiviral vectors. The recent emergence of lentiviral vectors, mainly derived from HIV-1, opened up the possibility to genetically modify cells which were not considered *a priori* to be targets for recombinant murine retroviruses or which were difficult to transduce with MLV vectors. In addition, because of the expertise previously obtained with MLV vectors, the design of lentiviral vectors was able to evolve very rapidly, leading to comparative experiments between MLV and HIV vectors. Several talks and posters presented data from such 'comparative' experiments designed to select the most appropriate vector. In particular, it was suggested that lentiviruses may be more efficient than MLV vectors for the transduction of hematopoietic stem cells and dendritic cells. With regard to hepatocytes, A. Weber (Clamart) presented results from preclinical studies in non-human primates which aimed to optimize a classical *ex vivo* strategy with MLV vectors for the treatment of familial hypercholesterolemia. These investigators are also developing protocols for transplantation of rapidly proliferating fetal hepatocytes into monkey livers. In contrast, it was emphasized by others that third-generation lentiviral vectors may allow *in vitro* transduction of unstimulated human hepatocytes (whereas transduction by MLV vectors requires the induction of cell cycling). However, it should be stressed that lentiviral vectors may not be required for efficient transduction of all cell types, as demonstrated by the positive clinical outcome in the recent X-linked SCID gene therapy trial (see below). Finally, several posters discussed two major issues – the level of transgene expression and up-scaling of the production procedures – that need to be addressed both for MLV and for lentiviral vectors.

Genetic diseases and degenerative disorders

Another group of presentations focused on gene therapy approaches for genetic diseases. First, M. Cavazzana-Calvo (Paris) presented an update on the well-known clinical trial for X-linked SCID (in which an MLV vector is used), the first really successful trial in clinical gene therapy! M. Cavazzana-Calvo also showed positive results obtained with an MLV vector in a mouse model of another SCID disease (RAG2 deficiency), while N. Taylor (Montpellier) presented interesting data obtained in ZAP-70-deficient mice (also a model of a human SCID disorder). These results strongly suggest that MLV-mediated gene

transfer may have a therapeutic effect in the corresponding human diseases.

The oral presentation by G. Meneguzzi (Nice) and several posters were devoted to gene therapy approaches for genetic diseases of the skin, especially epidermolysis bullosa (EB). It was emphasized that *in vitro*, retrovirus-transduced keratinocytes express the transgene permanently, an observation suggesting that epidermal stem cells can be stably corrected. Consequently, the feasibility of an *ex vivo* strategy involving retrovirus-transduced autologous keratinocytes is now being tested in natural dog models of EB.

J. M. Heard (Paris) discussed the treatment of β-thalassemia in mice via an indirect gene therapy strategy based on the induction of erythropoietin (Epo) secretion from muscle. Here, similar results were obtained by transferring the mouse Epo cDNA with an AAV vector or with naked DNA electro-transfer, except that correction was permanent with AAV vectors and transient with naked DNA. Interestingly, with regard to the mechanism by which Epo improves β-thalassemia, the data suggest that early progenitors are actively recruited and stimulated towards differentiation, this premature triggering to differentiation probably being responsible for the maintenance of a fetal-like erythropoiesis.

M. Peschanski (Créteil) presented data on two complementary approaches for the treatment of Huntington's disease: substitution of the degenerated neurons by homologous fetal cells (cell therapy) and continuous administration of a neuroprotective agent in the vicinity of the threatened striatal neurons. The neuroprotective therapy has already been applied to patients via implantation of encapsulated BHK cells engineered to express the ciliary neurotrophic factor (CNTF). In this pilot study, survival of the implanted cells was heterogeneous, an observation that prompted the investigators to evaluate other delivery systems. Interestingly, recent data in rats indicate that single intrastriatal administration of CNTF adenoviruses provides long-lasting protection of the entire striatum. As regards neurons, T. Galli (Paris) discussed the use of AAV vectors to study the role of TI-VAMP (a vesicle attachment protein) in neurite outgrowth of neurons in primary culture.

Several poster presentations focused on gene transduction into hematopoietic stem/progenitor cells for other genetic diseases, in particular adrenoleukodystrophy and congenital erythropoietic porphyria. In HIV infection, Y. Ataman-Onali (Nantes) presented a study designed to determine whether a recombinant AAV vector expressing the early HIV-1 proteins Tat and Rev could induce specific humoral and cellular immune responses in rhesus monkeys. Although the immunized monkeys developed antibodies against the vector, the induction of an immune response against Tat and Rev was unsuccessful.

Finally, C. Coutelle (London) provided an overview of the numerous issues (especially the choice of the appropriate vector system) associated with fetal somatic gene therapy, an approach that could potentially avoid early-onset disease manifestations and allow permanent somatic gene supplementation or, alternatively, may enable the avoidance of immune sensitization and facilitate repeated postnatal treatment.

Cancer gene therapy

Although cancer represents a major area of clinical gene therapy (see Clinical Trials Database, www.wiley.co.uk/genmed), few presentations were actually devoted to cancer gene therapy. G. Lazennec (Montpellier) presented data showing that adenoviral delivery of the estrogen receptor β (ERβ) to breast cancer cells inhibits their growth and motility, a finding highlighting the pivotal role of ERβ in breast cancer progression and invasion. A. Van den Broeke (Brussels) showed that while sustained oncogene expression is required for cell transformation by oncoretroviruses, it is the silencing of the expression of Tax that is linked with the onset of acute BLV-induced leukemia. An interesting poster reported that an HIV vector including the DNA flap and encoding a melanoma polyepitope can transduce up to 100% of immature mouse and human dendritic cells (DCs) and that such DCs elicit more efficient CTL responses against melanoma. Finally, C. Duplaà (Pessac) presented results demonstrating *in vitro* and *in vivo* (by using an Ad vector in a mouse model of hindlimb ischemia) the involvement of a frizzled related protein in the control of the cell cycle of vascular cells, the secreted frizzled-like protein (FrzA) delaying the G1 phase.

Conclusions and prospects: towards pan-European cooperation

The meeting ended by a round table to sum up the present situation of the GVPN and discuss its future prospects. There was a clear consensus that the GVPN is a successful initiative and that it is meeting the expectations of gene therapy researchers, as witnessed by the increasing number of requests and the number and quality of the posters presented at the meeting. However, the GVPN is still a young, fragile (and costly) structure. As outlined by M. Mezzina (GVPN coordinator), new partnerships at both the French and the European levels should help to secure the future of the GVPN and allow it to develop further. In France, the national research organizations INSERM and CNRS could soon become academic partners, especially as a substantial proportion of requests for GVPN reagents have come from INSERM and CNRS laboratories. At the European level, trans-national partnerships may be developed in the context of the European Framework Programs (FP) of the European Commission (EC). This would be consistent with the scientific policy of the EC, as emphasized by G. Joliff-Botrel (Health Research Directorate, EC, Brussels). Indeed, in the current Fifth FP (1998–2002), gene and cell therapy is a high priority, with a budgetary allocation of about 60 million euros so far. Several clinical trials are being

supported, illustrating the concept of research from bench to bedside (a concept similar to the objectives of the 'Great Endeavour' program of the AFM). Most importantly, in the new FP (2002–2006) currently under preparation, cell and gene therapy research remains a priority, but funded research projects will have to achieve a sufficient critical mass and ensure a high level of transnational integration. Such a favorable context should allow the GVPN to implement strong cooperation across Europe, not only with academic institutions but also with biotechnology companies, as the EC wants to ensure that European research serves the Union's economic and social objectives. Genethon has in fact already initiated cooperation with the Belgian biotechnology company Henogen (see above). In addition, F. Bosch (Barcelona) described the Vector Production and Gene Transfer Research Facility, a unit set up recently at the University of Barcelona, which may constitute an ideal partner for GVPN in a European context. To end the conference, O. Danos presented the awards of the best oral presentations (of selected posters) to G. Lazennec (Montpellier) and A. Van den Broeke (Brussels) and M. Mezzina announced the first 'Gene Vectors EuroLabCourse' to be held at the Genocentre/Genethon complex in Evry on 14–27 April 2002 (www.genethon.fr/vecteuronet).

M. Mezzina and
the Scientific Committee