Extract from the Communication Paper of the

Gene Therapy Discussion Group Meeting (GTDG)
30 Oct-1 Nov 2007 (Rotterdam)

REPORT
ICH WORKSHOP ON VIRAL / VECTOR SHEDDING
30 Oct 2007 (Rotterdam)

1. Purpose of the Workshop

The ICH Gene Therapy Discussion Group (GTDG) public workshop on viral / vector shedding was held on October 30, 2007. "Shedding" in the field of gene therapy means dissemination of the gene therapy product through excreta of the patient.

The objectives of the workshop were:

- to discuss the data available on shedding of diverse vector systems
- to discuss assays to detect vectors shed into excreta
- to discuss the current requirements in the different regions
- to discuss third party exposure and public health concerns, and
- to contribute information to the drafting of an ICH considerations paper on viral / vector shedding.

It is considered that the information gathered at this workshop will provide a better understanding of the contribution of shedding studies to the risk/benefit assessment of gene therapy products.

2. Background

The ICH Steering Committee (ICH SC) at their meeting in Chicago (October 2006) agreed to the GTDG's proposal to hold an ICH Workshop on Shedding in the EU in conjunction with the XVth Annual Congress of the European Society of Gene and Cell Therapy (ESGCT; http://www.esgct.org/index.cfm). ESGCT hosted the Workshop. The European Gene Therapy Network of Excellence (CLINIGENE, http://www.clinigene.eu/) supported the workshop financially. The workshop was held on the 30th October 2007 in Rotterdam.

This report summarizes the presentations and pertinent issues raised during discussion.

3. Workshop Overview

The workshop was separated into 3 sessions. The aim of the first session was to give an overview of the requirements for viral / vector shedding studies in each region, and how these studies are currently used for regulating gene therapy products. This session was chaired by Klaus Cichutek (EU/EMEA), with presentations given by Teruyo Arato representing Japan/MHLW, Sharon Longhurst representing the EU/EMEA, Daniel Takefman representing the USA/FDA and Andreas Marti representing EFTA/Swissmedic.

None of the regions have specific guidance on how and when viral / vector shedding studies should be carried out. In each region there is legal basis for the assessment of viral / vector shedding in relation to potential public health concerns, i.e. potential transmission of a

pathogen. In addition, viral / vector shedding data can be used to support environmental risk assessments. There is a stepwise and a case-by-case approach in all ICH regions.

Points discussed in relation to design of non-clinical viral / vector shedding studies included:
- relevance and selection of animal models,
- advantages and disadvantages of assays used for detection of virus or vector shed into excreta, i.e., assays detecting sequences and infectivity assays, and
- likelihood of transmission.

Points discussed for clinical viral / vector shedding studies included:
- regional views on isolation of patients
- number of patients to be evaluated
- when and how long to monitor patients,
- advantages and disadvantages of assays used for detection of virus or vector shed into excreta, including the potential for a stepwise approach to analysis, and
- what excreta samples to analyze.

Horizontal transmission studies have rarely been requested by regulatory agencies.

Session 2 focused on experience in the assessment of viral / vector shedding during non-clinical and clinical studies. The session was chaired by Stephanie Simek (FDA) with presentations given by Edwin van Amersfoort (Amsterdam Molecular Therapeutics), Samuel Wadsworth (Genzyme), and Didier Guilhem (World Courier France).

There was some discussion as to the suitability of animal models used for shedding studies. Examples were given from studies in mice, rats, rabbits, cats, dogs, and non-human primates.

Examples of samples taken in clinical studies included urine, feces, saliva, semen, plasma/ blood, sputum, buccal / throat swabs, and gargle. One presentation highlighted the importance of developing procedures for sample handling, storage, and shipping, and training of staff at all stages to ensure the quality of viral / vector shedding data.

The suitability of the assays used to assess shedding was discussed at some length. There was recognition of the value of infectivity data, as well as the technical limitations associated with these analyses. PCR-based detection of vector sequences is the most frequently used method.

Session 3, chaired by Teruhide Yamaguchi (MHLW, Japan) and Alan Boyd (Alan Boyd Consultants, UK), focused on specific experiences with different types of vectors, including both biodistribution and viral / vector shedding. Presentations relating to adenovirus vectors were given by Toshiyoshi Fujiwara (Okayama University, Japan), David Eckland (Ark Therapeutics, UK) and Ingrid Boltje (Genzyme, US); AAV vectors were covered by Caroline Le Guiner (Inserm, France) and Janneke Meulenberg (Amsterdam Molecular Therapies); Seneca Valley Virus was discussed by Paul Hallenbeck (Neotropix); Ellen Schenk-Braat (Erasmus Medical Center, The Netherlands) provided a summary of the available literature in relation to viral shedding.

Toshiyoshi Fujiwara presented vector shedding and biodistribution data following intratumoral injection of a recombinant adenovirus containing the human p53 gene. Analysis of gargle, urine and autopsy samples by PCR were presented. Biodistribution data confirmed transient systemic spread of the vector, and transgene expression was detected in the presence of neutralizing antibodies.

David Eckland presented biodistribution of an adenovirus vector containing HSV-tk (Cerepro).

Ingrid Boltje presented information related to the logistics of shedding studies as experienced in a trial where patients were treated with a recombinant adenovirus expressing HIF-1α/VP16. The vector was administered via intramuscular injection. Samples (throat swab, urine, semen) were taken 1, 3 and 7 days post treatment. The nature and timing of samples to be taken were defined by non-clinical studies. Clearance pattern from blood was also evaluated in order to determine the 'shedding risk' period post treatment.

Caroline Le Guiner provided a review of the literature and her laboratory's 8 years experience with respect to viral shedding studies of rAAV in large animal models. She concluded that regardless of the mode of delivery, the serotype or the dose, administration of rAAV in large animals (dog, cat, non-human primate) and in humans is associated with shedding in the urine.

Janneke Meulenberg provided biodistribution and shedding data from non-clinical studies and a clinical study where a rAAV expressing lipoprotein lipase was administered intramuscularly. Clinical samples taken were serum, saliva, urine and semen, as well as a muscle biopsy. Serum, saliva, urine and semen were tested until 3 consecutive samples were negative by PCR. Overall, vector DNA was transiently present in all body fluids, and there was no clear relationship between dose and vector DNA concentration or duration of persistence. In general the biodistribution and shedding data correlated well with the data generated in mice and cats.

Paul Hallenbeck described Seneca Valley Virus, a porcine picornavirus with no known pathogenesis. This oncolytic virus appears to show selective replication in neuroendocrine tumors. There is no evidence of detectable horizontal transmission in studies using mice. Data from both PCR-based and infectivity assays of patient samples showed that virus is shed for as long as 50 days.

Ellen Schenk-Braat's summary of the available literature in relation to viral shedding is published (*J Gene Med.* 2007. *9:* 910-921). She concluded that it was difficult to compare data from different study publications as the amount of detail was limited.

Klaus Cichutek noted the following issues in the session on workshop conclusions:

- the need to provide clear definitions of the terminology (shedding, biodistribution, transmission)
- current reliance on a stepwise approach to assessing the risk of transmission
- factors to consider in design of non-clinical and clinical shedding studies
- the suitability of assays for detection of vector sequence and infectious vector
- analysis and impact of resulting data in the assessment for risk of transmission and possible consequences
- lack of clarity on the nature of the material to be used in non-clinical shedding studies (GMP?) and on the type of non-clinical study (GLP?)
- regional differences in biosafety requirements including patient isolation, and
- differing opinions on the availability, quality, and quantity of virus / vector class shedding data and the potential value of these studies