Gene therapy clinical trials: what about the environment?

A comparison between the Netherlands and North America

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Foreword

The prospect of treating human diseases by gene therapy is an appealing one. To date approximately 1800 clinical studies have been performed worldwide, and the first gene therapy products are about to be registered for clinical use. In many of the studies infectious agents (bacteria, viruses) are used as vectors for the transfer of the therapeutic genes into the patients' cells. The use of these vectors bears a risk of inadvertent exposure of humans other than the patient. Specific legislation demands an environmental risk assessment (ERA) for describing such risks and the measures taken to reduce these.

In the United States of America and in Canada gene therapy seems to progress more swiftly to the stage of clinical evaluation. It is therefore of great interest for The Dutch GMO Office and the Netherlands Commission on Genetic Modification (COGEM) to understand how the different bodies involved in environmental risks deal with environmental risks. COGEM therefore commissioned a desk study to compare the ERA methodology in gene therapy in North America and in the Netherlands. In addition, the study should summarize the pertinent legislation in the three countries, with a focus on the legislation and procedures that relate to the ERA. The study reveals that while the data required for the ERA are similar in the US and the Netherlands, the formal ERA in the US can be postponed up to the market authorization phase. This limits the number of applications in the US for which a full ERA is required and increases the number of studies that can be undertaken while data are being collected for the ERA later on.

The study was performed by Patrick L.J. Rüdelsheim and Greet Smets of Perseus BVBA, whom I like to thank for their efforts and the stimulating discussions during the meetings.

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Summary

Gene therapy refers to a wide range of techniques that use genetic sequences, mainly for the treatment of diseases that have no alternative cures or for which available treatments turn out to be not effective. So far, most of the genetic sequences that have been tested were intended to replace a mutated, non-functional gene or to encode a therapeutic protein.

The majority of the gene therapy clinical trials is carried out in North America (the USA and Canada), a development that may be at least partly due to a more conducive regulatory approach. This study compares the regulatory approaches in North America and in the Netherlands and clarifies how the different risk assessors deal with potential environmental risks related with the use of genetically modified organisms (GMOs).

The USA, Canada and the European Union (EU) (as implemented in the Netherlands) have established very different regulatory frameworks addressing gene therapy clinical trials. This is already clear when comparing the legal definition of the scope of gene therapy (*e.g.* inclusion of recent techniques, covering vaccination …).

In all jurisdictions, gene therapy clinical trials are within the scope of existing regulatory systems that govern human clinical trials or biomedical research. These regulations in the first place aim at the safety of the human subject in the studies.

The product-based approach is most apparent in Canada where gene therapy clinical trials are assessed the same way as any clinical trial. The US Food and Drug Administration (FDA) considers gene therapy not fundamentally different from other types of medical treatment and regulates it as biological products. Nevertheless, NIH has developed guidelines for research activities with recombinant DNA, including clinical trials. Concerning the possible environmental impact, authorities in both the USA and Canada apply existing environmental legislation. No specific legislation has been developed for gene therapy.

In the EU, in addition to the requirements for organizing clinical trials with investigational medicinal products, the process-based GMO legislation necessitates that the risks for human health and the environment are assessed for every activity involving a GMO. The environmental risk assessment (ERA) provides the basis for identifying the need for and the type of risk management measures to reduce potential adverse effects.

Whereas the evaluation of risks for participating volunteers and healthcare professionals is a standard element of any clinical trial application, the importance that is given to the environment at large distinguishes the evaluation process between the countries. Also important differences are observed depending on the type of gene therapy product, the way of administration and the clinical trial phase.

In the EU, a specific ERA as described in the GMO legislation is required as soon as an application is made for the first clinical trial. In the USA and Canada only in specific cases a full assessment is necessary for early clinical trials. In the early phase of development of a gene therapy product (phase I and II clinical trials) the environmental impact is considered negligible due to the limited number of sites and timeframe. Only when the product nears commercialisation its environmental impact becomes important and will be assessed. This makes that, compared to the European situation, developers are less (or later) confronted with the need to produce information to conduct the ERA and may even be exempted.

The clinical trial protocol is evaluated centrally in USA and Canada similar to the approach taken in the Netherlands. However, in the early phases the environment is for a large part addressed by the local

institutional committees in the USA and Canada. Both FDA and Health Canada have exemption rules in place to 'avoid' a full ERA for research and development of gene therapy products. In consequence, the regulatory level at which environmental issues are discussed differs. Marketing approvals are centralised in all countries.

With regard to the type and amount of information to be provided for the ERA, in the Netherlands this is partly overlapping, partly additional to the information normally required for a clinical trial. In the USA and Canada this is usually not the case. In the USA NIH requires an extra form to be filled out. Only in specific cases an extensive dossier on environmental risks needs to be compiled.

The Dutch procedure, while limited to a legally defined time, may be extended due to request(s) for additional information or for other reasons. In the USA the process may be very short provided that the trial is exempt from a full ERA and no public review is required. This is the case for applications that are more or less familiar to the reviewers. With public discussions total time may be as long as for the procedures in the Netherlands as officially stated. The time frame for a trial assessment in Canada is usually short. However, pre-submission consultations are very often used to clarify questions and fine-tune the dossier so as to make a short review period possible. The applicants experience these consultations as very helpful in that both applicants and assessors cooperatively target at the development of safe medical treatments.

Notwithstanding the differences, there is a lot of similarity of the information that is required when approaching the commercial stage. Postponing or reserving the full ERA for specific cases, as is now routinely done in the USA and Canada, has the advantage that early in the development, when the clinical concept still has to be proven, no expensive studies are needed. The necessary information may be collected while conducting trials as they advance. The potential lower level of knowledge of the gene therapy product is compensated by standard and possible extra clinical trial safety instructions.

Samenvatting

Gentherapie omvat een brede waaier aan technieken waarbij genetische sequenties worden gebruikt, vooral met het oog op behandeling van ongeneeslijke ziekten of ziekten waarvoor bestaande behandelingen niet (meer) werken. Tot dusver werden de meeste van de geteste genetische sequenties bedoeld om een gemuteerd, niet-functioneel gen te vervangen of om een therapeutisch eiwit aan te maken.

Het merendeel van de klinische gentherapieproeven wordt uitgevoerd in Noord-Amerika (de VS en Canada), een ontwikkeling die op zijn minst gedeeltelijk zou kunnen te wijten zijn aan een meer bevorderlijke wetgeving. In dit rapport wordt de regelgeving besproken zoals die geldt in Noord-Amerika en Nederland en wordt uitgelegd hoe de verschillende beoordelingsorganen omgaan met de mogelijke risico's verbonden aan werken met genetisch gemodificeerde organismen (GGO's).

Het wettelijk kader dat van toepassing is op klinische gentherapieproeven verschilt nogal tussen de VS, Canada en de Europese Unie (EU) (zoals toegepast in Nederland). Dit komt reeds tot uiting in de wettelijke definities van gentherapie (bv. al of niet met insluiting van de meest recente technieken, toepassing als vaccin …).

In alle rechtsgebieden vallen klinische gentherapieproeven binnen de werkingssfeer van de wetgeving op klinische proeven met geneesmiddelen voor menselijk gebruik of biomedisch onderzoek. Deze wetten hebben in eerste instantie tot doel de bescherming van de proefpersonen.

De aanpak waarbij de wetgeving is gebaseerd op het uiteindelijke product komt vooral tot uiting in Canada, waar klinische gentherapieproeven op de zelfde manier worden beoordeeld als eender welke klinische proef. Ook in de VS beschouwt de FDA gentherapie als niet fundamenteel verschillend van andere medische behandelingen. Gentherapie valt dan onder 'biological products'. Toch is het zo dat NIH richtlijnen heeft geschreven voor activiteiten met recombinant DNA, waaronder klinische proeven. Voor wat betreft milieu-impact wordt in de VS en Canada de bestaande milieuwetgeving toegepast en werden geen wetten uitgevaardigd specifiek voor gentherapie.

In de EU maakt de procesgebaseerde wetgeving het noodzakelijk dat mogelijk schadelijke effecten voor de gezondheid van mens en dier en voor het milieu worden onderzocht bij alle activiteiten met GGO's, en dit bovenop de vereisten voor klinische proeven met geneesmiddelen in onderzoek. De milieurisicobeoordeling (MRB) kan aanduiden op welke punten maatregelen moeten worden genomen om potentieel schadelijke effecten te minimaliseren. Mochten die maatregelen nodig zijn, dan vormt de MRB een basis om beheersmaatregelen te ontwikkelen, en om de overblijvende risico's te evalueren wanneer die maatregelen zijn geïmplementeerd.

Terwijl mogelijke risico's voor de vrijwilligers die deelnemen en de medische staf standaard worden meegenomen in elke klinische proef, zal de mate waarin men belang hecht aan het milieu in de brede zin van het woord het beoordelingsproces beïnvloeden. Verder zijn erg grote verschillen waar te nemen naargelang het type en de toedieningswijze van het gentherapieproduct en de fase van ontwikkeling.

In de EU wordt van bij het begin een specifieke MRB gevraagd zoals voorgeschreven in de GGO-wetgeving. In de VS en Canada wordt alleen in specifieke gevallen een uitgebreide beoordeling noodzakelijk geacht voor klinische studies. Vroeg in de ontwikkeling van een gentherapieproduct (fase I en II klinische proeven) beschouwt men de milieu-impact verwaarloosbaar klein gezien de beperkte schaal in ruimte en tijd. Pas wanneer het product de commercialisatiefase nadert, wordt die mogelijke impact belangrijk en wordt die bestudeerd. Gevolg is dat, vergeleken met Europa,

onderzoek dat de nodige data moet leveren wordt uitgesteld of dat men zelfs op dat moment een uitzondering op deze verplichting krijgt.

Het eigenlijke protocol van de klinische proef wordt centraal geëvalueerd in de VS en Canada, net zoals in Nederland. Maar in de VS en Canada worden in de eerste fasen mogelijke milieueffecten voor een groot deel op het niveau van de lokale veiligheidscomités behandeld. Zowel FDA als Health Canada hanteren uitzonderingsregels om een volledige MRB te 'vermijden' bij onderzoek en ontwikkeling van gentherapieproducten. Dit heeft voor gevolg dat het beleidsniveau waarop milieu-gerelateerde zaken worden bediscussieerd verschilt. Toelatingen voor marktintroductie daarentegen worden centraal afgegeven in alle landen.

Wat betreft de aard en hoeveelheid gegevens die moeten worden aangeleverd voor een MRB, geldt voor Nederland dat die ten dele overlappen, deels extra zijn vergeleken met de informatie die men gewoonlijk vraagt voor een klinische proef. In de VS en Canada is dat meestal niet zo. NIH vraagt wel een bijkomend formulier in te vullen. Alleen in bepaalde gevallen moet een uitgebreid dossier dat de milieurisico's beoordeelt, worden samengesteld.

De Nederlandse procedure, hoewel die wettelijk begrensd is in tijd, kan uitlopen door vragen voor bijkomende informatie of om andere redenen. In de VS kan het beoordelingsproces snel worden afgerond op voorwaarde dat geen volledige MRB of een publieke discussie nodig wordt geacht. Dit is het geval voor toepassingen waarmee de beoordelaars redelijk vertrouwd zijn. Wanneer er een publieke discussie bijkomt, kan de totale proceduretijd oplopen gelijk aan die wettelijk in Nederland geldt. Het tijdspad om een proef in Canada te beoordelen is meestal zeer kort. Maar dikwijls wordt gebruik gemaakt van de mogelijkheid om voorafgaandelijk vragen en problemen te bespreken en het dossier aan te passen zodat de officiële beoordeling weinig tijd vraagt. De aanvragers ervaren deze besprekingen als zeer nuttig temeer daar beide partijen ernaar streven om op een veilige manier medische behandelingen te beproeven.

Niettegenstaande de verschillen is er veel overeenkomst in de gevraagde informatie naar commercialisatie toe. Het uitstellen of het voorbehouden van de MRB tot specifieke gevallen, zoals dat nu gebruikelijk is in the VS en Canada, heeft het voordeel dat vroeg in de ontwikkeling, wanneer de klinische werking nog moet worden aangetoond, er geen dure studies vereist zijn. De noodzakelijke informatie wordt dan vergaard tijdens het uitvoeren van de klinische proeven. Mogelijk is er aanvankelijk minder geweten over de risico's van het gentherapieproduct, maar dat wordt dan gecompenseerd door de standaard- en eventueel bijkomende veiligheidsinstructies tijdens de proeven.

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List of abbreviations

AAV	Adeno-associated virus
ATMP	Advanced therapy medicinal product'
BGTD	Biologics and Genetic Therapies Directorate (Canada)
BLA	Biologics License Application (USA)
CBER	Center for Biologics Evaluation and Research (USA)
CBTE	Centre for Blood and Tissues Evaluation (Canada)
CCMO	Centrale Commissie Mensgebonden Onderzoek (NL)
CDC	Centers for Disease Control and Prevention (USA)
CEPA	Canadian Environmental Protection Act
CERB	Centre for Evaluation of Radiopharmaceuticals and Biotherapeutics (Canada)
CFR	Code of Federal Regulations (USA)
COGEM	Commissie Genetische Modificatie (NL)
CTA	Clinical Trial Application (Canada)
CU	Contained use
CVE	Centre for Vaccine Evaluation (Canada)
DHHS	Department of Health and Human Services (USA)
DR	Deliberate release
DSL	Domestic Substances List (Canada)
EA	Environmental Assessment (USA)
EARs	Environmental Assessment Regulations (Canada)
EC	European Community
EMA	European Medicines Agency
EPA	Environmental Protection Agency (USA)
ERA	Environmental Risk Assessment
EU	European Union, Europese Unie
F&DA	Food and Drugs Act (Canada)
FDA	Food and Drug Administration (USA)
FFDCA	Federal Food, Drug, and Cosmetic Act (USA)
GGO	Genetisch gemodificeerd organisme
GM	Genetically modified
GMO	Genetically modified organism
HIV	*Human immunodeficiency virus*
IBC	Institutional Biosafety Committee (USA)
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IenM	Infrastructuur en Milieu (NL)
IND	Investigational New Drug (USA)
IRB	Institutional Review Boards (USA)
IREB	Institutional Research Ethics Board (Canada)
MRB	Milieurisicobeoordeling
NDA	New Drug Application (USA)
NEPA	National Environmental Policy Act (USA)
NIH	National Institutes of Health (USA)
NSN	New Substance Notification (Canada)
NSNR	New Substances Notification Regulations (Canada)
OBA	Office of Biotechnology Activities (USA)
ORA	Office of Regulatory Affairs (Canada)
RAC	Recombinant DNA Advisory Committee (USA)
RCL	Replication-competent lentivirus
VWS	Volksgezondheid, Welzijn en Sport

1 Introduction

Gene therapy refers to a wide range of techniques that use genetic sequences to treat or prevent disease. The genetic sequence may be intended to replace a mutated, non-functional gene or may encode a therapeutic protein. The genetic information is delivered into cells within the body usually via a vector, mostly a viral vector, or may be introduced directly. Once inside the cells the DNA is expressed using the human cell machinery. Alternatively, human cells are first 'collected' and treated *ex vivo* with the genetic construct. Later on the transformed cells are (re)introduced into the human body.

A distinction can be made between somatic gene therapy where the modification is restricted to the treated individual and germline gene therapy where the therapy will affect future generations. In general germline gene therapy is prohibited in humans.

Gene therapy trials are conducted since more than 20 years with the largest number performed in the USA (Figure 1). Gene therapy is mainly being studied for the treatment of diseases that have (almost) no alternative cures or for which available treatments turn out to be not effective. The majority of the diseases that are targeted are cancers followed by a relative equal amount of trials addressing monogeneic, vascular and infectious diseases (Figure 2). For cancer the strategies aim to selectively kill the cancer cells either directly or via immunomodulation. The trial participants usually are in an advanced stage of disease development, for which no cure is available anymore. Other studies aim at replacing a defective gene, like in sickle-cell anaemia or Pompe disease. Also vaccination is experimented.

Both academic and to a lesser extent industrial organisations investigate the possibilities of these techniques. Until now the most common vector to deliver therapeutic gene(s) are viruses (Figure 3). They are administered either directly or after transducing autologous or allogeneic cells that are then injected in the participant. Also, bacteria and liposomes are used as vehicles for gene delivery. Naked DNA is sometimes applied.

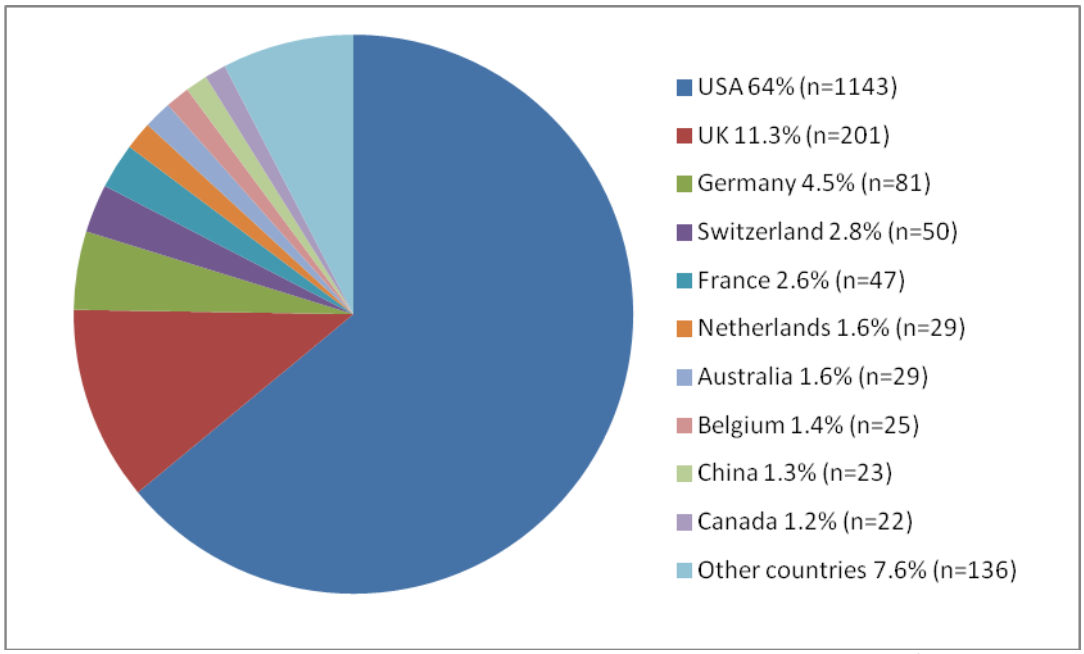

Figure 1 Geographical distribution of gene therapy clinical trials (Wiley[1], update Jan., 2012)

[1] http://www.wiley.com/legacy/wileychi/genmed/clinical/

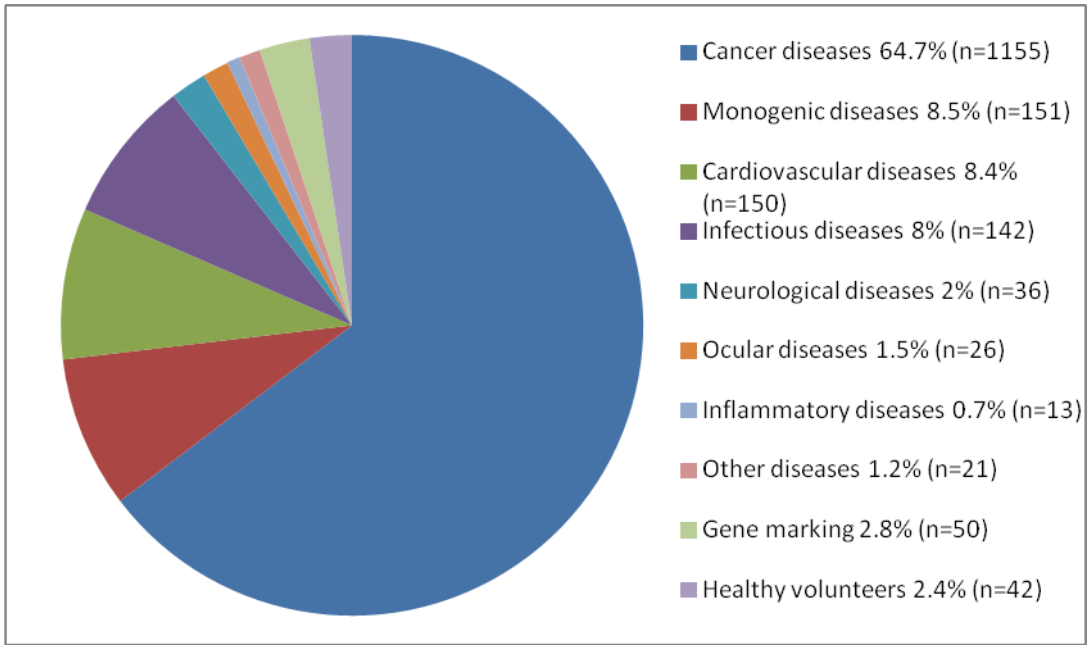

Figure 2 Diseases for which gene therapy is studied in clinical trials (Wiley, update Jan., 2012)

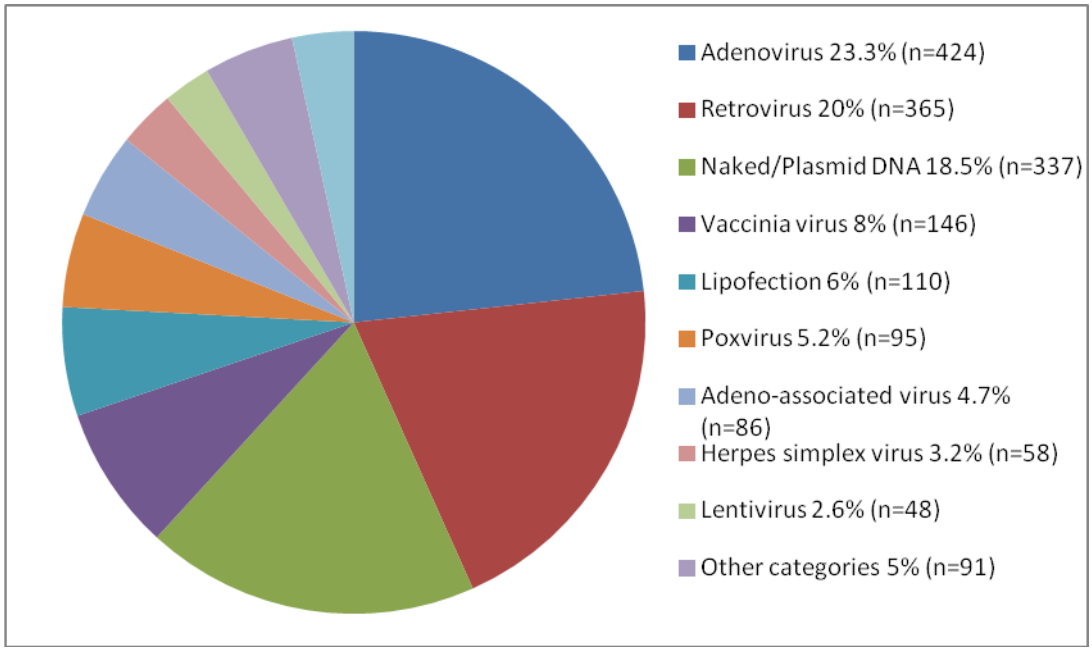

Figure 3 Relative contribution of vector types that are used in gene therapy clinical trials (Wiley, update Jan., 2012; trials with 2 vector types are counted twice)

Many strategies that look promising in pre-clinical trials are advanced to phase I clinical trials. However, few reach the next stages of drug development (Figure 4).

No gene therapy product has been placed on the market in Europe or North America.

European legislation requires that the risks for human health and the environment are assessed before performing activities with GMOs. The environmental risk assessment (ERA) provides the basis for identifying the need for risk management measures to reduce potential adverse effects, for designing risk management measures if needed, and for evaluating the remaining risk in case these measures are implemented. The way the ERA is handled in different countries, as a separate dossier

or integrated in the clinical trial application, and the importance that is given to the environment will make the approval process different.

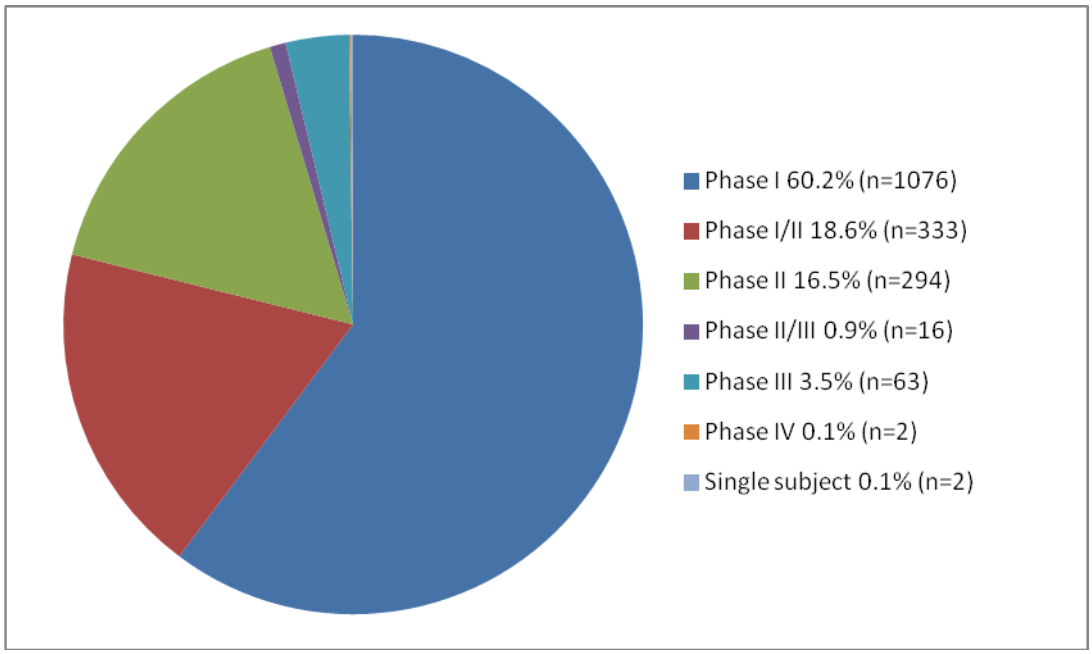

Figure 4 Relative distribution of the types (phases) of gene therapy clinical trials (Wiley, update Jan., 2012)

Whereas the evaluation of risks for participating volunteers and staff is a standard element of any clinical trial application, concerns about the environmental impact have received more attention since the advent of biotechnology, more specifically genetic engineering. In consequence, the experience with ERA remains relatively limited and few initiatives have been taken to harmonise approaches. Since the major part of the gene therapy clinical trials is carried out in North America, experience gained in assessing the applications, including how to deal with the environmental risks, may provide important indications for risk assessors in other countries.

Perseus BVBA was commissioned to perform this review of gene therapy clinical trials. Of the various aspects that are evaluated in the gene therapy clinical trial authorisation process only the assessment of the environmental risks of the involved GMOs is discussed.

2 The legal framework for gene therapy

2.1 Gene therapy legislation in the European Community

2.1.1 Definitions

In the European Community (EC) 'gene therapy' is one of the 'advanced therapies' that are regulated in Regulation (EC) No 1394/2007[2]. The definition of an 'advanced therapy medicinal product' (ATMP) is found in Article 2(1):

(a) 'Advanced therapy medicinal product' means any of the following medicinal products for human use:

- *a gene therapy medicinal product as defined in Part IV of Annex I to Directive 2001/83/EC*
- *a somatic cell therapy medicinal product as defined in Part IV of Annex I to Directive 2001/83/EC*
- *a tissue engineered product as defined in point (b).*

(b) 'Tissue engineered product' means a product that:

- *contains or consists of engineered cells or tissues, and*
- *is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue.*

A tissue engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices.

Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, shall be excluded from this definition.

(c) Cells or tissues shall be considered 'engineered' if they fulfil at least one of the following conditions:

- *the cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved. The manipulations listed in Annex I, in particular, shall not be considered as substantial manipulations,*
- *the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor.*

A gene therapy medicinal product is, as indicated, further defined in Directive 2001/83/EC[3] as amended by Commission Directive 2003/63/EC[4], in Annex I Part IV:

'gene therapy medicinal product' shall mean a product obtained through a set of manufacturing processes aimed at the transfer, to be performed either in vivo *or* ex vivo*, of a prophylactic, diagnostic or therapeutic gene (*i.e. *a piece of nucleic acid), to human/animal cells and its subsequent expression* in vivo*. The gene transfer involves an expression system contained in a delivery system known as a vector, which can be of viral, as well as non-viral origin. The vector can also be included in a human or animal cell.*

[2] Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. OJ L324, 10.12.2007, p.121-137.

[3] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, p.67-128.

[4] Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use. OJ L159, 27.6.2003, p.46-94.

…

Gene therapy medicinal products include:

- *naked nucleic acid,*
- *complex nucleic acid or non-viral vectors,*
- *viral vectors,*
- *genetically modified cells.*

Gene therapy is opposed to somatic cell therapy as defined in the same Annex I Part IV:

'somatic cell therapy medicinal products' shall mean the use in humans of autologous (emanating from the patient himself), allogeneic (coming from another human being) or xenogeneic (coming from animals) somatic living cells, the biological characteristics of which have been substantially altered as a result of their manipulation to obtain a therapeutic, diagnostic or preventive effect through metabolic, pharmacological and immunological means. This manipulation includes the expansion or activation of autologous cell populations ex vivo *(*e.g. *adoptive immuno-therapy), the use of allogeneic and xenogeneic cells associated with medical devices used* ex vivo *or* in vivo *(*e.g.*, micro-capsules, intrinsic matrix scaffolds, bio-degradable or not).*

Commission Directive 2009/120/EC[5] later updated the text of Annex I Part IV according to scientific and technical progress in the field of advanced therapies, and rephrased the definition into:

'Gene therapy medicinal product' means a biological medicinal product, which has the following characteristics:

(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;

(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products shall not include vaccines against infectious diseases.

The definition of a GMO can be found in Directive 2009/41/EC[6] in Art.2(b):

'genetically modified micro-organism' (GMM) means a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination

Micro-organisms also include viruses, viroids, and animal and plant cells in culture (Art.2(b)). A similar definition is given in Directive 2001/18/EC[7] for GMOs other than micro-organisms (Art.2(2)).

Annex I, Part A of Directive 2009/41/EC lists the techniques that are covered to alter a micro-organism:

1. Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.

2. Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism, including micro-injection, macro-injection and micro-encapsulation.

[5] Commission Directive/2009/120/EC of 14 September 2009 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products. OJ L242, 15.9.2009, p.3-12.

[6] Directive 2009/41/EC of the European Parliament and of the Council of 6 may 2009 on the contained use of genetically modified micro-organisms, OJ L125, 21.5.2009, p75-97.

[7] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, OJ L106, 17.4.2001, p1-38.

3. Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Whereas Commission Directive 2009/120/EC specifies that a gene therapy medicinal product may '*consists of a recombinant nucleic acid*', European Member States have diverging views on the inclusion of naked nucleic acid (comprising both DNA and RNA) in the scope of the GMO legislation.

2.1.2 Legislative framework

Clinical trials

Clinical trials in general, and therefore also trials with gene therapy medicinal products, are regulated by Directive 2001/20/EC[8]. The main objective of this directive is to protect the participants in a trial. '*The foreseeable risks and inconveniences have been weighed against the anticipated benefit for the individual trial subject and other present and future patients*' (Art.3.2(a)) and found to be justifiable before a trial can start. Also, participants have to give their 'informed consent' (Art.3.2(b & d)). The evaluation and authorisation of clinical trials is handled at the level of each individual EU Member State. The evaluation process of a request for authorisation may not exceed 60 days or 90 days in case of ATMP clinical trials (Art.9.4).

If GMOs are included, also the European GMO legislation needs to be observed. Some Member States choose to assess clinical trial applications under the CU legislation of Directive 2009/41/EC. Others follow Directive 2001/18/EC on DR or use a combination of both procedures.
The standard authorisation procedure according to Directive 2001/18/EC should be completed within 120 days (Art.6). For CU, whether a permit is needed or a notification is sufficient, depends on the risk class of the GMO.

Marketing

The procedure and requirements for market authorisation of ATMPs are described in Directive 2001/83/EC (and amendments), in Regulation (EC) No 726/2004[9] and in Directive 2001/18/EC in case GMOs are involved. The EU has a centralised marketing procedure. Marketing applications are submitted to the European Medicines Agency (EMA). The Committee for Advanced Therapies established in accordance with Regulation (EC) No 1394/2007 prepares a draft opinion on each ATMP application for the EMA, in order to adopt a final opinion. It also advises and evaluates scientifically in any matter that relates to quality, safety and efficacy of ATMPs.

2.1.3 Environmental risk assessment

Directive 2001/20/EC, Directive 2001/83/EC (and amendments) as well as Regulation (EC) No 726/2004 refer to the DR and/or CU legislation for those cases where GMOs are involved. Regulation (EC) N° 726/2004 states in the pre-amble (36) that:

'Environmental risks may arise from medicinal products containing or consisting of genetically modified organisms. It is thus necessary to subject such products to an environmental risk-assessment procedure similar to the procedure under Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms, to be conducted in parallel with the evaluation, under a single Community procedure, of the quality, safety and efficacy of the product concerned.'

[8] Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. OJ L121, 1.5.2001, p.34-44.
[9] Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. OJ L136, 30.4.04, p.1-33.

The requirements and the procedures for performing an ERA are laid down in Directive 2009/41/EC and in Commission Decision 2000/608/EC[10] for CU of GMOs, and in Directive 2001/18/EC and in Commission Decision 2002/623/EC[11] for DR.

An ERA encompasses the evaluation of risks to human health and the environment, whether direct or indirect, immediate or delayed, which the GMOs may pose. Directive 2001/18/EC explains in Annex II, D. that an ERA must consider:

- *'Likelihood of the GMO to become persistent and invasive in natural habitats …;*
- *Any selective advantage or disadvantage conferred to the GMO …;*
- *Potential for gene transfer to other species …;*
- *…;*
- *Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms …;*
- *Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s)';*
- *Possible immediate and/or delayed effects on animal health …;*
- *Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions …'*

An ERA consequently covers a broad field. It also comprises effects on healthcare workers, family members of the trial participants and the public at large. Healthcare workers are also protected by legislation on worker's protection, Directive 2000/54/EC[12]. The effect of the GMO on patients enrolled in a clinical trial is covered by Directive 2001/20/EC[13] and falls outside of the ERA.

Conceptually, risk has two components, one related to the possibility of adverse effects happening, and the other related to the consequences if the adverse effect occurs. Risk is also sometimes defined as the hazard combined with the likelihood that the hazard will occur:

Risk = Hazard & Likelihood

Concerning the type of adverse effects Commission Decision 2002/623/EC further elaborates that:

- *'Direct effects'* *refer to primary effects on human health or the environment, which are a result of the GMO itself and which do not occur through a causal chain of events.*
- *'Indirect effects'* *refer to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management; observations of indirect effects are likely to be delayed.*
- *'Immediate effects'* *refer to effects on human health or the environment, which are observed during the period of the release of the GMO. Immediate effects may be direct or indirect.*

[10] Commission Decision 2000/608/EC of 27 September 2000 concerning the guidance notes for risk assessment outlined in Annex III of Directive 90/219/EEC on the contained use of genetically modified micro-organisms, OJ L258, 12.10.2000, p43-48.
[11] Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, OJ L200, 30.7.2002, p22-33.
[12] Directive 2000/54/EC of the European Parliament and of the Council of 18 September2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). OJ L262, 17.10.2000, p.21-45.
[13] DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. OJ L 121, 1.5.2001, p.34-44.

- *'Delayed effects'* *refer to effects on human health or the environment, which may not be observed during the period of the release of the GMO but become apparent as a direct or indirect effect either at a later stage or after termination of the release.*

A risk assessment typically takes a step-wise approach:

- Potential adverse effects (hazards) are first identified based on the knowledge about the GMO (**hazard identification**).
- In the subsequent **hazard characterisation**, the potential consequences (harm), either as direct, or indirect, immediate and delayed effects, are evaluated. This involves the qualitative or, whenever possible and useful, quantitative description of the nature of the hazards and their respective accompanying uncertainties.
- Parallel to the hazard characterisation an assessment of the **likelihood** of occurrence (or exposure) of each of the identified hazards is prepared.
- Hazard and exposure characterisation lead to **risk characterisation** as the qualitative or quantitative estimate of the probability of occurrence and severity of adverse effect(s) or event(s). Often uncertainty about the severity of effects or their occurrence has to be dealt with. One way to solve this is to assume a worst-case scenario. If the risk of a worst-case scenario is found to be negligible, the risk of the 'less than worst-case' would also be negligible.
- The next step is **risk management** that aims to control identified risks and address remaining uncertainties.
- The final step is the overall **conclusion** concerning risks on the proposed activities. Whether the resulting overall risk is acceptable is the responsibility of the competent authorities, not the assessors.

Potential benefits for the targeted patient population are not taken into account in an ERA.

2.2 Gene therapy legislation in the Netherlands

2.2.1 Definitions

The definitions of a GMO are based on the European Directives as indicated in the previous section. In the Netherlands experiments with naked DNA are also subject to GMO oversight, based on the fact that naked DNA may lead to the formation of GMOs by uptake and integration in somatic cells, germline, bacteria, viruses etc.

2.2.2 Legislative framework

EC Directives are implemented in the Member States via national legislation.

Clinical trials

The protection of the participants of a clinical trial is taken care of by the Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek, CCMO) according to the Medical Research involving Human Subjects Act (Wet medisch-wetenschappelijk onderzoek met mensen) and implementing legislation, the Central Assessment of Medical Research (Human Subjects) Decree (Besluit Centrale Beoordeling Medisch Wetenschappelijk Onderzoek met Mensen). These pieces of legislation are the transposition of Directive 2001/20/EC.

The Ministry of Health, Welfare and Sport (VWS) checks for additional adverse effects on trial participants in the EudraVigilance Clinical Trial database of EMA (suspected unexpected serious adverse reactions; SUSARs).

The GMO aspect is regulated via the Genetically Modified Organisms Decree (Besluit genetisch gemodificeerde organismen milieubeheer)[14] and amendments that deal with both CU and DR. Art.5.1a and Art.24 of the Decree require an applicant to perform a risk analysis respectively before starting CU activities and before a DR in conformity with the European Directives. The regulation implementing the Decree is the Genetically Modified Organisms Regulation (Regeling genetisch gemodificeerde organismen)[15] and amendments that were last adapted in 2010 by the Wijzigingsregeling Regeling genetisch gemodificeerde organismen[16].

Marketing
The procedure for a marketing license is handled at the EU level. However, each individual Member State may appoint experts for the Committee for Medicinal Products for Human Use and the competent authorities for GMO of all Member States are informed and may comment on the application.

2.2.3 Environmental risk assessment

In the Netherlands the ERA for gene therapy clinical trials is based on Directive 2001/18/EC on DR of GMOs. The Genetically Modified Organisms Regulation specifically refers to Commission Decision 2002/623/EC in Art. 13.2 concerning the ERA for DR activities.

The Advisory Committee on Genetic Modification (Commissie Genetische Modificatie, COGEM), as the scientific advisory body to the Ministry of Infrastructure and Environment (Ministerie van Infrastructuur en Milieu, IenM), advises on the risks for human health and the environment associated with the use of GMOs. The COGEM may suggest additional containment measures. The Ministry eventually decides whether or not a permit is issued with or without extra conditions.

The decision on a gene therapy application must be signed by the Minister within 120 days after submission of the application, unless the clock is stopped while waiting for additional information from the applicant. The decision will take effect 6 weeks thereafter, being the period of appeal.

2.2.4 Procedural streamlining

As multiple permits need to be applied for in order to conduct a gene therapy clinical trial, a central Gene Therapy Office (Loket Gentherapie)[17] has been established to streamline the different national review processes. The process starts with a coordinated preliminary consultation. This informal and voluntary consultation facilitates the submission of the correct information and reduces delays due to additional requests for data once the official procedure has started. Also, the different application forms are combined to prevent duplication of information.

Guidance is provided in 'Guidelines for researchers and sponsors with regard to the assessment by official bodies of clinical research involving gene therapeutics in the Netherlands'[18]

[14] Besluit genetisch gemodificeerde organismen milieubeheer van 25 januari 1990 (BWBR0004703, Stb. 1990, 53) en wijzigingen
[15] Regeling genetisch gemodificeerde organismen van 28 mei 1998 (BWBR0009653, Stcrt. 1998, 108)
[16] Wijzigingsregeling Regeling genetisch gemodificeerde organismen (herziening bijlage 1 en actualisering indeling handelingen in procesinstallaties) BWBR0028026, Stcrt. 2010, 12420.
[17] http://www.loketgentherapie.nl
[18] Guidelines for researchers and sponsors with regard to the assessment by official bodies of clinical research involving gene therapeutics in the Netherlands, September 2011
http://bggo.rivm.nl/Documenten/Documenten%20IM/Guidelines%20gene%20therapy%20applications.pdf

A schematic presentation of the approval process is given in Figure 5. At first sight the GMO permit is the rate-limiting step in the procedure. In practice this is not always the case as the clock may be stopped when additional information is asked for. However, the IenM procedure is bound to a public consultation: during a fixed 6-week-period the public may comment on the draft decision. Therefore, an application for an IenM permit is sometimes submitted before other the applications.

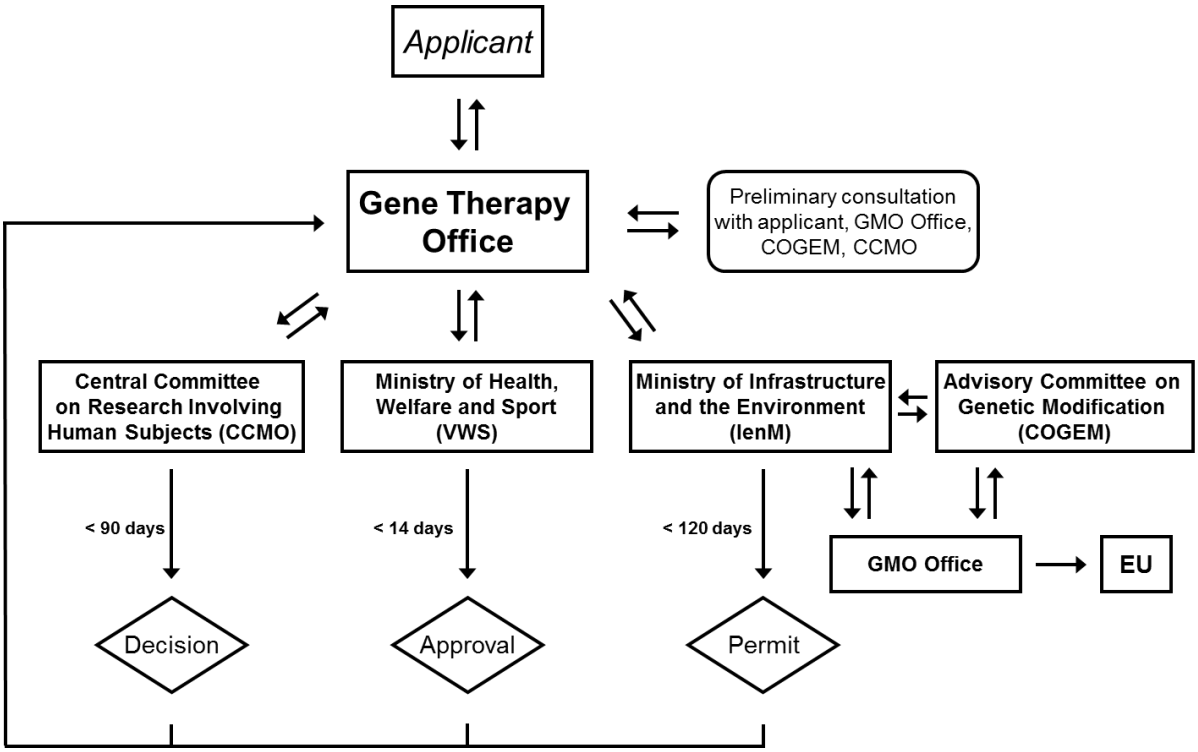

Figure 5 Gene therapy approval system in the Netherlands (Bleijs *et al*., 2007)

2.3 Gene therapy legislation in the United States

2.3.1 Definitions

The Food and Drug Administration (FDA) uses the following as a working definition for gene therapy[19]:

> *"Human gene therapy is defined as a medical intervention based on the administration of genetic material in order to modify or manipulate the expression of a gene product or to alter the biological properties of living cells. Cells may be modified* ex vivo *for subsequent administration or altered* in vivo *by gene therapy products given directly to the subject, including but not limited to autologous bone marrow stem cells modified with a viral vector, intramuscular injection of a plasmid DNA vector, use of antisense oligonucleotides to block gene transcription, ribozyme technology, and use of sequence-specific oligonucleotides to correct a genetic mutation."*

The National Institutes of Health (NIH) Guidelines[20] describe a human gene transfer experiment as (Section III-C-1):

[19] Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products.' Federal Register Vol. 58, No. 197, October 14, 1993, 53248
FDA, Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy, March 1998.
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm081670.pdf

[20] NIH Guidelines for research involving recombinant DNA molecules (NIH Guidelines), October 2011.
http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf

an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants.

A proposal has been discussed to adapt this definition to[21]:

Section III-C-1: Human Gene Transfer:
Human gene transfer includes all experiments involving the deliberate transfer of either:
1. Recombinant DNA, or DNA or RNA derived from recombinant DNA; or
2. Synthetic DNA or RNA that

- *Contains more than 100 nucleotides or base pairs in total; or*
- *Possesses biological properties that enable integration into the genome; or*
- *Have the potential to replicate in a cell; or*
- *Can be transcribed or translated.*

This change has not been implemented yet.

The NIH Guidelines define recombinant DNA molecules as either (Section I-B):

(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or
(ii) molecules that result from the replication of those described in (i) above.

According to these definitions using recombinant DNA or RNA derived from recombinant DNA regardless of the way they are administered to a clinical trial participant is considered gene therapy.

The Federal Food, Drug, and Cosmetic Act (FFDCA) contained in the Code of Federal Regulations (CFR), Section 21, defines an investigational new drug as:

a new drug or biological drug that is used in a clinical investigation. The term also includes a biological product that is used in vitro *for diagnostic purposes* (21CFR Part 312 Subpart A, General Provisions, Sec. 312.3 Definitions and interpretations[22]).

(21CFR Part 600 Subpart A, General Provisions, Sec. 600.3 Definitions):

(h) Biological product means any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man:

(1) A virus is interpreted to be a product containing the minute living cause of an infectious disease and includes but is not limited to filterable viruses, bacteria, rickettsia, fungi, and protozoa.

(2) A therapeutic serum is a product obtained from blood by removing the clot or clot components and the blood cells.

(3) A toxin is a product containing a soluble substance poisonous to laboratory animals or to man in doses of 1 milliliter or less (or equivalent in weight) of the product, and having the property, following the injection of non-fatal doses into an animal, of causing to be produced therein another soluble substance which specifically neutralizes the poisonous substance and which is demonstrable in the serum of the animal thus immunized.

(4) An antitoxin is a product containing the soluble substance in serum or other body fluid of an immunized animal which specifically neutralizes the toxin against which the animal is immune.

2.3.2 Legislation and Guidelines

Clinical trials

The CFR gives the Department of Health and Human Services (DHHS) authority to oversee clinical trials in general. Two organisations within DHHS, the Office for Human Research Protections and the U.S. Food and Drug Administration (FDA) have specific roles.

[21] Recombinant DNA Advisory Committee, Minutes of Meeting June 16-17, 2010. http://oba.od.nih.gov/oba/RAC/meetings/jun2010/RAC_Minutes_06-10.pdf
[22] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=312

The protection of the clinical trial participant is taken care of by the Office for Human Research Protections. The Office mandates that all research involving human subjects are reviewed and approved by Institutional Review Boards (IRB). An IRB is a committee of scientific and medical advisors and consumers (person who is not affiliated with the institution and who is not part of the immediate family of a person affiliated with the institution). The IRB is charged with evaluating research risk to subjects (patients and volunteers) and must approve research protocols and informed consent documents before starting a study (21 CFR Part 56).

The FDA is the primary government agency charged with protecting the health of U.S. citizens by ensuring that drugs, medical devices and biological products are safe and effective before they are used by healthcare professionals and consumers. The Center for Biologics Evaluation and Research (CBER) at FDA regulates human gene therapies, which fall under the legal definition of a "biologic".

Since the USA regulations require that medicinal products be approved for marketing before they are transported or distributed across states, an exemption is applied for this requirement of market approval when an investigational medicine enters the clinical trial phase[23]. The sponsor then submits an investigational new drug (IND) application to FDA.

The FFDCA documents all requirements for an IND (21CFR Part 312). The dossier contains animal pharmacology and toxicology studies, manufacturing data and the proposed clinical protocol. If within the review period of 30 days no objections are received from the authorities, the trial may start. It is possible to consult authorities before submitting an IND.

One of the instruments for FDA to assure the safety and rights of the participants is the possibility to call for a 'clinical hold', delaying a proposed clinical trial or suspending an ongoing investigation. This would be the case when the FDA finds that human participants are or would be exposed to an unreasonable significant risk or that not enough information is provided to the FDA to assess the risk to the participants.

The NIH, another DHHS agency, has published guidelines with additional requirements to those specified in the CFR. Clinical trials on gene transfer that are either directly funded by NIH or conducted at Institutions that receive funding from NIH for recombinant DNA research must meet these requirements (NIH Guidelines, Section I-C-1):

> *The NIH Guidelines are applicable to:*
> *Section I-C-1-a-(1). Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH Guidelines.*
> *Section I-C-1-a-(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.*

In practice most other gene therapy clinical trials align to these requirements as well[24].

The NIH established the Recombinant DNA Advisory Committee (RAC) over thirty years ago. All human gene transfer trials conducted at, or sponsored by, institutions receiving NIH funding for recombinant DNA research are registered with the Office of Biotechnology Activities (OBA). As discussed below, certain vaccine trials for infectious diseases are not required to register with OBA.

[23] Investigational new drug application
http://www.fda.gov/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/approvalapplications/investigationalnewdrugindapplication/default.htm
[24] http://oba.od.nih.gov/rdna/rdna_faq.html#RDNA_FAQ006

RAC is responsible for reviewing human gene transfer research and making recommendations regarding trial design and other matters to the Director of NIH. RAC discusses scientific, ethical, and legal issues raised by recombinant DNA technology and its basic and clinical research applications. However, as an advisory body to the NIH, the RAC has no mandate to approve or disapprove protocols.

Clinical gene therapy protocols that raise novel or particularly important scientific, safety or ethical considerations are discussed by the RAC in public meetings. Upon submission of a dossier to RAC, the Committee will first determine as to whether the human gene transfer experiment presents characteristics that deserve public RAC review and discussion (NIH guidelines, Section III-C-1) (Figure 6). Initial review is finalised within 15 working days. A protocol is generally taken for public review if more than three members of the RAC make such a recommendation to the NIH OBA Director. In addition, the NIH Director may ask for public review or a Federal agency other than NIH.

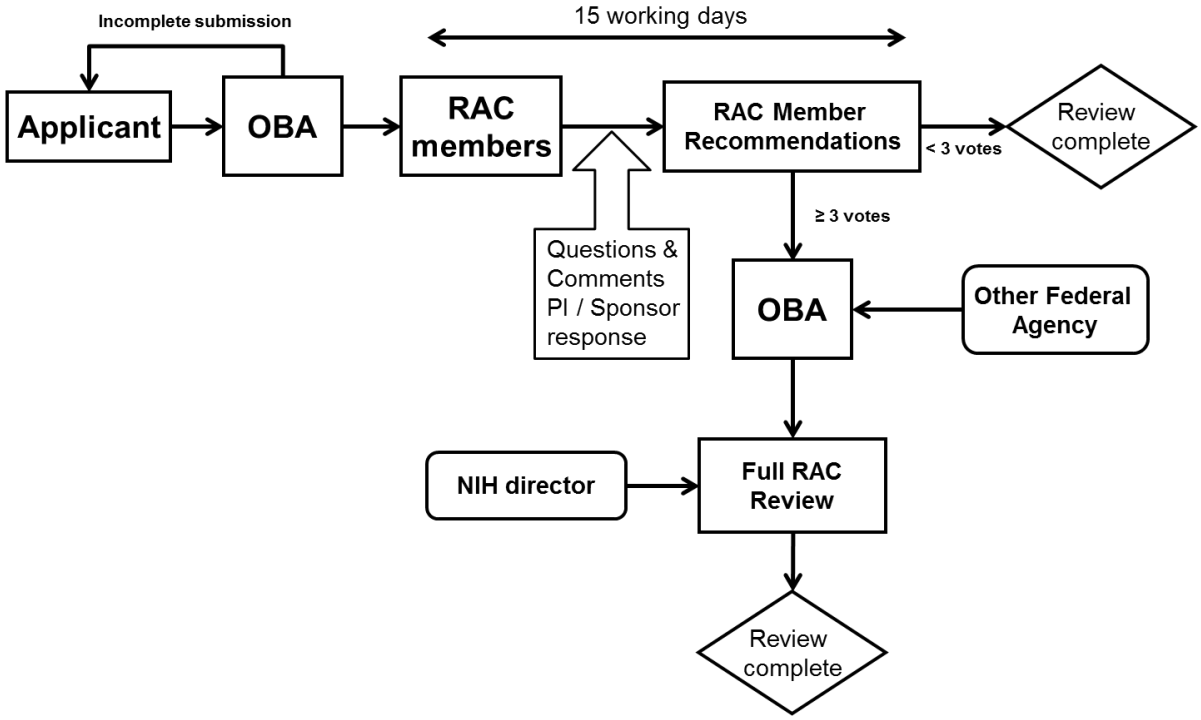

Figure 6 Summary of the human gene transfer protocol review process (after Corrigan-Curay, 2007[25])

RAC reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. Factors that may trigger public review and discussion include:

a) a new vector/new gene delivery system;
b) a new clinical application;
c) a unique application of gene transfer; and/or
d) other issues considered to require further public discussion.

The level of review is therefore based on the novelty of the application and not on the technique used.

RAC meets 4 times a year. The application dossier should be at OBA/RAC at least 8 weeks before a scheduled meeting to be discussed, if necessary, in that public meeting. The time before a meeting is used to already clarify issues and concerns in writing. At the meeting additional questions may be

[25] http://oba.od.nih.gov/oba/IBC/ASGT_2007_Training/RAC%20and%20Protocol%20Review.pdf

answered. The NIH OBA summary letter with recommendations is sent within 10 working days after the RAC meeting. Occasionally a dossier is submitted in an early stage to discuss potential issues and the need and type of additional pre-clinical studies.

Clinical trials of recombinant gene based vaccine constructs that are non-transmissible, fall under the so-called 'vaccine exemption' and do not require RAC review (NIH Guidelines, Appendix M-VI-A). These are:

Human studies in which:

- *induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, and*
- *such an immune response has been demonstrated in model systems, and*
- *the persistence of the vector-encoded immunogen is not expected,*

are exempt from Protocol Submission, Review, and Reporting Requirements.

They are not exempt from Institutional Biosafety Committee (IBC) review or general biosafety requirements of the NIH Guidelines. An investigator may however submit such a trial protocol on a voluntary basis, for example if he believes that a trial presents scientific, safety, or ethical concerns. The exemption was intended for the development of new vaccines against infectious diseases. If the principal goal is to treat for example cancer by generating an immune response to the cancer causing virus, the study does not fall under this exemption. Also, when combined with for example recombinant interleukin-2, such trials are also not exempt since the recombinant DNA encoding the cytokine is not of microbial origin[26].

FDA and NIH have complementary responsibilities regarding the regulation of human gene therapy.

- FDA's primary task is to ensure the quality and safety of gene therapy products and that these products are properly studied in human subjects.
- NIH's primary role is to evaluate the quality of the science in gene therapy research and to fund the laboratory scientists who do research in this domain.
- The IBC has oversight of recombinant DNA research at the institutional level. IBC reviews projects for compliance with the NIH Guidelines: it ensures that all NIH data requirements and aspects have been appropriately addressed prior to RAC protocol review, examines the preclinical animal data that supports the safety of the vector, ensures that RAC's recommendations, if applicable, are considered, ensures that the informed consent incorporates information regarding risks that arise from the biological nature of the agent, approves protocol only after RAC review process is complete, identifies new biosafety issues through analysis of adverse event reports and oversees compliance with all surveillance, data reporting and adverse event reporting requirements (NIH Guidelines, Section IV-B-2).

A clinical trial can only start when the RAC review process has been completed; the IBC approval (from the clinical trial site) has been obtained; the IRB approval has been obtained; and all applicable regulatory authorisation(s) (*e.g*. IND) have been obtained. IRB review and approval can occur before or after RAC review, but in practice IRBs await RAC findings. FDA authorisation of the IND application may be applied for at any time, but the optimal timing is after RAC review.

The American Society of Gene & Cell Therapy proposed to revise the role of RAC in reviewing gene therapy protocols (Genetic Engineering & Biotechnology News[27]; RAC meeting March, 2012). To streamline and shorten the procedure, instead of reviewing each individual protocol, the RAC may focus and discuss more generally new and emerging, sometimes controversial, scientific and safety issues in the field. Also, to avoid duplication the review may be left to FDA as well as IRBs and IBCs. On the other hand RAC is already limiting the in-depth review to the novel applications. The majority of

[26] http://oba.od.nih.gov/rdna/rdna_faq.html#RDNA_FAQ006

[27] http://www.genengnews.com/insight-and-intelligenceand153/gene-therapy-society-looking-to-nih-to-revisit-role-of-the-recombinant-dna-advisory-committee/77899620/

the protocols is processed within 3 weeks (15 working days). Additionally, other entities pronounce to benefit from the discussions in the public meetings. Exchange of thoughts and considerations are still ongoing.

Marketing

For market introduction of a medicinal product approval from FDA is applied for (New Drug Application, NDA; or in the case of a gene therapy product this would be a Biologics License Application, BLA). Gene therapy products must meet FDA requirements for safety, purity and potency before they can be sold. As with all investigational products, the gene therapy product is first tested in a laboratory and then in research animals. After conducting pre-clinical trials the next step are safety and efficacy studies in humans in clinical trials. The data gathered during the animal studies and human clinical trials of an IND become part of the NDA/BLA.
FDA has not yet approved any human gene therapy product for commercialisation.

2.3.3 Environmental risk assessment

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impacts of their actions and to ensure that the interested and affected public is informed of environmental analyses.

An IND is excluded from this assessment, and, therefore, normally does not require the preparation of an Environmental Assessment (EA) or an Environmental Impact Statement (21CFR Part 25 Subpart C--Categorical Exclusions, Sec. 25.31 Human drugs and biologics). FDA's reasoning is that an IND clinical trial does not individually or cumulatively have a significant effect on the human environment. Generally relatively small quantities of a drug or biologic product are involved and only a limited number of patients are treated. Likewise the IND safety reports (21 CFR §312.32) only relate to the patient or subject in the clinical trial (Investigational New Drug Safety Reporting Requirements for Human Drug and Biological Products and Safety Reporting Requirements for Bioavailability and Bioequivalence Studies in Humans (Federal Register /Vol. 75, No. 188, September 29, 2010 /Rules and Regulations, 59935)).

When extraordinary circumstances exist at least an EA is necessary (21 CFR Part 25.21). Possible exceptions may be when cytotoxics are used or when large volumes of waste are produced. Also, an EA is usually required for use of virulent organisms, organisms that are ecologically more fit than their wild-type counterparts, or organisms for which eradication is problematic or difficult to document. Where significant adverse effects cannot be avoided, FDA uses the submitted information as the basis for preparing an Environmental Impact Statement.

For market applications (NDA) an EA is usually required. In case of a gene therapy product a BLA also requires an EA unless exempt. The most important item is toxicity to organisms in the environment (fate and effect testing)[28].

Concerning the EA IBC's role in the review of human gene transfer trials is to identify and manage biosafety issues raised by gene transfer agents and any potential risk to public health or the environment (NIH Guidelines, Section IV-B-2). Whereas the IRB focuses on risk-benefit assessments relative to the individual research participants and other ethical issues, the IBC focuses more broadly on the risk to the environment and to public health, to close contacts, and to healthcare workers. Issues are for example the potential horizontal or vertical transmission risk, and safe handling and

[28] Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications, July 1998, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070561.pdf

administration of the gene therapy product (presentations by Corrigan-Curay at the September[29] and December[30] 2011 RAC meeting). IBCs also look at the risk to the individual participant, adequacy of facilities, standard operating procedures, training of personnel.

Concerning the risks for healthcare workers the National Institute for Occupational Safety and Health, as part of the Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services, is the federal agency making recommendations for the prevention of work-related injury and illness. Several documents and reports concern the safety of healthcare workers, and especially those confronted with biological hazards. Occupational Safety and Health Administration resides under the U.S. Department of Labor and is responsible for developing and enforcing workplace safety and health regulations. Both the National Institute for Occupational Safety and Health and the Occupational Safety and Health Administration are established by the Occupational Safety and Health Act of 1970 (29 CFR § 671). The Needlestick Safety and Prevention Act (HR.5178)[31] is relevant in this context.

2.4 Gene therapy legislation in Canada

2.4.1 Definitions

Food and Drugs Act (F&DA)[32] defines a 'drug' as (Art.2):

"drug" includes any substance or mixture of substances manufactured, sold or represented for use in

(a) the diagnosis, treatment, mitigation or prevention of a disease, disorder or abnormal physical state, or its symptoms, in human beings or animals,

(b) restoring, correcting or modifying organic functions in human beings or animals, or

(c) disinfection in premises in which food is manufactured, prepared or kept;

Art.12. *No person shall sell any drug described in Schedule C[33] or D unless the Minister has, in prescribed form and manner, indicated that the premises in which the drug was manufactured and the process and conditions of manufacture therein are suitable to ensure that the drug will not be unsafe for use.*

A schedule D drug is, amongst others:

Drugs obtained by recombinant DNA procedures
Drugs, other than antibiotics, prepared from micro-organisms
Immunizing agents, …

There is no definition for 'biologics' but it relates to products generally derived from or through the metabolic activity of a living organism and includes for example vaccines, blood (products), gene therapies, protein therapeutics (*e.g*. cytokines, hormones, antibodies), etc. (working definition BGTD) (Ridgway, 2008).

In the environmental legislation 'substance' is defined in section 3 of the Canadian Environmental Protection Act, 1999 (CEPA) [34] as:

[29] http://oba.od.nih.gov/oba/RAC/meetings/Sept2011/Agenda_Final_Sep2011_wBriefings.pdf and http://oba.od.nih.gov/oba/RAC/meetings/Sept2011/RAC_Minutes_09-11.pdf
[30] http://oba.od.nih.gov/oba/RAC/meetings/Dec2011/Agenda_Final_Dec2011_wBriefings.pdf and http://oba.od.nih.gov/oba/RAC/meetings/Dec2011/RAC_Minutes_12-11.pdf
[31] Pub. L. No. 106-430, 114 Stat. 1901, November 6, 2000.
[32] Food and Drugs Act, R.S.C., 1985, c. F-27, http://laws-lois.justice.gc.ca/PDF/F-27.pdf
[33] Schedule C drugs are drugs, other than radionuclides, sold or represented for use in the preparation of radiopharmaceuticals; and Radiopharmaceuticals.
[34] Canadian Environmental Protection Act, 1999 (S.C. 1999, c. 33) http://laws-lois.justice.gc.ca/PDF/C-15.31.pdf

any distinguishable kind of organic or inorganic matter, whether animate or inanimate, and includes

(a) any matter that is capable of being dispersed in the environment or of being transformed in the environment into matter that is capable of being so dispersed or that is capable of causing such transformations in the environment,

(b) any element or free radical,

(c) any combination of elements of a particular molecular identity that occurs in nature or as a result of a chemical reaction, and

(d) complex combinations of different molecules that originate in nature or are the result of chemical reactions but that could not practicably be formed by simply combining individual constituents.

This definition includes micro-organisms and probably also DNA or RNA as such.

Living organism is defined in section 104 of CEPA 1999 as "*a substance that is an animate product of biotechnology".* Biotechnology is defined in section 3 of CEPA 1999 as "*the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms".*
As defined in CEPA 1999, biotechnology is not limited to activities involving genetic engineering.

2.4.2 Legislation and Guidelines

Health Canada is responsible for regulating drugs for use in human clinical trials via the F&DA and the Food and Drug Regulations (Part C, Division 5)[35]. The Regulations define specific Clinical Trial Application (CTA) and Clinical Trial Application Amendment requirements for the sale and importation of drugs for use in human clinical trials in Canada.

Sponsors must file applications to conduct clinical trials in Phases I through III of drug development and comparative bioavailability trials. Health Canada reviews clinical trial protocols to assess the protection and safety of the participants. A risk-benefit analysis of the data submitted by the sponsor is carried out including an assessment of the production process and the manufacturing facility (on-site evaluation).

Health Canada's Biologics and Genetic Therapies Directorate (BGTD)[36] is the federal authority with regard to biological drugs - being products that are derived from living sources - and radiopharmaceuticals for human use in Canada. BGTD is responsible for the review and approval of all types of biological and radiopharmaceutical drug submissions, including New Drug Submissions and Clinical Trial Applications.

The relevant Centres and Office at BGTD are the Centre for Vaccine Evaluation (CVE), the Centre for Evaluation of Radiopharmaceuticals and Biotherapeutics (CERB), the Centre for Blood and Tissues Evaluation (CBTE) and the Office of Regulatory Affairs (ORA):

- The CVE is responsible for viral vaccines, bacterial and combination vaccines.
- The CERB is responsible for the following products: radiopharmaceuticals, hormones, cytokines, enzymes, gene therapies, monoclonal antibodies, and allergenic extracts. In addition, the CERB evaluates the safety and clinical data for these products and for products in CVE.
- The CBTE deals with products such as blood and blood components, coagulation factors, immune globulins and other plasma derivatives and their recombinant analogues, cells and cell-based medicines, tissues and organs, xenografts, and semen for assisted conception. The CBTE also evaluates the safety and clinical data for these products.
- The ORA serves as a secretariat to BGTD. It manages submissions and applications, is the primary contact with industry, coordinates and facilitates submission meetings with industry, etc.

[35] Food and Drug Regulations, C.R.C., c. 870, http://laws.justice.gc.ca/PDF/C.R.C., c. 870.pdf
[36] http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/hpfb-dgpsa/bgtd-dpbtg/index-eng.php

ORA is also responsible for registering data of all adverse events related to biological clinical trials.

For human cells, tissues and organs for transplantation no pre-market authorisation procedure has to be followed. The involved organisations, however, have to register with Health Canada and adhere to strict safety requirements.

Once a product may be commercialised, the BGTD, together with the Health Products and Food Branch Inspectorate, the Marketed Health Products Directorate and the Public Health Agency of Canada monitor the product's safety and effectiveness throughout the lifecycle of the product.

Concerning the procedure for clinical trials, a CTA must be filed before starting a trial (Figure 7). Usually a pre-CTA consultation meeting is advised for new active substances or applications with complex issues. The guidance for CTA[37] describes the pre-CTA information package that should be submitted at least 30 days before the meeting date. Within 14 days after the meeting the sponsor makes a meeting record to be added to the Central Registry file for the drug.

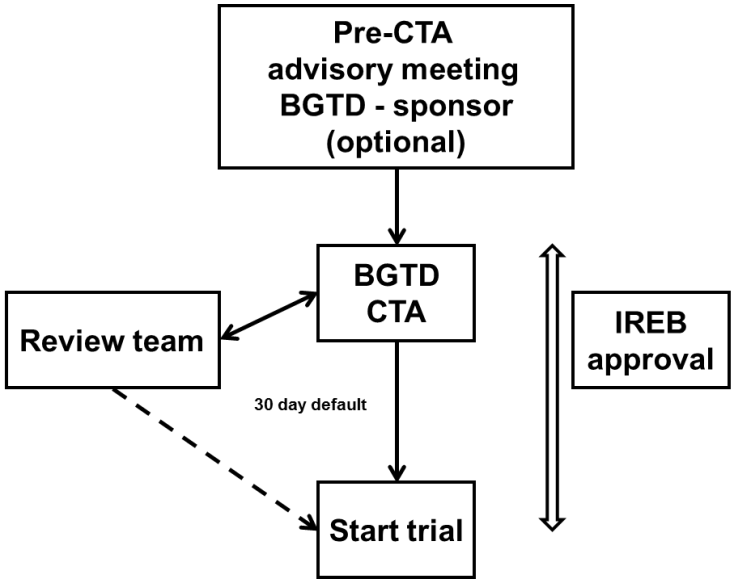

Figure 7 Canadian regulatory process for clinical trials (after Ridgway, 2009)

Health Canada reviews the CTA and notifies the sponsor within 30 days if the application is not complete (Regulations C.05.006(1)). Clarifications or additional information must be submitted within 2 days (Regulations C.05.009). A trial may start when a 'No Objection Letter' is received from Health Canada or when no notice, that would indicate that the sponsor may not sell or import the drug, has been received within 30 days. In practice, decisions are taken before the 30-day deadline. For each clinical trial site the Institutional Research Ethics Board (IREB) may still refuse to permit the clinical trial after a regulatory approval of a CTA. The IREB's role is to ensure the protection of the rights, safety and well-being of the trial participant. Members of the IREB should have knowledge either of biomedical science, or ethics, or biomedical laws. Also, at least one member of the 'community' (lay person) takes part in the IREB.

The Office of Laboratory Security, Centre for Emergency Preparedness and Response, Public Health Agency of Canada has jurisdiction over the importation of organisms and viruses into Canada, but the

[37] Clinical Trial Applications, Guidance for Clinical Trial Sponsors, http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/prodpharma/ctdcta-ctddec-eng.pdf

focus is on pathogenic organisms[38]. However, if the human pathogen is a pharmaceutical/drug or vaccine regulated by the Food and Drug Act, a permit to import the human pathogen is not required.

2.4.3 Environmental risk assessment

The CEPA requires that all new substances for use in Canada must be assessed for both direct and indirect impacts on human health and the environment. The approach aims to control new substances before they are manufactured or imported. An assessment needs to be performed to check whether they are potentially toxic and any appropriate or required control measures have to be taken. Only then they may be introduced into the Canadian marketplace. New substances are either notified under the New Substances Notification Regulations (Chemicals and Polymers) [NSNR (Chemicals and Polymers)] or the New Substances Notification Regulations (Organisms)[39] [NSNR (Organisms)] of CEPA, 1999.

Since 2001 manufacturers or importers of new substances in products regulated by the F&DA have to notify under the NSNR. Environment Canada is responsible for performing ERAs of CEPA toxic substances, including organisms and microorganisms that may have been derived through biotechnology. Part 6 of CEPA 1999 deals with new substances that are living organisms and that are products of biotechnology (sections 104-115). After receiving a notification under the NSNR (Organisms), Environment Canada performs the assessment jointly with Health Canada to determine whether there is a potential for adverse effects of the substance on human health, the environment or its biological diversity.

The NSNR implement Part 6 of CEPA 1999. 'New' means not yet on the Domestic Substances List (DSL). The DSL is a compilation of all reported substances (chemicals, polymers and living organisms) that were:

- in Canadian commerce between January 1, 1984, and December 31, 1986; or
- added to the list following notification and risk assessment, in accordance with CEPA 1999.

The Minister of the Environment is responsible for amendments to the DSL. The list currently contains about 23,000 chemicals, 67 microbial strains and 2 complex microbial cultures.

The NSNR were put in place primarily to assess the safety of industrial products. Since the assessment procedure does not always fit to substances under F&DA, Health Canada is developing new Environmental Assessment Regulations (EARs) through its Environmental Impact Initiative. The EARs will cover all new substances including biologics, cosmetics, food additives, medical devices, natural health products, novel foods, pharmaceuticals, radiopharmaceuticals and veterinary drugs. When the EARs come into effect, they will replace the NSNR only with respect to new substances that are used in products regulated under the F&DA.

The draft guidance document 'Preparation of Drug Submissions and Applications in the Common Technical Document (CTD) Format'[40] refers in Module 1.5 to the environmental assessment required for new substances in products regulated under the F&DA according to the NSNR of CEPA. This guidance document that is currently revised, applies to the preparation of all drug submissions and applications for human use, including CTAs, their amendments and Drug Master Files.

[38] Human Pathogens Importation Regulations http://www.phac-aspc.gc.ca/lab-bio/regul/reg-imp/index-eng.php
[39] New Substances Notification Regulations (Organisms), 2005 (SOR/2005-248) http://laws-lois.justice.gc.ca/PDF/SOR-2005-248.pdf
[40] Draft Guidance for Industry: Preparation of Drug Submissions and Applications in the Common Technical Document (CTD) Format, June, 2003, http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/prodpharma/ctdnds_ctdpdn-eng.pdf

A New Substances Notification package contains all information as prescribed in the NSNR. The type of information that is required and the timing of the notification depend on the type of substance, the quantity that will be imported or manufactured, the intended use of the substance and the circumstances associated with its introduction. Living organisms, either naturally occurring or genetically modified, are first classified as micro-organisms or as organisms other than micro-organisms. A microscopic living organism is either:

a) classified in the Bacteria, the Archaea, the Protista, which includes protozoa and algae, or the Fungi, which includes yeasts; or
b) a virus, virus-like particle, or sub-viral particle; or
c) a cultured cell of an organism not referred to in paragraphs (a) and (b), other than a cell used to propagate such organism; or
d) any culture other than a pure culture.

Then follow factors such as conditions or circumstances of introduction. The type and amount of information that is required depends on the classification:

- manufacture or import for introduction anywhere in Canada;
- manufacture or import for introduction in an ecozone where not indigenous;
- manufacture or import for introduction in accordance with confinement procedures;
- manufacture or import for introduction in an ecozone where indigenous;
- manufacture in a contained facility or import to a contained facility, and not for introduction outside the contained facility, or for export only;
- manufacture or import for introduction in an experimental field study; and
- manufacture and introduction at the same site from where isolated.

Micro-organisms that will be used in research and development in a contained facility, under certain conditions (below certain volumes, pathogenicity class) are exempt from the NSNR. Also exempt are living organisms regulated by another federal Act or Regulation that foresees in an assessment of whether it is toxic or capable of becoming toxic.

Most gene therapy clinical trials make use of this exemption as the investigational product is administered in a contained hospital room and only in relatively small quantities to a limited number of persons. A contained facility means a facility with physical and operational requirements aiming at preventing or limiting dispersal of the micro-organism. In the NSNR reference is made to the Canadian Laboratory Biosafety Guidelines and Appendix K of the US NIH Guidelines. Also, under certain conditions notifiers may submit a request to Environment Canada to waive the requirement for any of the prescribed information (subsection 106(8) of CEPA).

Several guidelines were compiled on the notification, testing and assessing new substances[41], amongst others for pathogenicity and toxicity against other organisms.

The assessment period again depends on the conditions or circumstances of introduction and varies between 30 and 120 days (Schedules 1, 2, 3, 4) (NSNR, Art.6). Pre-notification consultation with government officials is encouraged to resolve notification issues. The minister of the Environment and the Minister of Health share the ultimate authority and accountability for the decisions taken following the assessment. When the full complement of information requirements was submitted, the assessed micro-organism may be added to the DSL after a positive evaluation (CEPA, section 112).

A clinical trial may only start when all authorisations are given (BGTD, NSNR, and IREB).

[41] http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=66C60DFB-1

Other legislation that might be relevant for gene therapy trials is the Occupational Health and Safety Acts of the different Canadian provinces that apply to the safety of employees in clinical trials, and the Human Pathogens and Toxins Act[42].

2.5 International Conference on Harmonisation

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)[43] was created in 1990 as an agreement between the European Union, Japan, and the United States to harmonise different regional requirements for registering pharmaceutical drug products in order to reduce the need to duplicate the testing carried out during the research and development of new medicines. Scientific and technical aspects of product registration are discussed that may result in guidelines on quality, safety and efficacy.

Within ICH the Gene Therapy Discussion Group monitors emerging scientific issues in the field of gene therapy to proactively set out principles that may have a beneficial impact on harmonisation. This work has led to the issuance of consideration documents. For example the consideration document on virus and vector shedding[44] states that studies should be conducted to address potential public health concerns related to the potential risk of transmission to a third party. Although of concern, issues related to the environment have been excluded from the scope of the document as they are regulated differently in the various countries.

Competent authorities and agencies, as well as industry experts of several countries are involved in ICH as voting members or observers such as from Health Canada, the World Health Organization, and the European Free Trade Association. The Committee for Advanced Therapies contributes via its Gene Therapy Working Party to the activities of ICH.

Guidelines are not only implemented by the member regions but also by other countries. For FDA, once an ICH guideline is formalised, it becomes FDA guidance. The same is true for Health Canada. Although the ICH primarily deals with the marketing stage of pharmaceuticals (registration), the consideration documents are already followed at the clinical trial stage (development).

[42] Human Pathogens and Toxins Act, S.C. 2009, c. 24, http://lois-laws.justice.gc.ca/PDF/H-5.67.pdf
[43] http://www.ich.org/
[44] General Principles to Address Virus and Vector Shedding, June 2009, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Consideration_documents/GTDG_Considerations_Documents/ICH_Considerations_Viral-Vector_Shedding_.pdf

3 Inventory gene therapy trials

Worldwide 1786 gene therapy trials are being or have been conducted according to Wiley (The Journal of Gene Medicine Clinical Trial site, update Jan., 2012)[45]. The data in this list are compiled and regularly updated from official agency sources, published literature, conference presentations and posters and from information kindly provided by investigators or trial sponsors themselves. Given that data are acquired on a voluntary basis, this database is not presenting a complete picture.

Other databases include the ClinicalTrials.gov database[46] on clinical trials in general, performed in the US and worldwide. Federally or privately financed trials are registered. This database lists 377 studies related to gene therapy and gives detailed study data and recruitment information. The information is primarily meant for patients, family members and members of the general public. The NIH Genetic Modification Clinical Research Information System (GeMCRIS®)[47] makes searches on protocols, products and targeted disease available. The European JRC DR database for organisms other than plants[48] contains gene therapy trial summaries for those Member States that assess such a trial under the DR regulations. None of these databases claims to be complete.

3.1 Gene therapy in the Netherlands

In the Netherlands an advice has been formulated for the 24 trial applications that are registered on the COGEM website[49]. Wiley mentions 29 studies. The difference may be explained partly because COGEM advices issued before 2001 are not displayed, partly due to differences in counting method (multicentre trials). The JRC DR database has 23 records for studies in the Netherlands since October 17, 2002.

3.2 Gene therapy in the USA

Starting in 1988 the RAC evaluated 1131 gene therapy trial protocols (the number excludes protocols that were withdrawn or replaced; update Feb. 27, 2012[50]). Figuregure 8 presents the number of protocols over the years.
In the protocols with a therapeutic aim (1073) various diseases are targeted. The vast majority (772) deals with cancer. Infectious diseases account for 64 trials of which 55 caused by the *Human immunodeficiency virus* (HIV). Also, approaches addressing cardiovascular diseases are important with 80 studies. Ninety-five relate to inherited monogenic diseases and remaining 62 to several other diseases.

For treating various cancers the approaches that are mostly used involve immunotherapy either via *in vitro* transduction (283) or *in vivo* transduction (264) of cells.

Of the 1131 trials 798 were not selected for public review (submitted for the purpose of data monitoring and adverse event reporting), 7 were evaluated by an accelerated RAC review (this procedure is no longer used). One hundred seven protocols received a full RAC review (including NIH Director approval and FDA Investigational New Drug (IND) approval; procedure no longer used) and 204 a public review. Five protocols were voluntarily submitted to OBA/RAC although the research was not directly or indirectly NIH funded. For the rest the review level is still pending.

[45] http://www.wiley.com//legacy/wileychi/genmed/clinical/ , last accessed March 21, 2012
[46] http://clinicaltrials.gov/ct2/home
[47] http://www.gemcris.od.nih.gov/Contents/GC_HOME.asp
[48] http://gmoinfo.jrc.ec.europa.eu/, last accessed March 22, 2012
[49] http://www.cogem.net/index.cfm/nl/publicaties/categorie/advies, last accessed March 21, 2012
[50] http://oba.od.nih.gov/oba/rac/PROTOCOL.pdf

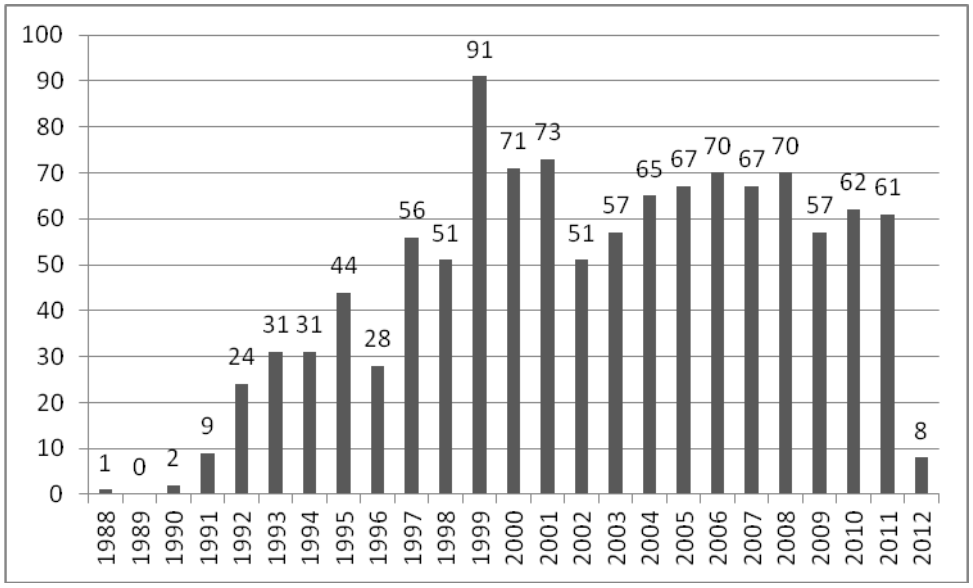

Figure 8 Gene transfer protocols in USA by year up to February 2012, ordered according to date of receipt by OBA/RAC (withdrawn and resubmitted protocols included)

If at least 3 members of RAC recommend public review because of novel scientific, clinical or ethical issues, protocols are selected for in-depth public review. Currently, approximately 15-20% of protocols are selected (Corrigan-Curay, RAC meeting December, 2011).

3.3 Gene therapy in Canada

Canada ranks fourth internationally in gene-transfer research (based on the number of gene therapy clinical trials) (Edelstein *et al*., 2007). Gene therapy protocol data are kept confidential and on the website of Health Canada no information can be retrieved on actual trials. The Wiley gene transfer clinical database compilers received limited information on the Canadian trials (Edelstein *et al*., 2007). In their 2007 review the authors mention 54 trials in Canada of which 17 contained enough information to include in the database. Today the Wiley database has 22 trials for Canada. Ridgway (2009) indicated that 65 trials were approved up to and including 2008 (Figure 9). Adenovirus vectors were mostly used followed by plasmid DNA (Figure 10).

On the DSL list none of the 69 microbial strains – bacteria and fungi - are thought to be related to clinical trials[51].

The list of Risk Assessment Summaries[52] in relation to the NSNR (Organisms) is still evolving with assessment decisions for new and past new substance decisions being added. Currently 13 summaries may be consulted, one of which is about a rotavirus-based vaccine and another on *Lactococcus lactis* for use as a vector in clinical trials.

[51] List of Organisms on the Domestic Substances List (DSL), Updated: September 21, 2011 http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=C4E09AE7-1

[52] Risk Assessment Decisions http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=8AD6A8C1-1

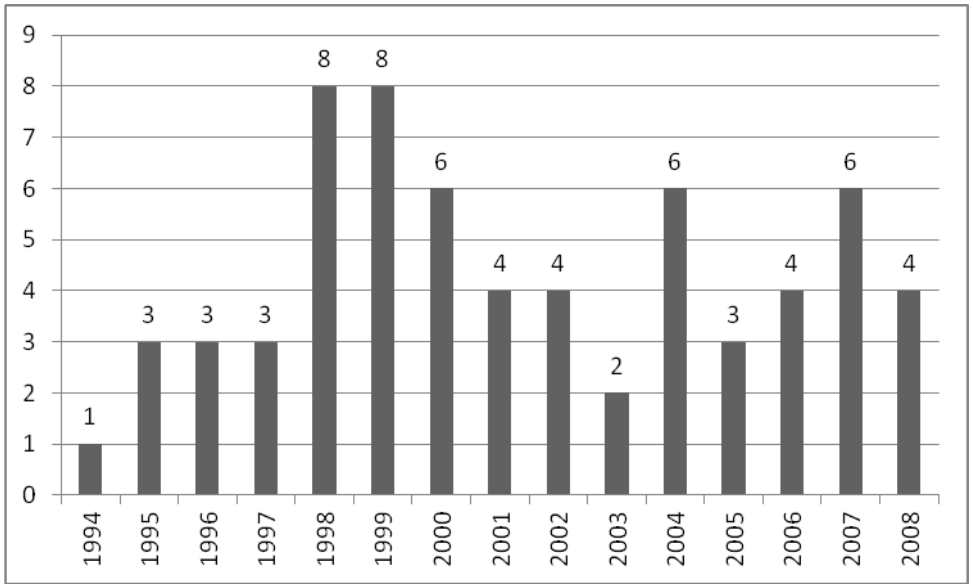

Figure 9 CTAs approved in Canada by year (adapted from Ridgway, 2009)

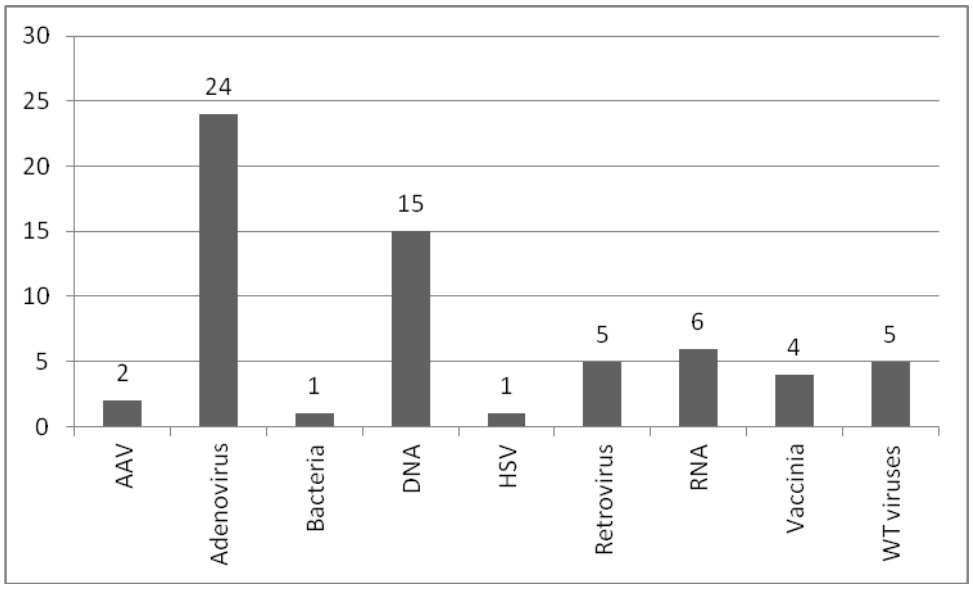

Figure 10 CTAs approved in Canada by vector type (adapted from Ridgway, 2009)

4 Environmental Risk Assessment

Many countries, including the EU, USA and Canada follow the guidelines and consideration documents on clinical trials issued by the ICH and accordingly require data in order to evaluate clinical trial protocols. Documents by the ICH Gene Therapy Discussion Group state that assessment of shedding can be utilised to understand the potential risk associated with transmission to third parties and the potential risk to the environment (ICH, 2009a). Shedding is here defined as the dissemination of the virus/vector through secretions and/or excreta of the patient.

In the ICH consideration documents the emphasis of the risk assessment is laid on the safety of the study participant and only in second instance attention is paid to human health other than the patient via virus shedding and presence of adventitious agents followed by shedding. It is stressed that caretakers should adhere to biosafety guidelines as available in the different countries and that family members should be instructed on how to minimise the exposure of others (ICH, 2009b). Furthermore reference is made to the fact *that all regulatory authorities require some form of barrier contraception for the duration of the clinical trial as a standard precaution to prevent person-to-person transmission* (ICH, 2009b).

Also, the potential for germline transmission is considered (ICH, 2006). This hazard depends on the biodistribution profile, the replication capacity and the integration potential of the vector.

The environment at large is not discussed.

4.1 ERA in the Netherlands

In order to assess the environmental risks the applicant needs to submit information as required by Directive 2001/18/EC Annex III A. These requirements are reflected in the 'Application form: Assessment of clinical research involving gene therapeutics in the Netherlands'[53], part A.
The stepwise approach is retained in the questions of the form. Both the spread of the investigational gene product into the environment as well as to third parties (including medical personnel) have to be evaluated.

In the COGEM advices the genetic constitution of the GMO is taken into account, the exposed environment (*e.g.* hospital), the health status of those likely to be exposed (*e.g.* caretakers, family members), the method of administration and the amount and frequency of use of the GMO.
Points of attention include the replication competency, the presence of free virus particles in a vector batch, the possibility to recombine with wild-type counterparts to regain pathogenicity or even worse, to become more pathogenic than the parent strain, the possibility to induce an immune response, host range, cell and tissue tropism, altered mobilisation, survival outside the host, etc. (Rüdelsheim and Smets, 2011).

4.2 ERA in the USA

There are no documents publicly available describing the procedure or considerations that regulators follow when conducting an environmental risk assessment, if any, in the development phase of a medicinal product.

[53] Application form: Assessment of clinical research involving gene therapeutics in the Netherlands, December 2010. http://bggo.rivm.nl/Paginas/form.htm

'The Guidance for Industry: Premarketing Risk Assessment' (FDA, 2005) is a tool for risk assessment during medicinal product development, including biological products, focusing on phase III clinical trials. However, the guidance only deals with trial design and safety information gathering to conduct a weighed risk-benefit assessment for the intended patient population.

Nevertheless, the data requirements for a clinical trial application as spelled out in the NIH guidelines already provide some evidence on aspects that are valued.
Appendix M relates to gene transfer trials. In Appendix M-II-B-1 basic information is asked for regarding the preparation, structure, and composition of the gene therapy product (*i.e.* molecular data, purity data, tests for contaminating materials, results from preclinical studies etc.), the gene delivery system, target cells, gene expression, stability of virus vectors, etc. Appendix M-II-B-4 'Public Health Considerations', describes the information that is needed to assess possible human health risks and the proposed risk management measures:

Appendix M-II-B-4. Public Health Considerations

Describe any potential benefits and hazards of the proposed gene transfer to persons other than the human subjects receiving the experimental treatment. Specifically:

Appendix M-II-B-4-a. On what basis are potential public health benefits or hazards postulated?

Appendix M-II-B-4-b. Is there a significant possibility that the added DNA will spread from the human subject to other persons or to the environment?

*Appendix M-II-B-4-c. What precautions will be taken against such spread (*e.g.*, subjects sharing a room, health-care workers, or family members)?*

Appendix M-II-B-4-d. What measures will be undertaken to mitigate the risks, if any, to public health?

Appendix M-II-B-4-e. In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to subjects? Are such concerns applicable to healthcare personnel?

Appendix M-III-B. deals with items that should be addressed in the informed consent document. Appendix M-III-B-2-a. on 'Reproductive Considerations' states that:

To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

The inclusion of pregnant or lactating women should be addressed.

It is clear that in these guidelines primarily public health is questioned, not the environment at large.

4.2.1 RAC review

In the RAC public meeting minutes the issues that somehow relate to risks for human health and the environment are only mentioned in 4 out of 10 protocol discussions. Most often they concern horizontal transfer of vector via intimate or close contact, or vertical transfer to be prevented via birth control. Other points of attention are the purity of the investigational drug (screening for contaminants; testing for absence of recombination-competent virus or feeder cells that may be used to culture human tissue *in vitro*), recombination with helper or wild-type strains, shedding via excreta, use of a biosafety cabinet to prepare the vector for administration, etc.

In Annex a summary is provided for approximately 120 protocols that were discussed in RAC meetings held from 2006 up to 2011. In Table 1 some examples of concerns that were discussed and proposals to manage the risk are provided.

Table 1 Examples of concerns identified during the RAC evaluation of clinical trial applications and management measures either proposed by the applicant or the RAC.

Protocol	Concern	Management
#0908-995	• Prevent spread of vaccinia virus to immune-compromised close contacts of the participants	• According to the proposed protocol, the participants are required to make arrangements to reside separately for a period of at least 3 weeks after the last dose to prevent contact. • The RAC further recommended that also clinical staff should be made aware of the risks of vaccinia exposure (RAC meeting December, 2009).
#0704-853	• Minimise risk of exposure of immune-compromised third parties from a trial with a live-attenuated form of the bacterium *Listeria monocytogenes*	• Individuals with immune-compromised family members were to be excluded from participating in the trial (RAC meeting June, 2007)
#0907-988	• Minimise risk of exposure of sensitive third parties from a trial with a human rhinovirus based vector	• Volunteers were to be quarantined or asked to refrain from attending large public gatherings, and participants having contacts with high-risk groups were to be excluded (RAC meeting September, 2009).
6 studies with a vaccinia virus vector	• Protect the workers that are administering the therapeutic	• Reference is made to CDC recommendations, *e.g*. the recommendations for routine vaccinia vaccination pustules (covering skin pustules). The participants in the trial must receive information according to the CDC recommendations to prevent contact transmission of vaccinia virus after vaccination. (RAC meeting September, 2010)
#1104-1101	• Protect the workers that are administering the therapeutic	• The RAC points to CDC contra-indications to vaccinia vaccination (pregnancy, immunodeficiency, immunosuppressive therapy, skin diseases). Personnel with such a contraindication should not participate in the study (RAC meeting June, 2011).
#0907-991	• Prevent transmission of the study bacteria, a *Salmonella*-derived vector, from the participants to others	• Instructions comprise for example the prohibition to prepare food for others, good-hand-washing techniques and disposal of potentially infectious faeces, and this for several weeks (RAC meeting September, 2009).

Requirements for extra studies

Occasionally a clinical trial protocol is provided to OBA/RAC early in the development phase before the preclinical stage is finalised. RAC's input in the experimental design and recommendations for safety, pharmacology and toxicology studies are then implemented in further research. Examples are study protocol #0802-905, a complex study with 3 agents to treat metastatic tumours, one of which is a modified adenovirus expressing interleukin-12 (RAC meeting June, 2008). In this case the discussions resulted in advice for additional studies not related to human health other than the research participant. For protocol #0807-927, about an oncolytic *Vesicular stomatitis virus* developed for treating cancer, serial passages of the virus under stress were suggested to establish reversion potential to the wild-type phenotype, next to other experiments (RAC meeting September, 2008). As a

public health precaution the protocol also requires isolation for all participants in a private room with a private bathroom for approximately 1 week after dosing or until it is established that no virus is present in the blood, in secretions, or in any vesicles that may develop.
Also for more advanced dossiers preclinical studies are sometimes recommended before a trial may start, when certain concerns according to the RAC are not yet solved.

Requirements for containment
NIH guidelines elaborate on risk group classification in Appendix B. The guidelines provide for physical (Appendix G) and biological (Appendix I) containment measures for laboratory activities. No containment requirements are indicated for hospital rooms, suggesting an approach for clinical trials with GMOs comparable to the European DR approach. Also, from the minutes of the public RAC meetings it is not clear what type of containment measures (physical, working procedures etc.) are proposed or taken. Only on one occasion a 'Class 2 isolation' is mentioned for a hospital setting to prevent exposure due to shedding (#1107-1120, RAC meeting September, 2011). Discussions on protocol #0908-995 using an oncolytic, replication-selective thymidine kinase-inactivated vaccinia virus refer to the responsibility of the local IBC on suitable containment measures (facility, administration methods) (RAC meeting December, 2009).

Safety measures covered
Although in the minutes of the RAC meetings environmental issues are not often mentioned, this does not exclude that human health and environmental risks are addressed in the dossier, but found to be satisfactory covered and not further discussed in public.

Guidelines
RAC published a 'Guidance on Biosafety Considerations for Research with Lentiviral Vectors' in 2006[54] to provide guidance to determine the appropriate containment at the laboratory phase via a risk assessment. The major risks in research are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis. Elements to consider are:

- *the nature of the vector system and the potential for regeneration of replication-competent virus from the vector components,*
- *the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care),*
- *the vector titre and the total amount of vector,*
- *the inherent biological containment of the animal host, if relevant,*
- *negative RCL testing.*

RCL testing at the research laboratory stage is not always needed or advisable. A risk assessment will give a decisive answer.

4.2.2 FDA review

The FDA requires that lentiviral vector stocks used in human clinical trials are tested for RCL. The 'Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)'[55] details on the assessment of the safety, identity, purity, and potency of a human gene therapy investigational product. One of the items that are discussed is testing for absence of replication-competent virus. Recommendations on how and when to test are given, next to approval criteria, for example, in master virus banks and final vector clinical lots.

[54] http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf
[55] Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs), April 2008 http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm078694.pdf

Further guidance on types of tests is found in 'Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors'[56].

Although only the safety of the study participant is envisioned, the safety and control measures also reduce or prevent risks for human health in general. Obviously, tests for absence of pathogens in allogeneic cells or other contaminating agents also protect the safety of third parties indirectly.

In the IND, the manufacturer explains how the study will be conducted, what possible risks may be involved and what steps will be taken to protect patients. Information is provided in support of the study. The IND application includes a list and description of all components used in manufacturing: vector, insert, sequence information, allogeneic (donor screening and testing for adventitious agents) and/or autologous cell components, cell bank system (master and working bank, packaging cell line), reagents, manufacturing process (*e.g.* purification steps), modification method, testing procedures, etc. From the guidance to complete an IND form it is clear that the amount, type and detail of data depends on the development phase ('Guidance for Industry: Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products'[57]).

The 'Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies[58]' states that regulations offer some flexibility in terms of the amount of data that need to be submitted with any IND application. Knowing that only a small fraction of Phase I studies lead to products on the market, a more extensive preclinical database, absorbing a lot of resources, is not required, especially for exploratory IND studies for treatment of serious diseases, whit only a limited human exposure and no therapeutic or diagnostic intent. As clinical development of a gene therapy investigational drug proceeds, applicants are advised to discuss the manufacturing data that will be needed to support the safe use of their products in Phase II and III trials with the appropriate FDA agency. In general applicants may always consult on the data that are necessary before giving in an IND application.

4.2.3 Local assessment

IBCs would pay attention to waste management, safe handling procedures etc. A document describing adequate procedures like *e.g*. an. 'Infection Control Manual', is mandatory for IBC approval.

OBA proposed to exempt some trials from IBC review:

> *"The OBA is considering exempting multisite Phase II or Phase III low-risk trials from IBC review. IBC review would not be required if the vector is a plasmid or a specified non-integrating vector derived from a Risk Group 2 virus and if a previous safety study in humans tested the proposed dose for the Phase II or Phase III study. In addition, the prior safety study should have resulted in no unexpected toxicities related to the investigational agent using the same delivery method at the dose proposed, the concomitant interventions must be*

[56] Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors', November, 2006
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm078723.pdf
[57] Guidance for Industry: Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products, November 1995
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071597.pdf
[58] Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies, January, 2006
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078933.pdf

comparable to the previous Phase I safety study or the previous Phase II study, and the study populations must be comparable" (RAC meeting December, 2011).

Criteria were further described in the March 2012 meeting. The virus vector should be an attenuated virus of the group of adenoviruses, HSV, poxviruses (but not vaccinia virus) or AAV and be non-replicating (RAC meeting March, 2012). Registration at OBA for all sites would still be required.

4.3 ERA in Canada

4.3.1 Environment Canada

The systematic steps used to conduct science-based risk assessments of micro-organisms are described in the Framework for Science-Based Risk Assessment of Micro-organisms regulated under the Canadian Environmental Protection Act, 1999[59]. This approach is used by Health Canada and Environment Canada for new substances notifications as well as for the screening of micro-organisms that are on the DSL. The ERA considers effects on the environment and human health, including pathogenicity and toxicity, and ecological effects (e.g. loss of biodiversity, loss of habitat, disease). Under 'human health effects' the potential for adverse effects is assessed for both occupational exposure as for exposure to the general public. The precautionary principle is included.

The consecutive steps are the identification and characterisation of the hazard, and the exposure assessment. Hazard characterisation and level of exposure each can be classified in 3 classes: low, medium or high. The risk characterisation follows from the previous steps. Risk is characterised as low, medium or high depending on the hazard severity and potential for exposure, also taking into account the weight of evidence and scientific uncertainties. Environment Canada recognises that it is difficult to quantify risks related to biological entities. A qualitative assessment is then performed. The weight of evidence considers several component lines of evidence (number, type and quality of studies) to reduce overall uncertainty. For medium or high-risk substances risk management or control measure are recommended.

For the DSL micro-organisms the ERA is based on literature reviews, experimental data from completed and ongoing research projects, decisions from other jurisdictions, etc. For the new micro-organisms the information provided in the new substance notifications is used.

As information on the ERA of only a limited number of organisms is disclosed, guidelines and application forms may give a general impression of points of attention. The 'Guidelines for the Notification and Testing of New Substances'[60] describe the kind of information and data needed to fill in the new substance notification form. Information requirements in the 'New Substance Notification Form for Micro-Organisms'[61] include a description of the micro-organism itself (taxonomic designation, identification criteria, parental and donor organisms, vector, modification mode, genotypic and phenotypic characterisation, etc.) to start with. Elements to provide related to the environmental assessment are, amongst others:

- the stability of the changes (genotypic and phenotypic), and the nature, source and function of any inserted genetic material.
- a description of the biological and ecological characteristics of the micro-organism, including:

[59] http://www.ec.gc.ca/subsnouvelles-newsubs/120842D5-16CB-4CD2-89DE-D73D9EC47095/Revised%20Risk%20Assessment%20Framework%20-%20EN.pdf
[60] Guidelines for the Notification and Testing of New Substances: Organisms, August, 2010, http://www.ec.gc.ca/subsnouvelles-newsubs/22FC25C8-2097-40D8-975C-5B0479D52BA8/NSNR%20%28Organism%29%20Guidelines%20-%202010%20-%20EN.pdf
[61] http://www.ec.gc.ca/subsnouvelles-newsubs/A1C6F4A5-B51E-4B85-9E8E-C689151D6B49/NSN%20Form%20for%20Micro-organisms%20-%20EN.pdf

i. its life cycle,
ii. its infectivity, pathogenicity to non-human species, toxicity and toxigenicity,
iii. its resistance to antibiotics and tolerance to metals and pesticides,
iv. its involvement in biogeochemical cycling,
v. the conditions required for, and conditions that limit, its survival, growth and replication, and
vi. the mechanisms of its dispersal and the modes of interaction with any dispersal agents;

- the dispersal by gene transfer of traits of pathogenicity to non-human species, toxigenicity and resistance to antibiotics, including a description of:
  i. the genetic basis for pathogenicity to non-human species, toxigenicity and resistance to antibiotics,
  ii. the capability to transfer genes, and
  iii. the conditions that might select for dispersal of traits of pathogenicity to non-human species, toxigenicity and resistance to antibiotics, and whether the conditions are likely to exist at the locations of introduction or within the range of dispersal of the micro-organism; and
- a description of the geographic distribution of the micro-organism

and information in respect of:

- the introduction of the micro-organism
- environmental fate of the micro-organism
- the ecological effects of the micro-organism
- the human health effects of the micro-organism

and a description of the confinement procedures and contingency plans.
However, not all topics need to be addressed, but depend on the intended use (Schedules 1, 2, 3, 4).

As an example the 'Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations (Organisms) (NSNR[O]) of the Canadian Environmental Protection Act, 1999 - EAU-308, 309, 310, 311, 312: Rotavirus strains W179-9 (G1), SC2-9 (G2), 178-9 (G3), BrB-9 (G4), 179-4 (P1)'[62] is further discussed:

- This New Substance is a mixture of 5 rotavirus strains that were each evaluated as a separate substance. They are components of a live attenuated oral vaccine for the prevention of rotavirus gastroenteritis in infants and children. As the vaccine will be used 'anywhere in Canada' the Schedule 1 information is required. The assessment does not include occupational health risks.
- The viruses were 'reassorted' and contain outer surface proteins from human and bovine rotavirus parent strains. They are grown in Vero cells. The natural strains are risk group 2 organisms.
- From the sequence analysis it was concluded that '*the potential for expression of unpredicted novel traits or for introduction of uncharacterized genetic materials appears to be significantly low*'. Due to low homology between human and animal rotaviruses, interspecies reassortment that would cause animal gastroenteritis is not likely.
- Pathogenicity is further assessed towards plants and invertebrates and found to be of no concern. However, for mammals and birds rotaviruses are highly pathogenic. Then again, the attenuation makes that '*the likelihood is considered low that the excreted notified strains would undergo reassortment that causes adverse effects in non-human species*'.
- In relation to human safety results from a phase III trial in the USA do not report adverse immunological reactions. Reversion to the parental strain is unlikely due to the fact that both human and bovine components were used.
- Vaccine validation and certification assure absence of residual Vero cell DNA or other contaminants.
- Process of spills and waste management procedures are in place. Furthermore, exposure of the environment via faeces is discussed. Natural rotaviruses are highly persistent and are dispersed

[62] http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=2DC9509D-1

e.g. via waste water. Given the low shedding rates and the host specificity of the vaccine etc., dispersal and persistence of the attenuated vaccine are expected to be limited.

Another example is the ERA for *L. lactis* strain of Case study 2 (Chapter 5.2).

4.3.2 Health Canada

For those clinical trials that are exempt from a new substance notification, Health Canada, when reviewing the CTA, looks at the potential for harm to third parties (Ridgeway, pers. comm.). Reference is made to the ICH consideration documents for the clinical trial phase (*e.g*. on virus shedding: ICH, 2009b). Health Canada developed a standard set of instructions for patients to use to help minimise exposure of family members and others. Sponsors are asked to adapt these instructions to the particular aspects of the vector and trial. Also, each institution has their own IREB and Biosafety Committee who examines the clinical protocol to ensure it meets the institutional requirements. Issues such as waste management (unused doses, wound dressings etc.) are addressed by the IREB and Biosafety Committee and are also under local municipal or provincial controls as implemented by the institutions.

5 Case studies

In this chapter 4 cases are chosen to be studied in more detail. For each of the cases an application dossier was submitted in the Netherlands and a similar one in the USA and/or Canada. The selection comprises different vector types (adenovirus, bacterium, retrovirus and adeno-associated virus) each with their specific points of attention. Also, for the Netherlands these gene therapy clinical trials were rather new as far as the vector system is concerned.

After a short presentation of the proposed trial the ERA as conducted by the COGEM is summarised. The COGEM advices were the source of information. Likewise, items that are important for human health and the environment were collected for the US and Canadian trials. These data were retrieved from the minutes of the RAC public meetings, clinical trial databases and websites of the authorities and committees. The applicants were contacted and invited to provide information on the timelines for approvals and the workload as they experienced this.

5.1 Case study 1: Clinical study with a conditionally replicating adenoviral vector.[63]

In this phase I/II clinical trial a conditional-replicating adenoviral vector (Ad5-Delta24-RGD) was proposed to be administered to patients with a brain tumour (glioblastoma multiforme). The wild-type virus from which the vector is derived is a group C serotype 5 adenovirus. Twenty-four base pairs from the viral *E1A* gene have been removed from the genome, limiting the replication of the vector to tumour cells. The viral E1A protein normally represses the Rb function releasing E2F, followed by cell division that allows virus replication. The mutant *E1A* gene does not bind to Rb and therefore the virus cannot replicate in healthy resting cells. Tumour cells have lost Rb function which makes them to proliferate thereby allowing virus replication. Furthermore the viral coat protein is modified by addition of the sequence coding for the arginine-glycine-aspargine-4C peptide (RGD motif). The RGD motif enables the vector to bind to and enter into cells that express specific integrines on their cell membrane like many tumour cells.

The concept underlying the clinical study is that infected tumour cells die due to lytic replication of the virus. The tumour cells that die, release progeny viral particles that will infect other neighbouring tumour cells. When all tumour cells are destroyed, the replication-selective viral vector will not be able to further replicate in or release progeny viral vectors from normal non-tumour cells in the absence of a functional adenoviral E1A.

In the Dutch trial a dose varying between $1x10^7$ and $1x10^{11}$ viral particles was proposed for administration by infusion via catheters for 50-68 hours in or around the tumour. The patients are expected to stay in an isolation room with negative pressure and antechamber until 24 hours after the treatment. After the removal of the catheters the wound is sutured and covered with an adhesive. Dexamethasone is administered to prevent oedema formation.

Patients with an active adenoviral infection are excluded from the trial. Tissue samples are taken to study persistence and shedding. Measures aiming at preventing dissemination during transport of samples are in place.

[63] COGEM advies CGM/090429-04 Klinische studie met een conditioneel-replicerende adenovirale vector.
COGEM advies CGM/091021-02 Aanvullende informatie over een klinische studie met conditioneel-replicerende adenovirussen.
COGEM-advies COGEM advies CGM/110112-01 Verzoek tot wijziging vergunning fase I/II klinische studie met conditioneel-replicerende adenovirussen.
EudraCT Number: 2007-001104-21. A phase I/II trial of a conditionally replication-competent adenovirus (Delta-24-RGD) administered by convection enhanced delivery in patients with recurrent glioblastoma multiforme.
https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-001104-21/NL
JRC: B/NL/08/008 B/NL/08/009

In the USA trial (protocol #0401-624) 2 groups of research participants with a brain tumour are taking part: one group with inoperable tumours, the second group with operable tumours. The latter undergo stereotactic injection of the virus using a permanently implanted catheter in the centre of the tumour. After 14 days the tumour is removed and examined for changes.

In protocol #0401-625 the same vector is used to study the effect in ovarian and extraovarian cancer patients. The gene therapy investigational product is delivered intraperitoneally.

Study #0401-625 has ended. The other studies are ongoing.

5.1.1 ERA in the Netherlands

a) <u>Hazard identification and characterisation</u>

- Pathogenicity

  As a class 2 pathogen the wild-type adenovirus infections cause mild disease symptoms and are self-limiting. The RGB-motif enlarges the cell host range, but the vector is only replication-competent in dividing tumour cells due to a deletion in the *E1A* gene and a defective Rb-pathway in tumour cells. However, infection of more cell types would not be followed by replication in resting cells and therefore not result in disease. In healthy dividing cells replication efficiency is much lower compared to wild-type and the virus only replicates for a few hours (Heise *et al*., 2000).

- New variants

  Recombination between the GMO and wild-type hAd5 virus, may lead to two new variant forms: wild-type adenovirus with a deletion in the E1 gene (Ad5-Delta24) and wild-type adenovirus with an insertion of the RGD-motif (Ad5-RGD). The first potential new variant recombinant Ad5-Delta24 is a deletion mutant of the wild-type hAd5, without new characteristics. This deletion mutant is no longer capable of replication in cells with a functional Rb-pathway, as present in 'healthy' cells. COGEM concluded that the potential risks of this Ad5-Delta24 are negligible low, as this recombinant is highly attenuated compared with hAd5. The second potential new variant recombinant Ad5-RGD consists of the wild-type adenovirus with an extended host range. Lacking the E1A deletion, this recombinant would be able to infect and replicate in healthy cells, possibly inducing disease. No predictions could be made about its pathogenicity as no information was available.

  Recombination may happen in patients as well as in other people that may become infected with both the GM and wild-type virus at the same time. However, both the GM and wild-type virus need to be present in the same cell. Adenovirus usually infects a small number of lung cells and is usually quickly cleared. The likelihood of co-infection of a cell is therefore very low.

- Altered tropism

  Introduction of the RGD-motif make that the modified virus can now infect, amongst others, endothelium cells, fibroblasts and epithelium cells. The recombinant Ad5-RGD would show an enlarged host range in comparison with the wild-type, possibly leading to more severe disease symptoms.

- Dissemination

  The modified virus may disseminate during administration, waste handling or via shedding.

b) Exposure assessment

- Pathogenicity

  Healthcare workers are most vulnerable of coming in contact with the virus vector. Normally the immune system of healthy people will be able to clear the virus very quickly. This natural defence system will also limit the period of potential shedding.

- New variants

  Recombination may happen in patients as well as in other people that may become infected with both the GM and wild-type virus at the same time. However, both the GM and wild-type virus need to be present in the same cell. The simultaneous presence of the GM and wild-type virus in patients is low due to patient pre-screening for the presence of an active adenoviral infection. Recombination leading to replication-competent adenoviruses has not been shown *in vivo*. Potential recombination during vector production is monitored.

  Administration of dexamethasone lowers the immune response and increases infection frequency in general and therefore masks a wild-type adenovirus infection. The same is true for immune-compromised persons. The simultaneous presence of the GM and wild-type virus in patients is low due to patient pre-screening for the presence of an active adenoviral infection.

- Dissemination

  The virus is not able to pass the brain–blood vessel barrier. However, brain tumours may disrupt this barrier directly (invasiveness) or indirectly (due to surgery) as indicated in bio-distribution studies.

  Shedding may occur via wound leakage, sample taking, care taking (urine, faeces), inhalation of airborne particles (*e.g*. when sneezing), and when handling waste. But, as replication in lung cells was deemed unlikely due to the presence of a functional Rb-pathway, aerosol formation is probably low. Wound leaking is taken care of by keeping the patient in isolation until the wounds have healed.

  The Dexamethasone treatment could also result in a prolonged shedding period.

c) Risk characterisation

- Pathogenicity

  Because of safe hospital practices and appropriate measures proposed by the applicant the risk of becoming ill because of the GM virus is negligible. Even for immune-compromised persons the risk is low.

- New variants

  The occurrence of recombination was estimated to be very low and was considered in the context of “worst-case scenarios”.

  Uncertainty exists about the consequences of the administration of dexamethasone in relation to the ability of the immune system to cope with the virus and as a result thereof about (the period of) shedding.

- Dissemination

  Chance for dissemination and exposure of people different than the patients (*e.g*. hospital staff, relatives) is limited because of the biosafety management measures installed.

d) Risk management

- New variants

  The possibility for simultaneous presence of wild-type adenovirus (and potentially leading to recombination in the patient) is reduced by:

- Excluding patients with fever and/or with reduced immune response,
- Testing participants for adenoviral infections shortly before the start of the trial.

- Limiting exposure

  Trial participants reside in an isolation room equipped with an antechamber and with negative pressure during treatment. The likelihood of exposure during treatment is reduced by implementing standard hospital hygiene and the following additional safety measures:
  - Contact with the patient should be as much as possible limited, in particular with other people that may be infected with wild-type virus.
  - Caretakers and visitors have to wear protective clothing (gloves, face mask, cap, and watertight gown). The protective clothing needs to remain within the isolation room.
  - Bedclothes, protective gowns and disposables need to be separately removed and sterilised.
  - In case the patient needs to leave the isolation room for another treatment, then he has to wear a mouth mask and clean clothes. Caretakers would need to take the same precautions as in the isolation room. In a similar way, conditions were formulated in case of emergency evacuations.

  After removing the catheters, the wounds are to be sutured and a watertight wound dressing preventing leakage is to be applied. If no wound leakage patients are allowed to leave this isolation chamber. Initially this period was set at 24h after removing catheters (CGM/090429-04). In the advice on an amendment (CGM/110112-01) the period of strict isolation was shortened to 3 consecutive inspections without wound leakage (*i.e.* after 6h at the earliest). The measures to protect healthcare workers and visitors have to be maintained for as long as shedding was detected. This means that patients stay in a single room with droplet isolation precautions until after 3 consecutive tests (*i.e.* 3 days) shedding in urine, faeces and mouth swabs is negative.

  If a patient decides to leave before the end of the trial, the patient needs to take care that the chance for dissemination is limited. Recognizing that it is difficult to impose measures on a patient that has left the trial, COGEM nevertheless recommended to make sure that such patients are professionally taken care of maintaining containment measures (*e.g.* single room). Material exposed to wound fluids and samples have to be packaged adequately and transferred to the hospital for analysis and/or destruction. Based on information provided by the applicant, COGEM accepted that it is very unlikely that patients would prematurely leave the trial.

e) <u>Overall risk evaluation and conclusion</u>

The GMO, the adenoviral vector Ad5-Delta24-RGD, is assessed to be less pathogenic than the wild-type adenovirus serotype 5 strain.

In the worst-case, recombination with wild-type adenovirus would result in a virus with unknown pathogenicity. This uncertainty triggers the implementation of further measures to make sure that no opportunities for recombination, either directly in the patient or after dissemination to other persons, are created.

5.1.2 ERA in the USA

The RAC meeting of March 2004 discussed the Human Gene Transfer Protocol #0401-624: A Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD) for Recurrent Malignant Gliomas, and the Human Gene Transfer Protocol #0401-625: A Phase I Study of a Tropism-Modified Conditionally Replicative Adenoviral Vector (Ad5-Delta-24-RGD) for Intraperitoneal Delivery in Ovarian and Extraovarian Cancer Patients. The main reason to publicly discuss these trials was the RGD motif leading to altered tropism that was used for the first time.

Investigators at the University of Alabama, Birmingham (protocol #0401-625), and the M. D. Anderson Cancer Center (protocol #0401-624) jointly developed the vector.

Questions that were asked relate to the attenuation of the virus, whether the RGD modification increased transduction of non-tumour cells, and biodistribution.

As the virus is attenuated in Rb+ cells, concern was expressed about whether other mutations could reduce that attenuation independent of the 24-base-pair mutation, since reduction in attenuation has occurred in prior experiments with replication-impaired viruses. Therefore, an assay to determine whether the virus has recombined or rearranged was promised.

Biodistribution studies on lab animals were not sufficient at the time of the discussion. The investigators would conduct 3 biodistribution studies using a nonhuman primate model, cotton rats, and nude mice. The FDA requires completion of these studies before they will grant an investigational new drug (IND) application. Biodistribution studies would focus on the liver, as the virus would have a tropism for the liver, if it would end up in the bloodstream.

It was also suggested that each potential research participant be tested for HIV and active hepatitis infection and infected individuals should be excluded from the trial.

The principal investigators were invited to submit these results to OBA for presentation to the RAC.

Risk management involved birth control.

In the June 2008 meeting the additional studies were briefly discussed.

On the IBC website of the University of Texas M.D. Anderson Cancer Center, Houston, Texas, study exclusion criteria that are mentioned are amongst others (from the study summary[64]):

> *"Female who is pregnant and/or nursing. Because of the potential risk of a recombinant virus containing a gene involved in cellular growth regulation and differentiation which could potentially affect a developing fetus or growing infant, females who are pregnant, at risk of pregnancy, or breast feeding a baby during the study period are excluded"*

Furthermore IBC looks at safe handling and administration of the gene therapy product, waste treatment etc.

5.1.3 Timelines

For the Netherlands:

The applicants consulted the GMO Office in March 2006. The application was submitted to the GMO office only in September 2008, due to extra legal requirements regarding clinical trials (Wet Medisch-wetenschappelijk Onderzoek met Mensen, effective as of March 1, 2006, implementing Directive 2001/20/EC).

The procedure for IenM was stopped 3 times during resp. 11, 4 and 3 weeks. Due to a request by the Ministry applicants provided extra information requiring COGEM's opinion a second time. This event also prolonged the procedure with 5 weeks. The permit finally arrived in January 2010. All dossiers (IenM, VWS and CCMO) started the procedure at the same time. CCMO approved on January 28, 2009. This decision, only valid for 1 year, expired before the trial could start.

For the USA:

The dossier was submitted to OBA: January 2004

RAC public discussion March 9, 2004; notice of additional information mentioned in the meeting of June 17, 2008.

[64] http://utm-ext01a.mdacc.tmc.edu/dept/prot/clinicaltrialswp.nsf/Index/ID01-310

Study approved by the IBC of the M. D. Anderson Cancer Center on May 11, 2005, no evaluation records available. The study was activated December 4, 2008.

5.1.4 Workload

For the Netherlands:

As the actual trial protocol was taken care of by an external contract research organisation, the environmental dossier was compiled by the principal investigator. Therefore, it was entirely felt as extra workload compared to a non-GMO dossier.

For the USA:

IBCs require an extra form to be filled out when recombinant DNA is involved.

5.1.5 Risk management

Due to the fact that a worst-case scenario was considered regarding the vector recombination potential, the risk management measures required in the Netherlands were conceived by the applicant as problematic and out of proportion. These measures not only affect the healthcare professionals but above all the research participants. In comparison, in USA working with the same vector almost no containment measures were taken.

5.2 Case study 2: Clinical study with a *Lactococcus lactis* strain expressing recombinant human interleukine-10 (hIL-10).[65]

The bacterium *Lactococcus lactis* strain AG011 has been genetically modified to produce the therapeutic protein human interleukin-10 (hIL-10). This protein is expected to reduce the symptoms (such as pain and bloody diarrhoea) of patients suffering from inflammatory bowel disease. Local expression of hIL-10 should limit and terminate inflammatory responses and regulate the growth of several immune cells.

Preceding studies had shown that this investigational medicinal product was safe and does not survive for a long time outside the human body. An initial clinical study with humans demonstrated safety and efficacy. In that study patients were administered 4 capsules with 10^{12} cfu a day for 1 week at the hospital.

The Dutch study is focussing on a second study in which patients administer themselves the freeze-dried powder orally and/or rectally at home. The trial aims at assessing safety, tolerability and efficacy of the drug. During 28 days, patients visit the hospital once a week for sample taking (blood, urine, faeces, and bowel biopsies), returning packages of the investigational product from the previous period as well as receiving the necessaries quantities for the subsequent week.

Also, in the USA study (Protocol #0804-917) and the Canadian study (NCT00729872) AG011 is administered both orally (capsules) and rectally (enema); participants receive either one of the three doses of AG011 or placebo during 28 days. Similar samples as in the Dutch trial are taken to evaluate safety, tolerability, pharmacodynamics, and efficacy parameters. The study is to enrol about 60 participants with moderate ulcerative colitis.

The US study is still ongoing. The Canadian and Dutch studies are completed.

5.2.1 ERA in the Netherlands

a) <u>Hazard identification and characterisation</u>

- Pathogenicity

  The parent strain is not pathogenic, widely used in the dairy industry and is not capable of colonizing the bowel. The presence of the hIL-10 gene would not change that.

- Dissemination

  The GMO may be dispersed in the environment, either via unused product or via shedding together with faeces and released into the sewage system. This could take on average 3 days.

  AG011 has lost all natural plasmids and the gene for thymine production was replaced by the gene coding for hIL-10, making it thymine/thymidine-dependant. AG011 is therefore highly biologically contained, which was confirmed in previous studies.

[65] COGEM-advies CGM/020823-02 Advies Klinische toepassing van *Lactococcus lactis* met daarin een recombinant humaan interleukine-10 (hIL-10) gen bij patiënten met de ziekte van Crohn.
COGEM-advies COGEM-advies CGM/080821-01 Fase 2a klinische studie met *L. lactis* stam AG011 tegen matige ulceratieve colitis.
EudraCT Number: 2008-000967-40. A Phase 2a Randomised, Placebo-Controlled, Double-Blind, Multi-Center Dose Escalation Study, to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of AG011, in Subjects with Moderately Active Ulcerative Colitis.
https://www.clinicaltrialsregister.eu/ctr-search/trial/2008-000967-40/NL
JRC: B/NL/08/004

b) Exposure assessment

- Dissemination

  Healthcare workers and family members may be exposed via empty product packaging and shedding. The environment will be exposed to the GMO via the sewage system. The duration of shedding is expected to be short.

  The freeze-dried investigational product is sensitive to heat, moist, UV etc. and will degrade quickly. In case of spillage decontamination with standard detergent or bleach will kill the bacteria.

c) Risk characterisation

Initially the effect of the GMO on healthy people was not clear. In the second trial this was no longer considered an issue.

Due to the biological containment and the limited exposure time the GMO was not expected to spread into the environment.

d) Risk management

Normal hygiene was deemed sufficient. In case of a spill cleaning with soap or bleach were indicated as adequate.

No further requirements were deemed necessary.

e) Overall risk evaluation and conclusion

The overall risk of the clinical trial with hIL-10-producing *L. lactis* for human health and the environment was deemed negligibly low.

5.2.2 ERA in the USA

The Human Gene Transfer Protocol #0804-917: A Phase IIa Randomized, Placebo-Controlled, Double-Blind, Multicenter, Dose-Escalation Study to Evaluate the Safety, Tolerability, Pharmacodynamics, and Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis, was publicly reviewed at the June 2008 RAC meeting. Key issues for public review included the use of a novel vector, the transgene, and the disease indication.

In the discussions the only question related to the environment was whether any gene transfer to other flora in the gut was observed, which was answered negatively.

The presenters of the study mentioned that AG011 is easily eliminated from laboratory animals after infection and that *L. lactis* is a non-colonizing, non-pathogenic, gram-positive bacterium that cannot invade cells or tissues and does not cause infection. Its residence time in the intestine is determined by intestinal transit as it moves along with the faecal stream. AG011 is susceptible to a number of antibiotics directed against gram-positive organisms.

At the time of the discussions no studies or data on the shedding of *L. lactis* in ulcerative colitis patients were available. It was expected that the bacterium travels through the intestines of patients at the same speed as in healthy individuals.

The study is performed at the University of North Carolina at Chapel Hill; Chapel Hill, North Carolina[66]. The IBC looks at safe handling and administration of the gene therapy product, waste treatment etc.

[66] http://ehs.unc.edu/committees/ibc.shtml)

5.2.3 ERA in Canada

A Phase 2a Study to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of AG011 in Ulcerative Colitis, NCT00729872[67], was conducted at 8 Canadian sites.

1. Health Canada:

Points of attention relative to human health (from the study description by ClinicalTrials.gov):

Woman of child bearing potential must use a hormonal (oral, implantable or injectable) or barrier method of birth control throughout the study.

2. Environment Canada:

A New Substances Notification was submitted as the clinical trial participants were to self-administer the investigational drug at home. Release into the environment was almost inevitable. Participants would be recruited from 8 hospital sites in British Columbia, Ontario and Quebec leading to a Schedule 1 notification (manufacture or import of micro-organisms for introduction anywhere in Canada). Health Canada conducted the ERA.

The Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations (Organisms) of the Canadian Environmental Protection Act, 1999 EAU-439: *Lactococcus lactis* subsp. *cremoris* strain sAGX0037[68] includes the following:

a) Hazard identification and characterisation

- Genetic stability

  Stability was demonstrated over 55 generations.

- Pathogenicity/toxicity

  Scientific literature shows that there is little evidence of any pathogenic potential of *L. lactis* and the hIL-10 protein in aquatic plants, fish or marine mammals. No toxic effects were seen in pre-clinical trials in mice and monkeys.

  The parent strain has a history of safe use in the food industry. *L. lactis* is not allergenic.

  hIL-10 was proven save in previous clinical studies (patients with Crohn's disease and healthy volunteers).

  L. lactis sAGX0037 is sensitive to a wide range of antibiotics.

- Gene transfer

  The modifications to the strain prevent gene transfer to the environment. And *L. lactis* is not known to competently take up exogenous DNA from the environment.

- Persistence

  The strain is dependent on thymine or thymidine supplementation as thymine starvation leads to rapid cell death. There is no selective advantage in the environment.

Altogether, its potential hazard to human health and the environment is considered low.

b) Exposure assessment

- Dissemination

  Healthcare professionals will adhere to containment procedures to minimise worker, bystander and wildlife exposure. All waste generated during the clinical trial will be discarded in approved biological waste containers and disposed according to provincial regulations.

[67] http://clinicaltrials.gov/ct2/show/NCT00729872?term=AG011&rank=1
[68] http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=10CE87F5-1

L. lactis release will occur mainly via faecal shedding. The sanitary sewer system will inactivate and remove the modified strain.

Exposure is therefore considered to be significantly low.

c) Risk characterisation

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that *L. lactis* subsp. *cremoris* strain does not cause harm to the Canadian environment or human health

d) Overall risk evaluation and conclusion

Given the above conclusion the import of *L. lactis* subsp. *cremoris* strain sAGX0037 for introduction anywhere in Canada may proceed.

The IREBs and Biosafety Committees of the clinical trial sites would examine the clinical protocol for compliance with local regulations (*e.g*. waste management).

5.2.4 Timelines

For the Netherlands:

After a pre-submission meeting the dossier was submitted May 15, 2008. The procedure started May 19, 2008. The COGEM advised on August 21, 2008, and the Ministry issued the permit on July 1, 2009. This was followed by an appeal period lasting from 14-07-2009 to 25-08-2009. The clock was stopped for 2 weeks for additional information.

Procedure time surpassed the legal 120 days due to administrative complications not related to the ERA.

For the USA:

The dossier was submitted to OBA on April 21, 2008 and was discussed on the RAC public meeting of June 17, 2008. The Recommendation letter followed on July 2, 2008.

IBC requests to submit the dossier 1 month in advance of a meeting.

For Canada:

Also in Canada a pre-submission meeting was scheduled for the New Substance Notification. The evaluation of the final dossier started June 09, 2008 and was completed October 06, 2008. This fits with the prescribed 120-days assessment period for Schedule 1 submissions and includes question-and-answer rounds.

5.2.5 Workload

For the Netherlands:

Total ERA dossier preparation time must have been 2-3 months.

For the USA:

The RAC procedure took 3.5 man-months to prepare and present. This was possible because the IND procedure started earlier and documents from that dossier were available.

Interaction with IBC took 1-2 weeks.

For Canada:

The dossier for the NSNR required 4.5-5.5 months to prepare (time to clarify and respond to questions not included). Especially the literature study used much time (key words provided by Health Canada; review over last 10 years).

5.3 Case study 3: Clinical trial with retroviral transduced T cells[69]

The retroviral vector MP71 is derived from a mutant of the *Molony murine leukemia virus* (MoMLV). The vector contains a gene for a melanoma-specific T-cell receptor. The study aims to investigate whether autologous T-cells transduced *ex vivo* with this virus may induce melanoma-specific T-cell immunity in participants with stage IV melanoma.

Because the *gag*, *pol* and *env* genes are lacking the vector is replication-deficient. The Long Terminal Repeat (LTR) sequences for replication and a leader sequence for high expression are taken from other murine viruses. The modified vector is not self-inactivating (mobilisation from host genome is still possible).

The working of the gene therapeutic is based on selective killing of the melanoma cells by the immune system. The immune system is triggered by the presence of T-cells presenting receptors that are specific for the patient's melanoma cells.

The study will enrol 20-25 subjects. The transduced T-cells are administered to the patient via intravenous infusion. This study just recently started.

In the USA several similar trials are being of have been conducted.

5.3.1 ERA in the Netherlands

a) <u>Hazard identification and characterisation</u>

- Pathogenicity

  MoMLV is an ecotropic virus causing T-cell lymphoma in new-born rodents.

  None of the viruses used to construct the virus vector are pathogenic to humans.

  Recombined virus may cause disease.

  Free vector particles that are replication-incompetent may infect lab workers, albeit only once resulting in a localised infection. Insertion in the genome, however, may lead to tumours depending on the integration site.

  Infection by transduced T-cells may cause mild disease symptoms that are easily treated.

- Dissemination

  Appearance and dissemination of replication-competent retrovirus or recombined virus and dissemination of transduced T-cells in the environment via shedding may cause disease in persons other than the participant.

b) <u>Exposure assessment</u>

- Pathogenicity

  Replication-competent retrovirus may be created at the vector production stage. For this to happen the construct has to recombine twice with helper plasmids. Vector batches are checked 3 times at different stages during production.

  Participants with retrovirus infections are excluded from the trial to prevent recombination with complementing sequences, although the GMO has no homology with human retroviruses.

[69] COGEM advies CGM/110831-01 Klinische studie met retroviraal getransduceerde humane T-lymfocyten
COGEM advies CGM/111012-03 Aanvullende informatie over de klinische studie met retroviraal getransduceerde humane T-lymfocyten
EudraCT number 2011-002941-36. Multicenter phase 1/2a study using T-cell receptor gene therapy in metastatic melanoma https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-002941-36/NL
JRC: B/NL/11/001

- Dissemination

  Lymphocytes are transduced in a class II biosafety cabinet in a BSL-2 lab with negative pressure (air circulates separately from other rooms).

  The presence of free vector particles is reduced depending on the duration of transduced cells before use and the number of wash steps. T-cells are grown for 8 days and washed 3 times prior to intravenous infusion of the participants.

  Participants reside in single bed hospital rooms at administration. Personnel may be accidently infected by T-cells at administration or sample taking. The immune system will clear the cells very quickly. It is very unlikely that a person unrelated to the participant has the same haplotype making it possible that disease symptoms would appear.

  The modified T-cells may reside several months in the participants. Outside the body (bleeding from an injury) they do not survive.

c) Risk characterisation

The risk for the creation of replication-competent or recombinant virus is negligibly small. However, test data need to be provided including limits of detection.

Free particles are reduced 50 times instead of the required 100.

d) Risk management

To reach the safe 100 reduction factor for free vector particles small modifications in the handling of cells are advised.

e) Overall risk evaluation and conclusion

At first instance the COGEM could not advise positively due to missing data on replication-competent virus tests and an inadequate reduction factor for free virus particles. In a later advice after receiving test data and a revised protocol COGEM concluded that the trial was safe to conduct as far as safety for human health and the environment is concerned.

5.3.2 ERA in the USA

Studies with similar gene-vector-disease therapies are (all performed at the NIH, Bethesda, Maryland):

- 0308-599 (Closed) Gene Therapy/Cancer/Melanoma/Immunotherapy/In Vitro/Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta Chain cDNAs/Intravenous Infusion.

  Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; Treatment of Patients with Metastatic Melanoma by Lymphodepleting Conditioning Followed by Infusion of TCR-Gene Engineered Lymphocytes and Subsequent Peptide Immunization.

  NIH/OBA Receipt Date: August 5, 2003. Not Selected for RAC Public Review: August 25, 2003.

- 0701-830 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta cDNAs/ Intravenous Infusion

  Rosenberg, Steven A.; National Institutes of Health; Bethesda, Maryland; Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-gp100:154-162 TCR-Gene Engineered Lymphocytes.

NIH/OBA Receipt Date: January 16, 2007. Not Selected for RAC Public Review: February 6, 2007.

- 0703-840 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta cDNAs (Anti-MART-1 F5 TCR)/Intravenous Infusion

  Rosenberg, Seven A.; National Institutes of Health; Bethesda, Maryland; Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 F5 TCR-Gene Engineered Lymphocytes.

  NIH/OBA Receipt Date: March 28, 2007. Not Selected for RAC Public Review: April 18, 2007.

- 0710-882 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta Chain cDNAs/Canarypox Virus/B7.1, ICAM-1, and LFA-3 (TRICOM) cDNAs/Intravenous Infusion

  Rosenberg, Steven A.; National Institutes of Health; Bethesda, Maryland; Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 F5 TCR-Gene Engineered Lymphocytes and ALVAC Virus Immunization.

  NIH/OBA Receipt Date: October 24, 2007. Not Selected for RAC Public Review: January 2, 2008.

- 0710-883 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta Chain cDNAs/Canarypox Virus/B7.1, ICAM-1, and LFA-3 (TRICOM) cDNAs/Intravenous Infusion

  Rosenberg, Steven A.; National Institutes of Health; Bethesda, Maryland; Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-gp100:154-162 TCR-Gene Engineered Lymphocytes and ALVAC Virus Immunization.

  NIH/OBA Receipt Date: 10-24-07. Not Selected for RAC Public Review: 1-02-08

- 0712-885 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta Chain cDNAs/Intravenous Infusion

  Rosenberg, Steven A.; National Institutes of Health; Bethesda, Maryland; Transfer of Autologous T Cells Transduced with the Anti-Mart-1 F5 T Cell Receptor in High Risk Melanoma.

  NIH/OBA Receipt Date: 12-02-07. Not Selected for RAC Public Review: 1-07-08

- 0809-939 (Open) Gene Therapy/Phase II/Cancer/Melanoma/Immunotherapy/In Vitro/ Autologous T Lymphocytes/Retrovirus/T Cell Receptor alpha and beta cDNAs Chains/ Intravenous Infusion

  Rosenberg, Steven A; National Institutes of Health; Bethesda, Maryland; Phase II Study of Metastatic Melanoma Using a Chemoradiation Lymphodepleting Conditioning Regimen Followed by Infusion of Anti-Mart-1 and Anti-gp100 TCR-Gene Engineered Lymphocytes and Peptide Vaccines

  NIH/OBA Receipt Date: 9-05-08. Not Selected for RAC Public Review: 9-26-08

No data are available on environmental issues.

The IBC at the National Cancer Institute would check procedures for safe handling and administration of the gene therapy product, waste treatment etc.

The NIH IBC uses a registration document for recombinant DNA work in general where details on the specifics of the gene-delivery method, use of viral or other vectors etc. are provided. With these data

the IBC determines if there is any safety concern associated with the particular gene-delivery agent. In addition, the clinical trial is reviewed by other committees and the clinical protocol is presented to the IBC and reviewed by the hospital's clinicians and then opened up for general discussions.

For the IBC the main point of concern is to ensure that a safe product is being administered to patients, with as little risk of transmitting an infectious agent as possible. In the case of the use of T cells modified by gamma-retroviral vectors, the risk is minimal. First, most vectors have extensive *in vitro* data to support both their efficacy and safety. Second, the vector is non-replicative and when transduced into mature lymphocytes, is considered a BSL1 reagent. There is little to no risk to the patient or of this agent being shed and spreading to others.

The NIH biosafety guidelines that determine the containment procedures including waste disposal are followed. For agents where there is risk of shedding, the clinical centre has special isolation wards for those trials.

5.3.3 Timelines

For the Netherlands:

The application was sent on April 21, 2011 and the permit was issued on October 31, 2011. The procedure was stopped several times allowing the applicant to provide additional information (4 weeks, 4 weeks and 10 days).

A consultation prior to submission was held with CCMO (not related to the ERA).

Although the procedure for the Ministry of Environment took quite some time, parallel procedures took even longer.

For the USA:

All study applications were reviewed in 3 weeks (15 working days) after submission to OBA.

In total an application can take 3-6 months to complete the review process including the filing of the IND with the FDA.

5.3.4 Workload

For the Netherlands:

More effort was put in the ERA dossier (was felt to be more difficult) compared to the CCMO part of the dossier. Due to the fact the some overlap exists in data requirements between the CCMO dossier and the ERA dossier it is hard to tell the exact workload. The time ratio CCMO:ERA would be around 60:40.

For the USA:

The information that applicants have to provide for a gene therapy clinical trial is different from one with a 'classical' investigational medicine. For a gene therapy trial the applicant has to register with the IBC the nature of the genetic modifications and gene delivery methods in order for the IBC to assess the biosafety.

5.4 Case study 4: Gene therapy for lipoprotein lipase (LPL) deficient patients with an adeno-associated viral vector coding for the LPL-protein (AMT-010)[70]

An adeno-associated viral (AAV) vector AMT-010 contains a gene encoding the lipoprotein lipase. Administration of AMT-010 aims at substituting the defective lipoprotein lipase in patients. Lipoprotein lipase deficiency inhibits the hydrolysis of triglycerides in blood. This may result in inflammation of the pancreas and arteriosclerosis.

The corrective gene is a human lipoprotein lipase gene driven by the cytomegalovirus promoter. This promoter in constitutively expressing the enzyme in all tissues. The vector backbone only contains the inverted terminal repeats for packaging. Other AAV genes are deleted. These functions are complemented using helper plasmids and cells at AMT-010 production. Tests confirm absence of replication-competent AAV, herpes virus or adenovirus.

The vector is administered intramuscularly. Fifty participants at maximum would be injected several times: first in the thigh, later in the lower limb and arms. The vector may infect cells and integrate in the genome, though at a low frequency.

In the USA in a similar study (Protocol #1201-1144) using an AAV vector to deliver a lipoprotein receptor gene the target is to recruit 12-18 trial participants. Three doses are proposed.

In Canada study NCT00891306 phase II and III was designed to test safety and efficacy of AMT-011. Five patients were enrolled. In study NCT01109498 phase II and III escalating doses of AMT-011 will be injected intramuscularly in 14 participants.

The trial in the Netherlands finished. In the USA the hospital is not yet recruiting. Canadian study NCT00891306 is completed, study NCT01109498 is ongoing.

5.4.1 ERA in the Netherlands

a) <u>Hazard identification and characterisation</u>

- Pathogenicity

  AAV infections are known worldwide but do not cause disease. The virus can only replicate using herpes virus or adenovirus as helper viruses.

  Overexpression of the lipoprotein lipase gene may lead to overweight in animal models (CGM/050531-01).

- Recombination

  The AMT-010 vector may recombine with wild-type AAV or may regain all its functions during vector production. This replication-competent recombinant virus may stay latent when stably integrated in the host's genome, but will replicate when the host cell is infected with herpes or adenovirus.

- Dissemination

  AAV is transferred via contact and aerosols.

  Via shedding other people might become infected.

[70] COGEM advies: CGM/050531-01 Gentherapie van lipoproteïne lipase (LPL) deficiënte patiënten met een adeno-associated virale vector coderend voor het LPL-eiwit (AMT-010)
JRC: B/NL/05/001

b) Exposure assessment

- Pathogenicity

  Replication is only possible when the deleted functions are complemented by wild-type AAV and when helper virus is present. All need to be present in the same cell.

  The vector administration dose is 10 to 100 times lower than in animal experiments. Caretakers that would accidently come in contact would be exposed to a much lower dose.

- Recombination

  The probability for recombination with wild-type is estimated to be low due to the limited sequence homology (inverted terminal repeats). Recombination would not result in AAV with the lipoprotein lipase gene, because this genome would be too large to be packed in the protein coat.

  Vector batches are checked for replication-competent AAV, herpes virus and adenovirus.

- Dissemination

  Animal studies show that the GMO stays in the muscles. Very low amounts are encountered in the blood. In a similar study no vector was found in serum, saliva or urine 7 days after treatment. Experiments with cats show presence in semen.

c) Risk characterisation

- Recombination

  Recombination with wild-type AAV, if it would happen, would most probably not lead to AAV carrying the lipoprotein lipase gene.

Given the very low frequency of replication-competent AAV production (below detection limit) and the absence of helper viruses, the risk for replicating recombinant AAV for human health and the environment is negligible.

- Dissemination

  It has been shown in animal studies that AAV-based vectors may be infectious only when excreted in serum, not in urine or saliva (Favre *et al*., 2001).

d) Risk management

Participants showing a herpes or adenovirus infection are excluded from the trial to prevent replication and dissemination.

Male participants are required to use barrier contraception until 3 consecutive tests no longer detect vector DNA, starting 75 days (= 1 cycle of male gametogenesis) after administration of the GMO.

Participants should not become donor for tissue or cell transplantation.

e) Overall risk evaluation and conclusion

COGEM concluded that with the extra management measures in mind the overall risk is negligibly small. Although the formation of replication-competent AAV cannot be excluded, the risks due to its presence are negligible.

5.4.2 ERA in the USA

The RAC meeting of March 7, 2012 discussed Study Protocol #1201-1144 titled: AAV8-mediated Low Density Lipoprotein Receptor Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia.

The reasons for public review are the novelty of the combination of vector, transgene, and disease application and the fact that for the research participant population with a chronic disease other therapeutic options exist. Finally, the possible future use of this approach in a paediatric population deserves further discussion.

No questions relative the human health or the environment were raised.

The Institutional Research and Safety Committee[71] of the University of Kansas Medical Center will have to review procedures for safe handling and administration of the gene therapy product, waste treatment etc. The Committee works in close collaboration with the Human Subjects Committee, the Institutional Animal Care and Use Committee and Environment, Health and Safety Office to ensure ongoing monitoring of research that can pose a significant risk to personnel and/or the community at large.

5.4.3 ERA in Canada

An Open-label Study to Assess the Efficacy and Safety of Alipogene Tiparvovec (AMT-011), Human LPL [S447X], Expressed by an Adeno-Associated Viral Vector After Intramuscular Administration in LPL-deficient Adult Subjects [72] (NCT00891306).

This study was started February 2009 and completed April 2011.

A Study to Determine the Safety and Efficacy in Lipoprotein Lipase-Deficient Subjects After Intramuscular Administration of AMT-011, an Adeno-Associated Viral Vector Expressing Human Lipoprotein Lipase S447X[73] (NCT01109498).

This study was started August 2007 and is ongoing.

Both trials were evaluating AMT-011 (Glybera) expressing the same variant of lipoprotein lipase gene as AMT-010.

Relevant data are obtained from the study description by ClinicalTrials.gov:

- Women of child bearing potential or with a positive pregnancy test or breast feeding are excluded. Women and their partner have to use barrier contraception 2 weeks before starting immunosuppressive therapy.
- Man have to use barrier birth control and their partner using appropriate contraception until three consecutive semen samples, taken at least 75 days after administration, are negative for AMT-011 vector DNA.
- Also, persons with active infectious disease of any nature, including clinically active viral infections are excluded.
- Shedding of vector will be monitored.

The gene therapy product was exempt from NSNR.

[71] http://www2.kumc.edu/researchcompliance/irsc.htm
[72] http://clinicaltrials.gov/ct2/show/study/NCT00891306?term=%22gene+therapy%22+OR+%22gene+transfer%22+OR+%22virus+delivery%22&cntry1=NA%3ACA&rank=3
[73] http://clinicaltrials.gov/ct2/show/study/NCT01109498?term=%22gene+therapy%22+OR+%22gene+transfer%22+OR+%22virus+delivery%22&cntry1=NA%3ACA&rank=4

5.4.4 Timelines

For the Netherlands:

The procedure was started on January 26, 2005 and the permit was issued on July 4, 2005. Other procedures were approximately as long.

The GMO Office was consulted several times prior to submission (first contact September 2004). The application dossier benefitted from extensive exchange of information during this period. Items were biosafety procedures for storage, transfer and inactivation of waste, timing of participant discharge relative to sample taking/analysis.

For the USA:

OBA received the application January 10, 2012. The protocol was discussed in a public RAC meeting March 7, 2012.

For Canada:

In Canada, the complete NCT01109498 CTA dossier, which includes a risk assessment was submitted in May 2007 and approved in June 2007. The study started in August 2007.

5.4.5 Workload

For the Netherlands:

Between the procedures a lot of overlap existed in the required information (product characterisation, administration procedures, and waste treatment).

For the USA:

For the of the Institutional Research Safety Committee of the University of Kansas Medical Center a Risk Assessment for Research Hazards form needs to be filled out, in which all types of potential hazards are collected and assessed. Also a Gene Therapy Registration Document is required to inform the responsible committee.

For Canada:

For Canada also a permit application for “Import of Pathogens and Drugs” needed to be filled out (2 pages) and submitted.

6 Comparison between North America and the Netherlands

6.1 Legislation

Gene therapy clinical trials on both sides of the Atlantic fall under the scope of existing regulatory systems that govern human clinical trials or biomedical research. These regulations in the first place aim at the safety of the human subject in the studies.

For the GMO aspects the approaches are very different:

- In the EU specific **legislation** is developed to consider potential environmental effects of GMOs, including those related to GMOs deployed in gene therapy. The product-based approach is most apparent in Canada where no GMO specific legislation exists. In the USA FDA considers gene therapy not fundamentally different from other types of medical treatment and regulates it as biologic products. The NIH has developed guidelines for research activities with recombinant DNA. While NIH has jurisdiction over gene therapy research limited to those institutions and researchers that receive federal funding, also private research in general voluntary follows these regulations.
- Regarding the **environmental impact assessment** of gene therapy studies, again differences are apparent. In the EU, a specific ERA is required as described in the GMO legislation. USA and Canada make use of their environmental legislation. However, only in specific cases a full assessment is necessary for clinical trials. Most of the trials, being exempted from in-depth review, may proceed with a limited amount of data submitted.
- There is a difference between the EU, and the Netherlands in particular, and North America in providing **definitions** for gene therapy, gene therapy products, etc. The definition in Commission Directive 2009/120/EC is rather broad and includes the latest technologies. Neither in the USA, nor in Canada gene therapy is defined in legislation. However, NIH guidelines do describe gene therapy and proposals have been put forward to delineate more clearly the characteristics of a gene therapy product (to capture synthetic biology research).

  It is important to stress that the novelty of the proposed study (including the perceived risks amongst others) is the determining factor and not the technique. The discussions within OBA/RAC illustrate the way of thinking (RAC minutes, 2012[74]). For example it is argued that oligonucleotides that have a short half-life, have more predictable pharmacokinetics. When they lack the ability to integrate into the genome, or to replicate, even inadvertently replicate, or to code for a protein, they resemble small molecule drugs rather than vector-mediated gene transfer. The risk is not comparable with recombinant DNA constructs (provided that *e.g*. no viral vectors are used for delivery) and similar to other therapeutics.

  In the EU all recombinant nucleotides regardless of their size or characteristics are within the definition of a gene therapy medicinal product. Only vaccines against infectious diseases, as usually administered to healthy people, are excluded.

  In Canada gene therapy clinical trials are assessed the same way as ‘conventional’ clinical trials. A gene therapy product fits within the existing definitions of a schedule D drug (see 2.4.1). Therefore, gene therapy products are regulated like any other biological drug; there are no specific regulations or additional steps in the review process. This is true also for investigational products using GMOs. There is no Canadian equivalent of the US NIH Recombinant DNA Advisory Committee (*i.e.* RAC) for the centralised ethical review of gene therapy protocols.

[74] http://oba.od.nih.gov/oba/RAC/meetings/jun2010/RAC_Minutes_06-10.pdf

In the EU, vaccination with viruses against infectious diseases is not considered gene therapy. In the USA likewise vaccination trials are exempt from RAC review, but still need IBC review as is the case with 'conventional' clinical trials.

In the USA it is also reflected in the way protocols are selected for public review. Although primarily patient safety, ethics, etc. are evaluated, most of the selected protocols are about a new vector/new gene delivery system or new clinical application. Once understood RAC review may pass without public discussions. Examples are the many studies with retrovirus transduced autologous T-cells to induce melanoma-specific T-cell immunity (see Case study 3, Chapter 5.3).

6.2 Information requirements

The basic information describing the gene therapy product and route of administration is required in all three countries. This information serves both the protocol evaluation with respect to safety, efficacy and scientific basis, and the ERA.

In the **Netherlands** gene therapy clinical trials are evaluated as DR activities. For all cases extra information is required specifically addressing environmental questions as outlined in Directive 2001/18 and Commission Decision 2002/623/EC.

In the **USA** for trials that have to register at OBA (NIH) the extra information is limited to the questions in Appendix M-II-B-4 of the NIH guidelines in most cases. Issues such as presence of replication-competent virus particles and other adventitious agents are addressed in the IND dossier (FDA). Again, these data are in the first place required to assess safety for the trial participant, but obviously also relate to third party's safety. Only in exceptional cases a detailed dossier is necessary to assess the environmental risks according to EPA.
Usually a full EA is only required when the application concerns a marketing approval (NDA/BLA). Even then so-called 'exclusions' are possible. Postponing the assessment has the advantage that early in the development, when the clinical concept still has to be proven, no expensive studies are needed. The necessary information may be collected while conducting trials as they advance. The potential lower level of knowledge of the gene therapy product is compensated by standard and possible extra clinical trial safety instructions.

Also in **Canada** products in clinical development are largely exempted from an ERA. Environmental regulations are usually applied only when the product is proposed for a marketing application. The underlying reason is, similar to the situation in the USA, the low potential for dispersal into the environment. In the confinement of a hospital room good clinical practices are adhered to and waste is adequately managed minimising contact with healthcare workers or release into the environment. Therefore, in most cases no extra information/dossier has to be compiled and no extra burden is put on the applicant.

However, although there is no specific regulation for gene therapy in Canada, in the evaluations of the protocols the route of administration, the replication competence of the vector, its potential pathogenicity, its ability to integrate into the host cell genome, the administered dose and expressed product dose are taken into account (Ridgeway, 2009). The same is true for the USA.

The *Lactococcus* case is clearly an exception as the trial participant was intended to be treated at home, which makes it almost inevitable that material is released into the environment (see Case study 2, Chapter 5.2). Several hospitals were involved in this trial making it necessary to go for a schedule 1 NSN (manufacture or import for introduction anywhere in Canada). The questions to be addressed in a NSN are similar to the environmental questions in Directive 2001/18 and Commission Decision

2002/623/EC. However, the required information may be extensive depending on the intended use, and whether the product is imported versus manufactured in Canada. A wide-ranging literature review may be required.

In summary the type of information related to ERA as performed in the Netherlands can be seen as partly overlapping, partly as extra to the information normally required for a clinical trial. In the USA and Canada this is usually not the case. Only in specific cases an extensive dossier on environmental risks needs to be compiled.

6.3 Points of attention

In the **Netherlands** the elements that are important are already described in the EU DR legislation. From the COGEM advices issues that are often raised are:

- molecular characterisation;
- the ability of the vector to recombine or revert to a pathogenic organisms (*e.g*. replication-competent virus);
- purity of the vector batch;
- integration potential, altered tropism;
- horizontal gene transfer via *e.g.* leakage at administration, shedding of the vector;
- immunogenicity;
- stability;
- dissemination potential in the environment.

In the **USA**, the NIH guidelines for protocol submission (Appendix M) point to the items that are thought to be important for safety of third parties and the environment. The RAC review considers several elements of a gene therapy clinical trial: the trial protocol itself (design, participant safety, scientific basis, risk-benefit) as well as ethic and social questions and safety of third parties. From the minutes of the public discussions it is not clear what is already addressed in the application dossier.
The most frequently found remarks concern (see overview RAC public meetings, in Annex):

- vertical gene transfer (birth control, barrier contraceptives);
- horizontal gene transfer (vector shedding, close and intimate contact with relatives);
- recombination between vector virus and helper virus.

Reproductive considerations normally are addressed in the informed consent document that is part of the RAC as well as the IBC review. Although sometimes reference is made to the CDC guidelines at the public meeting concerning healthcare worker's safety, this is the responsibility of the IBC. IBC also checks for compliance to local safe handling instructions for healthcare professionals, waste treatment, etc.
In the IND attention is paid to the manufacturing process with regard to safety, identity, purity.

Safety issues that are identified at Health **Canada** (CERB) when reviewing a CTA are, amongst others:

- altered tropism;
- shedding that may lead to third party exposure;
- unintentional generation of replication-competent virus, and
- immunogenicity.

For gene therapy clinical trials in general, there is concern regarding the potential for horizontal exposure or transmission of vector, during administration of product or due to vector shedding post-administration (Ridgeway, pers. comm.). Those conducting the study are expected to include steps to minimise such exposures. Minimising the exposure of healthcare workers and third parties (*e.g*. other

patients, family members or casual contacts) is important even if there is no risk associated with the vector. This is because it is not ethically acceptable to expose others to recombinant viral vectors without their consent.
Vector shedding is only reviewed in light of the potential for human-to-human transmission. CERB does not address release into the environment. Likewise, ICH guidance does not target the environment. Different regional laws and approaches cover this aspect (Ridgeway, 2009). Again, environmental issues like safe handling of the product by healthcare workers, family members, waste treatment, etc. is evaluated and checked locally. The Institutional Review (IREB) is critical in this context.

If a NSN needs to be submitted, potential for release into the environment and toxicity are the main drivers.

Not surprisingly the points of attention for safety of third parties are similar in all three countries. Nevertheless, the resulting containment requirements may be very divergent (see Case study 1, Chapter 5.1). With regard to the environment differences may be seen depending on the type of gene therapy product, the way of administration and the clinical trial phase.

Part of the data is collected not only for the ERA, but also for the more 'conventional' application procedure (CCMO, FDA, IBC, CERB, and IREB). Examples are the genetic stability, purity of the vector batch, possibility of production of replication-competent virus, vector shedding, waste treatment, etc.

6.4 Procedure

The clinical trial protocol is evaluated centrally in USA (RAC) and Canada (Health Canada) similar to the approach taken in the Netherlands (CCMO). However, the actual ERA is for a large part addressed by the local institutional committees (IREB, IBC). Both FDA and Health Canada have exemption rules in place to 'avoid' a full ERA for research and development of gene therapy products. In consequence, the level at which environmental issues are discussed differs. Only when the product nears commercialisation its environmental impact becomes important and will be assessed at the national level.

The length of the procedures for the different countries is compared in Figure 11. These are the theoretical and legal assessment periods. Interruptions for obtaining additional data are not considered ('stop-the-clock'). For FDA it is not necessary to assess the environmental impact in clinical trials, at least not in the early stages (phase I and II). Once promising and ready for market introduction these studies still need to be done. The time that would involve a full EA is not taken into account in this Figure.

The procedures for the different agencies may run in parallel to each other. To facilitate the process the GMO office in the Netherlands collects the whole application and passes it to the relevant agencies. However, this is not mandatory. One may start one procedure before another. In the USA and Canada procedures run almost independently. Though, while there is no prescribed sequence for proceeding, in the USA the IRB and IBC will await the RAC recommendations before a decision is taken. Also, IBC has the duty to review the application for completeness before it is submitted to OBA.

In the Netherlands the procedure at the Ministry of Environment (IenM) covering the ERA legally has the longest allowable evaluation time. Especially assessments for a first use in the Netherlands may take some time. In practice, the parallel procedure for CCMO may take longer. Obstacles of any kind may hinder a smooth progress in any procedure (see Case study 1, Chapter 5.1 and Case study 2, Chapter 5.2).

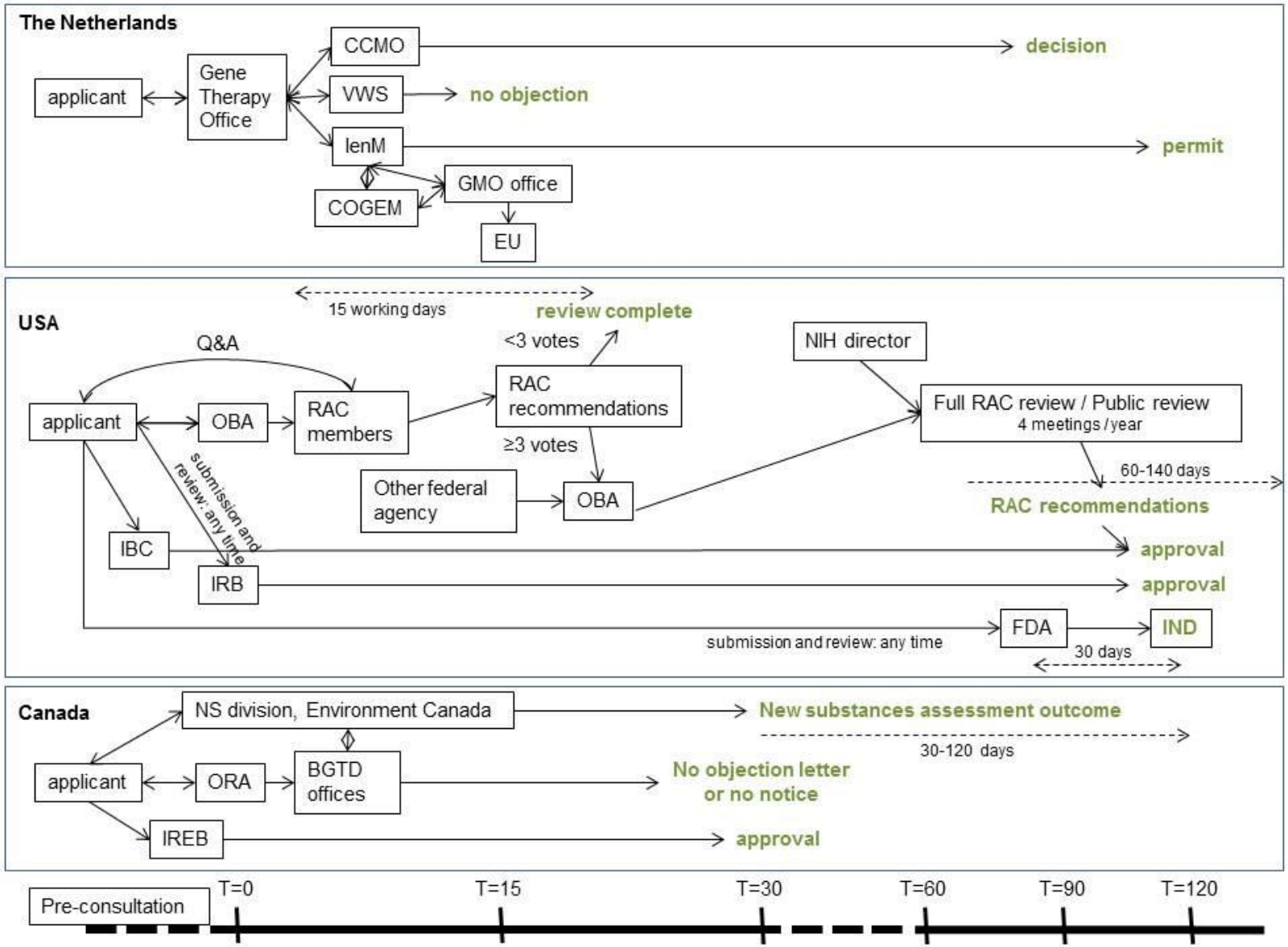

Figure 11 Comparison of the regulatory process for clinical trials in the Netherlands, the USA and Canada (T=0 is the point in time where the application is officially submitted; T=15 means 15 days after submission etc.)

In the USA the process may be very short provided that the trial is exempt from a full EA and no RAC public review is required. This is the case for applications that are more or less familiar to the reviewers (see *e.g.* Case study 3, Chapter 5.3). With public discussions total time may be as long as for the procedures in the Netherlands.

The time frame for a CTA in Canada is usually short. Almost all decisions are made close to the end of the 30 days. In practice, the default provision is never allowed to occur (Ridgeway, pers. comm.). With a NSN the evaluation time may increase to 120 days.

Still this comparison does not include the time applicants often spend consulting with agencies before a formal application is submitted. The pre-submission consultations are used to clarify questions and fine-tune the dossier, and may take as long as necessary to compile a solid application file. In this way the assessors are already acquainted with the clinical trial protocol at the time of official submission, saving time in the formal review.

Pre-consultation is possible in all countries. Especially in Canada it is almost mandatory, given the guidelines, pre-set timelines for a consultation procedure and the meeting minutes being added to the clinical trial file by the authorities. These pre-CTA meetings are held for any new active substance or application regardless of GMOs being involved. Likewise the CDER in the USA has a Pre-Investigational New Drug Application Consultation Program to foster early communications between sponsors and assessors. This possibility is often made use of.
In general applicants evaluate these meetings as very supportive. Applicants and assessors cooperatively discuss concerns, problems and opportunities.
Although available in the Netherlands as well, the procedure is focussed on clarifying on the type of data and the amount of detail that is required. Also, the fact that not all commission members are present on these meetings is felt as a disadvantage.

6.5 Clinical trials versus market introduction

Whereas oversight on clinical trials may be organised at different regulatory levels, registration for market introduction follows a centralised procedure in all three countries.

European regulations require an ERA report to be added to the marketing dossier, prepared on the basis of the information specified in Annexes III and IV of Directive 2001/18/EC and in accordance with Annex II of Directive 2001/18/ EC. Also a copy of any written consent or consents to the deliberate release into the environment of the GMO(s) for research and development purposes according to Part B of Directive 2001/18/EC should be included. The trials assessed in the Netherlands already have taken into account risks for human health and the environment. The clinical trials themselves may add additional information on *e.g*. dissemination via shedding through monitoring requirements.

In the USA an environmental assessment under EPA is usually only needed at the commercialisation stage (NDA/BLA). This makes that, compared to the European situation, data production to conduct the EA is postponed. Nevertheless, several data that would serve the EA already need to be present at clinical phase I. For example data and testing on the creation of replication-competent virus at the vector production stage are already included in the IND requirements. Shedding studies are performed in the pre-clinical stage on animals and further studied in the human trials whenever a risk is perceived or uncertainty exists. Also, information on manufacturing of the therapeutic (waste handling, procedures for spills) is provided in the IND.

Other topics would be addressed later, *e.g.* assessment of toxicity to environmental organisms (using a tiered approach of testing), the potential for persistence and spread, etc.

Even at the registration stage products may be exempt from the requirement of an EA when dosages and the produced quantities would not significantly affect the quality of the human environment (Sutton, 2008).

The situation in Canada relevant to an ERA is similar. In the majority of the cases the assessment is partly postponed to the marketing application.

6.6 Conclusion

Whereas legislation concerning GMO aspects may be very different in the studied countries, for initial assessment the basic data requirements (general basic data describing the product and administration mode) are very similar. Data and measures ensuring safety of healthcare professionals and the wider public are discussed early in the development phase of a gene therapy product. However, whereas risk management measures are imposed centrally in the Netherlands, this responsibility is left for a large part to the local authorities/committees in the USA and Canada.

The points of concern towards third parties, *e.g.* vertical and horizontal gene transfer, potential for vector recombination etc., are similar.

The procedure length is in most cases shorter in the USA and Canada compared to the Netherlands, not taking into account the occasional environmental assessment period, pre-submission consultations or public discussions at RAC.

The most striking difference is the environmental impact assessment. At the level of phase I, early phase II there is a clear difference between the USA and Canada on the one hand and the EU/the Netherlands on the other hand. The broad environment is very often not considered in these early stages in North America, since exposure is supposedly limited. Accordingly only a restricted amount of data on this aspect is required. Finally, when it comes to an application for market introduction a full environmental assessment targeting the broader environment is performed and similarities between the countries are observable. The in-depth ERA is thus postponed or sometimes even exempted.

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Annex

The table presents a summary of the gene therapy clinical trials that were publicly reviewed in the period 2006-2011. Reviewed protocols are ordered according to the vector that was used, the trial phase and finally the disease type. Items related to the risks for human health and the environment are summarised. The table only tabulates items that were mentioned in the minutes of the RAC meetings. Items related to the risks for human health and the environment may have been addressed in the dossier, but found to be satisfactory covered and not further discussed in public, are not included.

Table 2 Overview of RAC public discussions 2006-2011

Vector type	Protocol number	Title	Trial phase	Vector	Gene of interest	Disease	Disease type	Administration mode	Monitoring / risk management / questions human health and environment / recommendations / concerns / other comments
AAV	0504-705	A Phase I Dose-Escalation Study of Repeat Intra-Articular Administration of tgAAC94, a Recombinant Adeno-Associated Vector Containing the TNFR:Fc Fusion Gene, in Inflammatory Arthritis Subjects with and without Concurrent TNF-" Antagonists	I	AAV	human TNFR:Fc (antagonists of TNF-α)	Inflammatory Arthritis	autoimmune	local injection	
AAV	0707-864	An Open-Label Dose-Escalation Study of a Self-Complementary Adeno-Associated Viral Vector (scAAV-2/-8-LP1-hFIXco) for Gene Therapy of Hemophilia B	I	self-complementary AAV vector	human factor IX	Hemophilia B	genetic	into a peripheral vein	**From the protocol:** monitoring viral shedding into various body fluids. **Human health**: birth control.
AAV	0707-864	An Open Label Dose-Escalation Study of a Self-Complementary Adeno-Associated Viral Vector (scAAV2/8-LPl-hFIXco) for Gene Therapy of Hemophilia B	I	AAV-2	human factor IX	Hemophilia B; deficiency of clotting Factor IX	genetic	intravenous injection	**Update on trial**: Vector shedding was detected in a number of fluids but clears quickly within 2-3 weeks without reoccurrence.
AAV	0904-977	Direct Central Nervous System Administration of a Replication-Deficient, Adeno-Associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human CLN2 cDNA to Children with Late Infantile Neuronal Ceroid	I	AAV-10	CLN2 cDNA, coding for TPP-1 protein	Late infantile neuronal ceroid lipofuscinosis	genetic	catheter to the brain	
AAV	0904-977	Direct CNS Administration of a Replication-Deficient Adeno-Associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human CLN2cDNA to Children with Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL)	I	Replication-Deficient AAV	Human CLN2cDNA	Late Infantile Neuronal Ceroid Lipofuscinosis	genetic	intracranial administration	
AAV	1010-1068	An Open Label Dose-Escalation Study of a Self Complementary Adeno-Associated Viral Vector (scAAV2/8-LP1-hPPCA) for Gene Transfer in Subjects with Galactosialidosis	I	self-complementary AAV vector	Protective Protein/Cathepsin A, liver-specific expression	galactosialidosis, a lysosomal storage disease	genetic	infusion via peripheral vein	**From the protocol**: monitoring semen samples: PCR for vector presence. **Human health**: barrier contraception until 3 serial weekly semen samples have been declared as negative.

AAV	1010-1074	Phase I Clinical Intramuscular Gene Therapy of rAAV.FS344 Trial to Patients with Becker Muscular Dystrophy and Sporadic Inclusion Body Myositis	I	AAV-1	follistatin gene	Becker muscular dystrophy and sporadic inclusion body myositis	genetic	intramuscular injection	**From the protocol:** monitoring blood and urine tests; semen test for virus shedding. **Human health:** contraception.
AAV	1104-1104	AAV-BDNF Gene Therapy for Obesity	I	AAV	brain-derived neurotrophic factor	Severe obesity from genetic mutation of melanocortin 4 receptor; and Prader Willi Syndrome	genetic	intracranial administration	
AAV	0807-931	A Phase I/II Trial of Diaphragm Delivery of Recombinant Adeno-Associated Virus Acid Alpha-Glycosidase (rAAV1-CMV-GAA Gene Vector) in Patients with Pompe Disease	I/II	AAV-1 vectors	CMV-promoter - human GAA gene encoding acid-α-glucosidase	Pompe Disease	genetic	injection into striated muscle	**Comment:** needs further pre-clinical studies.
AAV	0401-623	Phase I/II Dose-Escalating Randomized Controlled Study to Assess the Safety, Tolerability, and Efficacy of CERE-110 (Adeno-Associated Virus [AAV]-Based, Vector-Mediated Delivery of Beta-Nerve Growth Factor [NGF]) in Subjects with Mild to Moderate Alzheimer's Disease	I/II	Adeno-Associated Virus	Beta-Nerve Growth Factor	Alzheimer's Disease	other	intracranial injection	**Comment:** unregulated transgene, no rescue strategy.
AAV	0904-981	A Phase I/II Trial Assessing the Safety and Efficacy of Bilateral Intraputamenal and Intranigral Administration of CERE-120 AAV-2-Neurturin in Subjects with Idiopathic Parkinson's Disease	I/II	AAV-2 (CERE-120)	Neurturin (NTN)	Parkinson's disease	other	intracerebral administration	
AAV	0610-809	A Phase I/II Randomized, Double-Blinded, Placebo-Controlled Dose Escalation Trial of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure	I/II	AAV1-based vector	Sarcoplasmic reticulum ATPase (SERCA2a) as a genetic enzyme replacement therapy	Heart Failure	vascular	intracoronary infusion	
AAV	0807-930	A Double-Blind, Placebo-Controlled (Sham Surgery), Randomized, Multicenter Study Evaluating CERE-110 Gene Delivery in Subjects with Mild to Moderate Alzheimer's Disease (Phase II)	II	AAV-2 (CERE-110)	nerve growth factor	Mild to Moderate Alzheimer's Disease	other	intracranial injection	
AAV	0910-1002	Multiple-Site, Phase II, Safety and Efficacy Trial of a Recombinant Adeno-Associated Virus Vector Expressing Alpha 1 Antitrypsin (rAAV1-CB-hAAT) in Patients with Alpha 1 Antitrypsin Deficiency	II	AAV-1	alpha-1 antitrypsin	alpha-1 antitrypsin deficiency	genetic	intramuscular injection	**From the protocol**: semen samples to establish whether or not there is the potential for inadvertent vertical transmission of vector DNA. **Human health**: risk of shedding virus in semen? In rabbits low levels of vector DNA were detected in semen only during the first week after injection and not thereafter. The informed consent document should clarify the risk of germline transmission.
AAV	0607-788	Multicenter, Randomized, Double-Blind, Sham Surgery-Controlled Study of CERE-120 (Adeno-Associated Virus Serotype 2 [AAV-2]-Neurturin [NTN]) to Assess the Efficacy and Safety of Bilateral Intraputamenal (IPu) Delivery in Subjects with Idiopathic Parkinson's Disease	II	AAV-2 (CERE-120)	neurturin	Idiopathic Parkinson's Disease	other	intrastriatal administration	**Human health**: screening pregnancy tests should be performed within 72 hours of dosing
AAV	0710-877	A Phase II Safety and Efficacy Study Evaluating Glutamic Acid Decarboxylase Gene Transfer to the Subthalamic Nuclei in Subjects with Advanced Parkinson's Disease	II	AAV	2 isoforms of glutamic acid decarboxylase (GAD 65 and 67)	Advanced Parkinson's Disease	other	intracerebral administration	

AAV	0807-930	A Double-Blind, Placebo-Controlled (Sham Surgery), Randomized, Multicenter Study Evaluating CERE-110 Gene Delivery in Subjects with Mild to Moderate Alzheimer's Disease	III	AAV	beta nerve growth factor	Alzheimer's Disease	other	intracranial injection	
AAV	0910-1005	A Safety and Efficacy Study in Subjects with Leber Congenital Amaurosis (LCA) Using Adeno-Associated Virus Vector To Deliver the Gene for Human RPE65 to the Retinal Pigment Epithelium (RPE) [AAV2-hRPE65v2-301]	III	AAV	retinal pigment epithelium - specific protein	Leber congenital amaurosis (retinal degeneration)	genetic	subretinal injection	**From the protocol**: virus shedding noticed in some participants in tears, blood and seminal fluid. **Human health**: abstain from unprotected sex for 4 months
AAV	0710-877	A Phase II Safety and Efficacy Study Evaluating Glutamic Acid Decarboxylase Gene Transfer to the Subthalamic Nuclei in Subjects with Advanced Parkinson's Disease.	III	AAV	glutamic acid decarboxylase	Advanced Parkinson's disease	other	intracerebral administration	
Adeno-virus	0704-843	A Phase I Study of Autologous T Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 in HIV-Infected Patients	I	autologous CD4+ T-cells modified by replication-defective Ad5/F35 vectors	SB-728 encoding 2 ZFNs	aids	infectious	infusion	
Adeno-virus	0401-624	A Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD) for Recurrent Malignant Gliomas	I	conditionally replication-competent oncolytic adenovirus	deleting 24 nucleotides from the E1a locus	Recurrent Malignant Gliomas	cancer	intra-tumoural injection	**Comment**: needs further pre-clinical studies.
Adeno-virus	0401-625	A Phase I Study of a Tropism-Modified Conditionally Replicative Adenoviral Vector (Ad5-Delta-24-RGD) for Intraperitoneal Delivery in Ovarian and Extraovarian Cancer Patients	I	conditionally replicating adenovirus, Ad5-delta-24-RGD	deleting 24 nucleotides from the E1a locus	Ovarian cancer	cancer	Intra-peritoneal injection	**Comment**: needs further pre-clinical studies.
Adeno-virus	0604-767	AdV/RSV-tk Followed by Valganciclovir for Treatment of Patients with Retinoblastoma Complicated by Vitreous Seeds	I	adenoviral vector	herpes thymidine kinase gene (AdV/RSV-tk)	Retinoblastoma	cancer	injection into the tumour	**Comment**: the RAC concluded by unanimous vote that the protocols should not move forward as currently conceptualized.
Adeno-virus	0604-768	Pediatric Phase I Study of AdV/RSV-tk Followed by Valganciclovir for Treatment of Patients with Retinoblastoma	I	adenoviral vector	herpes thymidine kinase gene (AdV/RSV-tk)	Retinoblastoma	cancer	injection to the vitreous seeds	**Comment**: the RAC concluded by unanimous vote that the protocols should not move forward as currently conceptualized.
Adeno-virus	0607-784	A Phase I, Open-Label, Dose- Escalation, Pharmacodynamic Study of Intranodal Injection of Adenovirus-CD154 (Ad-ISF35) in Patients with Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma	I	Replication-defective adenovirus	functional and stable chimeric ligand of CD40(CD154)	Chronic lymphocytic leukaemia and small lymphocytic lymphoma	cancer	intranodal injection	
Adeno-virus	0610-807	A Phase I Trial of Intratumoural Administration of Secondary Lymphoid Chemokine Gene-Modified Autologous Dendritic Cells in Advanced Non-Small Cell Lung Cancer	I	autologous dendritic cells modified by a replication-deficient adenoviral vector	secondary lymphoid organ chemokine CCL-21 gene	Advanced Non-Small Cell Lung Cancer	cancer	intra-tumoural, bronchoscope	**From the protocol**: RCA production checked .
Adeno-virus	0704-846	A Phase I, Dose-Ranging Study to Assess Safety and Distribution of GT-111 in Patients with Advanced Metastatic Cancer	I	non-replicating adenoviral vector	modified murine pre-proendothelin promoter + Fas-chimera transgene	advanced metastatic cancer	cancer	intravenous injection	**Comment**: needs further pre-clinical studies.

					(Fas and human Tumour necrotizing factor (TNF) receptor)				
Adeno-virus	0710-881	A Phase Ib, Open-Label Trial to Define the Safety, Tolerance, Transgene Function, and Immunological Effects of Intratumoural Injection(s) of Adenoviral-Transduced Autologous Dendritic Cells Engineered to Express hIL-12 Under Control of the RheoSwitch® Therapeutic System in Subjects with Stages III and IV Melanoma	I	Autologous Dendritic Cells modified by an adenoviral vector	human interleukin-12 (IL-12)	melanoma	cancer	intra-tumoural Injection, oral activator drug	**Comment**: needs further pre-clinical studies.
Adeno-virus	0802-905	Phase I Trial of Intravenous Recombinant Human 4-1BB Ligand Fusion Protein (hIg-h4-1BB-Ls) in Combination with Intratumoural Adenoviral Vector Expressing Human Interleukin-12 cDNA (Adv.hIL12) and Oral Sunitinib Malate in Patients with Metastatic Nonhematologic Neoplasms	I	modified adenovirus	interleukin-12 (Adv-hIL-12); hIg-h4-1BB-Ls, fusion protein	Metastatic Non-hematologic Neoplasms	cancer	intra-tumoural injection of Adv-hIL-12; intravenous (IV) administration of hIg-h4-1BB-Ls	**Comment**: needs further pre-clinical studies.
Adeno-virus	0810-952	Phase Ib Study of Autologous Ad-ISF35-Transduced CLL B Cells and Fludarabine, Cyclophosphamide, and Rituximab (FCR) in Subjects with Fludarabine-Refractory and/or del(17p) Chronic Lymphocytic Leukemia (CLL)	I	autologous B cells modified by a replication defective adenoviral vector	ISF35, the human CD40 ligand, CD154	Chronic lymphocytic leukaemia	cancer	intravenous infusion	**Human health**: studies should be performed to determine whether the vector replicates in CLL cells. If the vector does replicate, the risk of vector dissemination is increased. **Comment**: study was started with 4 subjects prior to RAC review and then put on hold.
Adeno-virus	1001-1026	A Phase I Neoadjuvant Study of In Situ REIC/Dkk-3 Therapy Followed by Prostatectomy in Patients with High Risk Localized Prostate Cancer	I	replication-competent adenovirus	reduced expression in immortalized cells (REIC/Dkk-3)	Prostate cancer	cancer	injection	**Human health**: barrier contraception
Adeno-virus	0704-849	A Phase I Study Evaluating the Use of Allodepleted T Cells Transduced with Inducible Caspase 9 Suicide Gene after Haploidentical Stem Cell Transplantation	I	T cells modified by a retroviral vector	a suicide gene, inducible caspase 9 (iCaspS) fused to a mutated human FK506- binding domain (to induce apoptosis) + marker gene	to improve the outcome of T-cell-depleted stem-cell transplantation	other	intravenous injection	**From the protocol**: washing steps, selection etc.; product lacks residual B cells, checked by phenotyping; real-time PCR to monitor recipients for EBV reactivation.
Adeno-virus	0801-896	A Phase I, Open-Label, Nonrandomized, Dose-Escalation, Multicenter Study to Assess the Safety and Cardiovascular Effects of the Implantation of Autologous Skeletal Myoblasts Modified to Express the SDF-1 Protein (MyoCell™ SDF-1) via Multielectrode Percutaneous Transendocardial Catheter (MyoStar™) with Cardiac Navigation Guidance (NOGA™) in Congestive Heart Failure Patients with Postmyocardial Infarction(s) with Prior Placement of an Implantable Cardioverter Defibrillator (ICD)	I	autologous myoblast cells modified with an adenoviral (Ad5) vector	CMV-promoter stromal cell-derived factor 1	myocordial infarction	vascular	injection in heart	**Comment**: preexisting Ad immunity - inflammation; additional preclinical studies are needed.
Adeno-virus	1101-1087	Randomized Phase I/II Trial using a GM-CSF Producing and CD40L-Expressing Bystander Cell Line (GM.CD40L) Vaccine in Combination with CCL21 for Patients with Stage IV Adenocarcinoma of the Lung	I/II	human bystander cell line (allogeneic) modified by a replication-	GM-CSF and CD40 ligand; CMV-promoter CCL21 (chemokine)	advanced adenocarcinoma of the lung	cancer	intramuscular injection	**Human health**: what is the likelihood that infectious CCL21-expressing adenovirus particles would be released from the transduced and irradiated cells? Cells will be allowed to incubate for at least 12 hours and are washed afterwards.

				incompetent adenoviruses					
Adeno-virus	0401-622	Adenylyl Cyclase VI Gene Transfer for CHF (Congestive Heart Failure)	I/II	replication-incompetent adenovirus E1/E3 deleted	adenylyl cyclase type VI	congestive heart failure	vascular	intracoronary injection	**Comment**: needs further pre-clinical studies.
Adeno-virus	1002-1029	A Phase II Study of Repeat Intranodal Injections of Adenovirus-CD154 (Ad-ISF35) in Subjects with Non-Hodgkin's Lymphoma (Follicular, Diffuse Large Cell, Mantle Cell, and Small lymphocytic Lymphoma/Chronic Lymphocytic Leukemia)	II	Adenovirus 5	ISF35, the human CD40 ligand, CD156	Non-Hodgkin's lymphoma	cancer	intranodal injection	**Comment**: study was started prior to RAC review and then put on hold.
Adeno-virus	1004-1028	Phase II Study of Repeat Intranodal Injections of Adenovirus-CD154 (Ad-ISF35) in Patients with Chronic Lymphocytic Leukemia / Small Lymphocytic Lymphoma	II	Adenovirus 5	ISF35, the human CD40 ligand, CD155	Chronic lymphocytic leukaemia	cancer	intranodal injection	**Human health**: 2 weeks of abstention from unprotected sex after the last administration of the vector. If there is replication (which might differ among individuals), the research participants might be put at risk, as might their family members due to the possibility of prolonged shedding. **Comment**: study was started prior to RAC review and then put on hold.
Adeno-virus	0407-661	A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter, Dose-Selection Study of Ad2/Hypoxia Inducible Factor (HIF)-1α/VP16 in Patients with Intermittent Claudication	II	Adenovirus 2	hypoxia inducible factor (HIF)	peripheral artery disease	vascular	intramuscular injection	
Adeno-virus	0704-842	A Randomized, Controlled Phase III Trial of Replication-Competent Adenovirus-Mediated Suicide Gene Therapy in Combination with Intensity-Modulated Radiation Therapy (IMRT) Versus IMRT Alone for the Treatment of Newly Diagnosed Intermediate-Risk Prostate Cancer	III	replication-competent adenoviral vector	cytosine deaminase (CD)/herpes simplex virus thymidine kinase (HSV-1 TK) fusion gene	Prostate cancer	cancer	intraprostatic injections	**Human health**: replication-competent vector could appear in saliva, transfer via close contact.
Adeno-virus	0612-821	A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study to Evaluate the Efficacy and Safety of Ad5FGF-4 in Female Patients with Stable Angina Pectoris Who Are Not Candidates for Revascularization	III	adenoviral vector (Ad5FGF-4)	human fibroblast growth factor-4	symptomatic coronary heart disease	vascular	intracoronary administration	**Human health**: RCA test.
bacterium	0704-853	A Phase I, Open-Label, Dose-Escalation, Multiple-Dose Study of the Safety, Tolerability, and Immune Response of CRS-207 in Adult Subjects with Selected Advanced Solid Tumours Who Have Failed or Who Are Not Candidates for Standard Treatment	I	live-attenuated form of *Listeria monocytogenes*	mesothelin gene	Malignant mesothelioma, non-small cell lung cancer (NSCLC), and cancers of the pancreas and ovary	cancer	intravenous infusion	**Human health**: individuals with immune-compromised family members should not enrol in this trial. **Environment**: agent is shed in urine: spread of the agent in the environment or possible recombination with the wild-type organism. **Comment:** The best estimates of an infectious dose of the wild-type organism for humans are provided by investigations of food-borne outbreaks. These studies suggest that the oral dose required to cause disease in 90%of the normal population is about $1x10^9$ cfu; the infectious dose in individuals with compromised cellular immune responses is estimated to be $1x10^7$ cfu. Based on the estimated infectious dose of wild-type *Lm* and the shedding observed in nonhuman primates, human-to-human transmission of an infectious dose of CRS-207 appears unlikely. Epidemiological studies have shown that the principal mode of transmission of *Lm* for both epidemic outbreaks and sporadic infections is contaminated food; although person-to-person spread via the faecal-oral route is also postulated as a route for transmission of *Lm*, such occurrences appear to be very uncommon. Regarding potential implications for spread in the environment, CRS-207 does not have a

									selective advantage for growth in the environment compared with the wild-type organism. The possible recombination of CRS-207 with wild-type *Lm* is also highly unlikely, as is reconstitution of a wild-type phenotype by incorporation of foreign DNA into CRS-207.
bacterium	0808-942	A Phase Ib, Multicenter, Single-Blinded, Placebo-Controlled, Sequential Dose-Escalation Study to Assess the Safety of Topically Applied AG013 in Subjects Receiving Induction Chemotherapy for the Treatment of Cancers of the Head and Neck	I	*Lactococcus lactis*	human trefoil factor 1	chemotherapy-induced Oral Mucositis with Cancers of the Head and Neck patients	cancer	oral administration	**From the protocol**: monitoring the presence of *L. lactis* in blood.
bacterium	0907-991	A Phase I Study of an IL-2 Expressing, Attenuated *Salmonella enterica typhimurium* in Patients with Unresectable Hepatic Spread from Any Non-Hematologic Primary Cancer	i	attenuated *Salmonella enterica typhimurium*	truncated human interleukin-2	Unresectable Hepatic Spread from Any Non-Hematologic Primary Cancer	cancer	oral administration	**Human health:** participants may have an attenuated immune system leading to multiplication of bacteria and shedding; to avoid transmission from the participants to anyone else, indicate that participants are not to prepare food for anyone; good-hand-washing technique and disposal-of-potentially-infectious-feces protocol will require meticulous application several times per day for several weeks to be effective; how to monitor participants? **Comment**: *Salmonella* is a facultative intracellular parasite; their survival in the relatively hypoxic areas of tumors and their ability to invade tumor cells; *Salmonella* containing the IL-2 gene appears to have a more substantial antitumor effect. To maximise safety, study participants will be instructed not to prepare food for anyone until their stool cultures indicate that the organism has cleared their system. Study participants will meet with a study nurse, one on one, to review the necessary guidelines for hygiene and handling of excrement. Universal precautions will be taught to these participants. Furthermore, subjects will be asked to keep a diary of these daily activities. Immunosuppression to a significant degree will be excluded from this study. This organism cannot survive on many carbon sources in the environment; it can survive only on glucose because it is a hyper-deletion mutant. Using white blood cell counts as a screen for compromised immune status. Longitudinal shedding studies should be undertaken to document whether the attenuated bacteria persist.
bacterium	0311-614	First Time in Human Safety Study of Streptococcus mutans Lactic Acid-Deficient Effector Strain (A2JM) Administered in Conjunction with Twice-Daily Dose of D-Alanine Mouthwash in Healthy Adult Male Subjects for Replacement Therapy as an Aid in the Protection Against Dental Caries	I	*Streptococcus mutans* Lactic Acid-Deficient Effector Strain (A2JM)	Instead of lactic acid the strain makes the neutral compounds ethanol and acetone	caries	healthy volunteers	oral administration	**Human health**: horizontal transmission via intimate contact.
bacterium	0907-989	A Phase 1 Open-Label, Escalating-Dose Study, of the Safety and Tolerability of Single Daily Doses of CEQ 508 an RNAi-Based Therapy for Familial Adenomatous Polyposis	i	non-pathogenic *E. coli* bacteria	transkingdom RNA interference (tkRNAi) to degrade β-catenin mRNA; invasin and listeriolysin O to enter cells	Familial Adenomatous Polyposis	genetic	oral administration	**From the protocol**: evaluation of CEQ508 shedding in stool samples. **Human health**: horizontal gene transfer: are calculations of the possibility of transfer of the pMBV43-H3 plasmid to other bacteria by conjugation, transduction, or transformation theoretical? Was this scenario tested in vitro? No evidence was found of horizontal transfer to other bacteria in mice. **Comment**: needs further pre-clinical studies.
bacterium	0707-868	A Phase 1 Safety Study of Heat/Phenol-Killed, E. coli-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) in Normal Volunteers Followed by Subjects Allergic to Peanuts	I	*E. coli* (encapsulating the proteins)	modified peanut proteins, Ara h 1 , Ara h 2 and Ara h 3, heat/phenol-killed (no genes)	peanut allergy	other	rectal administration	**Human health**: effective method of birth control.
bacterium	1107-1117	A Phase I/II Safety, Pharmacokinetic, and Pharmacodynamic Study of APS001F with Flucytosine and Maltose for the Treatment of	I/II	non-pathogenic obligate	cytosine deaminase	Advanced and/or Metastatic Solid Tumours	cancer	intravenous infusion	**From the protocol**: no shedding from the body in stool or urine in mice. **Human health**: horizontal gene tranfer via plasmid: effect restricted due to the fact that it consists of just three units: a CD-expression unit, a

		Advanced and/or Metastatic Solid Tumours		anaerobe *Bifidobacterium longum*					spectinomycin-resistant unit, and a plasmid-replication unit that is only active in *Bifidobacterium.*
bacterium	0804-917	A Phase IIa Randomized, Placebo-Controlled, Double-Blind, Multicenter, Dose-Escalation Study to Evaluate the Safety, Tolerability, Pharmacodynamics, and Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis	II	modified *Lactococcus lactis*	human immunomodulating cytokine Interleukin-10 (hIL-10)	Inflammatory bowel disease: Crohn's disease (CD) and ulcerative colitis (UC)	other	oral or rectal administration	**From the protocol**: monitoring stool samples for excretion of AG011 to validate the environmental containment measures. **Human health**: gene transfer to other flora in the gut? not observed.
bacterium	0807-932	A Randomized, Placebo-Controlled, Double-Blind, Dose-Escalation Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of Multiple Intravenous Doses of ANZ-521 in Treatment-Naive Hepatitis C Patients	III	live-attenuated *Listeria monocytogenes*	consensus sequence corresponding to portions of the NS5B and NS3 proteins	Hepatitis virus type C	infectious	intravenous injection	**Human health**: why must women participants be postmenopausal or surgically sterilised rather than required to commit to using birth control? Do not have a good understanding of the effects on pregnancy of live attenuated *Listeria*
Canary-pox	1110-1133	Phase I Clinical Trial of mTOR Inhibition with Sirolimus for Enhancing ALVAC(2)-NY-ESO-1(M)/TRICOM Vaccine Induced Anti-Tumour Immunity In Ovarian, Fallopian Tube and rimary Peritoneal Cancer	I	canarypox virus	tumour antigens NY-ESO-1 and its variant LAGE-1 (elicit an immune response)	Ovarian, Fallopian Tube, and Primary Peritoneal Cancer	cancer	subcutaneous injections	
herpes	1104-1100	A Phase I Study of the Treatment of Recurrent Malignant Glioma with rQNestin34.5v2, a Genetically Engineered HSV-1 Virus, and Immunomodulation with Cyclophosphamide	I	replication-competent HSV-1	ICP6 has been removed; ICP34.5 removed and replaced with nestin promoter	Recurrent Malignant Glioma (brain tumour)	cancer	peritumoural injection	**Human health**: Birth control, using barrier-type methods, should be practiced by research participants until there is no evidence of shedding in blood, saliva, or semen/vaginal secretions; HSV shedding assessment by vaginal or rectal swab
herpes	0811-955	Herpes Simplex Virus Gene Transfer of Glutamate Acid Decarboxylase for Painful Diabetic Neuropathy	I	non-replicating HSV	CMV promoter glutamic acid decarboxylase	Pain from nerve damage in patients with diabetes	other	skin inoculation	
lentivirus	0904-975	A Phase I Dose-Escalation Clinical Trial to Evaluate the Safety and Immunogenicity of a Replication-Defective HIV-1 Vaccine (HIVAX™) in HIV-1 Infected Subjects Receiving Highly Active Antiretroviral Therapy	I	replication-defective HIV-1	HIV-1HXB2 gag, truncated pol, vpr, rev, tat, nef, and envelope proteins	HIV	infectious	subcutaneous injection	**Human health**: possibility that the vector and helper recombine to restore any of the deletions introduced; possible interactions between the wild-type HIV and the vaccine strain. **Comment**: needs further pre-clinical studies.
lentivirus	1007-1056	A Phase I, Dual Cohort, Two Site, Clinical Trial Evaluating the Safety and Activity of Redirected Autologous T Cell Expressing a High Affinity TCR Specific for MAGE-A 3/6 or NYESO-1 Administered Post ASCT In Patients With Advanced Myeloma	I	autologous CD4 and CD8 T cells modified by a lentiviral vector	a3a or c259 T cell receptors	Advanced Myeloma	cancer	intravenous infusion	
lentivirus	1007-1057	Phase I Study to Assess the Safety and Activity of Enhanced TCR Transduced Autologous T Cells Against Cancer-Testis Antigens In Metastatic Melanoma	I	autologous CD4 and CD8 T cells transduced by a lentiviral vector	a3a or c259 T cell receptors	Metastatic Melanoma	cancer	intravenous infusion	**Human health**: birth control.
lentivirus	1010-1076	Phase I Clinical Trial of Autologous Alpha-Folate Receptor Redirected T Cells Administered Intravenously in Ovarian Cancer Patients	I	autologous T cells modified by a lentiviral vector	chimeric anti-alpha-folate receptor immunoreceptors	Epithelial ovarian cancer	cancer	intravenous infusion	
lentivirus	0602-758	Lentiviral-Mediated, Hematopoietic-Directed Gene Therapy for Mucopolysaccharidosis Type VII	I	autologous hematopoietic progenitor cells modified by a lentiviral	beta-glucuronidase	lysosomal storage disease mucopolysaccharidosis type VII	genetic	intravenous injection	

				vector					
lentivirus	0704-852	Phase I Open-Label Clinical Trial for the Treatment of β-Thalassemia Major with Autologous CD34+ Hematopoietic Progenitor Cells Transduced with ThalagenTM, a Lentiviral Vector Encoding the Normal Human β-Globin Gene	I	Autologous CD34+ hematopoietic progenitor cells modified by a lentiviral vector	normal human β-globin gene	β-thalassemia major	genetic	intravenous infusion	**From the protocol**: screening for contaminants in the vector batch. **Human health**: birth control (because of the chemotherapy agent busulfan to prepare for gene transfer).
lentivirus	0801-895	A Phase I Study of Gene Transfer for Patients with Fanconi Anemia Complementation Group A	I	autologous hematopoietic stem cells modified by a lentiviral vector	Fanconi gene	Fanconi anemia (blood disease)	genetic	intravenous injection	
lentivirus	0901-963	A Pilot Feasibility Study of Gene Transfer for X-Linked Severe Combined Immunodeficiency (X-SCID) in Newly Diagnosed Infants Using a Self-Inactivating Lentiviral Vector to Transduce Autologous CD34+ Hematopoietic Cells	I	bone marrow CD34+ cells modified by a SIN lentiviral vector		X-linked severe combined immunodeficiency (SCID-X1)	genetic	intravenous infusion	
lentivirus	0901-964	Lentiviral Gene Transfer for Treatment of Children Older Than 1 Year of Age with X-SCID	I	bone marrow CD34+ cells modified by a SIN lentiviral vector	IL2RG gene encoding the common gamma chain (γc)	X-linked severe combined immunodeficiency (SCID-X1)	genetic	intravenous infusion	
lentivirus	1007-1052	Pilot and Feasibility Study of Hematopoietic Stem Cell Gene Transfer for Wiskott-Aldrich Syndrome	I	autologous bone marrow derived CD34+ hematopoietic stem cells modified by a lentiviral vector pseudotyped with the VSV glycoprotein envelope	human WAS cDNA under control of the WAS promoter	Wiskott-Aldrich Syndrome	genetic	intravenous infusion	
lentivirus	1007-1061	A Phase I Dose Escalation Safety Study of Subretinally Injected RetinoStat®, a Lentiviral Vector Expressing Endostatin and Angiostatin, in Patients with Advanced Neovascular Age-Related Macular Degeneration	I	non-replicating Lentiviral Vector (Equine Infectious Anaemia Virus)	genes for Endostatin and Angiostatin	Advanced Neovascular Age-Related Macular Degeneration	vascular	subretinal injection	**From the protocol:** in the rabbit study, no vector shedding was detected in urine, saliva, and contralateral eye tear swabs. Vector DNA was detected in other sample types sporadically at early time points but only in a minority of animals and never above the lower limit of quantification. **Human health**: Urine samples and blood plasma will be analyzed for vector RNA; in addition, peripheral blood mononuclear cells will be tested for integrated provirus. women must be surgically sterile or postmenopausal, men have the options of being “surgically sterile or agreeing to use two forms of contraception including one barrier method for at least 3 months following RetinoStat® administration if their partner is of childbearing capacity.
lentivirus	1110-1130	An Adaptive Phase I/II Study of the Safety of CD4+ T Lymphocytes and CD34+ Hematopoietic Stem/Progenitor Cells Transduced with CAL-1, A Dual anti-HIV	I/II	human hematopoietic stem cells modified by a	siRNA: a short hairpin (catalytic) RNA directed to human c-c motif chemokine	HIV	infectious	subcutaneous injections	

		Gene Transfer Construct, in Busulfan Conditioned HIV-Infected Adults previously exposed to ART		lentiviral vector	receptor 5, CCR5 (sh5) + C46, glycoprotein gp41				
lentivirus	0910-1006	Treatment of Subjects with Adenosine Deaminase (ADA) Deficient Severe Combined Immunodeficiency (SCID) with Autologous Bone Marrow CD34+ Stem/Progenitor Cells After Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector	I/II	human hematopoietic stem-progenitor cells modified by a lentiviral vector	Adenosine Deaminase	Adenosine Deaminase Deficient Severe Combined Immunodeficiency	genetic	intravenous infusion	
lentivirus	1001-1023	Gene Transfer for Patients with Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open Label Phase I/II Pilot Study	I/II	autologous bone marrow CD34+ cells modified by a lentivirus	γ-globin exons and β-globin non-coding regions and regulatory elements	Sickle cell disease	genetic	intravenous infusion	
lentivirus	1010-1073	An Open Label, Non-Randomized, Single Dose, Multi-Center Phase 2/3 Study of the Safety and Efficacy of Lenti-D Modified Autologous Stem Cells (Lenti-D Drug Product) for the Treatment of Subjects with Childhood Cerebral Adrenoleukodystrophy (CCALD)	II/III	lentivirus based autologous cell therapy	ABCD-1 gene encoding the ALD protein - a peroxisomal transporter	Childhood Cerebral Adrenoleuko-dystrophy	genetic	intravenous infusion	
lentivirus /vaccinia	1104-1106	A Phase I Placebo Controlled Clinical Trial To Evaluate the Safety and Immunogenicity of a Prime-Boost Vaccine Regimen of GEO-D03 DNA and MVA/HIV62B Vaccines in Healthy, HIV-1-Uninfected Vaccinia Naïve Adult Participants	I	human immunodeficiency virus = DNA vaccine; and modified vaccinia Ankara	Gag, PR, RT, Env, Tat, Rev, and Vpu, and human granulocyte macrophage colony-stimulating factor (GM-CSF) on HIV plasmid and gag, pol, and env on vaccinia	HIV	infectious	intramuscular injections	
liposomes	0804-913	Phase I Study of BikDD Therapy in Advanced Breast Cancer	I	liposomes	CMV promoter - mutant Bik (Bcl-2 interacting killer), a pro-apoptotic BH3-only protein	Advanced Breast Cancer	cancer	intravenous infusion	**Comment**: use specific CT-90 promoter that is selectively expressed in BC cells to avoid off-target effects.
liposomes	0804-914	A Phase I, Open-Label, Dose-Escalation Study to Assess the Safety and Tolerability of the BikDD Nanoparticle in Patients with Advanced Pancreatic Cancer	I	liposomes	cholecystokinin type A receptor promoter - mutant Bik (Bcl-2 interacting killer), a pro-apoptotic BH3-only protein	Advanced Pancreatic Cancer	cancer	intravenous infusion	
liposomes	0808-936	A Phase I Trial of the Immunostimulant JVRS-100 for the Treatment of Patients with Relapsed or Refractory Leukemia	I	cationic liposome and plasmid DNA complexes derived from *E. coli*	No foreign genes are encoded in the plasmid DNA for expression in human cells	Relapsed or Refractory Leukaemia	cancer	intravenous injection	
liposomes	0808-934	A Phase I, Open-Label Study of the Safety, Tolerability, and Therapeutic Activity of JVRS-100 Cationic Lipid-DNA Complex in Patients with Chronic Hepatitis C Infection Who Relapsed After Receiving Interferon-Ribavirin Treatment	I	cationic liposome and plasmid DNA complexes derived from *E. coli*	No foreign genes are encoded in the plasmid DNA for expression in human cells	Chronic Hepatitis C Infection	infectious	intravenous injection	

liposomes	0807-923	Compassionate Trial of Nanocomplex-Mediated GNE Gene Replacement in Hereditary Inclusion Body Myopathy-2	single subject	liposomes	CMV-promoter - bifunctional enzyme UDP-GlcNAc2-Epimerase/ManNAc kinase	Hereditary inclusion body myopathy-2	genetic	intramuscular, later also intravenous injections	**Comment**: needs further pre-clinical studies.
plasmid	0512-752	Phase I Trial to Assess Safety and Immunogenicity of Xenogeneic CD20 DNA Vaccination in Patients with B-Cell Lymphoma	I	DNA plasmid	mouse extracellular domain of CD20	lymphoma	cancer	intramuscular injection	
plasmid	0704-848	A Phase I Study of Intratumoural Administration of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Alloclone-002 Modified for Glucocorticoid Resistance and Interleukin-2	I	CD8+ cytotoxic T lymphocyte (CTL); electroporat.	IL13Rα2-specific IL13-zetakine chimeric immunoreceptor, HyTK selection suicide fusion protein; alter both alleles of the glucocorticoid receptor (GR) zinc finger nucleases (ZFNs)	brain tumours	cancer	intra-tumoural administration	
plasmid	0801-890	A Phase I, Single-Center, Open-Label, Dose-Escalation Study to Evaluate the Safety and Tolerability of GHRH DNA Plasmid (VGX-3200) + Electroporation in Adults with Cancer Cachexia	I	DNA plasmid	human growth hormone releasing hormone (hGHRH)	Cancer cachexia	cancer	intramuscular injection + electroporation	**Human health**: birth control.
plasmid	1007-1049	Phase 1/2 Open-Label, Single-Center, Multiple-Dose, Dose-Escalation Study to Evaluate the Safety and Tolerability of SNS01-T Administered by Intravenous Infusion in Patients with Relapsed or Refractory Multiple Myeloma	I	polyethyleneimine cationic polymer to form nanoparticles	non-hypusinable mutant of translation initiation factor 5A protein; and small interfering RNA targeting an untranslated region of native human eIF5A mRNA	Relapsed or Refractory Multiple Myeloma	cancer	intravenous Infusion	**Comment**: needs further pre-clinical studies.
plasmid	1007-1050	Phase I Study of an Active Immunotherapy for Asymptomatic Phase Lymphoplasmacytic Lymphoma with DNA Vaccines Encoding Antigen-Chemokine Fusion	I	DNA vaccine	idiotype protein: autologous lymphoma-derived immunoglobulin variable region genes combined into a single chain antigen format; fused with chemokine	lymphoplasmacytic lymphoma	cancer	intramuscular injection	**Human health**: birth control (as precaution, since the vaccine is new).
plasmid	1108-1122	Phase I Trial of the Safety and Immunogenicity of a DNA Plasmid Based Vaccine Encoding the Amino Acids 1-163 of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) in atients with Advanced Ovarian Cancer	I	DNA Plasmid	Amino Acids 1–163 of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2)	Ovarian Cancer	cancer	intradermal injections; GM-CSF: intradermal as a vaccine adjuvant	
plasmid	1110-1127	Pilot Clinical Trial of Autologous Met Redirected T cells Administered Intratumourally and Intravenously in Patients with Operable Triple Negative Breast Cancer	I	autologous T cells modified by electroporat. → transient expression	chimeric antigen receptor specific for c-Met	Operable Triple Negative Breast Cancer	cancer	intra-tumoural followed by intravenous injections	**Comment:** Phase 0 trial.

plasmid	0604-769	A Phase I, Randomized, Placebo-Controlled, Open-Label, Cross-Over Safety and Pharmacodynamic Study of BHT-3021 in Subjects with Recent Onset Type 1 Diabetes Mellitus	I	DNA plasmid	CMV-promoter human proinsulin protein	Type 1 diabetes	other	intramuscular injection	**Human health**: birth control.
plasmid	0901-966	A Prospective, Randomized, Controlled, Multicenter, Unblinded, Safety, and Early Efficacy Trial of ExpressGraft™Enhance Skin Tissue Versus Wet-to-Dry Dressings in the Treatment of Recently Occurring, Noninfected Foot Ulcers in Diabetic	I	human allogeneic epidermal keratinocytes modified with plasmid DNA	cathelicidin (hCAP-18/LL-37) host defense peptide	Diabetic foot ulcers	other	skin graft tissue: epidermal layer on infected ulcers (allograft)	**From the protocol**: each lot of final tissue product is tested for the presence of residual murine feeder cells using a proprietary, species-specific PCR-based assay.
plasmid	0610-810	Phase I, Open-Label, Rising-Dose Study of the Safety and Tolerability of Single Doses of NUC B1000, an RNAi-Based Therapy for Chronic Hepatitis B	I	DNA plasmid	short-hairpin RNAs against HBV type 1	Chronic Hepatitis B	infectious	intravenous injection	**Comment**: needs further pre-clinical studies.
plasmid	0907-988	A First-in-Human Safety and Dose-Finding Study of a New Type-16 Human Rhinovirus (RG-HRV16) Inoculum in Healthy Volunteers	I	plasmid derived from HRV		Human rhinovirus infections	infectious	intranasal administration	**Comment:** this is only to study the virus. **Human health**: college-student participants, who come into contact with others transiently during class, at mealtimes, in a dormitory, and while sharing materials; exposing family members and close contacts; quarantine at least the first cohort of participants to see if not more virulent than parent strain; suggestion: concept of "social distancing" so that research participants are not quarantined but that they do refrain from attending large public gatherings. participants in or having contacts with high-risk groups will be excluded: High-risk groups are defined as the very young, elderly individuals, and people with chronic lung disease. specific instructions on how not to spread common colds.
plasmid	0604-774	A Phase I, Multicenter Study Evaluating the Safety and Potential Activity of Three Escalating Doses of hMaxi-K Gene Transfer in Female Participants with Overactive Bladder Syndrome and Detrusor Overactivity: Double-Blind, Imbalanced, Placebo-Controlled Design within Three Sequential Active Treatment Groups	I	DNA plasmid	hMaxi-K - potassium channel	overactive bladder syndrome	other	urethral catheter in the bladder lumen	**Human health**: refrain from sexual intercourse for 24 hours following administration of the study agent.
plasmid	0910-1004	An Open Label Dose Escalation Study to Evaluate the Safety of a Single Escalating Dose of ACRX-100 Administered by Endomyocardial Injection to Cohorts of Adults with Ischemic Heart Failure	I	DNA plasmid	stromal cell-derived factor-1	Ischemic Heart Failure; ischemic cardiomyopathy	vascular	delivered to the myocardium via direct catheter-guided injection	
plasmid	1007-1053	A Phase 1/2 Randomized, Blinded, Placebo-Controlled, Sequential Dose Escalation Study of the Safety and Pharmacodynamics of BHT-3034, an Acetylcholine Receptor Tolerizing Plasmid	I/II	DNA plasmid BHT-3021	mammalian promoter, gene for the α-chain (Chrna1) of the human nicotinic acetylcholine receptor	Myasthenia gravis	auto-immune	intramuscular injections	
plasmid	0901-967	A Phase I/IIa, Dose-Escalation, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Intraperitoneal Administration of DTA-H19 in Subjects with Advanced-Stage Ovarian Cancer	I/II	DNA plasmid	H19 promoter diphtheria toxin A chain (selective expression)	Ovarian cancer	cancer	intra-tumoural injection (intra-peritoneal catheter)	**Comment**: plasmid excreted in urine: no questions about this!
plasmid	0710-880	A Phase I/IIA Study of the Safety and Efficacy of Neuroprogenitor Cells (SB623) in Patients with Stable Ischemic Stroke	I/II	allogeneic adult human bone marrow stromal cells (neuroprogenitor cells)	human Notch-1 intracellular domain, a gene involved in neuronal differentiation	Stable Ischemic Stroke	vascular	intracranial administration	**Comment**: needs further pre-clinical studies.

				transiently transfected with a plasmid					
plasmid	0703-838	A Randomized Double-Blind Placebo-Controlled Parallel Group Study of the Efficacy and Safety of XRP0038/NV1FGF on Amputation or Any Death in Critical Limb Ischemia Patients with Skin Lesions	III	DNA plasmid	human acidic fibroblast growth factor (FGF1)	critical limb ischemia (CLI) amputation	other	intramuscular injection	
retrovirus	0610-813	A Pilot Study of Genetically Modified Haploidentical Natural Killer Cell Infusions for B-Lineage Acute Lymphoblastic Leukemia	I	haploidentical natural killer cells modified by a murine retroviral vector	signalling receptor that binds to CD19: single-chain; variable region of a murine anti-CD19 monoclonal antibody and signalling domains of 4-1BB and CD3 zeta	Acute lymphoblastic leukaemia	cancer	infusion	**From the protocol**: replication-competent retroviral testing in blood.
retrovirus	0704-849	a Phase I Study Evaluating the Use of Allo-Depleted T Cells Transduced With an Inducible Caspase-9 Suicide Gene After Haploidentical Stem Cell Transplantation	I	Allo-depleted T cells modified by a retrovirus	Inducible Caspase 9 Suicide Gene	blood disorders; cancer	cancer	Intravenous injection	
retrovirus	0710-878	A Pilot Feasibility Study of Oral 5-Fluorocytosine and Genetically Modified Neural Stem Cells Expressing Escherichia coli Cytosine Deaminase for Treatment of Recurrent High-Grade Gliomas	I	immortal neural stem cells modified by a replication-incompetent retroviral vector	*E. coli* gene for cytosine deaminase; v-myc oncogene to immortalise	Recurrent High-Grade Gliomas	cancer	intracerebral administration; oral for 5-fluorocytosine	**Comment**: needs further pre-clinical studies.
retrovirus	0904-976	A Phase I Ascending-Dose Trial of the Safety and Tolerability of Toca 511 in Patients with Recurrent Glioblastoma Multiforme	I	replication-competent mouse leukaemia virus	cytosine deaminase (CD) gene to convert the antifungal drug flucytosine to the cell-killing drug fluorouracil	Glioblastoma multiforme (malignant brain tumour in adults)	cancer	single intra-tumoural injection	**Human health**: avoid recombination between xenotropic MLV-related virus and Toca 511 in infected individuals.
retrovirus	1001-1020	Administration of EBV-Specific Cytotoxic T Cells Expressing HER2 Chimeric Antigen Receptor to Subjects with Advanced Osteosarcoma (ECHO)	I	autologous EBV-specific T cells modified by retroviral vector SFG	chimeric antigen receptor targeting the tumour associated antigen HER2	Osteosarcoma	cancer	intravenous injections	
retrovirus	1001-1024	Lymphodepletion Plus Adoptive Cell Transfer with CXCR2 and NGFR Transduced T-Cells Followed by High Dose Interleukin-2 in Patients with Metastatic Melanoma	I	autologous tumour infiltrating lymphocytes modified by retroviral vectors encoding CXCR2	chemokine CXCR2 and truncated nerve growth factor receptor (NGFR) marker gene	Metastatic Melanoma	cancer	intravenous injections	
retrovirus	1004-1034	Phase I Study of the Administration of EBV-CTLs Expressing CD30 Chimeric Receptors for Relapsed CD30+ Hodgkin's Lymphoma and CD30+ Non-Hodgkin's Lymphoma	I	autologous EBV-CTLs modified by a Moloney retroviral	artificial receptor for the CD30 antigen	Hodgkin's lymphoma and non-Hodgkin's lymphoma	cancer	intravenous injections	

				vector					
retrovirus	1102-1091	A Phase I Dose Escalation Trial Using In Vitro Expanded Allogeneic Epstein-Barr Virus Specific Cytotoxic T-Lymphocytes (EBV-CTLs) Genetically Targeted to the B-Cell specific Antigen CD19 positive Residual or Relapsed Acute Lymphoblastic Leukemia After Allogeneic Hematopoietic Progenitor Cell Transplantation	I	allogeneic T-cells selected to target Epstein-Barr virus	19-28z chimeric antigen receptor recognising CD19	B-cell acute lymphoblastic leukaemia	cancer	intravenous injections	**From the protocol**: replication-competent retrovirus testing. **Human health**: use contraceptive
retrovirus	1107-1120	Phase I Ascending Dose Trial of the Safety and Tolerability of Toca 511, a Retroviral Replicating Vector, Administered to Subjects at the Time of Resection for Recurrent High Grade Glioma and Followed by Treatment with Toca FC, Extended-Release 5-FC	I	replicating retrovirus vector (Moloney murine leukaemia virus)	yeast-derived cytosine deaminase	Recurrent High Grade Glioma	cancer	intra-tumourally via stereotactic, transcranial injection, followed by repeated cycles of orally administered 5-fluorocytosine	**From the protocol**: monitoring for virus levels in blood, urine, and saliva (PCR signal in blood was the most sensitive predictor of shedding in the mouse). **Human health**: barrier contraceptive; test lymph nodes, vaginal fluid, and semen for infectious particles; given that the lifespan for these individuals is approximately 6 months past enrollment in this clinical trial, the emphasis on viral risk should be with the contacts, not with the participants. **Comment:** the investigators described the universal precautions (Class 2 isolation) under which these research participants would be kept while in the hospital.
retrovirus	0810-950	Gene Therapy for SCID-XI Using a Self-Inactivating (SIN) Gammaretroviral Vector	I	autologous hematopoietic progenitor cells modified by a self-Inactivating gamma-retroviral vector	interleukin-2 receptor (IL-2RG) gene	X-linked severe combined immunodeficiency (X-SCID)	genetic	intravenous infusion	**Comment**: revision to also include infants less than 3,5 months of age: accepted.
retrovirus	1004-1036	Phase I/II Study of Metastatic Cancer Using Lymphodepleting Conditioning followed by Infusion of Anti-VEGFR2 Gene	I/II	autologous T-cells modified by a retrovirus	anti-VEGFR2 genes and T-cell receptor genes	metastatic cancer	cancer	intravenous infusion	
retrovirus	1004-1037	Phase I/II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of CD8 Enriched Tumour Infiltrating Lymphocytes Genetically Engineered to Express IL-12	I/II	autologous tumour-infiltrating lymphocytes modified by a retroviral vector	interleukin 12 (IL-12) genes	metastatic melanoma	cancer	intravenous infusion	**From the protocol**: rtPCR test for xenotropic MuLV prior to administering the vectored cells and test for RCR.
retrovirus	1103-1095	A Phase I/II Study of the Safety and Feasibility of Administering T Cells Expressing Anti-EGFRvIII Chimeric Antigen Receptor to Patients with Malignant Gliomas Expressing EGFRvIII	I/II	autologous T-cells modified by a retrovirus	chimeric antigen (CAR) receptor that targets EGFRvIII	glioblastoma multiforme with EGFRvIII expression	cancer	intravenous infusion	
retrovirus	0701-827	Phase I or Phase I/II Single-Center Trial of Gene Transfer for Recessive Dystrophic Epidermolysis Bullosa	I; I/II	autologous keratinocytes modified by a retroviral vector, pLZRSE-Col7A1	type VII collagen	Recessive dystrophic epidermolysis bullosa	genetic	skin graft	

retrovirus	0912-1016	A Phase II Study to Determine the Efficacy and Safety of Allogeneic Human Chondrocytes Expressing TGF- β1 in Patients with Grade 3 Degenerative Joint Disease of the Knee	II	3: I mixture of non-transduced allogeneic human chondrocytes and irradiated allogeneic human chondrocytes transfected with a retroviral vector	transforming growth factor-β1 (TGF-β1)	Degenerative arthritis	auto-immune	intra-articular administration	**Human health**: birth control.
Sendai	0801-897	A Phase I/II, Multicenter, Open-Label, Dose-Escalation Study to Evaluate the Safety and Tolerability of DVC1-0101 Administered Intramuscularly in Subjects with Stable Peripheral Artery Disease	I/II	Sendai virus	human fibroblast growth factor 2 (hFGF-2),	peripheral artery disease	vascular	intra-muscular injection	**Human health**: birth control.
transposon	0804-922	Adoptive Immunotherapy for CD19+ B-Lymphoid Malignancies Using Sleeping Beauty Transposition to Express a CD19-Specific Chimeric Antigen Receptor in Autologous Ex Vivo Expanded T Cells	I	CD19-specific autologous T cells transduced by the Sleeping Beauty transposon system (analogous to retrovirus)	chimeric antigen receptor (CAR)	advanced B-lymphoid malignancies	cancer	intravenous injections	
Vaccinia	0401-629	A Phase I Dose-Escalation Trial of vvDD-CDSR (Double-Deleted Vaccinia Virus Plus CD/SMR) Administered by Intratumoural Injection in Patients with Superficial Injectable Tumours	I	vvDD-CDSR virus (from vaccinia virus): oncolytic, replication-selective virus	cytosine deaminase to convert a safe drug to a toxic drug, somatostatin receptor to visualise	injectable superficial tumours	cancer	intra-tumoural injection	**From the protocol**: test for viral spread in blood, shedding into the urine or throat. **Comment**: concern about pre-existing vaccinia immunity.
Vaccinia	0908-995	A Phase I Open-Label, Dose-Escalation Trial of JX-594 (Thymidine Kinase-Inactivated Vaccinia Virus Plus GM-CSF) Administered by Intratumoural Injection in Pediatric Patients with Unresectable Refractory Solid Tumours	I	oncolytic, replication-selective viruses: thymidine kinase-inactivated vaccinia virus	granulocyte macrophage colony stimulation factor and humanised *E. coli* β-galactosidase	unresectable Refractory Solid Tumours	cancer	Intra-tumoural injections	**From the protocol**: semen samples (noted that most of the individuals enrolling in this trial would likely be sterile from their prior high-dose cytotoxic chemotherapy); Q-PCR is used to measure JX-594 genomes in the blood. The protocol addresses the risks of inadvertent spread of vaccinia virus to immuno-compromised close contacts of the participants by requiring alternative living arrangements for a period of at least 3 weeks following the last dose of the study medication. **Human health**: Clinical staff should be made aware of the risks and potential contraindications for vaccinia exposure both to them and any close contacts, especially individuals with compromised immune systems. **Comments**: Under the NIH Guidelines, vaccinia viruses other than monkeypox and restricted poxviruses – such as alastrim, smallpox, and whitepox – are classified as RG 2 agents. Experiments with such agents generally require a containment level of Biosafety Level (BL) 2, even for

									attenuated vaccine strains. As always, however, the local institutional biosafety committee is required to do a thorough risk assessment for such constructs and to review the administration protocols.
Vaccinia	1101-1088	Phase I Study of Intra-pleural Administration of GL-ONC1, a Genetically Modified Vaccinia Virus, in Patients with Malignant Pleural Effusion: Primary, Metastases, and Mesothelioma	I	Vaccinia Virus replicating in tumour cells	fluorescent/luminescent fusion protein	Malignant Pleural Effusion	cancer	intra-pleural administration	**Human health**: use procedures that will minimize generation of aerosols when preparing the vector for administration (biosafety cabinet or using ethanol soaked gauze); healthcare workers who will be administering this vector, or interacting with participants who have received the vector, should understand the CDC contraindications to vaccinia vaccination.
Vaccinia	1101-1089	Phase I Trial of Attenuated Vaccinia Virus (GL-ONC1) Delivered Intravenously with Concurrent Cisplatin and Radiotherapy in Patients with Locoregionally Advanced Head and Neck Carcinoma	I	Vaccinia Virus replicating in tumour cells	fluorescent/luminescent fusion protein	Locoregionally Advanced Head and Neck Carcinoma	cancer	intravenous injection	**Human health**: use procedures that will minimize generation of aerosols when preparing the vector for administration (biosafety cabinet or using ethanol soaked gauze); healthcare workers who will be administering this vector, or interacting with participants who have received the vector, should understand the CDC contraindications to vaccinia vaccination.
Vaccinia/ fowlpox	1104-1101	A Randomized, Double-Blind, Phase III Efficacy Trial of PROSTVAC ± GM-CSF in Men with Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer	III	vaccinia vector, PROSTVAC-V, or a fowlpox vector, PROTVAC-F	gene for the tumour associated antigen prostate specific antigen; and genes for three different T cell co-stimulatory molecules	prostate cancer	cancer	subcutaneous injections	**Comment**: Recognizing potential risks for workers administering this vaccine, Dr. Gulley stated that the investigators will follow the CDC guidelines. They are working with all the sites on specific procedures. At Dr. Gulley's site, preparation of the vaccine will be done in a biosafety cabinet. minimise generation of aerosols; understand the CDC contraindications to vaccinia vaccination.
VSV	0807-927	Phase I Translational Trial of Oncolytic Virotherapy with Recombinant Vesicular Stomatitis Virus (rVSV(MΔ51)-M3) by Hepatic Arterial Delivery in Patients with Primary Hepatocellular Carcinoma or Metastatic Colorectal Carcinoma in the Liver	I	oncolytic Vesicular Stomatitis Virus; replicating in tumour cells	deletion in M protein; and heterologous viral chemokine binding gene from murine gammaherpesvirus-68	Primary Hepatocellular Carcinoma or Metastatic Colorectal Carcinoma in the Liver	cancer	Hepatic Arterial Delivery	**Human health**: only a relatively small fraction of the population has been exposed to VSV: after virus injection, participants would need to stay in isolation (±1 week) because of the potential risk for spreading the virus; blood, urine, and nasal swabs will need to be negative for VSV prior to participant release from the clinical center. **Comment**: needs further pre-clinical studies.
VSV	0808-946	Phase I Trial of Intratumoural Injection of Vesicular Stomatitis Virus Expressing Interferon Beta in Patients with Hepatocellular Carcinoma	I	oncolytic Vesicular Stomatitis Virus (VSV)	Interferon Beta	hepatocellular carcinoma	cancer	intra-tumoural injection	
yeast	1107-1119	An Open Label Phase I Study To Evaluate the Safety and Tolerability of GI-6301, a Vaccine Consisting of Whole, Heat-Killed Recombinant Saccharomyces cerevisiae (yeast) Genetically Modified to Express Brachyury Protein in Adults with Metastatic Carcinoma	I	whole heat-killed yeast	Brachyury protein	Metastatic Carcinoma	cancer	subcutaneous injections	**Human health**: exclude pregnant or breastfeeding women, but also any female of childbearing potential.