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Biosafety and Biotechnology Unit

Injection of CD34+ hematopoietic stem cells transduced with lentiviral vector to immunodeficient mice

1. Aim of the project

Wiscott-Aldrich Syndrome (WAS) is a rare and severe recessive X-linked immunodeficiency characterized by a profound microthrombocytemia, recurrent infections, autoimmunity, eczema and increased susceptibility to develop tumors, in particular B and T lymphomas. The disorder almost exclusively affecting males is caused by mutations in the gene encoding for the WAS protein.

The only curative treatment to date is the hematopoietic stem cells transplantation with matched human leucocyte antigen (HLA) donor or allogeneic bone marrow transplantation.

In this project, an hematopoietic stem cells gene therapy approach based on lentiviral-mediated gene transfer of WAS gene was developed. Lentiviral-transduced CD34+ cells were transplanted in immunodeficient mice.

2. Vector and vector production system

- Vector: Replication deficient [third-generation self-inactivating (SIN)] lentiviral vector (LV).
- Vector production system: LV were generated in human embryonic kidney 293 T (HEK-293T) packaging cell line. This cell line is stably transfected with *gag*, *pol*, vesicular stomatitis virus G (VSV-G) *env* and *rev* genes that are located on different plasmids in such a way that three different recombination events are required to generate replication competent lentiviruses (RCL) particles (packaging cell line of the third generation). Various assays for RCL contamination all failed to detect the presence of RCL, underscoring the relative safety of such packaging systems of the third generation. In the framework of this project, the packaging cell line is cotransfected with the viral envelope protein VSV-G allowing the production of amphotropic replication defective vectors.

3. Inserts

The insert encodes for WAS protein. *In vivo*, the gene encoding WAS protein is only expressed in hematopoietic cells and involved in actine cytoskeleton reorganization, signal transduction and apoptosis.

4. Localization of inserted genetic material

- Insertion at a predefined localization in the LV.
- Stable integration into the genome of the transduced CD34+ cells.
- From PCR-assays investigating potential mobilization of the vector into murine cells or tissues, no evidence of vector sequences integrated in the murine genome was reported (Scaramuzza *et al.*, 2013).

5. Receptor organisms

CD34+ cells were isolated from human umbilical cord blood. These cells were transduced with the recombinant LV. Human primary cells are of class of risk 2.
For more information on the risk assessment of cell cultures (see http://www.biosafety.be/CU/animalcellcultures/mainpage.html).

6. Biological hazards and/or considerations related to the manipulation of the resulting genetically modified micro-organism (GMM)

Two types of GMM are discussed in this example, a recombinant replication defective LV and CD34+ cells transduced with the recombinant vector.

Considerations related to the production and the use of the recombinant LV:

- While LV are used for their ability of stable integration of transgenes of interest into the receptor genome, LV have the potential to disrupt transcriptional active genes or to transactivate neighbouring genome sequences, potentially resulting in adverse effects.
- Due to the use of third generation SIN vectors, the probability of generating RCL is very low.
- Wild-type HIV is not stable in aerosols. There is no documented case of aerosol mediated transmission of HIV. Therefore, no such risk for aerosol mediated transmission is envisioned for LV.
- The use of VSV-G as a pseudotype to generate viral particles with increased stability has the disadvantage of prolonged persistence in the environment. However, lentiviral particles are not stable at room temperature, furthermore VSV-G are inactivated by human serum complement (DePolo *et. al.*, 2000).
- Exposure to the biological hazards of the recombinant vectors could be generated during their manipulation, *e.g.* by accidental parenteral inoculation or exposure of broken skin or mucous membranes to contaminated material.

Considerations related to the use of the CD34+ transduced cells:

Human cells (genetically modified or not) hardly survive in non-optimized conditions of growth, in other words, in hostile environment where control of temperature, CO_2 concentration and osmolality is lacking or where cell-specific nutrients (e.g. glucose, vitamins, lipids) are not balanced or missing. Therefore, the survival of the transduced cells outside of proper conditions is unlikely to occur.

Considerations related to the use of animals injected with the transduced cells:

- Injection of CD34+ transduced cells to mice enhances the risk of accidental parenteral inoculation and enhances the risk of generation of infectious aerosols.
- Before transduction with LV, the absence of homologue, helper virus and complementary viral sequences in CD34+ cells should be tested. Virus p24 concentration should be measured by enzyme-linked immunosorbent assay (ELISA).

7. Class of risk of the resulting GMM

Vector producing cells HEK-293T and recombinant replication deficient LV in a reduced laboratory scale (research and development) are of class of risk 2.
CD34+ transduced cells are of class of risk 2.

8. Class of risk of the activity

During this operation, CD34+ cells, have been harvested, purified and genetically modified, before injection to mice. This *ex vivo* phase of the treatment on one hand clearly reduces the risks related to *in vivo* delivery of LV, but on the other hand in itself harbour potential biosafety risks during cell manipulation. After the transduction, remaining free viral particles from the inoculum may be present for a certain period of time which depends on different factors (*e.g.*, incubation time and temperature, the VSV-G envelop, incubation with serum, viral load) (Higashikawa and Chang, 2001; COGEM, 2009).

The activity involves on one hand, standardized manipulations (*e.g.*, cell culture, production of recombinant viral vectors) on laboratory scale and on the other hand, non-standardized manipulations (other than routine activities) such as injection of transduced cells to animals. These manipulations enhance the probability of exposure to the LV.

The activity can be assigned to class of risk 2.

9. Recommended containment measures

The manipulation of LV and transduced cells requires the implementation of a containment level (CL) 2 added with the following specific measures and work practices:

- LV and cell cultures should be manipulated in a class II biosafety cabinet (BSC).
- The use of gloves is required to manipulate LV and transduced cells.
- Work with needles and other sharp objects should be strictly limited and workers should never recap nor remove needles from syringes. Removal of the syringe should occur by means of hand free operations (i.e. hands do not touch the needle) into a closer container.
- Work surfaces are cleaned and decontaminated with an appropriate disinfectant after work is finished and after any spill of biological material. Appropriate disinfectants for inactivating lentiviral vectors on surfaces include 1% sodium hypochlorite, 2% alkaline glutaraldhehyde or 70% ethanol.
- Transport of LV or LV transduced cells within the facility must occur in double packaging. Primary leak-proof receptacle must be packed in secondary packaging in such a way that, under normal transport, it must be unbreakable.
- Injection of CD34+ transduced cells to mice is performed in a class II BSC under CL2 conditions. Appropriate personal protective equipment should be worn, including double gloves and protective gown. Animals are preferably anesthetized or puncture resistant gloves are worn.

Mice injected with LV transduced cells are allowed to be kept in a CL1 animal house and housed in filter top cages because the absence of vector shedding has been demonstrated by different approaches (Scaramuzza *et al.*, 2013).

10. Reference and further reading

COGEM, Inschaling van laboratoriumwerkzaamheden met lentivirale vectoren (*090331-03*), 2009, available at: http://www.cogem.net/index.cfm/nl/publicaties/publicatie/inschaling-van-laboratoriumwerkzaamheden-met-lentivirale-vectoren

DePolo NJ, Reed JD, Sheridan PL, Townsend K, Sauter SL, Jolly DJ, Dubensky TW Jr. VSV-G pseudotyped lentiviral vector particlules produced in human cells are inactivated by human serum. *Molecular Therapy*, 2000, 2, 218-222.

Higashikawa F and Chang L-J. Kinetic analyses of stability of simple and complex retroviral vectors. *Virology*, 2001, 280, 124-131.

Marangoni F, Bosticardo M, Charrier S, Draghici E, Locci M, Scaramuzza S. Evidence for long-term efficacy and safety of gene therapy for Wiskott-Aldrich syndrome in preclinical models. *Molecular Therapy*, 2009, 17, 1073-1082.

Pauwels K, Gijsbers R, Toelen J, Schambach A, Willard-Gallo K, Verheust C, Debyser Z, Herman P. State-of-the-art lentiviral vectors for research use: risk assessment and biosafety recommandations. *Current Gene Therapy*, 2009, 9, 459-74. Available online: http://www.eurekaselect.com/70629/article

Rothe M, Modlich U, Schambach A. Biosafety challenge for use of lentiviral vectors. *Current Gene Therapy*, 2013, 13, 453-68.

Scaramuzza S, Biasco L, Ripamonti A, Castiello MC, Loperfido M, Draghici E, et al. Preclinical safety and efficacy of human CD34+ cells transduced with lentiviral vector for the treatment of Wiskott-Aldrich Syndrome. *Molecular Therapy*, 2013, 21, 175-184.