UNIVERSITY of GLASGOW

ADVance Adenoviruses as clinical treatments
Marie Curie Initial Training Network Contract n° FP7-290002

http://www.gla.ac.uk/researchinstitutes/icams/postgraduateresearchopportunities/mariecurieitnadvance/

ADVance Newsletter n° 3

Summary of this issue:

Editorial of the Coordinator

Welcome to the third ADVANCE newsletter.

We are now a little more than half way through the ADVance programme and have a number of important scientific achievements under our belt. The midterm of the programme was marked by a review with the European Commission (REA) and an external expert. This was a one day intensive meeting with all ESR/ERs and PIs in Paris and the overall conclusion was that ADVance is progressing very well. Such reviews also represent the chance to learn and build on our network's strengths and opportunities, in order to maximize the potential for the remainder of the programme. This will be reflected in the upcoming training events, journal publications and exhibitions. Since the last newsletter, three important training events have taken place; "Academy meets Industry" Training Course in Leiden (Sep 2013), "Switching Career from/to Academia or Industry" in Evry, Paris (Mar 2014) and most recently "Adenovirus vectors for gene transfer and therapy - Application in animal models of myocardial ischemia" Practical Training Course in Kuopio, Finland (April 2014). Feedback from our ESR/ERs after each event has been excellent, indicating the calibre of speakers, tutors and organisers involved. In the following pages we provide a more in-depth look at what happened during the aforementioned training events, updates on ESR/ER research projects, dissemination activities undertaken and news about upcoming meetings, as well as an updated list of our ESR/ER's publications.

We thank you for taking the time to keep up to date with ADVance and remind you that for frequent updates, you can like and follow ADVance on Facebook and Twitter:

https://www.facebook.com/ADVance ITN

https://twitter.com/ADVance_ITN

We look forward to seeing everyone at the next consortium level meeting, to be held in Barcelona in November 2014!

Until then, let's keep the communication and adenovirus science going well!

Best wishes,

Andy & Karen

ADVance Events

Past trainining events

- Workshop: ***Virus Infection Biology & Adenovirology***
  Zurich, March 12 – 22, 2013.

Organizers: Urs Greber & Andrew Baker

This was the first ADVance theoretical and practical workshop organised since the beginning of the ITN. In the theoretical part of the course, several lectures delivered by ADVance PIs and external speakers gave an overview of the scientific and technical aspects of viral infection focusing on the involved factors. The first complementary skills were provided to the ESRs and ERs with tutorials on research management and scientific writing (aricles and grant applications). The practical part delivered siRNA-based technologies by using reverse transfections and quantification of metabolic/infection state of cells with high throughput approaches, data analyses and data interpretation.

An extended report on the workshop, written by Rodinde Hendrickx and Nicole Stichling, is downloadable from the Glasgow University and EASCO websites.

- Workshop: ***Academia meets Industry***
  Leiden, September 23-27, 2013

Organizers and main tutors: Jerome Custers, Menzo Havenga & Joanna Lubelska

In this Batavia/Crucell co-organized workshop, the ESRs and ERs spent one week in the environment of two Leiden companies. Keynote speakers developed industry-related topics (entrepreneurship, intellectual property, translation of science into business, valorization, regulations, quality insurance…) with scientific subjects related to adenoviruses and other basic and applied virology. The issues related to careers in both industry and academia were also presented during the half-day session organised by EASCO. Practical aspects of industrial scale Ad technologies were shown in the facilities of the two companies. The ESRs and ERs had the opportunity to give oral presentations - one showing their research data and another one talking about concepts of the quality of research and on their own future career. Finally, for the social event on the last evening, dinner was split into three lovely restaurants in Leiden and combined with a boat tour through the city.

Dragomira Majhen (Post-doc at Crucell) and Karsten Eichholz (PhD student at CNRS in Montpellier) wrote an **excellent extended report** on this workshop, as an annex of this Newsletter issue, that is also dowloadable from the *ADVance* webpages of the Glasgow University or from EASCO websites[1].

[1] If you do not get the link by clicking directly of the text, copy and paste the following links:
http://www.gla.ac.uk/media/media_329364_en.pdf or
http://easco.org/home/sites/default/files/files_rep/pdf/Leiden%20workshop%20report%20final.pdf

▪ Workshop: ***Switching career from / to academia or industry***

Evry-Paris, March 5-6 2014

Organizers: Karine Charton, Otto Merten & Mauro Mezzina

This workshop was organized in connection with the *ADVance* mid-term review meeting held on 4th March at the Hôpital Pitié Salpêtrière in Paris.

During this event we further developed the issues related to careers in academia and in industry, which we started to discuss with the fellows during the EASCO session in the abovementioned workshop in Leiden.

The program was established according to the training needs that the *ADVance* fellows expressed in their presentation in Leiden. Therefore, in the workshop in Evry-Paris we developed the following approaches:

(i) Firstly, the *ADVance* fellows met professionals from bio-entrepreneurship, two of them belonging to CELLECTIS (**CD**, see the list of teachers below) and OBE Therapy (**IH**) and a third one (**PR**) is a company builder/developer, who shed some light on the world of business and shared their experience.

(ii) Secondly, the fellows learned the basic rules of scientific writing through: (i) an overview (by **EL**) on the system of scientific publications today, dealing with the present communication environment and the requirements of the scientific journals. (ii) How to exploit scientific writing skills for a competitive grant application during the session on 6th March, namely, long/short-term fellowships (Marie Curie, EMBO, BIF, FEBS and FEMS fellowships).

(iii) Thirdly, the fellows visited the facilities of the **Généthon Bioprod**, and were exposed directly to a pharma company (i.e., the work organization, technologies and equipments), in the Evry's campus; the place where the genomic era flourished and gene therapy technologies were pioneered.

(iv) Finally, two seminars, by **AB** and **SP** provided the fellows with information on: (i) the scenario of careers in academia and industry, (ii) methods to set up a career plan to move on into the labor market and (iii) how to manage a research project and a research team the strategy of research.

List of teachers (name, initials organization and city) :

Amandine BUGNICOURT (**AB**), ADOC TALENT MANAGEMENT, Paris
Christophe DELENDA (**CD**), VP Program Planning & Scientific Audit at CELLECTIS, Paris
Itzik HAROSH **(IH),** CEO at OBE Therapy, Evry
Eric LICHTFOUSE (**EL**), Chief Editor, Head of Communication Department at INRA, Dijon
Otto-Wilhelm MERTEN (**OWM**), Dpt of Applied Vectorology at GÉNÉTHON and EASCO Secretary
Mauro MEZZINA (**MM),** Research Director at CNRS / EASCO manager
Sophie PELLEGRIN (**SP**), Association AGB-Intelli'agence, Paris
Partick ROUSSEAU (**PR**), SPRL-S Rousseau, Charlton & Associés, Gosselies (B) and EASCO manager

An **extended report** on this workshop, describing the content of the seminars and discussions, the profile of lecturers and the visit of the Généthon Bioprod, is available as an annex of this Newsletter and it is dowloadable from the *ADVance* webpages of the Glasgow University or from EASCO websites[2].

[2] If you do not get the link by clicking directly of the text, copy and paste the following links:
http://www.gla.ac.uk/media/media_329364_en.pdf (Glasgow web site) or
http://easco.org/home/sites/default/files/files_rep/pdf/Evry_Paris2014_workshop_report_final.pdf (EASCO web site)

▪ Practical course: ***Adenovirus vectors for gene transfer and therapy - Application in animal models of myocardial ischemia***

University of Eastern Finland, Kuopio, 22-25 April 2014

Organizers: Minna Kaikkonen

and **Seppo Ylä-Herttuala**

In this section we provide an overview of the scientific and technological content of the course and summary of the activities done during the four days in Kuopio. Further description on the activities is available in the **course book** that the colleagues of Kuopio have produced for the attendees. The book contains details of the experimental approaches used, relevant slides of the first lecture on the ethics on animal experimentation and that of on the production and purification of adenoviral vectors. The book is available online at **http://www.gla.ac.uk/media/media_329364_en.pdf**.

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Context of the course: Cardiovascular gene therapy is no longer a dream, but an emerging reality as has been seen in recent clinical trials. Among the successes, Ad-mediated myocardial gene transfer has proven its potential for the treatment of ischemic heart disease, which is among the leading causes of mortality in the industrial world. Pre-clinical studies using animal models played an important role in the evaluation of efficacy and safety of gene therapy before entering into human clinical studies.

The aim of the *ADVance* course in Kuopio was to provide an overview of large-scale, clinical-grade Ad vector production and to introduce students to mouse and pig

animal models of myocardial ischemia. Imaging technologies, such as angiography, ultrasound and magnetic resonance imaging, were also presented in order to demonstrate efficacy and heart function. The ESRs and ERs acquired expertise on the latest approaches of Ad-mediated gene transfer into the cardiovascular system and, at the same time, enjoyed the natural beauty surrounding the University campus as well as the friendly conviviality of the tutors and organizers of the AIV Institute.

Agenda of the activities

Time	Tuesday 22.4	Wednesday 23.4	Thursday 24.4	Friday 25.4
9.00- 11.00	Ethics lecture (1h) Introduction to **Demo I** (1h) **BT2 2440**	ER/ESR presentations (5 x 15') Introduction to **Demo III** (30') **BT2 2440**	ER/ESR presentations (8 x15') **BT1 3208**	Seminar **TTA**
11.00-12.00	Lunch			
12.00 - 14.00	**Demonstration I** **Lab Animal Centre**	**Demonstration III** **Lab Animal Centre**	**Demonstration IV** (2h30) **Tehnopolis S**	Seminar **TTA**
14.00-14.30	Coffee break **Snellmania**	Coffee break **Snellmania**		Farewell coffee **Tietoteknia**
14.30- 17.00	**Demonstration II** **BT2 2440**	**Demonstration III** **Lab Animal Centre**		
17.00-18.30			Poster session + aperitif **Bioteknia 1 lobby**	
18.30			Dinner **Tiukanlinna**	

1. Presentation of the demonstrations of the course

Demonstration I: mouse myocardial ischemia and ultrasound imaging

In this demonstration we first saw (via video), how an acute myocardial ischemia is caused in a mouse by permanently ligating the left anterior descending (LAD) coronary artery (Gao et al. 2010). The progression of the disease model and the morphology of the myocardium at different points in time after the operation has been shown. After going through the operation we visited the animal facility to see how myocardial ischemia corresponds to the function of the heart. This was visualized by echocardiography with small animal ultrasound Vevo2100 (Visual Sonics Inc., Ontario, Canada). After assessing the function of the heart, we performed a gene transfer experiment with an angiogenic factor to increase the perfusion in the ischemic myocardium. Gene transfer was carried out in ultrasound guidance in a closed-chest manner and visualized by echocardiography (Huusko et al. 2010).

Images of acute myocardial infraction in mouse and of the effect of injection of AdV vectors into mouse myocardium are available in **pp. 25-27 of the course book**.

References cited in the text:

Gao E., Lei Y.H., Shang X., Huang Z.M. Zuo L., Boucher M., Fan Q., Chuprun J.K., Ma X.L. & Koch W.J. 2010. *A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse*. Circulation Research, vol. 107, no. 12, pp. 1445-1453.

Huusko J., Merentie M, Dijkstra M.H., Ryhänen M-M., Karvinen H., Rissanen T.T., Vanwildemeersch M., Hedman M., Lipponen J., Heinonen S.E., Eriksson U. Shibuya M. & Ylä-Herttuala S. 2010. *The effects of VEGF-R1 and VEGF-R2 ligands on angiogenic responses and left ventricular function in mice*. Cardiovascular Research, vol. 86, no. 1, pp. 122-130.

Demonstration II: mouse cardiac magnetic resonance imaging

Magnetic resonance imaging (MRI) has superior soft tissue contrast when compared to many other medical imaging methods, such as computed tomography and ultrasound. Cardiac MRI is a powerful tool to characterize anatomy and function of myocardium.
Small size, high heart rate and respiration movement make mouse cardiac imaging challenging. Usually, cardiac MRI requires the data acquisition to be synchronized to the heart movement. It is necessary to acquire the data at the same phase of the cardiac cycle at each heartbeat. Typically, cardiac MRI relies on the ECG signal for synchronizing the acquisition. It is also possible to use retrospective self-gating method for cardiac MRI. There, no ECG signal is needed for the imaging and the motion synchronization signal is obtained from the non-triggered MR data and used in the image reconstruction phase. Usual cardiac MR images are cine images which are movies of the beating heart revealing the anatomy and function of the heart. From the cine images, one can calculate for example the end-systolic and end-diastolic volumes, stroke volume and ejection fraction, which give direct information about the heart function. In this demonstration, cine imaging of a normal mouse heart has been performed. The demonstration showed how the mouse is anesthetized, ECG needles are placed, respiration triggering is done, and how to obtain short axis cine images, what needs to be taken into account in the measurements and how to analyze the data.

See the **course book** **pp. 27-34** for details and pictures relative to this demonstration

Demonstration III: Ad gene transfer to ischemic porcine myocardium

This demonstration began with a brief introduction to porcine myocardial ischemia models. We proceeded over acute and chronic models of myocardial ischemia, and performed these operations. The current data and histology about angiogenic adenoviral gene transfers to ischemic porcine myocardium were presented. In the beginning of the demonstration, we performed the catheterization of a porcine heart. First, an introducer sheath was placed in the femoral artery of a pig, after which catheters were introduced into the coronary arteries for coronary angiography. This was followed by imaging of the left ventricle using left ventricular cine angiography (LV-CINE) during rest and pharmacological stress. These operations were performed under fluoroscopy using GE Innova 3100 angiography system. Finally, angiogenic adenoviral gene transfer to the ischemic porcine myocardium using MyoStar™ injection catheter was performed. See **pp. 34-41 of the course book** for details and images on this demonstration.

Demonstration IV: production and purification of clinical-grade adenoviral vectors

Many commonly used viral vectors are produced for gene therapy purposes using HEK293

based producer cells. Small-scale production can be performed in a T-flask using plasmid transfection, viral infection or stable cell lines. However, large animal models and clinical studies require large quantities of viral vectors. For this purpose, the only practical way to produce viral vectors is to use bioreactors. After virus production, vectors need to be concentrated and purified. For research purposes viral vectors are routinely used after simple concentration by ultracentrifugation without any following purification steps. However, several modern purification methods, such as anion exchange chromatography, affinity chromatography, and ultrafiltration have been developed to attain better quality of the vectors after large-scale production. This demonstration introduces wave bioreactor for viral vector production. In the bioreactor HEK293 can be grown in suspension to ease the up-scaling of the production. In addition, a demonstration of tangential flow filtration and anion exchange chromatography in viral vector purification, concentration and diafiltration is provided. Finally, we were able to become familiarized with the GMP facilities of Finvector Vision Therapies developed for viral vector production for clinical trials.

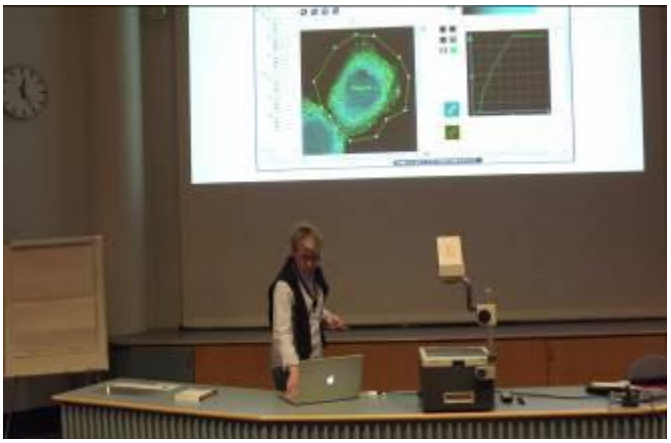
The presentation of Hanna Lesch (**pp 43-51 of the course book**) illustrated the sequences and details of the overall Adv process

Images of the clinical grade production-purification process of AdV vectors in the GMP facilities of the course in the UEF.

Image of five *ADVance* fellows who enjoyed the practical course in Kuopio!

2. SEMINAR PROGRAM on April 25th

9.00-9.10	**Seppo Ylä-Herttuala** (UEF) Opening words
9:10-9:50	**Andrew Baker** (Univ. Glasgow UK), Models of acute vascular injury and their use for intervention studies in gene and RNA therapy
9:50-10:30	**Eric Kremer** (CNRS, France): Gene transfer to the brain for an orphan neurodegenerative disease
10:30-11:10	**Ari Hinkkanen** (UEF): Tumor restrictions to oncolytic virus
12:10-12:45	**Maija Vihinen-Ranta** (Univ. Jyväskylä): Viral strategies for nuclear delivery of their genomes
12:45-13:20	**Varpu Marjomäki** (Univ. Jyväskylä): Quantitative tools and probes to study virus entry and targeting
13:20-13:40	**Hanna Sallinen** (UEF): Ovarian carcinoma clinical trial at Kuopio University Hospital
13:40-14:00	**Hanna Lesch** (FKD Therapies): Large scale AdV production for clinical trials



Coming events in 2014

- Management-based science meeting: ***Assessment of ADVance scientific progress at management level.***

Hotel HCC Montblanc, Barcelona, Spain, 6-7th November 2014

Organizers and main tutors: Andrew Baker, Karen Trofimova & Ramon Alemany

This meeting aims to assess the scientific progress of ADVance at the management level with the interaction of all PIs and trainees. All participants in the ITN (PIs and trainees) will attend this meeting. More information about this meeting will follow.

ADVance research news

In this section the authors describe the status of their projects (rationale, approaches, methods, data obtained and/or expected, future perspectives, etc…), including the collaboration(s) foreseen or in progress during the secondments scheduled in the ADVance program. The articles are written by the ESRs and ERs and supervised by their respective PIs and by the PIs of the receiving laboratories in the case of the secondments.

Research updating

▪ *In vitro and in vivo Mouse Adenovirus characterization and construction of their oncolytic vectors*

Fellow : **Rodinde HENDRICKX**

PI : PD Dr. Silvio Hemmi

Institute: University of Zürich, Institute of Molecular Life Sciences

In the months after the first AdVance workshop in Zürich the research focus was on: (i) the production of Mouse Adenovirus (MAdV), (ii) a pilot study for *in vivo* tropism and (iii) a first study for the development of a luciferase expressing virus. Here we will briefly discuss the most important findings. An outlook of the expected work in the coming months is given as well.

Production optimization of MAdV. Production of MAdV is a relatively lengthy process (6-10 days) with unsatisfactory low yields (output/input 100) as compared to human adenovirus production (3-4 days output/input up to 10^5). Using quantitative PCR (qPCR) we first determined the output of the MAdVs (MAdV-1/-2/-3) that we could obtain with the cell lines described in the literature for this purpose (CMT93, 3T6) Here we found output/input ratios of maximal 100. To optimize the viral yields, i.e., bringing the output/input value to at least 10^3, we decided to follow two approaches: screening cell lines for best infection rates and modifying the innate immune response. Therefore, first, we screened additional cell lines that were found to be well infectable by MAdV-1 in an initial big screen done by S. Hemmi. In this new screen we also analyzed the PancO2 and Trampc cells (received from R. Alemany, IBIDELL, Barcelona, Spain). As an outcome of this screen, the Panc02 cell line was determined to represent an optimized production cell line for MAdV-1. However for the production of MAdV-2 and MAdV-3 we could not identify a cell line exhibiting a better production yield than we had before. In the second

approach, i.e. modifying part of the innate immune response, we hypothesized that the down regulation of the Interferon Regulatory Factor 3 (IRF3) would represent a possible approach to reduce innate immune responses. Phosphorylated IRF3 is a transcription factor involved in induction of interferon-β expression. It has been shown previously that using a lentivirus (LV) construct expressing the NPro protein of Bovine Viral Diarrhea Virus, the IRF3 expression can be knocked out in human A549 cells. We received this LV construct from Dr B. Hale (Center for Virus Research, University of Glasgow, UK) and used it to transfect A549 human and CMT93 and 3T6 mouse cell lines. By Western immunoblotting experiments, we could indeed show a total suppression of IRF3 expression in the human cell line and a down regulation by a factor of 2 for the mouse cell lines. We are currently testing the production of the different virus strains in these IRF3-knockdown cell lines.

Pilot in vivo study. In collaboration with Prof. Dr. P. Stäheli at the Uniklinikum (Freiburg, Germany), we setup a pilot experiment to investigate the *in vivo* replication sites of the three MAdVs. Our aim is to visualize the replication sites by a luciferase output. In a first experiment we used mice that express the firefly luciferase gene under the interferon-β promoter. We infected three adult mice (5 weeks old) and 3 pups (10 days old) with one virus (9 adult and 9 pups in total) and luciferase expression was assessed by imaging these mice every 24 hours as described below. The infection was initiated through intra peritoneal (i.p) injection for pups and adults and additionally intra nasal (i.n) administration for the adult animals. Three control adults and two control pups were part of the pilot experiment as well. Every 24 hours the animals were aneasthesized with isofluran, injected with 200ul of substrate (100ul for the pups) and imaged in an IVIS using different exposure times (10 seconds – 2 min). The weight of the animals was monitored as well. As expected the mice infected with MAdV-1 showed the most obvious disease symptoms (loss of weight, no grooming and long recovery time after aneasthesia) and had to be sacrificed after 8 days. Of the three pups infected with MAdV-1 two were found dead in the cage. The third pup survived and was sacrificed at the end (day 10). Mice infected with MAdV-2 gained weight normally and did not show any disease symptoms. MAdV-3 infected mice did neither gain weight, nor did they lose weight, and they also did not show any disease symptoms.

The reporter mice we used here gave a considerable amount of background signal, most prominently in the liver area. This had also been noticed in earlier studies with other viruses for which these mice were used. In addition, in these studies they reported a high background signal in the thymus.

After ten days all animals were sacrificed and the organs harvested and processed. We will further characterize these tissues by performing qPCR analysis on these samples. Combined with the imaging results we can provide a first preliminary characterization of the *in vivo* tropism of these viruses both in adult and neonate mice.

Luciferase expressing virus. For the *in vivo* characterization of the three viruses described above we relied on an indirect signal induced in the mouse. As discussed above, this can lead to a considerable amount of background signal. One way to circumvent this would be to incorporate a luciferase signal within the mouse adenovirus genome. When introducing any

gene within the viral genome it is crucial that this extension does not lead to a too large genome size incapable of properly incorporating into the capsid. For this and other reasons we proposed to work with Gaussia luciferase (GLUC), a 19.9 kDa protein with a reported 1000x higher luciferase signal in comparison with Firefly or Renilla luciferase. However, since the native form of GLUC is secreted, this would not be optimal for us. In the literature, altered intracellular and membrane bound GLUC variants have been described. These forms together with the native form and a newly constructed membrane bound GLUC by our lab were tested in an initial *in vitro* experiment for their proper localization and signal strength. We found that the newly constructed membrane-bound GLUC was indeed located at the cellular membrane. In addition, the membrane-bound GLUC revealed the most intense signal strength, similar to the parental secreted form. Using this new construct we are now investigating how this GLUC variant can be efficiently incorporated into the viral genome. For this purpose we would like to use protein-fusions constructs with a build-in cleavage site. This would allow complete retention of the wt viral genome and thus hopefully also maintain the wt virulence. Once the initial studies have been completed, we will start with engineering of the MAdVs constructs encoding the optimal luciferase reporter.

Outlook In the upcoming month we aim to focus on the further *in vitro* characterization of MAdVs, most prominently to look at the cellular innate immune response upon virus infection. Also, we would like to characterize infection by identifying the time points of protein expression of the different viruses. Furthermore, we would like to focus on the development of a MAdV-1 expressing luciferase virus.

Collaboration and secondments

Secondment n°1: from **Batavia Bioservices** (Leiden, NL) to the Institute of Cardiovascular and Medical Sciences at the **University of Glasgow** (UK)

Title of the project: ***Analysis of the action mechanism of small molecules in enhancing AdV-mediated gene transfer***

Fellow involved: **Agnieszka LIPIEC**, Ph.D, Batavia Bioservices, Leiden

Dates: October 1st – December 31st, 2013

Supervisor(s): Dr Raquel GARCIA and Prof Andrew BAKER

Background The safety and effectiveness of Adenovirus-5 in intravascular delivering is hampered by the high propensity of the Ad5:FX complex to transduce the liver. However, we can take advantage of this high transduction capacity if we deliver the Ad5:FX complex *ex-vivo*, instead of intravascular injection. This type of strategy can be very attractive for the treatment of cardiovascular diseases (vein graft failure), because delivery the gene of interest *ex vivo* before surgery, enable to avoid the problems related with the intravascular injection. The coating stents with AdV5:FX:compound enable direct virus uptake in the vessel wall.

Professor Andrew Baker's virus group have been identifying small molecules enhancers of FX-mediated Ad5 gene transfer using a high throughput approach to screen a library of over 10000 compounds in search of the ones which increase virus transduction. The compound T5998260 (Figure 1) has been discovered to increase the virus transduction in three different cell lines significantly.

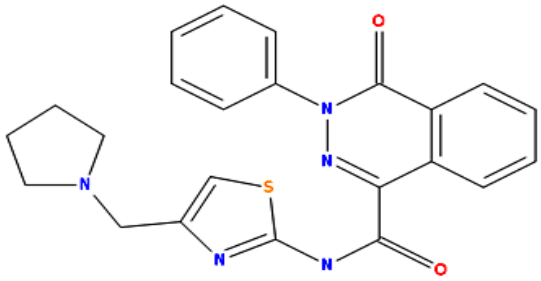


Figure 1: Structure of T5998260 (mw: 431.5). this compound has been identified to enhance virus transduction

Approaches/methods We shall follow different strategies to understand how a compound enhances the transduction of the Ad5. For this, the following *in vitro* assays are being performed and will be used in the project of my secondment.

- **Transduction assay** - to confirm the results obtained in previous experiments. Infection of SKOV3 cells with Ad5-LacZ will be performed in the presence or absence of the

compound and the FX. β-gal colorimetric assay is used for quantifying β-gal activity in cells after Ad5 transduction.

- **Trafficking assay** - to observe the enhanced properties of the compound in the colocalization of the virus with Microtubule Organization Center (MTOC). Virus infection (Ad5 labelled with Alexa red) of SKOV3 cells will be performed in the presence or absence of the compounds and the FX after incubation at 37° C and at 4° C for 1h. The confocal microscopy will be used for visualization of the complex.
- **Binding assay** – to understand if the compound helps the virus in the initial steps of transduction. SKOV3 cells will be infected with Ad5-LacZ and incubated at 4° C for 2 h in the presence or absence of the compound and the FX. QPCR method will be used for quantification of viral particles.
- **Human Primary cells (smooth and endothelia cells) transduction assays** - This approach will be employed to study if this compound enhances the transduction in vascular cells and to envisage eventually the use of this molecule as a drug helping the delivering of the virus in vein graft.

Competences/complementarities of the sending (BATAVIA) and receiving (UGLA) institutions. This secondment will exploit the following approaches and competences.
Batavia Bioservices is a for profit organization with unique expertise, knowhow and technologies to develop Biopharmaceuticals such as antibodies, proteins, vaccines or vectors. The company is capable of taking a project from concept idea (DNA cloning) to delivery of clinical material for phase I studies in humans. The field Batavia Bioservices operates in is highly controlled and compliance to set quality parameters is of the utmost importance to deliver biological candidate medicines for clinical testing. A glimpse of that regulated world was shared with all participants in the *ADVance* ITN workshop in Leiden (September 2013). During the first year of my tenure at Batavia Bioservices, I have acquired the full skill set in manufacturing recombinant replication deficient adenoviral vectors for vaccine and Gene therapy purposes. This includes production, purification and quality testing. Also, I have been trained by the company on multiple aspects regarding good research practice and GMP documentation which are skills pivotal in a product R&D and manufacturing environment.

At Glasgow University, the team of Prof Andrew Baker represents one of the leading academic groups pioneering novel cardiovascular intervention strategies. One of the research programs in the Baker lab is to investigate the safety and efficacy of recombinant replication deficient adenoviral vectors. The complementarities may thus be evident: The tools I have learned to generate at Batavia Bioservices are being tested in the Baker lab for their safety profile in cardiovascular disease settings. Likewise, the Baker lab is equipped with all models know and expertise to demonstrate efficacy in halting or reversing the onset or disease progression in the field of cardiovascular anomalies. In this program I have thus learned all aspects of adenoviral vector manufacturing and have the opportunity to test such vectors in pre-clinical settings for their efficacy and safety.

Results. Our goal was to find out the mechanisms of the enhancement by the hit compound of Ad5 transduction that may modulate gene delivery into human cells in view to

develop new drugs exploitable in medicine. The figures below resume the approaches and results obtained.

Figure 2.

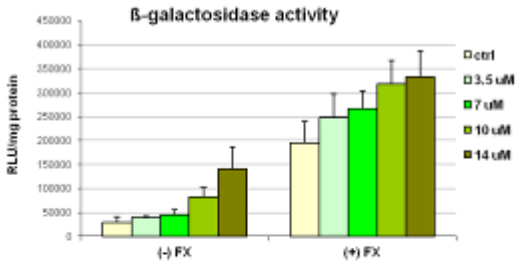


Figure 3.

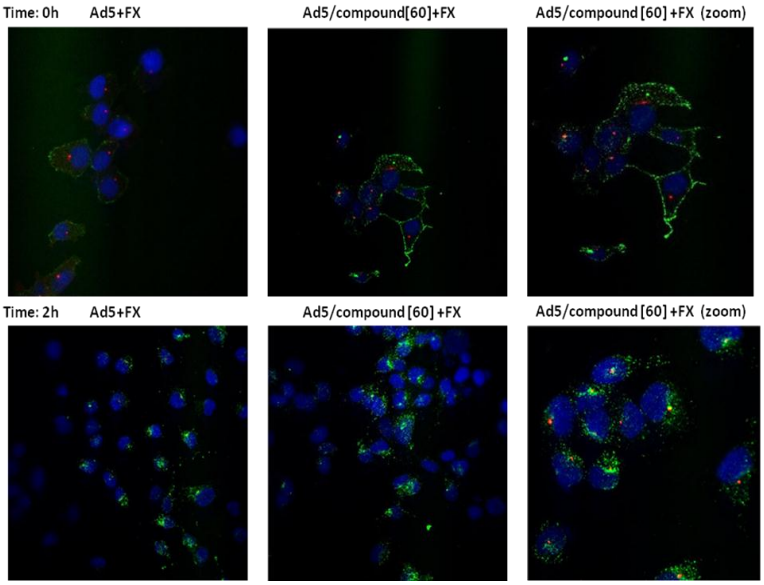


Figure 4.

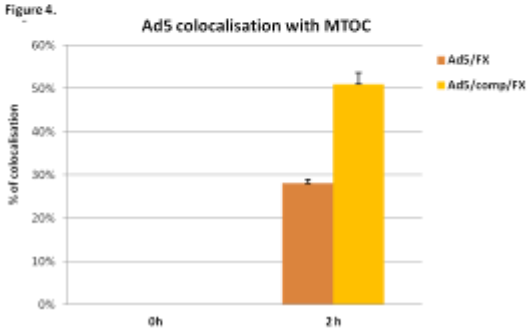


Figure 2: B-galactosidase activity quantification in SKOV3 cells infected with Ad5 +/-FX with different concentration,

Figure 3: Trafficking assay - SKOV3 cells infected with Ad5 in the presence FX and with or without compound T5998260 [60],

Figure 4: Quantification of the collocalization of Ad5 with pericentrin protein, a major component of the Microtubule Organization Centre (MTOC), in the presence/absence of compound T5998260 [60].

Personal comments The secondment at the University of Glasgow has been an excellent chance for me to meet more international people in the field of the adeno-vectorology and their applications in cardiovascular system. Although this tenure in Prof Andrew Baker Institute was short, my interaction with Dr Garcia and all other members of the team will broaden my scientific culture with new methods/techniques used in adenovirus studies. I am sure that scientific environment and the spirit of multinational cooperation will surely open my mind to new challenges and innovative working approaches. Moreover, staying in Glasgow is a unique opportunity to visit Scotland, improve my English and learn more about the Scottish people and their culture.

Proposed secondment n° 2: from the **University of Zurich** (CH) to the **VMRI**, Budapest (HU)

Title of the project: **Identification and characterization of new mouse adenovirus species**

Fellow involved: **Rodinde Hendrickx**, University of Zurich (Prof. Silvio Hemmi)

Dates: March 10th- 21st 2014

Supervisor(s): Prof. Maria Benko

Within the ADVance network, exchange of expertise among the participating laboratories has been highly encouraged. For this and other reasons, we organizeda short stay at the VMRI to learn about the molecular genetic characterization and isolation procedure of (new) mouse adenovirus types and variants from wild mice.

Mouse adenoviruses (MAdVs) are the vectors of interest for my thesis project. The *in vitro* and *in vivo* characterization of the three currently known MAdVs (MAdV-1/-2/-3) is within the scope of this project. MAdV-1 and MAdV-2 have been isolated in the 1960s from the house mouse (*Mus musculus*), then a third MAdV was discovered in 2009. MAdV-3 was isolated from a wild field mouse (*Apodemus agrarius*). Although originating from different hosts, MAdV-1 and MAdV-3 were found to share a high degree sequence similarity. Compared to them, MAdV-2 has a significantly longer genome. Phenotypically the three viruses differ substantially. Whereas MAdV-1 has been found to be fatal in some strains for both adult and neonate mice, MAdV-2 and MAdV-3 have not been associated with any disease so far.

In line with my project, my secondment in the group of Prof. Dr. Maria Benkö at the VMRI was aimed to get a thorough introduction into the procedure of detecting and isolating viruses from wild mice. In the two week stay at the institute I gained insight in to sample gathering, handling and the analysis of (new) adenoviral sequences. Furthermore the more thorough alignment analysis and the generation of phylogenetic trees was practiced.

The acquired knowledge on how adenoviral screenings are performed has broadened the quality and diversity of experiments in Zürich and further strengthens the collaborations between the two laboratories.

Dissemination articles

The two articles in this section aim at disseminating the scientific information to non-specialists readers. The articles were contributed by two ESRs, who made the effort to writeusing simple and clear statements understandable by everybody to convey the scientific message: from high-school students, for instance, to professionals of all fields and backgrounds and to other readers who are unfamiliar with the scientific language.

The ADVance readers will recognize how different the style is here compared to that of a scientific paper destined to specialists and the ESRs and ERs – but not only them! – are invited to contribute to such dissemination articles in the next Newsletter.

Polioviruses: new candidates anticancer gene therapy

Lukasz Kuryk, Oncos Therapeutics Ltd., Helsinki, Finland

1. What viruses are: Viruses are very small infectious organisms that replicate only inside the living cells they infect. Viruses can infect all types of living organisms e.g. bacteria, plants, animals and humans, which, once infected, are the hosts of viruses. When viruses infect host organisms, the physiological equilibrium of the latter is altered and most viruses may cause diseases. For example, influenza, the very common disease amongst all people worldwide commonly known as the flu, is due to the infection of one of these organisms called *influenza virus*.

The shapes of viruses range from simple forms to more complex structures (figure 1, upper row). Their size is 1/10-1/100 of the average size of a bacterium, i.e. 20-500 nm. Therefore, they can be visualized only by the electron microscope (figure 1, lower row).

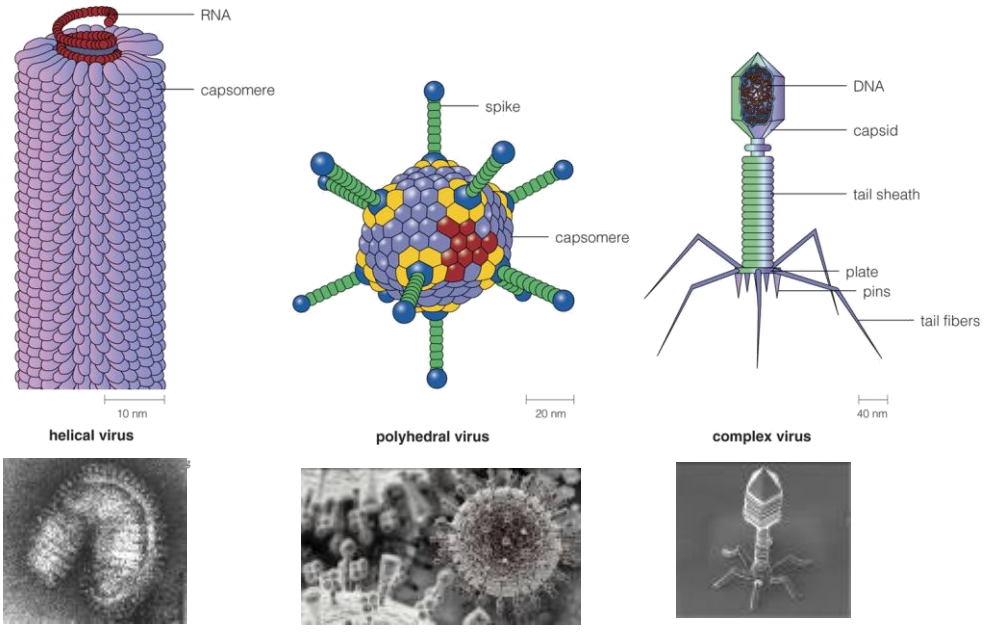


Figure 1:

Upper row: Shape of (from left to right) helical, polyhedral and complex viruses. The main parts of the viruses (DNA or RNA, coat, capsid and other parts of the complex viruses) are indicated.

Lower row: Electron microscopy photographs of the corresponding shaped virus.

A virus consists of the following parts: 1: the genetic material of DNA or RNA that carries genetic information, 2: a protein coat that protects the genetic material and, in some cases, 3: a protein capsid which is the external cover of the viral particle.

Therefore, according to their structure and biology, viruses infect different species of living organisms and may induce different pathological conditions. However, although many viruses are dangerous and cause life threatening diseases, scientists have developed new approaches/techniques that now allow us to use several viruses as therapeutic tools to cure many human diseases and, namely, degenerative disorders such as **cancer**.

2. Virotherapy. This is the name of the abovementioned approach that enables the use of viruses as therapeutic tools in the experimental medicine. Basically, this approach consists of genetically modifying the viruses in a such a way that they lose some functions and acquire some other ones. Indeed, virotherapy can be split into three main branches: 1: The development of oncolytic viruses, to selectively kill cancer cells and to be used thus as antitumor agents, as described in the chapter 4. 2: Gene therapy, that involves the use of different viruses and techniques to deliver genes or factors that change the gene expression of the host cells and tissues to cure genetic diseases and other disorders (see Rodinde's article in this section of the newsletter). 3: Viral immunotherapy is a third branch where viral vectors deliver immune system-targeting factors to change the immune responsiveness of the hosts. Thus, new vaccines may be generated and many diseases may be cured with the immunotherapy.

3. The viruses-to viral vectors transition. What is a viral vector? Is it a virus? **Yes and no**. Yes, because it has the same component and structure of a virus: DNA or RNA, the protein coat and/or the capsid, and it infects organisms with the same efficiency of viruses. No, because it has lost the pathogenicity that most viruses have, i.e. they no longer induce diseases in the hosts. Therefore, we may use viral vectors in exploiting their viral properties to efficiently infect organisms in order to deliver exogenous genetic materials into them. This process, called commonly **gene transfer**, can be performed directly into tissues and organs or into cells cultured outside the body. In other words, these vectors, like the viruses, are able to efficiently transport their genomes inside the organisms they infect and, unlike viruses; they do not induce diseases in the host organisms. When we read papers in the literature on this topic, the whole gene transfer procedure is called *transduction* and the cells or tissues that have been received the exogenous genes in this way and they express them are termed *transduced cells* or *transduced tissues*.

Paul Berg, an American biochemist and virologist Nobel Prize in 1980, was the first scientist in the world who, in the 70's, modified the genome of a virus that had some primate species as host (called SV40 virus) to extend its infectious spectrum to cells and tissues of monkeys and humans. At that stage, Pr Berg initiated the molecular virology era to convert viruses into gene transfer vectors.

4. Oncolytic viruses. Let us focus now on those viruses used (or that may be used) in cancer therapy. Such viruses are called **oncolytic viruses**, because they infect and kill cancer cells

only. This property is linked to their biological property to proliferate, according to their normal lytic cycle, in rapidly dividing cells, as cancer cells, whereas they are unable to replicate in resting normal cells.

As shown in figure 2, when the virus particle infects cancer cells, it proliferates and the infected cells are destroyed by the lysis as result from the viral cycle. The lysed cells then release new virus particles that infect surrounding cells and, thus, they help to destroy the tumour. Oncolytic viruses are used also to stimulate host anti-tumor immune responses and, thus, they are also used in an immunotherapy approach.

In fact, some oncolytic viruses are very immunogenic and may, by infecting the tumours, they elicit a strong anti-tumour immune response, especially the viruses that deliver cytokines or other immune-stimulating factors

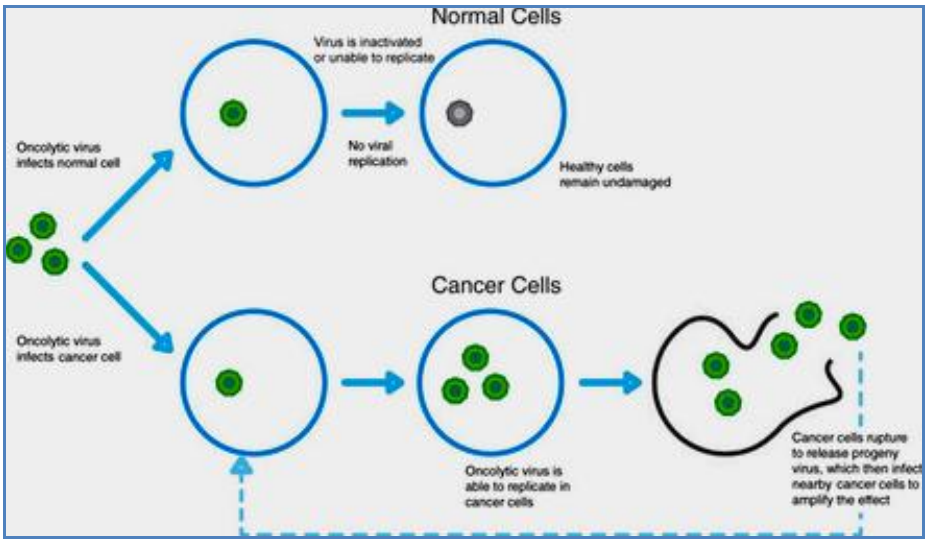
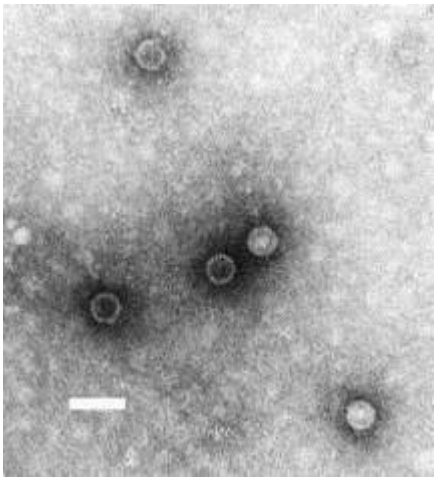


Figure 2. Life cycle of oncolytic viruses.

5. Poliovirus. Among the many candidates to be used in virotherpy, one of them is poliovirus, a virus hosted by humans. It is member of the family of viruses called *Picornaviridae* and it consists of RNA genome and a protein capsid , as shown in figure 3.



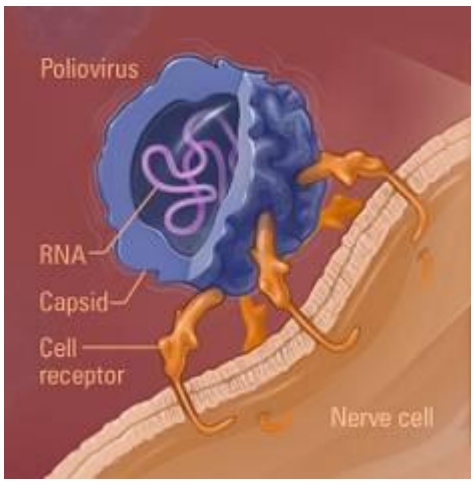


Figure 3.

Left image: electron microscopy micrograph of poliovirus virions (white bar: 50 nm).

Right image: schematic representation of the poliovirus parts combined with host nerve cell.

5.1. Poliomyelitis. The popularity of poliovirus and the interest of the scientific community at global level on it is because this virus is responsible for the occurrence of the poliomyelitis, a highly contagious and devastating disease that, spreading in epidemic mode, disabled or killed several millions people worldwide between the late XIXth century and half of the XXth. The virus attacks the nervous system, and can cause total paralysis. The virus enters the body through the mouth (fecal-oral route) and multiplies in the intestine. Typical symptoms

are fever, fatigue, headache, vomiting, stiffness in the neck and pain in the limbs. One in 200 infections leads to irreversible paralysis. Among those paralyzed, from 5% to 10% die when their breathing muscles become immobilized. There is no cure for poliomyelitis and it can only be prevented by vaccination by administering early in children anti-polio vaccines multiple times, and the vaccination protects a child for all life.

. In 1988, the World Health Assembly adopted a resolution for the worldwide eradication of poliovirus. It marked the launch of the Global Polio Eradication Initiative (GPEI), spearheaded by national governments, WHO, Rotary International, the US Centers for Disease Control and Prevention (CDC), UNICEF, and supported by key partners including the Bill and Melinda Gates Foundation.

Overall, since the GPEI was launched, the number of cases has fallen by over 99%. In 2013, only three countries in the world **remain "polio-endemic"**, i.e. that the disease is regularly found among particular populations and/or in certain geographical areas: Nigeria, Pakistan and Afghanistan. Additionally, the disease can be transferred from endemic countries into other countries such as Ethiopia, Cameroon, Somalia, Kenya, Niger, Chad, Syrian Arab Republic, as it has been reported this year (Figure 4). The total number of polio cases have been rated on 359 (as of December 11, 2013).

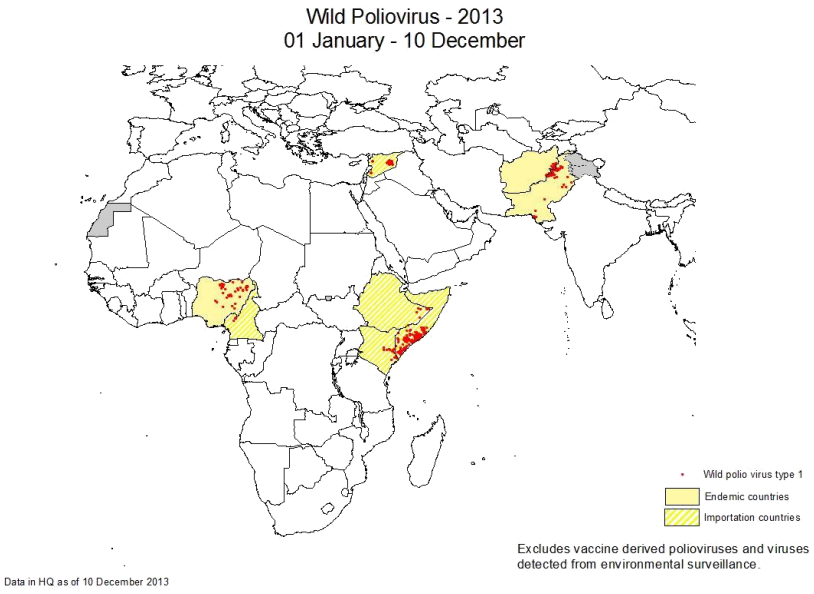


Figure 4: polio cases worldwide today

5.2. Poliovirus as oncolytic vector. As a natural neuropathogen, poliovirus replicates in tumours derived from neuronal cells. The translation of its RNA genome depends on a given sequence that is tissue-specific and is called Internal Ribosome Entry Site (IRES). This sequence is active in cells of neuronal origin and allows translation of the viral genome. If this IRES sequence is modified, we may change the tissue-specificity of poliovirus and allow it thus to replicate in other host cells. This is in fact, what some scientists have performed with their experiments of replacing the poliovirus IRES sequence with that of the Rhinovirus, another virus of the same *Picornaviridae* family, known to cause the common cold . Thus, thanks to this modification, the poliovirus is able now to selectively destroy malignant glioma cells, while leaves normal neuronal cells intact. This opens exciting perspectives to use the poliovirus as an efficient neuro-oncolytic vector .

5.2.1. Is it too beautiful to be true? Although many laboratory experiments demonstrate that polioviruses are able to kill tumour cells, these viruses are still far from being used currently as anticancer agents in clinic. Oncolytic vectors in general are not perfect and a considerable work is still necessary before their use as new anticancer therapeutics. The following criteria are necessary to meet:

(i) *Safety*: when viral vectors are derived from pathogenic viruses, they must be modified in such a way as to minimize the risk of residual pathogenic effect in host body.

(ii) *Low toxicity*: The viral vector must produce maximal killing effect of target cancer cells and minimal side perturbations of resting cells physiology.

(iii) *Stability*: Some viruses have a fragile physical structure (weak cover envelope and/or capsid), which reduces their therapeutic effect and may increase their genetic instability, with increased risk of becoming pathogenic for host organism. Engineering physical components of the viruses must be achieved to overcome these drawbacks.

(iv) *Cell type specificity*: The infectivity of oncolytic vectors must target tumour cells instead of normal ones.

(v) *Identification*: Optimization of marker genesis is necessary to follow the fate of vectors in transduced cells and tissues.

Indeed, the approach of viral vectors to deliver genetic material into cells comes with many problems that are still to be solved. The progress of knowledge of the biology of polioviruses and of the responses of host organisms to their infection (namely, the immune response), as well as of the virus technology to maximize the therapeutic benefit and minimize the side effects in patients, will pave the road for the development of these viruses as new anticancer medicines.

An introduction to Gene Therapy

Rodinde Hendrickx, University of Zürich

You, me and all other living organisms have a lot in common when we zoom in. More than you would think. Biologically we are all built up from small building blocks called **cells**. The smallest organisms we know, bacteria, usually consist only of one cell (but they come in large numbers!). Other small organisms like worms can contain up to 2000 cells, whereas for humans the number of cells is more like 1,000,000,000,000 (one trillion)!

Cells are too small to be seen by naked eye. The size of an average human cell is about 30 micrometers, 33 xs smaller than a millimeter! Yet everything that happens in it is crucial for us to live. That is because cells can be thought of as very small factories that produce energy and define what we look like. Cells have a boundary, called a **cell membrane**, which protects the inside from the outside. Through membrane interactions with other cells it stays in place and is also able to communicate with its nearest neighbors. The inside of a cell is very crowded. This factory is filled with all kinds of different particles (molecules) that work continuously to make sure the organism is alive and kicking. The most prominent molecules are the **proteins**. Proteins are molecules that can execute specific tasks with the help of energy. This energy comes from molecules called "**ATP**" (**A**denosine **T**ri-**P**hosphate) that donate their energy when they change their composition by losing a phosphate, i.e., changing from Adenosine Tri-Phospate to **A**denosine **D**i-**P**hosphate and are then called "**ADP**" molecules. There are proteins that form networks inside the cell (such that it is able to keep its characteristic shape), proteins that take care of transport between two different ends of the cell and so on and so forth.

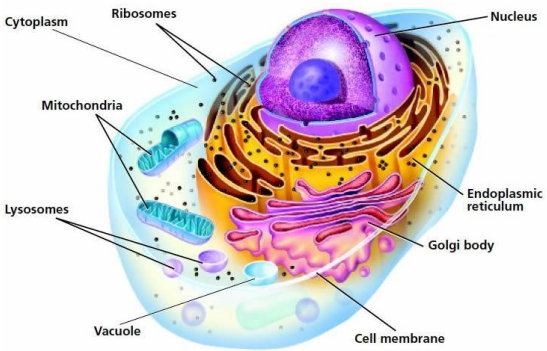


Figure 1: Cartoon of an animal cell. Image taken from *http://www.shwartzlucas.com/*

Not all of the cells in our body are identical. The cells in your eye for example, are very different from those in your lungs. But how do the cells know what kind of cell they should be? Almost every cell has a **nucleus**, the "heart" of the cell. The nucleus is a region inside the cell with a separate membrane. In the nucleus we find a blueprint for determination of the celltype, in the form of a code scientists call the **DNA** (**D**eoxyribo**N**ucleic **A**cid). DNA is a very long string of four different units, which are labeled by the letters **A**, **T**, **G** and **C** (for **A**denine, **T**hymine, **G**uanine and **C**ytosine). You should imagine a string of billions of letters, only using those four units: A, T, G and C! It is almost like a language that has an alphabet of only four letters, but this is enough information for the cell to function.

This blueprint contains the information about our eye color, hair color, the shape of our ears, etcetera. For each of those pieces of information there is a separate 'section' in the DNA

describing it. This section is called a **gene**, and so there is a gene for eye color. This gene may encode that you have blue eyes, but in another person DNA this gene may encode for green eyes.

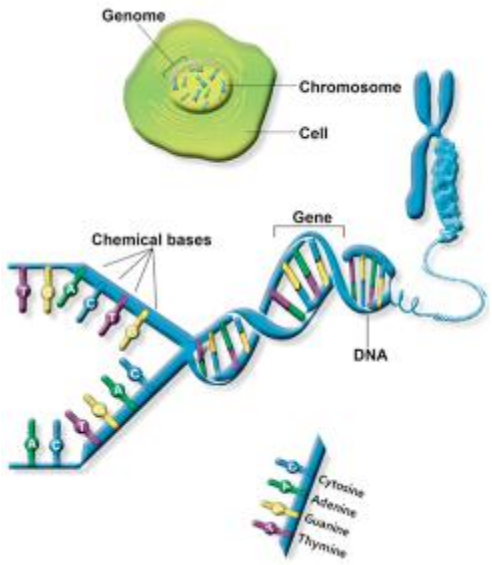


Figure 2: Cartoon showing the localization and structure of DNA. Image adapted from *http://www.coriell.org*

Every cell in our body has the same copy of the DNA, the difference between them are defined by which genes of it are “active”. “Active” means that the DNA is first transcribed into **RNA** (**R**ibo**N**uceic **A**cid) and the RNA is then translated into proteins. So the cells in your lungs know that they should ignore the gene for your eye color. In lung cells, only the genes that contain information for the lungs are switched on and, thus, the proteins necessary for lung functions are produced. This principle applies to every part of our body.

Ever since the discovery of DNA in the ‘50s, scientists have been very eager to unravel its code, and to understand how this seemingly random sequence of A, T, G and C’s define us. Today, a lot can be explained already, but most certainly we will not completely understand this code for a long time to come.

For over 40 years the interest in DNA has shifted from understanding what it is used for, to how can we handle it in laboratory, change it and move it from an organism to another one. Why would one want to change and handle the DNA? There are certain diseases, so called **genetic diseases**, which occur because there is a mistake in one or several of the genes in our DNA. This mistake can have serious consequences when it leads to the faulty production of an important protein in your cells. In these cases the cell will not function normally anymore, and when this happens to many cells in our body at the same time we will get sick. Only when the mistake in the gene(s) is corrected, the patient suffering from one of these diseases can be cured and become healthy. This can be achieved if we are able to handle easily the DNA.

One of the most known diseases is **cancer**. Cancer is the condition in which there are many cells in your body that no longer know how to control themselves, and start multiplying uncontrollably as a consequence. This leads to the death of neighboring healthy cells and therefore makes us very sick. Currently there are only few treatments for cancer, and moreover those therapies are very harsh: they make you feel even more sick. Those therapies are aimed at killing cells that multiply rapidly, because that is what the cancer cells do. Unfortunately other healthy cells in your body divide rapidly too, as your hair for example, and so they too get killed by the therapies.

The knowledge of the genome has helped scientists understand what can go wrong in the case of cancer, namely that cell regulator genes are switched off and the cell dividing

(multiplying) genes are switched on. The approach with gene therapy is that we can insert into the cancer cells genes that tell them to commit suicide. Delivering to the sick cells this killer gene would be like giving them a pill with a poison in it. When this gene is taken up in the cell and gets translated in the nucleus, the cell will die and the good neighboring cells will have enough space again to proliferate.

How to correct a mistake in the DNA, in the case of the genetic diseases and how to introduce into cancer cells a suicide gene? In both cases, we need to introduce genes into the cells: either the correct copy of DNA to produce the proteins that are defective in patients suffering from a genetic disease, or the suicide gene to kill cancer cells in patients suffering from cancers. The techniques to deliver these genes refer the **gene therapy**.

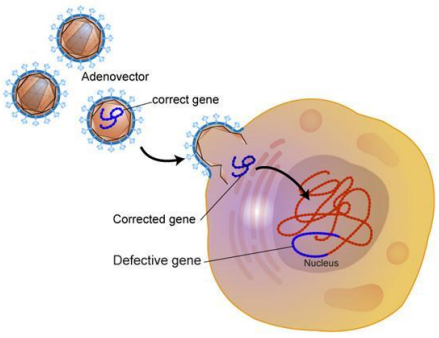


Figure 3: Cartoon explaining gene therapy in its most simple form using Adenovirus as a vector. Image taken from *www.genomebc.ca*

This new therapeutic and promising approaches has been extensively studied for 25 years. However, there are many hurdles to be overtaken before it can be applied. One of the main research topics is what tools to use to bring the gene into the cell. It is very important that the gene gets delivered to the correct cell (only to the “sick” cells) and that it will not be filtered out of your body by either the kidney/liver or immune system before it has a chance to activate the gene.

For this reason one of the most studied tools to deliver genes into the cells and tissues are **viruses**. Viruses are small packages that contain DNA, as the adenoviruses and other smaller associated viruses, or RNA, as retroviruses and lentiviruses. Viruses are not alive alone; they need to get into the cells to transcribe their DNA into RNA, to translate their RNA into their proteins and to make a lot more viruses. However, viruses cannot be used before having been depleted of their pathogenicity, such as respiratory illness and immune deficiencies for example, but they retain intact their infectivity. Therefore, because they can specifically infect cells and bring their DNA to the nucleus they are seemingly ideal for the purpose of gene therapy.

Virus-based gene therapy is and will be extensively studied in the upcoming decade. It is aimed that this boost in research will help scientist to develop new and better cures for diseases like cancer, but also other genetic diseases.

Meetings, Conferences, Seminars

Recent meetings:

- ***Gene, Cell and Molecular Therapies for Inherited Metabolic Disease***

  March 27th 2014 London. Annual meeting of the British Society of Gene and Cell Therapy (http://www.bsgct.org/en/92-bsgt-news/meetings-a-events/408-gene-and-cell-therapy-for-inherited-metabolic-disease-meeting-2015.html)

- 8th International Conference ***Oncolytic Virus Therapeutics***,

  Oxford, UK April 10-13, 2014 http://oncolyticvirusmeeting.weebly.com/

Next meetings:

- ***11th International Adenovirus Meeting***,

  San Diego July 16-20 2014
  http://iam.scripps.edu/_web_iam_docs/Announcement.html

- ***IUMS XVIth International Congress of Virology***,

  Montreal, July 27-Aug 1 2014
  http://www.montrealiums2014.org/side_organization/national_organizing_committee/scientific_program_committees/xvith_international_congress_virology_e.shtml

- ***Early Events in Virus Infection***

  Mte Verita, Ascona, Switzerland August 25-28, 2014.

  Organizers: Urs Greber and Ari Helenius - http://events.mnf.uzh.ch/index.php?id=63

  Travel information: http://www.csf.ethz.ch/travel/index

  Besides well known speakers from the field of virus cell biology, the meeting features high profile interdisciplinary talks, which explore systems biology and screening technologies to unravel the complexity of virus-host interactions.

  The meeting will have an EMBO lecture given by Dr. Steve Harrison from Harvard Medical School.

  *In addition, we will have **11 short talks** chosen from the abstracts. The best poster or talk from a scientist early in his / her career will receive a special CSF (Centro Stefano Franscini) award. Limited travel support will be available for young scientists.*

  Maximal number of participants: 120.

- ***Modern DNA concepts and tools for safe gene transfer and modification***

  Université d'Evry-Val d'Essonne, Evry, France, March 30 – April 03, 2015.

Organizers: Karine Charton (Genethon), Fulvio Mavilio (Genethon), Otto Merten (Genethon /EASCO) and Mauro Mezzina (EASCO), Evry/Paris.

*The most internationally renowned scientists will be gathered in a 5-day **symposium/workshop** to review concepts on the organisation and dynamics of the genome and to show and discuss tools and methods to perform safe gene transfer and modification for therapeutic purposes. **16 short talks** will be selected by the participants' abstracts. Furthermore, networking activities between students, PhDs and senior researchers will be organized before and during the workshop to foster collaborations and career opportunities to young researchers.*

Publications of ADVance researchers (over the last 6 months)

PIs

A direct and versatile assay measuring membrane penetration of adenovirus in single cells Suomalainen, M., Luisoni, S., Boucke, K., Bianchi, S., Engel, D.A. & **Greber, U.F.** (2013).. J. Virol. 87, 12367-12379.

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Adenovirus E3 protein modulates leukocyte functions. **Arnberg N**. Proc Natl Acad Sci U S A. 2013 Dec 10;110(50):19976-7.

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Antiviral and antitumor T-cell immunity in patients treated with GM-CSF-coding oncolytic adenovirus. Kanerva A, Nokisalmi P, Diaconu I, Koski A, Cerullo V, Liikanen I, Tähtinen S, Oksanen M, Heiskanen R, Pesonen S, Joensuu T, Alanko T, Partanen K, Laasonen L, Kairemo K, **Pesonen S**, Kangasniemi L, Hemminki A. Clin Cancer Res. 2013 May 15;19(10):2734-44. doi: 10.1158/1078-0432.CCR-12-2546. Epub 2013 Mar 14.

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Assessment of a novel, capsid-modified adenovirus with an improved vascular gene transfer profile White KM, Alba R, Parker AL, Wright AF, Bradshaw AC, Delles C, McDonald RA and **Baker AH.** Journal of Cardiothoracic Surgery, 2013, Aug 9; 8(1):183.

Canine adenovirus type 2 vector generation via I-Sce1-mediated intracellular genome release Ibanes S & **EJ Kremer**. 2013. *PLoS ONE*. 2013;8(8):e71032.

Canonical transforming growth factor-β signalling regulates disintegrin metalloprotease expression in experimental renal fibrosis via microRNA-29 Ramdas V, McBride M, Denby L and **Baker AH**. American Journal of Pathology, 2013, In Press.

CNS delivery of helper-dependent canine adenovirus corrects neuropathology and behavior in mucopolysaccharidosis type VII mice Ariza L, Giménez-Llort L, Cubizolle A, Pagès G, García-Lareu B, Serratrice N, Cots D, Chillón M, **Kremer EJ** & A Bosch. 2013. *Hum Gen Ther.* Dec 3, PMID 24299455

Combined anti-apoptotic and anti-oxidant approach to acute neuroprotection for stroke in hypertensive rats Ord ENJ, R Shirley J D. McClure, C McCabe, **E J. Kremer**, I. M Macrae, & LM Work. 2013. *J Cereb Blood Flow Metab* May 1. doi: 10.1038/jcbfm.2013.70.

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