UNIVERSITY of GLASGOW

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

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ADVance Newsletter n° 2

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Editorial of the Coordinator

Welcome to the second ADVANCE newsletter. I hope everybody is enjoying the summer. Even Glasgow has been radiant! Not bad, our 4th good summer in the 14 years that I have been north of the English border. Who needs to live in Montpellier to get the sun??! We are over 12 months into the ADVANCE programme and time is moving on quickly. In the enclosed pages we have some really important reports and information about ADVANCE. Our biggest event so far was the recent meeting in Zurich and I am very grateful to **Rodinde HENDRICKX** and **Nicole STICHLING** from the Zurich labs for putting together a fabulous report on the event. It was very exciting to have everyone together for the first time and to see the success of the event. Well done all (especially Urs). Please click on the link on the next page for the full report. As we approach the meeting in Leiden, organized by Menzo Havenga and Jerome Custers, we enclose an overview about the meeting and its objectives. It is excellent to gain experience from both academic and industrial partners in ADVANCE and I am grateful to our partners in Leiden for organizing such a strong event where experience of adenovirus development towards clinical applications will be discussed.

We also enclose detailed outlines of all the ESR/ER projects. This follows on from the first newsletter where we introduced each researcher and their background. Now it is important to detail the science. Understanding and help each of these projects will help maximise productivity and engagement within the consortium, aid secondments and create future opportunities. Please take the time to read each in detail. We will have a scientific event at the end of the Leiden meeting with the concept that we focus on ensuring the overall objectives of ADVANCE at the science level are moving well and that individual projects are vibrant and proceeding efficiently.

I hope you all enjoy NL2 and look forward to meeting up again in Leiden. Until then, enjoy the last weeks of the summer sun!

Andy

ADVance Traininig

▪ Report on the workshop in Zurich on March 2013 (organized by Urs Greber & Andrew Baker)

Downloadable by clicking here*

This report (written by Rodinde Hendrickx and Nicole Stichling, who are ESRs at Zurich University) has been designed to be a supplement of the Newsletter (*Zurich_workshop_report*), and can be downloaded separately The report is composed of sections describing the activities and events during the workshop. After a short preface that outlines the training objectives of the workshop, the authors provide a synopsis of the presentations delivered by the 16 keynote speakers at the international symposium *Virus Infection Biology & Adenovirology* held on March 12. A further section focusses on the seminars and workshops on complementary skills (research management, career development and scientific writing), which took place on March 11th, 14th and 17th. The activities of the ESRs and ERs during the practical lab course and the results of the experiments they performed are described in the fourth section. Finally, the social and networking activities (meals out and trip to the mountains) are described in the last section. All sections are very clearly written, using simple language which can be understood by non-specialists and there are also some photographs showing the main events of the workshop

** or copy paste the link http://easco.org/home/sites/default/files/files_rep/pdf/Zurich%20workshop%20rep_final.pdf*

▪ Workshop in Leiden on 22-27 September 2013: *ADVanced development of vaccines based on adenoviral vectors: from bench to clinic and beyond*

Place: CRUCELL and BATAVIA, Leiden, The Netherlands

Organizers and main tutors: Jerome Custer, Menzo Havenga, Joanna Lubelska

The two Dutch companies will organize and host a training event focused on the applied approaches of the AdV vectors as vaccines and detailed manufacturing aspects. The scientific aspects will be combined with the main issues of the management of intellectual property in the field of vaccines. The upstream/downstream industrial development of vectors and vaccines as well as their business development will be described. Visits to the CRUCELL and BATAVIA laboratory facilities will give the ESRs/ERs direct exposure to the industrial environment.

The **main learning objectives** of the event are focused on:

(i) the different issues of the innovation and entrepreneurship of vaccines and related research field, focusing in particular on

- intellectual property and valorization
- quality and regulatory aspects of clinical development
- commercial/marketing strategies

(ii) theory and practice of the upstream/downstream vector processes, namely:

- how to culture suspension cells, (CHO or PER C6)
- advantages/disadvantages of different cell counting methods (manual, CASY and nucleocounter)
- scaling up cell cultures: growth cells in 2 liter bioreactors and collect samples for analysis
- preparation and purification of Ab (IgM or IgG) by using column chromatography
- protein purification from cell suspensions
- characterization of the purity of the final product by SDS-PAGE
- finally, students will figure out from literature how to select an appropriate purification method and purification column, as well as learning they will find out and discussing how to choose applicable product characterization criteria.

(iii) career development issues of PhD students and post-docs

Provisional program

Sunday September 22 *- Arrival of ER/ESRs*	
Monday September23	
Morning	*Innovation & Entrepeneurship, speaker to be azppointed (tba)*
Afernoon	*Workshop Upstream process development, speaker tba*
Tuesday September 24	
Morning	*Intellectual property speaker Guus Hateboer* *Valorization, speaker Ada Kruisbeek*
Afernoon	*Workshop Downstream process development, speaker tba*
Wednesday ***September 25***	
Morning	*Clinical development & Regulatory affairs, speaker tba,* *Quality assurance in development, speaker Jolanda van Vliet*
Afernoon	*Commercial/strategic marketing & Product deployment, speaker tba*
Evening	*Social event*
Thursday September 26	
Morning	*Career development (EASCO)*
Afernoon	*Scientific event: presentations by ER/ESRs, and lecture by SAB member tba*
Evening	*Joint dinner PIs/ERs/ESRs*
Friday September 27	
Morning	*Scientific event: presentations by ER/ESR, and lecture by SAB member tba*
Afernoon	*Closure and departure*

Tropism-modification of adenoviral vectors for targeted gene delivery

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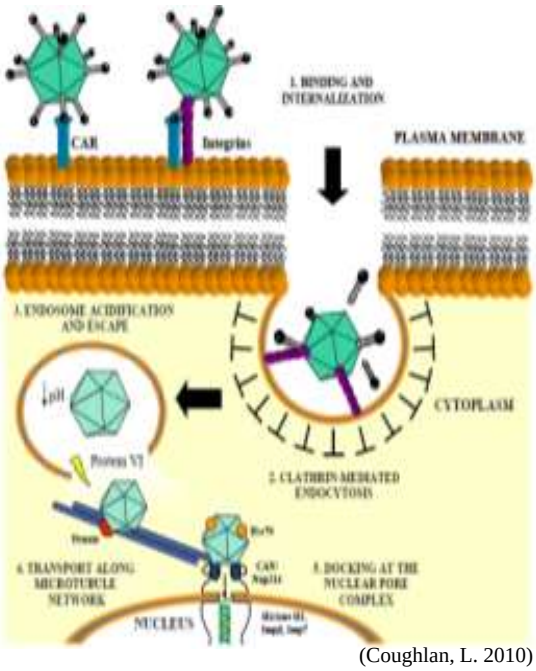
Currently, one of the most promising gene transfer vehicles for gene therapy is viral vectors. Several different viral vectors have been safely used in gene therapy clinical trials, but adenoviral vectors have been the ones most frequently used because of their efficiency as gene transfer vehicles[1].



(Coughlan, L. 2010)

Adenoviruses serotypes have been classified into different groups mainly according to neutralization assays performed *in vitro* and sequencing analysis (human species A to G). The adenovirus DNA encodes 3 major structural proteins: hexon protein (II), the most abundant in the viral capsid, penton base protein (III) and fiber protein (IV), a spike anchored in the penton base (reviewed by Coughlan et al.[2]). During the *in vitro* entry of the Ad5 (C species) to the target cell, the fiber knob domain recognizes the transmembrane Coxsackie and adenovirus receptor (CAR)[3]. Then, the Arg-Gly-Asp motif (RGD) of the penton base interacts with $\alpha v\beta_{3,5}$integrins, leading to clustering of integrins followed by activation of signalling pathways and viral internalization by clathrin-mediated endocytosis[4,5].

However, there are other factors involved in cellular recognition such as alternative receptors and co-receptors. Particularly, when the adenoviral vector is administered by intravascular delivery, coagulation factor X (FX) has been defined to bridge the hexon protein from the adenoviral vector to the heparan sulphate proteoglycans (HSPGs)[6,7] of the hepatocytes, determining its native hepatic tropism. Further studies by Alba R. et al.[8] identified the critical amino acids implicated in FX-binding and through genetic engineering the first-generation Ad5HvR5*HvR7*E451Q, which does not bind FX, was successfully generated. In this way, Ad5HvR5*HvR7*E451Q hepatocytes uptake is substantially diminished, increasing the bioavailability of the gene transfer vector for the desired *in vivo* target tissue.

(Alba, R. 2009)

(Waddington, SN. 2008)

(Lowenstein, PR. 2008)

Despite that this was a step forward in the development of safe gene delivery vectors, recent studies have demonstrated that, in addition to acting as a bridging molecule between Ad5 hexon and HSPG, FX has the ability to enhance liver transduction also by protecting Ad5 from attack by the classical complement pathway which involves natural IgM antibodies[9]. These findings suggest that the abolishment of FX-hexon interaction should be carefully reconsidered since it might not be the most suitable strategy for Ad5 liver de-

targeting.

Once de-targeting of the adenoviral vector from its native tissue is achieved, it is crucial to increase the tropism for the desired *in vivo* target tissue or generate new tropisms in order to gain specificity. With this aim, in the last decade several strategies have been developed based on genetic modifications such as the insertion of heterologous binding ligands, peptides that home to the *in vivo* target tissues (reviewed by Coughlan et al.[2]). The preferable localization for these heterologous peptides is the HI loop of the fiber knob domain due to its flexibility, exposure and unconserved characteristics, and it has been shown to maintain the viral structure and the viral titer after peptide incorporation. In order to identify novel heterologous peptides, techniques including *in vitro* and *in vivo* phage display have proven useful as they allow isolation of highly efficient and selective targeting sequences[10]. With the aim to optimise adenoviral vectors for specific delivery to the kidney, an organ for which there is no efficient gene transfer vehicle available, we will re-target the liver de-targeted Ad5HvR5*HvR7*E451QKO1 (AdT*KO1)[11] adenoviral vector by introducing selective peptides (found by *in vitro* phage display) in the HI loop of the fiber knob domain that home to claudin16 and nephrin in the kidney[12]. Once the adenoviral vectors are generated and characterized, we will determine their therapeutic potential by assessing their renal specificity *in vitro* and biodistribution and inflammatory profiles *in vivo.* Furthermore, we will investigate the implications in liver and spleen adenovirus uptake of the documented interaction between natural antibodies and Ad5 in presence or absence of FX-binding. Also, we will evaluate the role of possible receptors that could be involved in Ad5 hepatocyte transduction in immunocompromised mice, such as CAR and $\alpha v\beta_{3,5}$ integrins, in the absence of FX-binding. Nevertheless, further studies need to be performed in order to increase our understanding of the complex adenovirus biology, essential for developing novel tissue-specific adenoviral vectors and, in that way, increase their applications flexibility.

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How do adenovirus immune complexes induce maturation of human monocyte derived dendritic cells?

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Human adenovirus type 5 (Ad5) has a high seroprevalence in the human population and is often applied as a vector for vaccination and gene therapy. The persistence of neutralizing antibodies in the serum and presence of memory T and B cells thereby creates an environment close to a secondary infection whenever adenoviral based vectors are used. We use monocyte derrived dendritc cells to study the interaction of the virus immune complexes (IC) and innate immune cells. Monocytes circulate in the blood in high numbers and they inflitrate infectious tissue, differentiate into dendritic cells (DC) and, after a maturation step they are able to stimulate both naive and memory T and B cells. Thus, they play a pivotal role in the sensing of infection and activation of the adaptive immune response.

We recently showed that Ad5 opsonized with immunglobulins triggers several innate immune sensors in human DC and induce their maturation whereas the virus alone is far less potent. Understanding the initial activation of DC. We will investigate these interactions mainly in human primary cells to work as close to the clinics as possible.

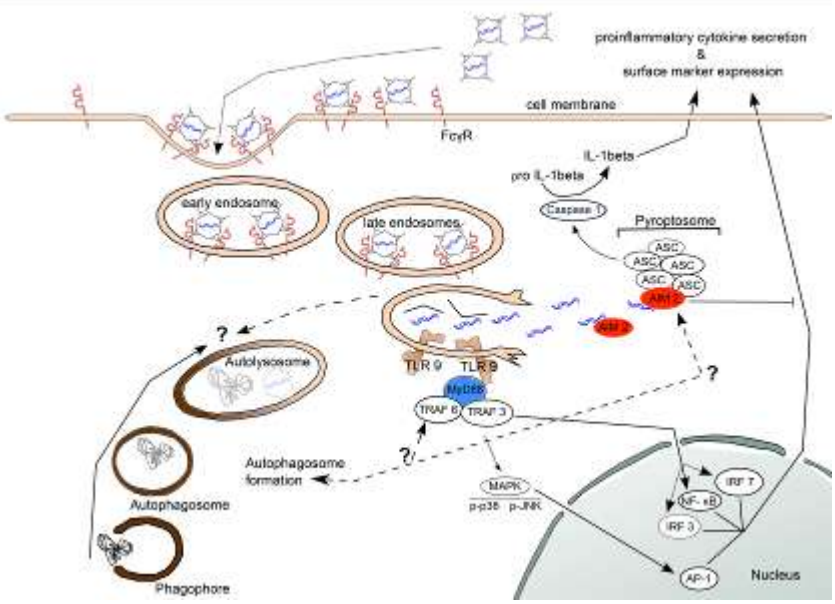

Figure 1: Intracellular processes involved in adenovirus immune complex (IC) induced maturation of dendritic cells (DC). The IC enter the cell through Fcγ receptor mediated phagocytosis and travels through different endosomal compartments until it reaches an environment which destablizes the immune complex and the capsid. The free viral DNA then activates through both cytosolic (TLR 9) and vesicular (AIM 2) DNA sensors several signaling cascade and an inflammasome like structures and thus induces DC maturation. We are now interested how autophagic processes orchestrate the maturation as autophagy has been shown to be involved in direct pathogen elimination, to be regulated by innate immune sensors and to be involved in inflammasome activation in immune cells. Dotted lines represent the interactions to be investigated.

Currently, we are focusing on understanding the trafficking of the IC and Ad5 alone after they have been phagocytosed by the DC. We want to know the fate of the capsid and the viral DNA on its journey through endosomal compartments and if they separate at one point (Fig. 1). Beyond that, there is increasing evidience that both inflammasome like activation and autophagic processes are involved in the maturation. Autophagy is an evolutionary ancient vesicular degradation pathway during which a part of the cytosol can be engulfed through the *de novo* formation of a double membrane layered vesicle. These vesicles mature and can fuse with lysosomes to form autophagolysosomes and are thus also connected to endo-lysosomal trafficking (Fig. 1) and are involved in several processes in immune cells (Fig. 1). It is therefore one of our objective to understand the trafficking of the IC and the role of autophagy during the maturation of DC. To further dissect this interaction, we will combine biochemical assays, imaging and genomic approaches.

In general, our studies might improve the future development of adenoviral vaccine strategies shaping the immune response towards a cellular or humoral adaptive response as desired. In addition, knowing the human immune response to adenoviral vectors is essential to precisely define the efficiency and biosafety settings in order to overcome drawbacks in clinical gene therapy.

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Innate immune sensing mechanisms during Adenovirus entry

Nicole STICHLING

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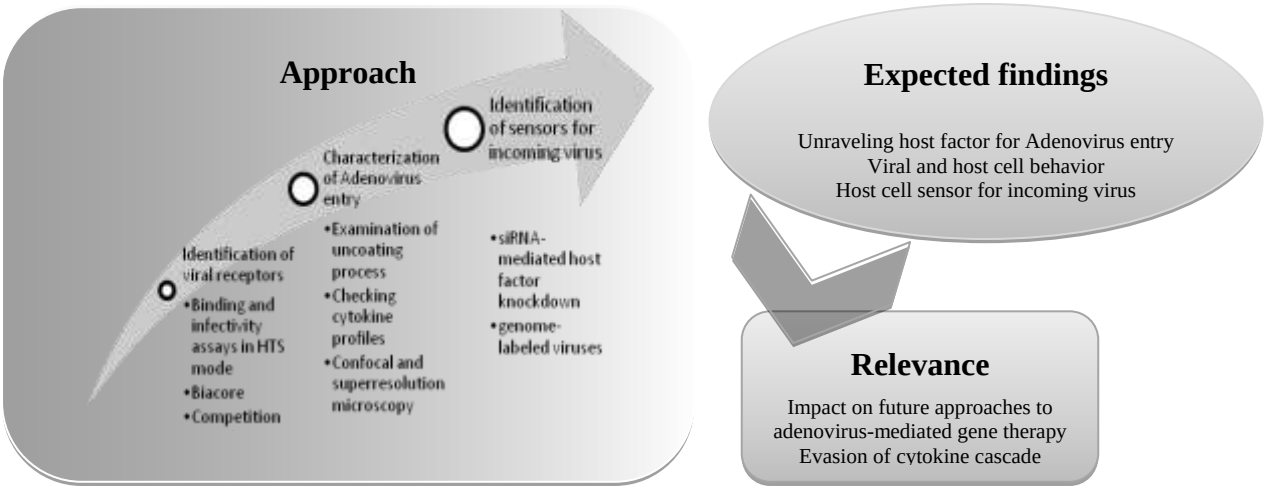
With the system of a self-renewing, non-transformed, GM-CSF dependent cell line that resembles alveolar macrophages, we aim to identify viral receptors on the cell surface of this model macrophage cell line, characterize the adenovirus entry pathway into these cells by identifying cellular mediators of the entry. In addition, we aim to identify 'sensors' for the incoming virus that triggers the innate response. Thus, another aim will be to investigate on how virus trafficking is linked to innate immunity-triggered IFN responses. Methods for gaining insights include confocal and high-throughput-fluorescence microscopy, electron microscopy, siRNA-induced knockdown of host factors and conventional and super resolution confocal fluorescence microscopy. Also fluorescence and whole-genome labeled adenoviruses will be used to unravel the mechanisms of viral particle entry and sensing in this specific cellular context.

We expect to find new host factors for Adenovirus entry and intracellular life style of the virus with respect to innate immunity.



Findings of this project may help to overcome limitations by innate sensing that gene therapy with adenoviral vehicles faces up to date. Determining the nature of the adenoviral structures that induce cytokine responses will aid designing future approaches to adenovirus-mediated gene therapy. This will also impact on the use of adenoviruses and its vectors as vaccines.

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Study on in vivo Mouse Adenovirus biology and construction of their oncolytic vectors

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Cancer is a life-threatening disease that affects millions of people worldwide. The scientific community is constantly searching for new ways to treat this disease. Over the last years, the focus has been mainly on non-invasive measures that can be applied in the early stages of cancer development. However, once cancer has progressed, treatment outcomes are often unsatisfactory.

A new type of cancer treatment includes the use of oncolytic viruses. Such natural or genetically modified viruses only replicate in cancer cells and thereby kill the malignant cells, without harming cells of healthy tissues. Viruses are of many different species that evolved to invade cells of humans, animals and plants. Depending on the virus type, and the cell type that is infected, virus replication can lead to actual clinical disease.

Adenoviruses are non-enveloped, double stranded DNA viruses that are well-suited for potential use in gene therapy. Adenoviruses infect both quiescent and dividing cells, they do not incorporate their genome into the host cell genome and the viral genome can be easily modified. Human adenoviruses have been in the forefront for development of oncolytic viruses. Various strategies have been used to restrict replication of adenoviruses to cancer cells. In order to make oncolytic adenoviruses more potent, they have been armed with therapeutic genes, including genes that lead to degradation of extracellular matrix, as well as robust immune responses against cancer cells.

In our laboratory we try to contribute to this research using mouse adenovirus. Mouse adenoviruses (MAdV-1/-2/-3) have been suggested to be relatively old members of the genus *Mastadenoviridae.* MAdV-1/-2 have been isolated in the 1960s and MAdV-3 in 2009, but their biology has only been poorly characterized compared to human adenoviruses. We therefore intend to first characterize the infectious behavior or tropism of reporter and/or wild type virus in cell cultures, followed by *in vivo* studies using a bioluminescence imaging system.

Subsequently, we will develop oncolytic viruses based on mouse adenoviruses. The main purpose to develop and study armed mouse oncolytic adenoviruses is that it allows us to appreciate the effect of immune stimulators in an immunocompetent syngeneic mouse tumor model. Pre-clinical models for armed human adenovirus-derived oncolytic vectors are handicapped in the sense that these viruses are mostly tested in nude mouse models with a deprived immune system, not adequately reflecting the human situation. However, in order to determine the immune-boosting efficacy of new gene candidates, a syngeneic tumor model is much more suited to evaluate potential benefits and risks. In addition, a well-established and tractable mouse adenovirus replication system could be used to test anti-viral drug candidates derived from initial drug library screenings.

Taken together, this work will contribute to our understanding of the infection biology of mouse adenovirus in its natural host and to the establishment of highly improved syngeneic model systems that allow thorough evaluation of armed oncolytic viruses in the treatment of cancer.

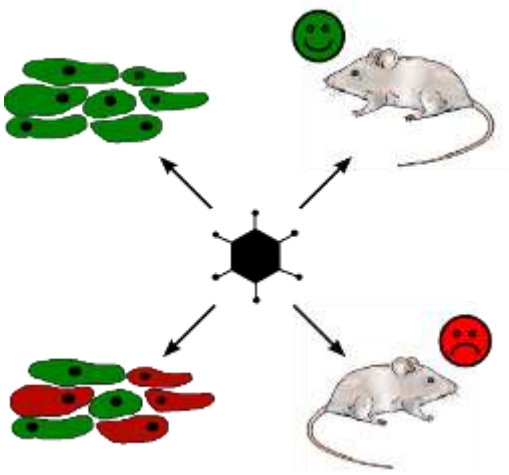

Figure 1: Schematic representation of the project outline. In black a mouse adenovirus (center), green indicates healthy cells (top left) and healthy mouse (top right), red indicates tumor cells (bottom left) and a mouse affected with cancer (bottom right). In this project the influence of MAdV will be investigated on all four situations.

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Identifying binding partners for human adenovirus 40 and 41 penton base proteins, and characterizing their roles in the Ad40/41 life cycle.

Anandi RAJAN

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Description – Human adenoviruses are known to cause respiratory illnesses, conjunctivitis (inflammation of the outermost layer of the eye and the inner surface of the eyelids) and gastroenteritis in children. Different types are associated with different illnesses. Eg.: Adenovirus B and C cause respiratory illnesses, species B and D cause conjunctivitis while gastroenteritis is mainly (but not only) caused by species F adenoviruses, type 40 and 41. These enteric adenoviruses are one of the chief causes of viral infantile gastroenteritis.

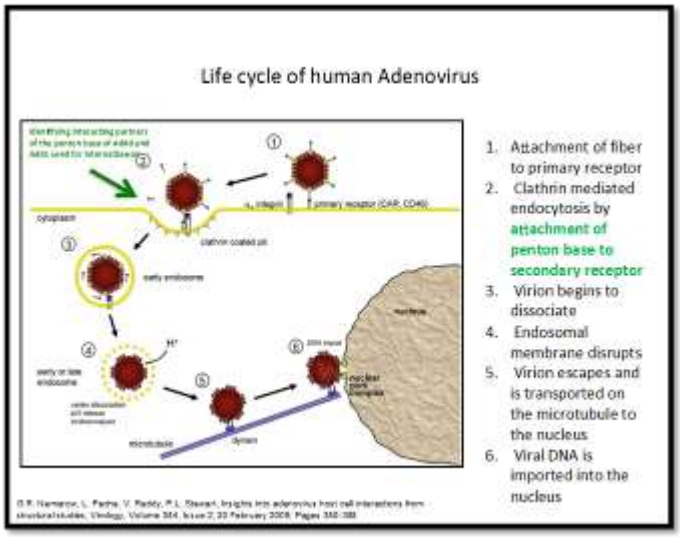
Adenoviruses enter the host cell in two steps – interaction of the primary receptor to the fiber knob for attachment and interaction of the secondary receptor to the penton base for internalization. The RGD (Arginine – Glycine – Aspartic acid) motif on the penton base is known to interact with integrins on the host cell surface to mediate internalization. However, the penton bases of Ad40 and Ad41 are different. They lack these RGD motifs. But Ad40 and Ad41 contain other motifs such as LDV (Leucine – Aspartic acid – Valine), LDA (Leucine – Aspartic acid – Alanine), SDI (Serine – Aspartic acid – Isoleucine) and ADI (Alanine – Aspartic acid – Isoleucine) which indicate that they might bind to integrins. These motifs have been shown to bind to alpha 4 integrins and to some extent alpha 5 integrins. Thus, the aim of this project is to identify the interacting partners of the penton base for the species F adenoviruses..

Methods - Flow cytometry; pull down experiments, binding and infection/internalization experiments to identify integrins to which the penton base protein might bind to. CHO cells that express specific integrins will be used.

If integrins are not receptors, proteomics approches will be performed in order to identify other cellular co-receptors. The NCI-60 cell library and corresponding microarray database will also be used. NCI-60 is a library of around 60 cell lines with available profiles of their gene expression. Binding experiments with each cell line could provide hits of putative receptors.

Also, to determine the kinetics and affinity of the interaction with surface plasmon resonance and determine structure by crystallography once an interaction has been observed.

Implications - Determining the receptors that the enteric adenoviruses bind for internalization would be useful in order to better understand adenovirus tropism. Moreover, these interactions may constitute targets for antiviral drugs. Finally, identification of receptors/coreceptors for Ad40 and Ad41 will also be helpful in improving and development of gene and cancer therapy vectors, since these viruses have a low seroprevalence (antibody titers in the population will be low thus increasing the probability of introducing the target gene) as compared to the more commonly used Ad5 based viral vectors



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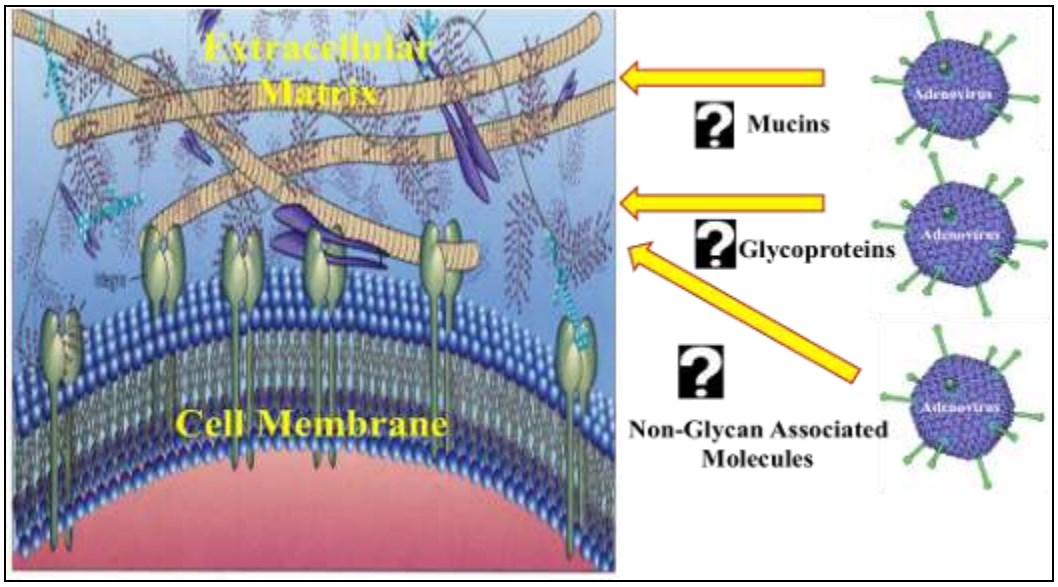
1. Identification and characterization of soluble components that regulate adenovirus tropism. There are more than 60 human adenovirus (Ad) types that are classified into seven species (A-G). Different human adenoviruses cause diverse diseases. Members from groups C and D, specifically causes respiratory and/or ocular infections, respectively. Recent development in the identification of cellular Ad receptors shows that these viruses use receptors such as the coxsackie and adenovirus receptor (CAR), and sialic acid monosacharides, which are usually expressed by a broad range of host cells. This makes us question which factors are responsible for Ad tropism. In this study, we explore the role of soluble components from different body fluids in Ad tropism.



2. Antiviral drug development against adenovirus type 37 causing epidemic keratoconjunctivitis *(Manuscript submitted).* Group D adenovirus contains more than 30 members and commonly causes ocular diseases among which the most severe is epidemic keratoconjunctivitis (EKC), mainly caused by highly contagious Ad8, Ad19 and Ad37, and includes symptoms such as pain, edema, lacrimation and decreased vision for weeks or even months. Worldwide, 10-25 million people suffers from EKC each year. Since there are no vaccines or antiviral drugs available, identification of novel targets for antiviral treatment is highly desirable. The terminal, trimeric knob domain of the capsid fiber protein of Ad37 binds to cellular receptors. Our group has already shown that sialylated proteins serve as cellular receptors for EKC-causing Ads. This study opened the doors to a new approach for structure based drug design. In the collaboration with Department of Chemistry, Umeå University, we have designed and synthesized compounds having three terminal intact or modified sialic acids. Antiviral evaluation of these compounds was carried out by molecular interaction studies including X-Ray crystallography and surface plasmon resonance (SPR) as well as by performing *in vitro* binding and infection assays. We have found that two out of five TSA compounds are efficient inhibitors of Ad37 binding to and infection of human ocular cells.

These studies would be helpful to explain the tropism of Ads and further can be implicated to design sialic acid-containing antiviral drugs. Moreover, Ads are commonly used as viral vectors for cancer and gene therapy, so identification of such soluble components that interact with Ads, and characterization of corresponding interactions will be useful to design efficient Ad-based vectors and would also be useful to explore new evolving mechanism of host pathogen interactions.

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Effects of TNF and LTA on colorectal cancer and the tumor microenvironment

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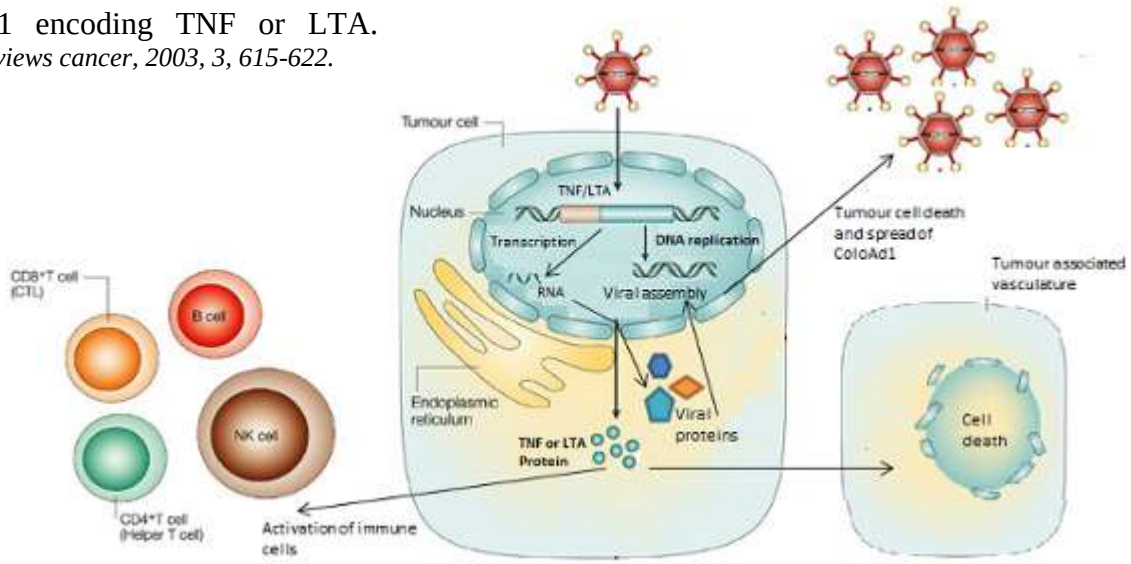
Cancer is one of the leading causes of death in the developed world. Colon and rectum cancers (CRCs) are the third most prevalent cancers as well as the third cause of cancer related death around the globe [1]. Cancer is caused by multiple mutations that allow cells to divide uncontrollably, spread to other parts of the body, attract new blood vessels to tumour site, avoid recognition and destruction by the immune system and become insensitive to signals that cause cell death or growth suppression [2].

Replicating oncolytic viruses are viruses that infect tumour cells, multiply within them and kill the cell as new viruses are released from the cell, free to infect adjacent cells and repeat the process. Using oncolytic viruses to treat tumours has several benefits to conventional chemotherapy, including better therapeutic index and the possibility to overcome acquired drug resistance mechanisms. Additionally, oncolytic viruses can be engineered to express therapeutic proteins with further anti tumour activities. Finally, because these viruses can replicate inside tumours, high concentrations of virus and of the proteins they produce can be achieved inside tumour. [3]

The aim of my project is to make an oncolytic adenovirus (ColoAd1) that produces tumor necrosis factor α (TNF) or lymphotoxin α (LTA). Both TNF and LTA are proteins discovered in the 1980s, which show strong anti tumour effects. However, in humans severe shock-like side effects were seen after injection of TNF into the blood stream [4].

By producing TNF and LTA inside the tumour I hope to see anti tumour effects, while avoiding the toxic side effects that were caused by injection into the blood. To study the effects of high levels of TNF and LTA inside tumours, I made cell lines that inducibly express these proteins. I am especially interested in the role TNF and LTA have on the blood vessels associated with the tumours and on immune cells inside of tumours. Based on these studies, we will decide whether to engineer a virus that makes TNF or LTA or whether we would like to combine this new therapy with other therapeutics.

Figure 1: ColoAd1 encoding TNF or LTA. *Adapted from Nature Reviews cancer, 2003, 3, 615-622.*



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Arming oncolytic Adenoviruses to improve antitumor immunity

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Cancer is the second leading cause of death worldwide. Despite advances in cancer therapies over the past decades, many types of cancer remain resistant to new or conventional treatments. There is therefore an urgent need to develop alternative and efficient cancer therapies.

Surprisingly, one of these alternatives came from what has been considered an enemy for many years: viruses. These tiny intracellular parasites infect cells to hijack their energy-, protein- and gene-machineries in order to make more copies of themselves. Although viruses can cause a wide variety of diseases, the vast knowledge of their biology has allowed scientist to genetically engineer them to selectively infect and kill cancer cells without harming healthy ones. This approach, known as virotherapy, holds promise as a novel cancer treatment. In fact, many of these so-called oncolytic viruses are currently being tested in clinical trials. From these, oncolytic Adenoviruses (OAds) are the most frequently used for virotherapy. Adenoviruses have ideal features, making them attractive for therapeutic applications. They are low pathogenic, they can infect dividing and non-dividing cells, they can be easily manipulated by genetic engineering, they are highly stable, and they have a high capacity to carry foreign DNA. This latter characteristic allows us to "arm" OAds with therapeutic genes.

Despite the potential of virotherapy, clinical trials with OAds have identified the immune system as a limiting factor for their success in the clinics. For instance, the immune system has evolved mechanisms to recognize and eliminate viruses. Thus, OAds can be easily cleared from the organism, reducing their efficacy. Additionally, cancer cells have evolved mechanisms to switch off the immune system at the site of the tumor in order to avoid their rejection from the organism. This inhibition of the immune system is, however, not beneficial for the virus, as observed in virotherapy clinical trials, in which the immune system eliminates the virus in the tumor but rarely the cancer cells.

With this in mind, we have asked ourselves whether we could generate an OAd which can overcome these hurdles. To answer this question, we will engineer an OAd carrying a gene encoding an immunomodulatory molecule which can activate and direct the immune response towards the tumor, rather than to the OAds. These molecules are intended to be secreted from infected cancer cells. Thus, we want to turn infected tumor cells into factories of therapeutic molecules which complement the oncolytic effect of the virus in order to improve the overall antitumor efficacy (Figure 1).

With this project we expect to obtain new OAds with potential to be translated into the clinics as an alternative to treat tumors for which conventional cancer therapies are not effective.

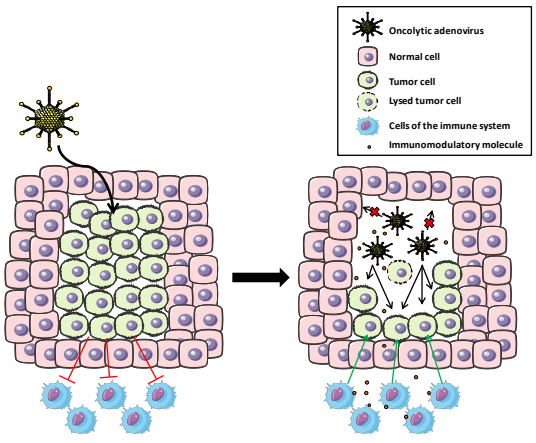

Figure 1. **Immunovirotherapy approach**. Cancer cells are able to switch off the immune system at the tumor site (red lines, left scheme). Engineered oncolytic viruses can selectively infect cancer cells without harming healthy ones (red crosses). These infected tumor cells will simultaneously produce new viruses and an immunomodulatory molecule which will activate the immune system (green lines), leading to the eradication of cancer cells. The figure was produced using Servier Medical Art.

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Modulation of gene expression via ncRNA induced epigenetic modifications for the treatment of cardiovascular diseases

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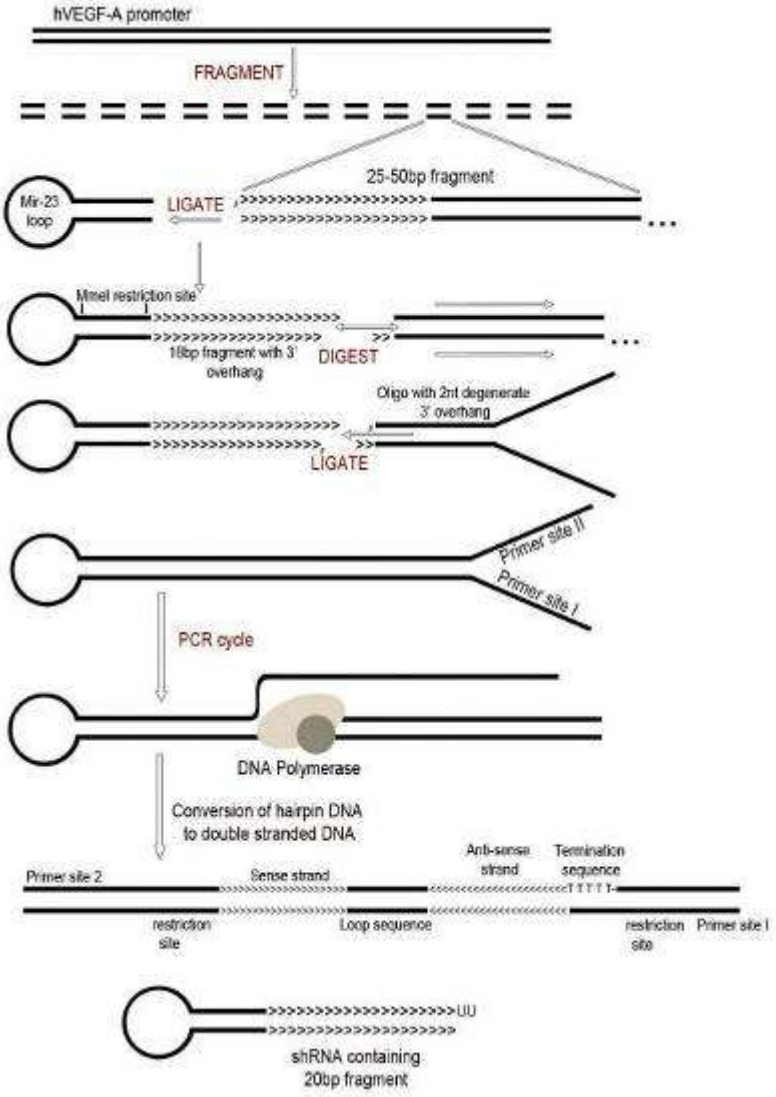
The research group I'm currently working in primarily focuses on gene-therapy based therapeutic angiogenesis for the treatment of cardiovascular diseases. In relation to this, there has also been parallel development of plasmids encoding viral vectors optimised for *in vivo* and clinical use. My work is based on utilising non-coding RNAs to modulate epigenetic statuses in target tissues as an alternative to traditional transgene based gene therapy methods. With recent transcriptomic analyses it has been shown that up to 90% of eukaryotic genome is transcribed, the majority of which are thought to be non-coding RNAs as only 1-2% are encoding proteins. Non-coding RNAs are emerging as major regulators of gene expression and epigenetics through various mechanisms at levels of chromatin remodelling, transcriptional and post-transcriptional regulation.

Using specifically designed non-coding RNAs complementary to regions of the target gene promoter, we have previously shown, *in vitro* and *in vivo*, that these RNAs can either stably or transiently, upregulate or downregulate endogenous target gene expression. The epigenetic modifications to the promoter responsible for the modulated gene expression have also been shown *in vivo* as proof of concept for this novel therapeutic approach. This method of utilising RNA-mediated gene expression modulation holds several advantages over transgene based therapeutic strategies. Indirect modulation of gene expression, as opposed to episome protein overexpression, is less likely to result in perturbations to the complex systems that make up cell signalling networks. Consequences of perturbations can unpredictably manifest in terms of immunogenicity and altered cellular functions. Additionally, the cassettes encoding these RNAs used in the viral vector are significantly smaller than protein expressing transgenes, allowing multiple RNAs to be expressed from the same vector, if required. Furthermore, specifically modifying the epigenetic state of the target gene promoter could potentially reverse pathological epigenetic modifications that have been identified as a contributing factor in several disease states.

A major hurdle in developing these non-coding RNAs for therapeutic purposes is that there is no reliable method to design RNA sequences that can epigenetically modulate a target gene promoter. The current process is largely trial and error, with varying degrees of up- or downregulation and diverse tissue specific responses. Consequently, we have developed a library of short dsRNA sequences specific for VEGF-A as a prototype therapeutic gene of interest. In order to efficiently screen the library for sequences with potential activity, we will use a novel engineered reporter cell line that can quickly and accurately quantify gene expression at multiple time points. Further characterisation of selected sequences can be performed initially *in vitro* using primary cell culture methods and subsequently *in vivo* using porcine models. Molecular characterisation of promoter epigenetic modifications and transcription will be performed using chromatin immunoprecipitation, chromosome conformation capture and global run-off sequencing.

It is hoped that the library will identify novel RNAs with increased potency for clinical application as well as helping to further elucidate the mechanisms behind RNA regulation of epigenetic marks. The possibility for multiple cassette payloads also opens up possibilities of targeting gene expression regulation at several points, amplifying therapeutic benefit whilst minimising cellular resistance or compensation to the therapy. As with preceding angiogenic gene therapy treatments, there would also be the opportunity to test this epigene-therapy in the clinic using adenovirus vectors.

Fig. 1. shRNA library construction - Target gene promoter is randomly fragmented and ligated to a hairpin oligo containing the Mir-23 loop and an MmeI restriction site in the stem. MmeI digestion produces hairpin fragments with an approximately equal stem length suitable for shRNA. The degenerate 3' overhangs is then ligated to an adaptor sequence that contains primer and restriction sites required for extension and cloning. A polymerase is then used to convert the hairpin into a double stranded template. The primer sites are then removed using the restriction sites and ligated into an expression vector in order to produce the library.



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Elucidating the mechanism of action for the oncolytic adenovirus ColoAd1

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Cancer is one of the most common causes of death worldwide, World Health Organization accounts 7.6 million deaths in 2008 and studies forecast an incidence of 22.2 million people suffering the disease by 2030 (F. Bray et al., 2008). Conventional cancer therapies have considerably increased survival rates, but some cancers depending on the type or stage of detection still have a poor prognosis. For this reason novel therapeutic strategies for combating cancer have emerged in recent years. Virotherapy through the development of oncolytic viruses, which have the ability to selectively infect and kill tumour cells, is making notable progressions in this field and a large number of oncolytic viruses are currently undergoing clinical trials.

ColoAd1 is a chimeric oncolytic adenovirus that is currently in phase I/II clinical trials. Unlike other oncolytic viruses, which are usually generated by rational design ColoAd1 was created by a process of directed-evolution. For this, a pool of adenoviruses representative of groups B-F was passaged on a colorectal tumour cell line. After 20 passages, ColoAd1 was selected as the most potent virus. Sequence analysis revealed that ColoAd1 was Ad11p, a group B virus, with a nearly complete E3 region deletion, a smaller deletion in the E4 region, and a chimeric Ad3/Ad11p E2B region (**Figure 1**) (Kuhn I et al., 2008). In vitro studies showed ColoAd1 to be 2-3 logs more potent than either of its parent serotypes (Ad11p and Ad3), Ad5, or the most clinically advanced oncolytic Ad, ONYX-015 (Kuhn I et al., 2008). Importantly, this increase in potency on cancer cells did not translate to increased potency on normal cells resulting in a therapeutic window that is 3-4 logs greater than Ad5 or Onyx-015. However, because ColoAd1 was not generated by rational design its mechanism of action (MoA) is not well understood.

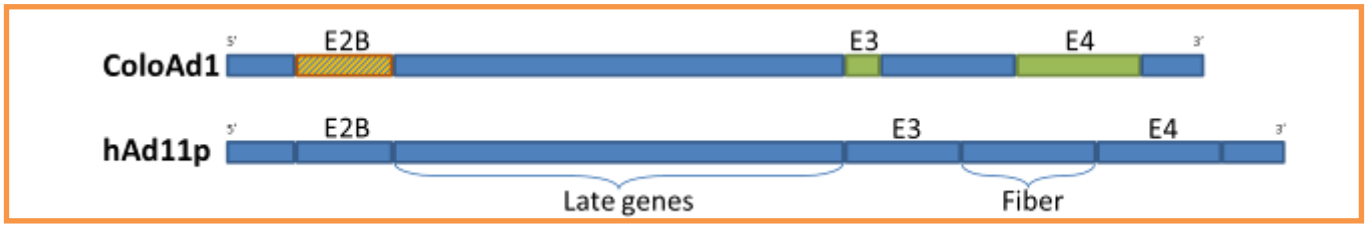

Figure 2. ColoAd1 and parental hAd11p genome scheme. Blue is for parental sequence. Orange represents the chimeric ColoAd1 E2B region. Green represents E3 and E4 regions with deletions.

We aim to investigate the virus factors and cellular factors which may be responsible for ColoAd1's MOA in terms of the virus potency, selectivity and interactions with immune system. To investigate viral factors we have generated a panel of chimeric Ad11/ColoAd1 viruses that each contain only one of the modifications present in the ColoAd1 genome (either E2B, E3 or E4). These will be used in comparative studies with the parental virus Ad11p. In addition we will be carrying out high throughput screening (HTS) using a commercial drug panel and technology learnt during the ADVance's International Workshop in Zurich, March 2013. Through the screen we expect to learn more about ColoAd1's MOA in cancer cells as well as understanding virus synergy with other therapeutics.

We hope that obtaining a better understanding of ColoAd1 MOA including its delivery, selectivity and interaction with blood and immune components will ultimately aid the development of the next generation ColoAd1 viruses.

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Kuhn I, et al., 2008 doi:10.1371/journal.pone.0002409

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Translation of oncolytic AdV: development and utility of AdVs as oncolytics

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INTRODUCTION, IMPORTANCE OF THE PROJECT

Cancer is a class of diseases characterized by out-of-control cell growth. According to WHO, cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (13% of all deaths) in 2008. Metastatic tumors often become resistant to conventional therapies like chemotherapy, radiotherapy, which cause serious side effects and therefore new approaches in cancer treatment are urgently needed. Oncolytic viruses have been determined to mediate beneficial immunologic responses, which have been suggested to contribute to reduction of tumor cells and improve healing process. CGTG-401 is a double-targeted chimeric adenovirus (Figure 1). CGTG-401 was constructed to provide cancer cell–restricted replication and increases tumor selectivity and immunologic activity against tumor cells. Therefore this is a very promising tool in cancer treatment.

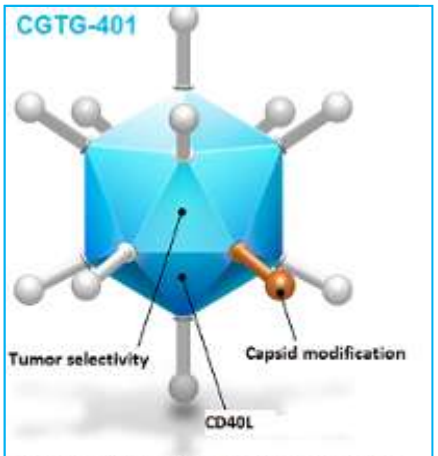

Figure 1. Structure of the replication-competent oncolytic adenovirus CGTG-401 that combines targeted oncolytic killing of tumor cells and simultaneous production of anticancer drug CD40L

BIOPHARMACEUTICAL PRODUCTION

The production of biopharmaceuticals is strictly regulated; the process should be performed according to Good Manufacturing Practices (GMP), the process must be reproducible, and produce repeatedly safe and potent drug. The development of a biopharmaceutical production process requires careful development and optimization work. It involves not only the development of the production process but also a set of specific analytical methods that are used to control the process and characterize the product.

In brief, my work at Oncos Therapeutics includes optimization study for CGTG-401 in order to maximize efficiency of virus production to obtain the highest yield of pure and active final bio-product. To evaluate quantity, quality, functionality, genetic stability is a must to design and implement analytical assays. In connection with this, my second challenge is to design and optimize analytic tools for CGTG-401 (Figure 2).

Figure 2. The order of actions

EXPECTED RESULTS

Optimized production process ensures quality and the quantity of final bio-product, thus allows manufacturing of the viruses in GMP for clinical trials.

PERSONAL NOTE

If you ask anyone who works in the biotech industry what their main motivation is, why do they work there, it's certain you'll get the same answer. People want to make people's lives better. Not many jobs offer you more of a chance to do this. I am happy to be a part of Oncos and work on my research, which gives me the opportunity to get professional knowledge of inter alia biopharmaceutical process development.

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Molecular characterisation of simian adenoviruses

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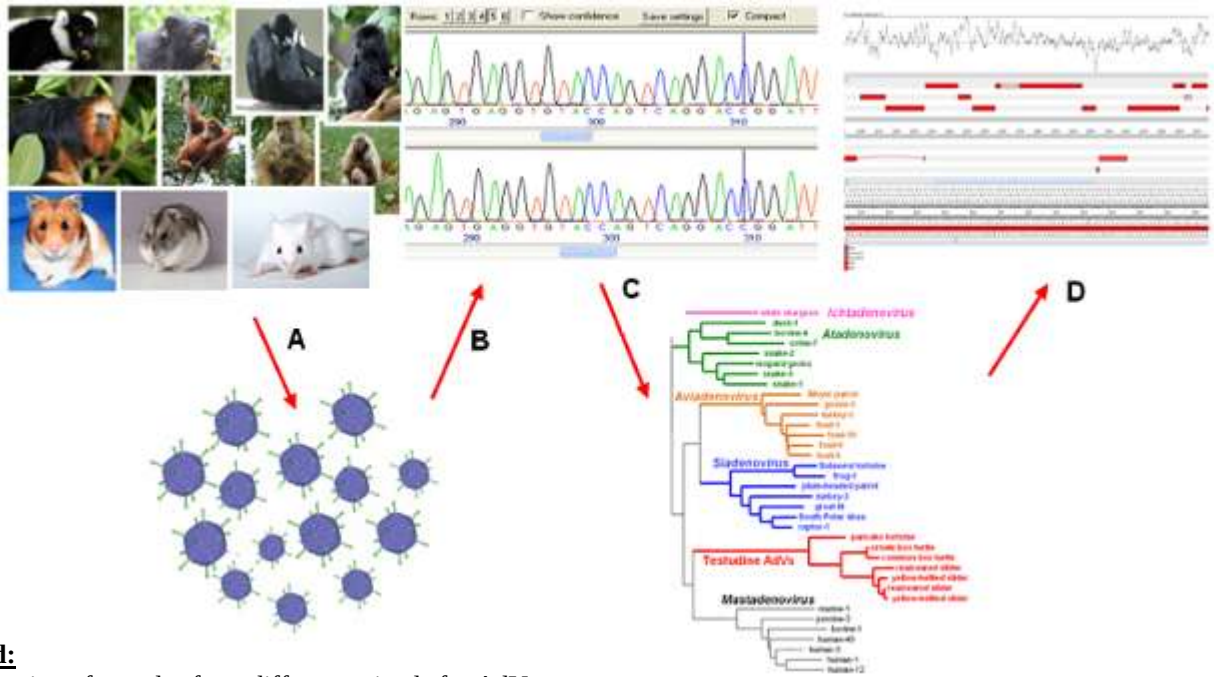
Adenoviruses (AdVs) have a growing popularity as gene delivery vectors and possible anti-cancer therapeutic agents. However, existing specific antibodies in humans significantly limit the medical usage of human AdVs. For this reason there is an increased interest in the possible use of non-human, especially simian AdVs. While human AdVs are among the best-studied viruses and we have relatively much knowledge about the ape AdVs, the AdVs of the more ancient simian lineages, especially that of the New World monkeys are barely known. The chimpanzee AdV vector is sufficiently similar to human serotypes, but different enough to avoid significant cross-neutralization by antibodies directed against human serotypes. First New World monkey AdV was found only two years ago, and no AdV has been reported from any prosimian host. Recent studies have suggested that some simian AdV-derived vaccine carriers can potentially circumvent immunological barriers, which inactivate human AdVs, and establish effective protection against the targeted pathogens.

The basic research goal is the screening of samples from prosimians, New World monkeys and some less studied Old World monkeys for the presence of novel AdVs in order to obtain better understanding of AdV biodiversity. The other goal, which orients to possible medical purposes, is the comparative analysis of simian AdV genomes to facilitate their application as vectors. Genome-wide sequencing and comparative genome analysis of the different simian AdVs will be performed. The E3 region will be especially studied, as there are the greatest differences among the AdVs. As a model, rodent AdVs will be studied, too.

The expected results of this work may help in preliminary characterization of the phylogenetic place and variability of simian AdVs, as well as their availability in nature. Isolation of PCR identified virus strains and the whole genome sequence is a prerequisite for vectoring. Understanding the organization of the virus genome is very important to get more knowledge about non essential genes which could be replaced. The expected results will be useful for producing animal AdVs based vectors for use in human medicine.



Legend:
A) Screening of samples from different animals for AdVs
B) Genome sequencing of simian AdVs
C) Understanding the evolution and diversity of novel AdVs
D) Full genomes annotations

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1. *IDENTIFICATION OF SMALL MOLECULES ENHANCERS OF FACTOR X-MEDIATED ADENOVIRUS 5 (AD5) TRANSFER*

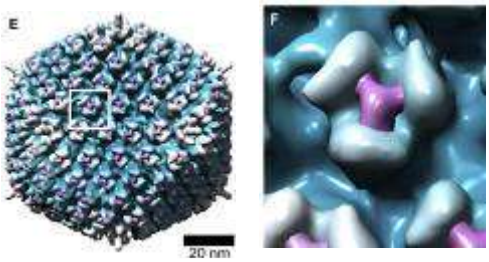

Figure 1. Analysis of Ad5 hexon binding to FX (Waddington *et al*., 2008)

Introduction: There is a high affinity interaction between coagulation Factor X and Ad5 (Figure 1) However, the safety and efficacy of Ad5 by intravenous delivered is hampered due to the high propensity of the complex Ad5:FX to transduce the liver.

We can take advantage of the high transduction capacity of the Ad5:FX complex if we deliver the virus *ex-vivo* instead of through intravascular injections. That is why vein graft failure is so attractive to gene therapy, because we can deliver a gene of interest *ex vivo* before surgery (Figure 2), thus avoiding the problems related with the intravenous delivery.

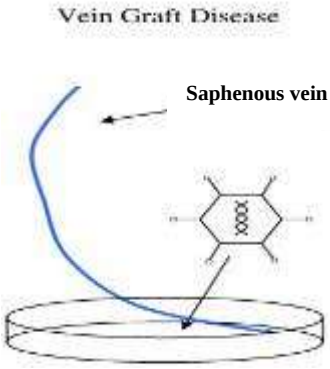

Figure 2. *Ex-vivo* incubation pre-grafting (Robertson *et al.*, 2012)

Objectives and methods To increase the transduction of Adenovirus into the cells of interest, we are implementing a pharmacological approach to enhance adenovirus transduction pathways. We are developing a high throughput screening (HTS) platform to identify the small molecule enhancers of FX-mediated Ad5 gene transfer. The high throughput screening methodology basically is as follows. We infect SKOV3 in the presence and absence of FactorX with Ad5-GFP. 48h after infection cells are fixed in paraformaldehyde and analyzed using IN Cell analyzer 2000 (Duffy *et al*., 2013). Nuclei, cells and GFP expression are segmented based on their staining, size, shape and intensity. Finally, we obtain the GFP intensity related to the number of viable cells. Our aim is to narrow down a library of 10,000 compounds to identify a top five which significantly increase the transduction of the Ad5.

2. *ADENOVIRUS AS A TOOL FOR miRNA THERAPEUTICS IN VEIN GRAFT FAILURE*

Introduction Coronary artery bypass surgery is a common procedure in acute coronary syndromes. This surgery has the potential complication of blood vessel blockage following the grafting and this limits the effectiveness of the therapy. Briefly, the processes involved in vein graft failure are, at the early stages, thrombosis and, at late stages, extra cellular matrix remodeling and smooth muscle cell migration. We are involved in studying novel therapies *ex vivo* that prevent vein graft failure and can be delivered at the same time as the procedure is performed. We would like to use the miRNAs as possible therapeutics *ex vivo* pre-grafting.

miRNAs are 22 nt long RNAs which repress complementary mRNA targets. They are transcribed as long precursor *pri-mir* and *pre-mir* that are processed in nucleus and cytoplasm till the maturation of the miRNA which binds to the target mRNA (Figure 3). Many studies have related the regulation of miRNAs with cardiovascular disorders

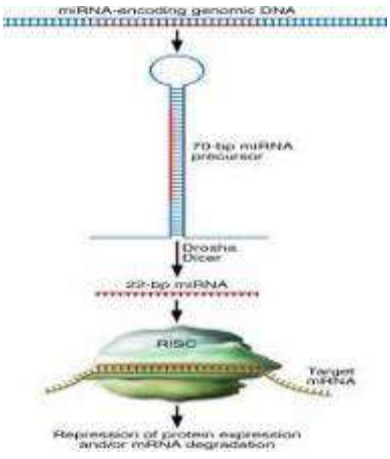

Figure 3. Maturation of miRNA from precursors pri-mir & pre-mir

such as hypertrophy, heart failure and vascular injury. Thus, the manipulation of the expression and/or function of miRNA is an attractive candidate for therapeutic manipulation.

Objectives and methods In this project we study the use of a specific cluster of miRNAs as a therapeutic strategy to treat vascular injury. We would like to construct an Ad5 recombined with Cluster 99b to enhance the expression of these miRNAs. This cluster is an evolutionary conserved miRNA cluster which contains mir-125-5p, mir-99b, mir-let-7e. We decided to work with this cluster of miRNAs because our group previously found out that the expression of these microRNAs in saphenous vein of our pig vein graft model was downregulated 28 days after the graft. We want to study if the overexpression of these three miRNAs using a recombined Ad5 could reduce the processes involved in the development of vein graft failure.

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3. Robertson KE et al. *Prevention of coronary in-stent restenosis and vein graft failure:does vascular gene therapy have a role?* Pharmacol. Ther. 2012 Jul 14. 136(1):23-34

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Characterization of the cell entry pathway used by Ad26 and Ad26 based chimeric vectors

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BACKGROUND Adenovirus type 5, the best studied human adenovirus so far, elicits potent antigen-specific immune responses (1). However, its utility is strongly hampered and limited by preexisting antibodies in human population. One of the possibilities to deal with this obstacle is developing vectors based on adenovirus serotypes with low seroprevalence in human populations. Among the rare serotypes AdV vectors, the AdV26 vector proved to be the most immunogenic and useful for generating a vaccine vector (2). AdV26 belongs to subgroup D and has a short fiber of only 8 β-repeats. In contrast to AdV5 and AdV35, AdV26 does not bind FX (3). By browsing the literature with regard to receptor usage, one can note that adenoviruses belonging to subgroup D have been correlated with CAR (4), integrins (5), and sialic acid (6). Very recently it was shown that AdV26 transduction is CD46-dependent and is efficiently blocked by anti-CD46 but not by anti-CAR antibodies, demonstrating that AdV26 utilizes CD46 as a primary cellular receptor for transduction of human peripheral blood mononuclear cells (7). It is generally accepted that AdV5 (subgroup C) after docking to the CAR receptor binds αv integrins and subsequently enters the cell by clathrin mediated endocytosis. For adenoviruses belonging to subgroup B, AdV3 and AdV35, was reported that they use macropinocytosis as infectious uptake pathway (8, 9). Both AdV3 and AdV35 bind CD46, although AdV3 with lower affinity than AdV35. These data suggest that adenovirus internalization pathway is determined by the receptor used for binding. Due to different entry pathways, once in the cell cytoplasm, adenoviruses can encounter different members of the host innate immune response. Depending on the vector purpose this can either present significant hurdles or be rather attractive as in the case of vaccine development.

OBJECTIVE Once attached to the receptor on cell surface, efficacy of gene transfer mediated by adenoviral vector correlates to the capacity of viral particle to correctly arrive to the nucleus. To ensure

that newly designed vectors will functionally allow gene expression it is necessary to consider the effects of vector's subcellular trafficking. Even though in the literature there is an important amount of data regarding AdV26 based vaccine vectors, there are only few studies dealing with AdV26 receptor usage, and none regarding the endocytic pathway of AdV26. Due to the lack of information concerning the AdV26 cell entry pathway, the main objective of this study is to investigate in more details the infection pathway used by AdV26 & AdV26 based chimeras, namely:

1) AdV26 binding, entry and internalization
2) AdV26 intracellular trafficking

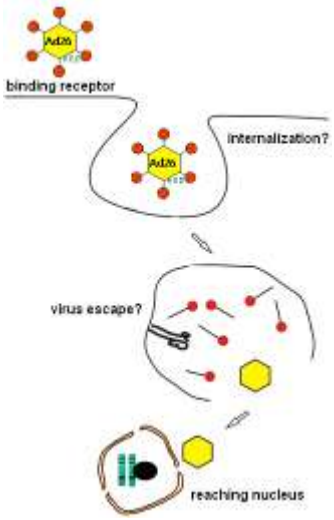

Figure 1: Schematic representation of the project objective. Adenovirus entry pathway is influenced by the receptor used for binding to the cell. In this project we aim to investigate in more details binding, internalization and intracelular traffic of Ad26.

SPECIFIC AIMS

1. *Defining in more details the role of receptor/s in AdV26 binding, entry and internalization*
2. *Analyse intracellular trafficking of AdV26 in respect of used receptor/s*

EXPECTED RESULTS Since recombinant adenoviruses are increasingly used as gene delivery vectors, it has become obvious that improved understanding of adenoviral entry could increase the efficiency of these vectors. Major part of knowledge concerning adenovirus basic biology is granted by adenovirus type 5 research. Limitation that AdV5 has in regards to host immune response diverged research towards other adenovirus serotypes. AdV26 based vector proved to be safe and immunogenic with high potential for furher clinical development as a vaccine vector (10, 11). Important difference between different Ad serotypes is not only presence of neutralizing antibodies but also receptor usage that subsequently influence viral perticle entry and internalization pathway. The data we would like to obtain in this project will provide us with insight into AdV26 cell entry and intracellular trafficking in regards to used receptor, and will allow additional improvement of AdV26 based vaccine vectors.

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Helping to accelerate biopharmaceutical development from discovery to the clinic

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Introduction Vaccines save millions of people each year from death, chronic disease, or permanent disabilities. It is widely known that the effectiveness of vaccines depends heavily on the type of immune response induced and all approved vaccines today elicit protective antibody responses. For many diseases though it is expected that a T-cell response must be induced and therefore scientists are researching recombinant vector technology whereby the vector delivers DNA or RNA encoding the pathogenic antigen to the host resulting in potent anamnestic T-cell induction. According to the gene therapy clinical trials databases (http://www.abedia.com/wiley/) more than 23% of vectors used in vaccine and gene therapy clinical trials are Adenovirus (AdV) vectors. The main advantages of AdV vectors are: large cloning capacity, relative ease of genetic manipulation, high transduction efficiency of professional antigen presenting cells, established production platform and an excellent safety record in human phase I trials.

Relevance of the project Adenoviral vectors are being studied, amongst others, as vectors for development of vaccines against *mycobacterium tuberculosis*, (TB), *plasmodium falciparum* (Malaria), and HIV (AIDS). Naturally, this requires hundreds of millions of doses to be produced annually and the challenge is to develop a manufacturing process for AdV that is able to deliver the annual doses at a very low price (the vaccines are to be used mainly in developing countries). This project aims to identify improvements in AdV manufacturing to increase yield and reduce cost. Hereto, we optimize, on lab scale, the process looking for instance at optimal multiplicity of infection (MOI), harvest parameters, correct medium (animal component free), choice of cells, improving cell density, increasing efficiency of purification. Likewise, we optimize important assays such as the TCID50 assay, VP/IU determination assays, as well as other critical assays.

My contribution to the program involves improving the production using small scale cell–based (HEK293) production techniques (2-10 Liter bio-reactor) for AdV vectors and working on novel as well as existing AdV purification techniques. For instance, using chromatography to purify AdV through charge and size rather than using classical CsCL density gradient purification. Also, I am involved in the development and improvement of assays that allow release testing for novel AdV vectors. These include the replication competent AdV assay (RCA), and q-PCR based infectivity assay. Finally, I learn about the required paperwork, preparation of documentation and batch release criteria for clinical grade AdV vectors, a process

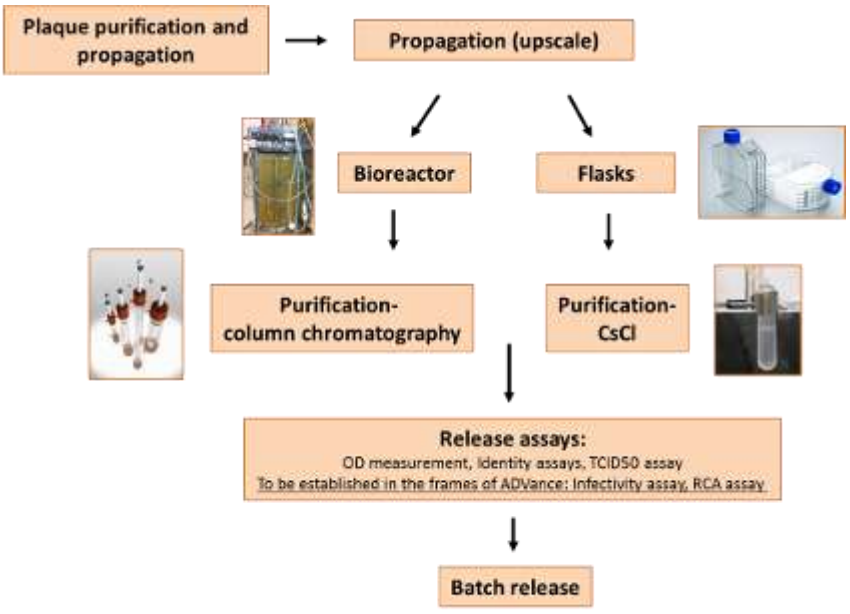

Figure 1. Adenoviral production process

mandatory for entering into clinical trial testing of AdV. As part of the project I will be stationed at Glasgow University (secondment) where I will be involved in the biological validation and mechanistic analyses of candidate host factors and small molecule modulators that impact AdV transduction**.** At Glasgow, I will also carry out infection assays and perform high throughput siRNA screens on SKOV3 cells (in 384 well format for small molecule inhibitors of FX binding to HAdV-5).

Expected results

1. The optimization of recombinant AdV manufacturing process with the following innovations :

- A bio-reactor protocols to increase AdV yield per volume, which will significantly reduces the production cost
- A scalable purification protocol that is cost effective and crucial for large scale product deployment
- A fast and highly sensitive AdV RCA assay (that will meet approval from the FDA), which will increase the bio-safety of the AdVs.

2. Identification of novel (small) compounds that could act as antiviral agents or promote AdV transduction (Glasgow University).

Personal note The mission of Batavia Bioservices is to contribute significantly to ease human suffering from disease by improving the success rate in the translation of candidate medicines from discovery to the clinic. Batavia offers novel technologies and know-how to help its customers to complete their preclinical phases in product development at higher speed, reduced cost, and with higher success rate.

When working for Batavia I feel I contribute personally to this mission, giving me a sense of meaningfulness. The project is framed within the strategy of Batavia, which, in implementing the quality and market competitiveness of the AdV-based products and processes, will help the bench-to-clinic development of gene therapy approaches for many diseases and disabilities, with great impact for the public health in general.

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Meetings, conferences, seminars on AdVs

Alemany

- XII National Virology Congress. Burgos, Spain. June 10 to 13, 2013.
- ESGCT Congress. Madrid October 25-28, 2013.

Arnberg

- 10th Swedish Summer Symposium in Virology. Smögen, Sweden. August 22-24.
- European Congress of Virology, Lyon France. September 11-14.
- 2nd ADVance meeting. Leiden, The Netherlands. September 23-27.

Our group will present on adenovirus-host interactions, i.e. molecules and mechanisms that are involved during adenovirus attachment to human host cells.

Benkő, Harrach

- 9th International Symposium on Viruses of Lower Vertebrates, Yeosu, Korea. October 6-11, 2013 http://www.9thisvlv.kr/.

Our group will present the partial genome characterization of adenoviruses of a novel lineage found in read-eared sliders (and the molecular characterization of novel reptilian and fish parvo- and herpesviruses).

Kremer

- 19th European Study Group on Lysosomal DiseasesWorkshop and Graduate Course on Seggau Castle/ Graz, Austria, September 25-29, 2013 http://www.esgld.org/13/workshops

Prior to the main workshop, we are also offering a **Course on Lysosomes and Lysosomal Diseases** on 25-26 September, for Graduate Students and Young Postdocs

Greber

Invited lectures abroad 2013:

▪ Thun, Switzerland, 02-05	Swiss Workshop in Fundamental Virology
▪ Greifensee, Switzerland, 02-14	Keynote lecture: EU FP7_ITN 'Project Virus entry'
▪ Hannover, Germany, 02-20	4th Adenovirus workshop Hannover
▪ Paris, France 03-21	Keynote lecture: French Society of Gene and Cellular Therapy (SFTCG)
▪ Hong Kong, HK_China. 04-18	5th HKU Pasteur Cell Biology Course, Hong Kong
▪ Basel, Switzerland, 06-18	Novartis Pharma
▪ Thun, Switzerland, 06-26	Swiss Society for Microbiology

▪ Madrid, Spain, 07-21	XX International Summer School 'Nicolás Cabrera' Biomolecules & single molecule techniques
▪ Seili, Finland, 08-19	Virus-cell interactions
▪ Woods Hole, USA, 10-18	Nucleo-cytoplasmic Transport
▪ Heidelberg, Germany, 10-23	EMBO Members Meeting
▪ Utrecht, The Netherlands, 10-31	Students invitation: Institute of Biomembranes, Utrecht University

Seymour

▪ 7th International Conference on Oncolytic Virus Therapeutics, Quebec, Canada June 18-22 2013
ESGCT Conference, Madrid, November 2014

▪ 8th International Conference on Oncolytic Virus Therapeutics, Oxford, UK April 12-15, 2014

Relevant publications of ADVance (last 6 months)

PIs

Papers are ordered alphabetically according to the first word of the title

1. ***Adenoviruses - from pathogens to therapeutics: a report on the 10th International Adenovirus Meeting.*** **Greber UF**, **Arnberg N,** Wadell G, **Benkő M**, & **Kremer EJ**. *Cell Microbiol*. **2013 January**; 15(1):16-23. doi: 10.1111/cmi.12031. (Review)

2. ***Adenovirus signalling in entry*** Wolfrum, N. & **Greber, U.F**. (**2013**). *Cell Microbiol*. 15, 53-62. doi: 10. 1111/cmi.12053. Epub 2012 Nov 13. (Review)

3. ***Bioprocess development for canine adenovirus type 2 vectors.*** Fernandes P, Peixoto C, Santiago VM, **Kremer EJ**, Coroadinha AS, Alves PM *Gene Ther.* **2013 April**; 20(4):353-60. doi: 10.1038/gt.2012.52. Epub 2012 Jul 5

4. ***Canine adenovirus type 2 vector generation via I-Sce1-mediated intracellular genome release*** Ibanes S & **EJ Kremer**. *PLoS ONE*. **2013** in press

5. ***Cavitation-enhanced delivery of a replicating oncolytic adenovirus to tumors using focused ultrasound*** Bazan-Peregrino M, Rifai B, Carlisle RC, Choi J, Arvanitis CD, **Seymour LW**, Coussios CC., *J Control Release.* **2013** Jul 10;169(1-2):40-7.

6. ***Combined antiapoptotic and antioxidant approach to acute neuroprotection for stroke in hypertensive rats.*** Ord EN, Shirley R, McClure JD, McCabe C, **Kremer EJ**, Macrae IM, Work LM. *J Cereb Blood Flow Metab*. **2013 May 1**. doi: 10.1038/jcbfm.2013.70. [Epub ahead of print]

7. ***Combined fiber modifications both to target avB6 and detarget CAR improve virus toxicity profiles in vivo but fail to improve anti-tumoral efficacy over Ad5*** Coughlan L, Vallath S, Gros A, Giminez-Alejandre M, van Rooijen N, Thomas G, **Baker AH**, Cascallo M, **Alemany R**, Hart I. , *Human Gene Therapy*, **2012,** 23(9):960-79.

8. ***Combining Virotherapy and Angiotherapy for the Treatment of Breast Cancer*** Bazan-Peregrino M, Sainson RCA, Carlisle RC, Thoma, C., Morrison, J., Waters RA, Arvanitis C, Harris, AL, Hernandez-Alcoceba R, and **Seymour LW** *Cancer Gene Therapy* (in press **2013**)

9. ***Comparative proteomics using iTRAQ-8plex-based and label-free procedures for studies of human adenovirus infections*** Trinh, H.V., Grossmann, J., Gehrig, P., Roschitzki, B., Schlapbach, R., **Greber, U.F.** & **Hemmi, S**. (**2013**) *Int. J. Proteomics*, **2013**: 581862. doi: 10.1155/2013/581862.

10. ***Differentiated neuroprogenitor cells incubated with human or canine adenovirus, or lentiviral vectors have distinct transcriptome profiles*** Piersanti S, Astrologo L, Licursi V, Costa R, Roncaglia E, Gennetier A, Ibanes S, Chillon M, Negri R, Tagliafico E, **EJ Kremer** & I Saggio.. *PLoS ONE*. **2013** in press.

11. ***Genome sequence of a waterfowl aviadenovirus, goose adenovirus 4.*** Kaján GL, Davison AJ, Palya V, **Harrach B, Benkő M** **2012 November** J Gen Virol 93(11) 2457-2465

12. ***Identification of Novel Small Molecule Inhibitors of Adenovirus Gene Transfer Using a High Throughput Screening Approach*** Duffy MF, Parker AL, Kalkman ER, White K, Kovalskyy D, Kelly SM, **Baker AH** *Journal of Controlled Release* **2013**, 170: 132–140

13. ***Impact of E1 and Cre on Adenovirus Vector Amplification: Developing MDCK CAV-2-E1 and E1-Cre Transcomplementing Cell Lines.*** Fernandes P, Santiago VM, Rodrigues AF, Tomás H, **Kremer EJ**, Alves PM, Coroadinha AS. *PLoS ONE.* **2013**; 8(4):e60342. doi: 10.1371/journal.pone.0060342. Epub **2013** Apr 2.

14. ***Intraductal delivery of adenoviruses targets pancreatic tumors in transgenic Ela-myc mice and orthotopic xenografts.*** José A, Sobrevals L, Miguel Camacho-Sánchez J, Huch M, Andreu N, Ayuso E, Navarro P, **Alemany R**, Fillat C. *Oncotarget*. **2013** Jan;4(1):94-105.

15. ***Investigating endocytic pathways to the endoplasmic reticulum and to the cytosol using SNAP-trap*** Geiger, R., Luisoni, S., Johnsson, K., **Greber, U.F**. & Helenius, A. (**2013**). *Traffic*, 14, 36-46. doi: 10.1111/tra.12018. Epub **2012** Nov 12.

16. ***TRAQ-based and label-free proteomics approaches for studies of human adenovirus infections*** Trinh HV, Grossmann J, Gehrig p, Roschitzki B, Schlapbach R, **Greber UF**, **Hemmi S.** *International Journal of Proteomics* (**2013**) http://dx.doi.org/10.1155/2013/581862

17. ***Regulation of a viral proteinase by a peptide and DNA in one-dimensional space. I. binding to DNA and to hexon of the precursor to protein VI, pVI, of human adenovirus.*** Graziano, V., McGrath, W.J., Suomalainen, M., **Greber, U.F**., Freimuth, P., Blainey, P., Luo G., Xie, X.S. & Mangel, W.F. (**2013**). *J. Biol. Chem.* 288, 2059-67. doi: 10.1074/jbc.M112.377150. Epub **2012** Oct 7

18. ***The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus Aviadenovirus.*** Marek A, Kosiol C, **Harrach B**, Kaján GL, Schlötterer C, Hess M Vet Microbiol **2013** (in press) doi:10.1016/j.vetmic.2013.05.017

19. ***Uncoating of non-enveloped viruses.*** Suomalainen, M. & **Greber, U.F.** (**2013**). *Curr. Opinion Virol.* doi:pii: S1879-6257(12)00192-7. (Review)

20. ***Virus interactions with endocytic pathways in macrophages and dendritic cells*** Mercer, J. & **Greber, U.F**. (2013).. *Trends in Microbiol.*, in press. (Review)

21. ***Viruses in cancer treatment***. **Alemany R**. *Clin Transl Oncol.* **2013** Mar;15(3):182-8.

ESRs and ERs

CagA EPIYA polymorphisms in Colombian Helicobacter pylori strains and their influence on disease-associated cellular responses **Fajardo CA**, Quiroga AJ, Coronado A, Labrador K, Acosta N, Delgado P, Jaramillo C, Bravo MM.. World Journal of Gastrointestinal Oncology. 2013. 5(3):50-59.

Summary: The research of this paper was performed during my Bachelor thesis at the National Cancer Institute in Colombia. It is based on *Helicobacter pylori*, a bacterial pathogen which has been associated with the development of chronic gastritis, duodenal ulcers and gastric cancer. One of the most important virulence factors *of H. pylori* is the protein CagA which can trigger disease-associated changes in the host cell. Specific molecular variations of the CagA protein have been linked to cancer development in Asian and some Western countries. We evaluated several *H. pylori* strains from Colombia, isolated from patients with different gastric pathologies, on the basis of the CagA molecular variations and their possible association with disease-associated cellular responses *in vitro*. We found no association between these molecular variations and disease-associated cellular responses or to disease severity. These results suggest that other factors (*i.e.* host, bacterial or environmental) may play a more important role than *H. pylori* CagA protein molecular variations in gastric cancer development in Colombia.

The Detection of Enteroviruses in Sewage Using Caco-2 Cells, Magdalena Wieczorek**, Łukasz Kuryk,** Agnieszka Witek, Anna Diuwe, Bogumiła Litwińska, Pol J Microbiol., 62(1):97-100, 2013

The work demonstrates the utility of Caco-2 cells to detect enteroviruses in sewage. Viruses were concentrated by beef extract elution and organic flocculation prior to analysis by cell culture assays and RT-PCR. Enteroviruses were detected in all sewage samples, but only one sample was positive solely in RT-PCR assay. We have demonstrated that Caco-2 cells were more effective in enterovirus isolation than RD and L20B cells, depending on procedures used in inoculation process.

Polio-mysterious virus, **Łukasz Kuryk**, Magdalena Wieczorek, Bogumiła Litwińska, Advances in Microbiology, 52, 2, 143-152, 2013

Poliovirus is a very small virus; single stranded RNA of positive polarity constitutes its genetic material. It belongs to the *Picornaviridae* family and may cause the development of poliomyelitis (viral inflammation of the anterior horn of the spinal cord). At the beginning of the twentieth century, the epidemics of polio were very frequent and this fact has caused an increased interest in this virus. At present, there are two types of polio virus vaccines: OPV (oral polio vaccine), it contains live attenuated three serotypes of virus; and inactivated vaccine IPV (inactivated polio vaccine) containing inactive virus particles. In 1988, the World Health Organization (WHO) introduced a program of global eradication of wild-type polio virus around the world (Global Polio Eradication Initiative). The outbreaks of wild-type polio still occur in Asia and Africa. Possibility of reversion of vaccine strains of polio virus to wild type carries the fear of re-transmission of the pathogen. It's the most intensively studied enterovirus, but still remains a "virus-puzzle".

The work for the two above papers paper was performed at Department of Virology, National Polio Laboratory at the National Institute of Public Health in Warsaw (Poland) from July 2011 till November 2012 during my work (of **Ł. Kuryk**) *at the Institute, which was thus a part of my PhD research. These studies demonstrate the utility of Caco-2 cells to detect enteroviruses in sewage and show new methods for enteroviruses isolation and subsequently identification and characterization. The review paper presents the most relevant issues about poliovirus like inter alia: cellular life cycle, pathogenesis of the organism, neuro-virulence, poliovirus tissue tropism, vaccines against poliovirus and eradication program. The work draws attention to unexplained and still unclear issues referring to the biology of poliovirus.*