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

The forth ADVance training event took place this year 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.

**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 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**

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<tr>
<th>Time</th>
<th>Tuesday 22.4</th>
<th>Wednesday 23.4</th>
<th>Thursday 24.4</th>
<th>Friday 25.4</th>
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<td>9.00-11.00</td>
<td>Ethics lecture (1h) &lt;br&gt;Introduction to Demo I (1h) &lt;br&gt;BTT 2440</td>
<td>ER/ESR presentations (5 x 15') &lt;br&gt;Introduction to Demo III (30') &lt;br&gt;BTT 2440</td>
<td>ER/ESR presentations (8 x 15') &lt;br&gt;BTT 3208</td>
<td>Seminar TTA</td>
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<td>11.00-12.00</td>
<td>Lunch</td>
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<td>12.00-14.00</td>
<td>Demonstration I &lt;br&gt;Lab Animal Centre</td>
<td>Demonstration III &lt;br&gt;Lab Animal Centre</td>
<td>Demonstration IV (2h30) &lt;br&gt;Tehnopolis S</td>
<td>Seminar TTA</td>
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<td>14.00-14.30</td>
<td>Coffee break &lt;br&gt;Snellmania</td>
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<td>14.30-17.00</td>
<td>Demonstration II &lt;br&gt;BTT 2440</td>
<td>Demonstration III &lt;br&gt;Lab Animal Centre</td>
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<td>17.00-18.30</td>
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<td>Poster session + aperitif &lt;br&gt;Bioteknia 1 lobby</td>
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<td>18.30</td>
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<td>Dinner &lt;br&gt;Tiukanlinna</td>
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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.
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.
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