

Symposium and EMBO Practical Course: Viral Vectors in Gene Therapy: Applications & Novel Production Methods

A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland - August 26th – September 4th, 2010

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AGENDA

Day 1 – Thursday 26th Aug

Reception of participants															
8:00 – 10:00	<p>Set up posters and exhibits in the hall of the conference building.</p> <p><i>The posters will be available all the time from Aug 26th to 29th. The coffee breaks (each morning and afternoon) and the lunch will be of 30' and 90', respectively, or otherwise indicated</i></p>														
Opening session <i>Chairpersons: E. Makkonen and S.Ylä-Herttuala</i>															
10:00 – 11:00	<table border="1" style="width: 100%;"> <tr> <td style="text-align: center;"><i>Welcome addresses</i></td> <td style="text-align: center;">Hanna LESCH / Mauro MEZZINA (Kuopio/Paris), Ari HINKKANEN (A.I.V. Institute) and Timo RISTOLA (Ark Therapeutics)</td> </tr> <tr> <td style="text-align: center;"><i>History of the baculovirus system</i></td> <td style="text-align: center;">Loy VOLKMAN (Berkeley, Ca-USA) (30')</td> </tr> </table>	<i>Welcome addresses</i>	Hanna LESCH / Mauro MEZZINA (Kuopio/Paris), Ari HINKKANEN (A.I.V. Institute) and Timo RISTOLA (Ark Therapeutics)	<i>History of the baculovirus system</i>	Loy VOLKMAN (Berkeley, Ca-USA) (30')										
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12:45 – 14:15 Lunch															
State-of-the-art lectures <i>Chairpersons: O. Danos and M. Collins</i>															
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Vector development <i>Chairpersons: Boro Drobolic and M. Kaikkonen</i>															
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19:00	Welcome dinner cocktail														

Day 2 – Friday 27th Aug

Vector applications I: Baculovirus system <i>Chairpersons: K. Airene and M. Van Oers</i>	
9:00 – 10:30	<i>Tailoring the baculovirus expression system for recombinant protein and virus-like particle production</i> Monique VAN OERS (Wageningen, NL) (30')
	<i>AAV vector production using baculovirus expression system – Comparison with traditional methods</i> Robert M. KOTIN (Bethesda, MD-USA) (30')
	<i>Baculovirus-mediated gene delivery for tissue regeneration</i> Yu-Chen HU (Hsinchu, TW) (30')
10:30 – 11:00	Coffee break
11:00-12:00	<p style="text-align: center;">Selected communications (15')</p> <p><i>Production and purification of lentiviral vectors generated in 293T suspension cells with baculoviral vectors</i> Anna LAITINEN (Kuopio, FI)</p> <p><i>Baculovirus deleted for chitinase, cathepsin and p10 genes improves purified rAAV8 quality</i> Lionel GALIBERT (Evry, F)</p> <p><i>Baculovirus-mediated sustained expression of human endostatin-angiostatin fusion gene for cancer therapy</i> Wen-Yi LUO (Hsinchu, TW)</p> <p><i>Development of Baculovirus Vectors for micro-RNA Expression and sustained Gene Suppression</i> Chiu-Ling CHEN (Hsinchu, TW)</p>
12:00 – 13:00 Workshop (part 1)	<p style="text-align: center;">International Efforts for Recombinant Baculovirus Reference Materials</p> <p>Panel members: Amine KAMEN (NRC, Montreal, CA), Otto Merten (Genethon, Evry, FR), Mauro MEZZINA (CNRS-EASCO, Paris, FR), Yu-Chen HU (Hsinchu, TW), Kari AIRENNE (Ark Therapeutics, Kuopio, FI), Paula ALVES (IBET, Oeiras, PT), Yoshifumi HASHIMOTO (Protein Sciences Corp. USA), GSK-Biologics (to be confirmed)</p> <p><i>Presentation of the project / Implementation of the project /role of the participants</i> <10' minutes panel-list remarks. Panel members will focus on selection of robust recombinant baculovirus construct, method of production, concentration and/or purification , formulation and quantification/characterization of Baculovirus Reference Material (BRM)</p> <ul style="list-style-type: none"> • <i>Baculovirus molecular design</i> (Yoshifumi Hashimot, Protein Sciences Corp. USA) • <i>Large Sccale Production</i> (Otto Merten, Genethon, FR) • <i>Baculovirus concentration/purification and Formulation</i> (Paula ALVES (IBET, PT) • <i>Baculovirus quantification and characterization</i> (Amine Kamen, NRC, CA)
13:00 – 14:30	Lunch
14:30 – 15:30 Workshop (part 2)	<p style="text-align: center;"><i>Interactive discussion with panel-lists-participants</i></p> <ul style="list-style-type: none"> - Responses to questions: <i>Which recombinant baculovirus construct? Marker gene? Quantity to produce and mode of production? Series of downstream processing steps? Formulations? Do we agree on methods for quantification and characterization? How to store and distribute the BRM?</i> - Define strategies to engage other institutional and Industrial partners in the initiative? - Establish a Project Roadmap (deliverables/milestones) - Conclusions and perspectives
15:30 – 16:30 Round table I	<p style="text-align: center;">From education to the innovation in the development of gene therapy products <i>Chairpersons: M. Mezzina and A-M. Määttä</i></p> <p>Kalervo VÄÄNÄNEN, Academic Rector of the University of Eastern Finland (UEF): <i>The role of UEF in the education, research and innovation in Kuopio Science Park</i> (15')</p> <p><i>Presentations on the state-of-the-art of the GT development and market by the participants from academia and industries (Ark, IBET, Lentigen, Molmed, UEF...)</i></p>
16:30 – 17:00	Coffee break

17:00 – 18:00	Selected communications on animal model and stem cells approaches Chairpersons: H. Lesch and M. Mezzina
	A new mouse model for Crigler-Najjar syndrome and possible therapeutic approaches Giulia BORTOLUSSI (Trieste, I)
	Bcl-2 Expression Enhances Myoblast Sheet Transplantation Therapy for Acute Myocardial Infarction Antti SILTANEN (Helsinki, FI)
	The effect of CDF gene delivery using recombinant AAV2 vector in a rat partial lesion model of Parkinson's disease Susanne BÄCK (Helsinki, FI)
	Antagonizing HSA21-synthetic miRNAs effects in the Ts65Dn mouse model of Down syndrome by a lentiviral mediated approach Luciano SOBREVALS (Barcelona,SP)
19:30	Bus transportation to Koivumäki Manor
20:00 –	Gala dinner at the Koivumäki Manor

Day 3 – Saturday 28 th Aug	
9:00 – 10:30	Vector applications II: AAV and LV in human diseases Chairpersons: J. Chamberlain and A. Aiuti
	Viral-mediated gene therapy for muscular dystrophies Jeffrey CHAMBERLAIN (Seattle, WA-USA) (30')
	Diagnostic, transgenic and translational medicine of muscular dystrophies Bjaerne UDD (Helsinki, FI) (30')
	Recombinant AAV-mediated gene delivery to the central nervous system Liliane TENENBAUM (Brussels, B) (30')
10:30 – 11:00	Coffee break
11:00 – 13:00	Application of AAV vectors for the treatment of cardiovascular disease Richard J. SAMULSKI (Chapel Hill, NC-USA) (30')
	Development of lentiviral gene therapy for SCID Alessandro AIUTI (Milan, I) (30')
	Novel treatments for human diseases using lentiviral vectors Boro DROPULIC (Gaithersburg, MD-USA) (30')
	Directed integration of new insulated lentiviral vectors to the heterochromatin towards safer gene transfer to stem cells. Odile COHEN-HAGUENAUER (Cachan, F) (30')
13:00 – 14:30	Lunch
14:30 – 16:30	Production and purification Chairpersons: O-W. Merten and A. Kamen
	Vaccine & Viral Vector Manufacturing – Strategies for the Future Robert MOHRENWEISER (Freiburg, D) (30')
	Advances in the purification of baculoviruses: implications for enveloped viruses Manuel CARRONDO (Oeiras, PT) (30')
	Strategies for manufacturing gene therapy applications Robert M. KOTIN (Bethesda, USA) (30')
	Purification of AAV-based vectors for use in clinical studies J. Fraser WRIGHT (Philadelphia, PA-USA) (30')
16:30 – 16:50	Coffee break

16:50 – 18:10	<i>Efficient manufacturing of viral vectors using the large scale transfection technology</i>	Amine KAMEN (Montreal, CA) (30')
	<i>Advances in the large scale production and purification of LV vectors</i>	Otto-Wilhelm MERTEN (Evry, F) (30')
	<i>Monolithic chromatography supports – technology platform for the analysis and production of new generation of drug candidates like plasmid DNA and viral vector particles</i>	Ales STRANCAR (Ljubliana, SI) (20')
18:10 – 19:30 Round table II	From bench to the bed side: Regulations & Ethics Chairpersons: O. Cohen-Haguenuer and R. Shaw	
	<i>Clinical development, regulations and European concerted actions</i> Robert SHAW (Kuopio, FI) and Tiina PALOMÄKI (FIMEA, Kuopio, FI) (30') <i>Ethics and gene therapy</i> Raymond SPIER (Surrey, UK) (30') <i>Discussion</i> (20')	

Day 4 – Sunday 29th Aug

8:30 – 10:30	Analytical methodology Chairpersons: P. Alves and R. Kotin	
	<i>Development of quality control methods for baculovirus vectors</i>	Paula ALVES (Oeiras, PT) (30')
	<i>Quality control for AAV-vectors: efficacy and safety considerations</i>	J. Fraser WRIGHT (Philadelphia, PA-USA) (30')
	<i>Development and validation of analytical methods for lentiviral vectors for clinical applications</i>	Giuliana VALLANTI (Milan, I) (30')
	<i>In vivo assays to address the genotoxic potential of gene therapy vectors</i>	Eugenio MONTINI (Milan, I) (30')
10:30 – 11:00	Coffee break	
11:00 – 12:30	Closing session Chairpersons: F. Bushman, H. Lesch and M. Mezzina	
	Presentations of posters by topics (15') and of 3 selected posters* (10' each)	
	<i>Future prospect of viral gene therapy tools</i>	Frederic BUSHMAN (MD-USA) (35')
	Concluding remarks and presentation of the prizes to the two best posters** by F. BUSHMAN and H. LESCH (10')	
END of the SYMPOSIUM		

* selected by the organizers ** selected after the vote of the participants

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August 30 – September 4, 2010: *EMBO Practical Course*

25 students will be shared into 3 groups (8-9 students for each) involved in the technology of the production and purification of the BV, AAV and LVV vectors, as described in (i), (ii) and (iii), respectively, below. The three groups will produce and test the vectors preparations with *in vitro* (titration, quality control) and *in vivo* functional assays with mice (iv). In order to show the whole technologies within the 6 days, parts of the experiments will be prepared in advance, e.g. cells transfections, infections and *in vivo* transfer into animals, namely, which take several days or weeks.

(I) Baculovirus production, (purification/concentration) and characterization (**experiments for group 1**).

(II) AAV production, (purification) and characterization using regular and baculovirus-based methods (**experiments for group 2**)

(III) Lentivirus production, (purification) and characterization using regular and baculovirus-based methods. (**experiments for group 3**)

(IV) *In vivo* applications of last generation of AdV, (LV), AAV and baculovirus into models for cardiovascular diseases/cancer. (**experiments for all groups**) Gene transfer will be performed into cell cultures and into animal models (mice). The AdV and BACV will be the reference vectors systems which will be prepared in advance in Kuopio laboratory. The expression of the transgenes (GFP and other therapeutic genes to be decided) with the SPECT/CT imaging approach

(V) Meet the experts: experts in purification, quality control and *in vivo* transfer (this will include also the good laboratory practices for animal experimentation) of vectors will meet the groups of the students.

Day 5 – Monday 30th						
Day 5 30.8.	Group 1 (Bac) <i>Tiago VICENTE</i> <i>Emilia MAKKONEN</i> <i>Joonas MALINEN</i>		Group 2 (LV) <i>Hanna LESCH</i> <i>Petri MÄKINEN</i> <i>Anna LAITINEN</i> <i>Juha RUUSKANEN</i>		Group 3 (AAV) <i>Elisa VÄHÄKANGAS</i> <i>Jarmo ASIKAINEN</i> <i>(Emilia MAKKONEN)</i> <i>(Juha RUUSKANEN)</i>	
9-10	Overview of the work		Overview of the work		Overview of the work	
10-11	Cell division for transfection /infection	Cell division for transfection /infection	Cell division for production (adh 293T)	Cell division for production (Suspension)	Cell division for production (293T)	Cell division for production (Sf9)
11-12			Cell division for production (Suspension)	Cell division for production (adh 293T)	Cell division for production (Sf9)	Cell division for production (293T)
12-13	Lunch					
13-14	Primary transfection	Large scale production: infection	Transfection (293T)	BV Transduction	Transfection (293T)	Infection (Sf9)
14 -15	Large scale production: infection	Primary transfection	BV Transduction	Transfection (293T)	Infection (Sf9)	Transfection (293T)
15 - 15.30	Coffee break					
15.30- 17	Meet the experts: Vectorology and vector production systems <i>Otto MERTEN, Tiago VICENTE, Hanna LESCH, Emilia MAKKONEN</i>					

Day 6 – Tuesday 30th

Day 6 31.8.	Group 1 (Bac) <i>Tiago VICENTE</i> <i>Emilia MAKKONEN</i> <i>Joonas MALINEN</i>	Group 2 (LV) <i>Hanna LESCH</i> <i>Petri MÄKINEN</i> <i>Anna LAITINEN</i> <i>Juha RUUSKANEN</i>	Group 3 (AAV) <i>Elisa VÄHÄKANGAS</i> <i>Jarmo ASIKAINEN</i> <i>(Emilia MAKKONEN)</i>
9:00- 9:30	Basics of chromatographic purification methods <i>Tiago VICENTE</i>		
9:30- 10:30	Virus collection and clarification	Virus chromatographic purification demo	Transfection efficacy by microscopy Virus collection and clarification
10:30- 11:30	Virus concentration	Transfection efficacy by microscopy Virus collection and clarification	Virus chromatographic purification demo I (plasmid produced AAV)
11:30- 12:30	Lunch	Virus concentration by ultracentrifugation	Lunch
12:30- 13:30	Virus chromatographic purification demo II (BV produced AAV)	Lunch	Virus chromatographic purification demo II (BV produced AAV)
13:30- 14	Virus polishing, formulation and storage	Virus polishing, formulation and storage	Virus polishing, formulation and storage
14-15	Virus transduction for titration	Virus transduction for titration	Virus transduction for titration
15- 15.30	Coffee Break		
15.30- 17	Meet the experts: Downstream processing of viruses and viral vectors <i>Otto MERTEN, Mikko KARJALAINEN, Tiago VICENTE</i>		
17- 17:30	Clean room working / GMP (SAKKY)		
17:30- 18	Tour at GMP facilities (SAKKY)	Tour at Ark Therapeutics	
18- 18.30	Tour at Ark Therapeutics		Tour at GMP facilities (SAKKY)

Day 7 – Wednesday 1st

Day 7 1.9.	Group 1 (Bac) <i>Tiago VICENTE</i> <i>Emilia MAKKONEN</i> <i>Joonas MALINEN</i>	Group 2 (LV) <i>Hanna LESCH</i> <i>Petri MÄKINEN</i> <i>Anna LAITINEN</i> <i>Juhan RUUSKANEN</i>	Group 3 (AAV) <i>Elisa VÄHÄKANGAS</i> <i>Jarmo ASIKAINEN</i> <i>(Emilia MAKKONEN)</i>
9-10	Overview of the standard analytical methods for baculoviruses	Overview of the standard analytical methods for lentiviruses	Overview of the standard analytical methods for AAV
10-11	Genomic DNA extraction	Preparation of qPCR run	Genomic DNA extraction
11-12	Preparation of qPCR run	Titration (FACS) <i>Hanna-Riikka KÄRKKÄINEN</i>	Preparation of qPCR run
12-13	Lunch		
13-14	Virus titration (microscope)	p24 ELISA (demo)	Titration (FACS) <i>Hanna-Riikka KÄRKKÄINEN</i>
14-15	Virus titration(FACS) <i>Hanna-Riikka KÄRKKÄINEN</i>	p24 ELISA/qPCR end	qPCR end
15-15.30	Coffee break		
15.30-17	Analysis of titration results	Analysis of titration results	Analysis of titration results
17-19	Meet the experts: Basic bioinformatics approaches to study vector integration <i>Eugenio MONTINI (Milan, I)</i>		

Day 8 – Thursday 2nd

Day 8 2.9.	Group 1 (Bac)	Group 2 (LV)	Group 3 (AAV)
9-10.30	Meet the experts: Good Practices of Animal Experimentation <i>Jere PIKKARAINEN, Tommi HEIKURA, Taina VUORIO</i>		
10.30-12	Tour at the animal facilities	Tour at the animal facilities	Tour at the animal facilities
12-13	Lunch		
13-14	Gene transfer to pig heart– demonstration <i>Krista HONKONEN</i>	Gene transfer to rat brain tumor –demonstration <i>Jere PIKKARAINEN</i> <i>Haritha SAMARANAYAKE</i>	Gene transfer to mouse heart -demonstration <i>Mari MERENTIE,</i> <i>Riina RISSANEN</i>
14-15	Gene transfer to rat brain tumor –demonstration <i>Jere PIKKARAINEN</i> <i>Haritha SAMARANAYAKE</i>	Gene transfer to mouse heart -demonstration <i>Mari MERENTIE,</i> <i>Riina RISSANEN</i>	Gene transfer to pig heart– demonstration <i>Krista HONKONEN</i>
15-15.30	Coffee break		
15.30-16.30	Gene transfer to mouse heart –demonstration <i>Mari MERENTIE,</i> <i>Riina RISSANEN</i>	Gene transfer to pig heart– demonstration <i>Krista HONKONEN</i>	Gene transfer to rat brain tumor –demonstration <i>Jere PIKKARAINEN</i> <i>Haritha SAMARANAYAKE</i>
16.30-18.30	Meet the experts: Pre-clinical studies in Cancer and Cardiovascular diseases <i>Haritha SAMARANAYAKE, Ann-Marie MÄÄTTÄ, Tommi HEIKURA</i>		

Day 9 – Friday 3rd

Day 9 3.9.	Group 1 (Bac)	Group 2 (LV)	Group 3 (AAV)
9- 10.30	Lecture: Histological Methods <i>Jere PIKKARAINEN</i>		
10.30- 12	Analysis of histological stainings <i>Taina VUORIO</i>	Analysis of histological stainings <i>Tommi HEIKURA</i>	Analysis of histological stainings <i>Jere PIKKARAINEN</i>
12-13	Lunch		
13- 14.30	Meet the experts: Imaging techniques <i>Olli GRÖHN, Jere PIKKARAINEN, Tuulia HUHTALA or Jussi RYTKÖNEN</i>		
14.30- 15	Coffee break		
15.00- 16.10	SPECT/CT <i>Tuulia HUHTALA or Jussi RYTKÖNEN</i>	Analysis of ultrasound and angiographic data <i>Mari MERENTIE, Krista HONKONEN, Henna KARVINEN</i>	MRI, tumor size <i>Jere Pikkarainen, Haritha SAMANAYAKE</i>
16.10- 17.20	MRI, tumor size <i>Jere PIKKARAINEN, Haritha SAMANAYAKE</i>	SPECT/CT <i>Tuulia HUHTALA or Jussi RYTKÖNEN</i>	Analysis of ultrasound and angiographic data <i>Mari MERENTIE, Krista HONKONEN, Henna KARVINEN</i>
17.20- 18.30	Analysis of ultrasound and angiographic data <i>Mari MERENTIE, Krista HONKONEN, Henna KARVINEN</i>	MRI, tumor size <i>Jere PIKKARAINEN, Haritha SAMANAYAKE</i>	SPECT/CT <i>Tuulia HUHTALA or Jussi RYTKÖNEN</i>

Day 10 – Saturday 4th

Day 10 4.9.	Group 1 (Bac) <i>Emilia MAKKONEN</i>	Group 2 (LV) <i>Hanna LESCH</i> <i>Petri MÄKINEN</i>	Group 3 (AAV) <i>Elisa VÄHÄKANGAS</i>
9- 10.30	Debriefing of the course		
11-13	Evaluation of the course <i>Hanna LESCH, Emilia MAKKONEN, Petri MÄKINE, Mauro MEZZINA, Jere PIKKARAINEN, Elisa VÄHÄKANGAS and Tiago VICENTE</i>		
13-14	Lunch		

END of the EMBO practical course