Training Event:
Academia meets Industry

23-27 September 2013,
Leiden, The Netherlands

Preface

The second *ADVance* training event took place this year in Leiden, gathering the ESRs and ERs together for one week at the *Batavia Bioservices* enterprise. The event involved theoretical and practical training on adenoviruses (AdV), focusing mainly on the management, exploitation and commercial valorization of the innovations, which were the core topics of the seven lectures delivered over the first 3 days. Scientific lectures were also delivered on the 4th day, which presented modern basic virology approaches, in terms of basic evolution and therapeutic applications. The ESRs and ERs had the opportunity to present their work and to talk about their career. The practical workshop was dedicated to downstream and upstream development processes whose objective was to familiarize the ESRs and ERs with production of biological material on an up-scaled level. Finally, networking between all participants in the event (the ESRs and ERs among themselves as with PIs) was fostered by social events including an enchanting cruise in *grachten* of Leiden, which allowed the workshop to be scientifically excellent in a pleasant environment.

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1. Reports on the presentations

As for the theoretical part of the workshop, 10 plenary lectures were delivered throughout the whole week by alternating the sessions with seminars and discussions with the practical laboratory sessions described in the section 2 of this report. Therefore, this first section has been divided into 3 subsections, as follows, (**1.1**) lectures on entrepreneurship, intellectual properties, business development and other transversal issues related to the exploitation of the research innovation and

industry-related topics, (**1.2**) seminars on scientific topics related to adenoviruses and virology I general and (**1.3**) the presentation of the ESRs and ERs on their scientific projects, orally or with a poster as indicated.

1.1. Lectures on entrepreneurship, intellectual property and other industry-related topics

- **Leon KRUIMER**, Former Chief Financial Officer at Crucell, Leiden.

Title of the lecture: ***Leadership in biotech***

He started the session on leadership and entrepreneurship in biotech with a very inspirational presentation on how to create and lead a company. Based on personal experience he emphasized several features which are necessary to become a successful organizational leader: the importance of vision, the capacity to make a decision when needed, well defined goals, ambition, achieving the impossible. As with every company, a biotech company also relies on persons who are working for it, so having a strong team of innovative, inspired and motivated people, ready to meet "stretched goals" is indispensable for growing a competitive organization. Of course, a good leader also has to be competent in his/her field, and possesses excellent communication and interpersonal skills. The main take home message from his presentation was Winston Churchil's quote: "*Never, never, never give up!*" that can easily be applicable in the field of biotechnology.

- **Menzo HAVENGA**, President & Chief Executive Officer, Batavia Bioservices, Leiden)

Title of the lecture: ***Entrepreneurship***

He introduced us to first hand entrepreneurship by telling the story of *Batavia Bioservices*. He described in more details some of the points tackled in the previous lecture: 1. uniqueness – having concept of originality and ingenuity, 2. creativity – readiness, willingness, being able to, 3. risk taking – nothing is impossible, 4. business survey, 5. developing potentials, 6. adaptability – being able to adapt faster than the competition. He underlined the importance of market analysis in order to identify the need of the market, emphasizing that a highly growing market is more likely to grant opportunity for success. Since starting up a biotech company requires important investment, good starting points as a source for funding are semi-governmental organizations, as proved to be the case for *Batavia Bioservices*.. In the second part of his talk, he gave us an overview of preparing a business plan, which is a prerequisite for conducting a good business. A business plan is an essential roadmap for business success that generally projects 3-5 years ahead and outlines the route a company intends to take in order to grow revenues. In short: what you plan to do and how you plan to do it. By preparing a business plan one makes oneself comfortable with one's own ideas and projects, and perceives if their realization is financially realistic and feasible. A business plan comprises several sections: executive summary, introduction and background information, company profile (mission, vision, strategy, partners..), market analysis (become familiar with all

aspects of the market so that the target market can be defined), financial analysis (funds, investors opportunities), competitive analysis (determine the strengths and weaknesses of the competitors within the market), risk assessment (SWOT, i.e. Strengths, Weaknesses, Opportunities, and Threats), appendices (references, IP reports..).

- **Bart VAN WEZENBEEK**, Patent Attorney, V.O. Patents & Trademarks, The Hague

  Title of the lecture: ***Intellectual property***

Dr van Wezenbeek outlined the importance of protecting intellectual property, and how it is handled from a legal point of view. By definition, a patent is a set of exclusive rights granted by a sovereign state to an inventor or their assignee for a limited period of time, in exchange for the public disclosure of the invention. One of the goals for protecting intellectual property is to keep and profit from your lead and to bring your product to the market, while requirements for patentability include novelty, inventive step and industrial applicability. The overall rule in patenting is the principle of novelty, according to which everything that is already publicly available cannot be patented. The lecturer also drew a parallel between patent application procedures in Europe and US, as well as what is considered patentable in Europe and what in US. The last part of the presentation was dedicated to examples aimed at explaining priority and prior rights.

- **Ada KRUISBEEK**, Founder, CSO, *DCPrime B.V.* a spin-off company of the VU University Medical Center, Amsterdam

  Title of the lecture: ***Translation of science into business, valorization***

She started her career in academic settings, and after being successful as a scientist, she proved that university research can set a good foundation for developing small companies. Her arguments are the remarkable reservoir of knowledge present at universities, smart and well educated people who come up with new ideas during their research, multidisciplinary teams covering different areas. The development of novel therapeutics in itself is a rather inefficient process that requires large investments. Her opinion is that more than a half of all innovative medicines and diagnostics are generated by small companies, which are then developed further by big pharmaceutical companies. *DCPrime B.V* is dedicated to developing a novel type of therapeutic DC-based vaccines. While founding her own company was gratifying on a professional level, it is the translation of her work into products and services, as well as in patients in future, which is giving her personal satisfaction. This is for her the main difference between scientific research in academic and industrial settings.

- **Lorenz SCHEPPLER**, Director Regulatory Affairs, Crucell, Leiden

  Title of the lecture: ***Regulatory affairs***

The main role of regulatory affairs (RA) is to protect public health by controlling the safety and efficacy of products in areas including pharmaceuticals, veterinary medicines, medical devices, pesticides, agrochemicals, cosmetics and complementary medicines. The RA also assure that the studies are performed accordingly to ethical standards. The regulatory professional's job is to keep

track of the ever-changing legislation in all the regions in which the company wishes to distribute its products, to advise on the legal and scientific restraints and requirements, and to present registration documents to regulatory agencies in order to obtain authorization for the products concerned. There are two major regulatory affairs agencies in the world: Food and Drug Administration (FDA) and European Medicines Agency (EMA). Both FDA and EMA are involved in clinical trial authorization, market authorization application and delivering guidelines from The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and International Organization for Standardization (ISO).

- **Jolanda VAN VLIET** (Quality Assurance manager, *Batavia Bioservices*, Leiden
  Title of the lecture: ***Quality assurance in development***

This lectures convinced us that very often we do not realize that quality control can be applied in all aspect of life and not only in production processes. Jolanda presented us, in a very understandable manner, that quality control is very important whether it concerns preparing pizza in pizzeria, writing our lab journals or producing flu vaccines. Quality is not there as a threat but is there to protect. By definition quality means “fit for use” and assures that the user of the product is happy when using it. That product may be called quality product. In pharmaceutical terms quality means identity, strength (dose), safety and purity. According to the FDA quality should be built into the product and controls should be in place at every step of the manufacturing: people, facilities, equipment, processes, testing, and documentation. Quality of a pharmaceutical product should start already at the level of research. Tools to achieve quality in drug development include: common sense, good documentation practices, GxP (Good Research Practice (GRP), Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), and Good Clinical Practice (GCP)). Even though it sometimes takes time and seems senseless, good documentation practice is essential for research, development and GMP: if it is not documented, it did not happen. In the second part of the presentation we had a practical exercise. We divided ourselves into three groups and each group had to define a process for the delivery of pizza based on the CTQ (Critical To Quality is an attribute of a part, assembly, sub-assembly, product, or process that is literally critical to quality or more precisely, has a direct and significant impact on its actual or perceived quality.) tree. We had to define critical process parameters which should be set during development. All three group presented results at the end and successfully organized efficient pizza delivery.

- **Oliver GUILLAUD** Government Affairs and Public Health Advisor, *Crucell*, Leiden
  Title of the lecture: ***Product deployment and global access***

He introduced us to the field of product deployment and global access for pharmaceutical products. He talked about the stakeholders like political institutions, manufacturers, the civil society and public health authorities (e.g. World health organization) who have an interest in distributing a vaccine on a global scale. He compared how a vaccine can be introduced into to the market and what the differences in Europe (sovereignty of state, but the national institutions follow the EU

laws) and in the US are. There is a lack of treatments for orphan or rare diseases as these are often not lucrative for the pharmaceutical industry to invest in profound research and development. Therefore, he informed us that there is funding available from the European Union to develop cures against rare diseases. .

1.2. Scientific topics related to adenoviruses and virology



- **Ab OSTERHAUS** Professor of Virology, Erasmus MC, Rotterdam

Title of the lecture: ***Emerging viruses in a changing world***

With a perspective on megacities, climate change and increasing traveling around the world the spread of new emerging viruses is easier than ever before. Ab Osterhaus' talk on virus discovery and vaccine development showed the need for 'online' surveillance of emerging viruses. For instance, he described how his laboratory was involved in the quick identification of SARS-associated coronavirus in 2002/2003. Therefore, fast identification of the causative agent of an upcoming epidemic is the first step in preventing it.
In the second part, he showed that viruses can easily overcome species borders and this is a permanent threat for new emerging viruses. In this regard, he pointed out the increasing number of infections of animal poxvirus in humans since the eradication of the small pox virus and the controversy around the 'gain of function' experiments on the avian flu virus H7N9 in the beginning of 2013.

- **Rob HOEBEN** Professor of gene transfer, Leiden University, Leiden,

Title of the lecture: ***Reverse and forward genetic strategies to improve oncolytic viruses.***

Pr Hoeben gave a talk on the TWINS (Therapy with infectious anti-cancer agents) he accommodates in his lab. He tries to improve the efficacy of reoviruses and adenoviruses using various approaches. For example, albumin coating of the viral capsid improves the uptake rate of tumor cells. He also showed an alternative approach to improving virus targeting of certain tissues by using a cell line, which is hard to infect but expresses an error-prone viral DNA polymerase. Thus, during several viral replication cycles, there is a positive selection on those mutations that facilitate further infections.

- **Sarah GILBERT** Professor of Vaccinology, Jenner Institute, University of Oxford

Title of the lecture: ***Exploiting synergies in human and livestock vaccine development***

Malaria is one of the world's deadliest killers, responsible for in excess of 1 million fatalities and between 300 and 500 million clinical cases each year. T-cell-mediated responses against the liver-stage of *Plasmodium falciparum*, the causative agent of malaria are critical for protection in

the human irradiated sporozoite model and several animal models. Sarah Gilbert () group developed a heterologous prime-boost approach against the highly immunogeneic multiple epitope-thrombospondin-related adhesion protein (ME-TRAP) employing both plasmid DNA and modified vaccinia virus Ankara (MVA) or simian adenovirus AdCh63 based delivery of malaria DNA. This 'synergistic' prime boost immunization induced a substantially greater T cell response than either treatment alone and is currently undergoing Phase I/IIa clinical trials.

1.3. Reports on the presentations of the ESRs and ERS (oral and posters)

The 14 *ADVance* fellows (see their picture in the composition here) presented their work either as a formal oral presentation (9 of them) or with a poster (5 fellows). The latter also presented their poster orally, with a very informal presentation by using the poster as the visual support and the PIs and other persons participating in the work -shop were the audience staying around the poster and the fellow who presented his/her poster. This created an environment where the fellows adapted the communication settings of their presentation to a public staying just at less than one meter around.

• **Rodinde HENDRICKX** ESR, Zurich University – Urs Greber's team

Title of poster presentation: ***Mouse Adenovirus Biology and construction of their oncolytic vectors.***

Rodinde's project is aimed at the further characterisation of Mouse Adenovirus (MAdV) and the development of an oncolytic virus, which can be tested in a syngeneic tumor model. The

progress of her research was outlined with a poster presentation. In the months following the Zürich meeting, Rodinde focused on two main topics: (1) improving the conditions to produce large(r) amounts of virus and (2) the further development of a Gaussia luciferase expressing virus. For the first topic, two approaches are tested to improve the viral production: firstly, the possibility to knock down part of the innate immune response of mouse cells *via* the inhibiton of IRF-3. This method is currently being developed. Secondly, screening different mouse and human cells to identify the lines exhibiting the highest viral production yields. In the latter approach Rodinde found that the Panc02 cells (a kind gift of Dr. R. Alemany) lead to a high yield of production of MAdV-1. The second topic is the development of a luciferase expressing virus. Rodinde tested different Gaussia luciferase constructs *in vitro*, since the Gaussia model is a small easy-to-handle protein and it has been successfully tested *in vivo*. The progress of the ongoing *in vivo* analysis of MAdV was described.

- **Anandi RAJAN** ESR, Umeå University – Niklas Arnberg's team

Title of oral presentation: ***Identifying binding partners of the penton base of Adenovirus types 40 and 41***

The penton base of AdV40 and AdV41 lacks the Arg-Gly-Asp tri-peptide (RGD) motif that is known to play a role in internalization of the virus. However, other tri-peptides motifs such as LDV, LDA, SDI, ADI and EGD are present in the Ad40/41 penton base, which could potentially mediate binding to other integrins such as α4β1 (LDV, LDA, SDI, ADI), α4β7 (LDV, ADI), α5β1 (SDI) and α2β1 (EGD, present in Ad40 penton base only). The aim of Anandi's project is to identify binding partners for the penton base of Adenovirus type 40 and 41. There are several approaches she is implying in her research: 1. use recombinant penton base protein to check binding with CHO cell lines expressing specific integrins, 2. use recombinant penton base protein to check binding with NCI60 cell library, 3. perform infection experiments in competition with peptides containing integrin-binding motifs, 4. perform infection experiments in competition with recombinant penton base protein, 5. pull down experiments with tagged penton base protein and cell membrane extracts of small intestinal cell lines (FHs74Int, Caco-2, HT-29).

- **Iva PODGORSKI,** ESR, Hungarian Academy of Science, Budapest - Maria Benk and Balasz Harrash's team

Title of oral presentation: ***Genomic and bioinformatics analysis of simian adenovirus 19 confirms the need to establish a new adenovirus species***

Species Simian adenovirus A (SAdV-A) is so far the only species officially approved exclusively for monkey adenoviruses (AdVs). Therefore, Iva is looking for novel AdVs in non-human primates, which could also be used as vectors in human medicine as they are very close to humans, but far enough that there are no antibodies against them. The purpose of her work was to examine the genetic content and phylogenetic relationships of an Old World monkey AdV (SAdV-19) isolated from yellow baboon (Papio cynocephalus). After sequencing, the SAdV-19 genome was found to consist of 34,063 bp. Among the 38 putative genes characteristic for the genus

Mastadenovirus, her team found two genes of different lengths predicted to code for the adenoviral cellular attachment protein, the fiber. For the first time in SAdVs, the two other exons belonging to the so-called U exon were also identified. Phylogenetic calculations based on the hexon implied that SAdV-19 and recently published baboon adenovirus 2 and 3 (BaAdV-2 and BaAdV-3) represent three different (sero)types within the proposed species SAdV-C. However, significant divergence was found between the shorter fiber proteins (fiber1) of SAdV-19 and BaAdV-2 or BaAdV-3. The closest relative of fiber1 of SAdV-19 in the GenBank was that of SAdV-1, sharing ~47% amino acid sequence identity. This finding may reflect that a usually rare, inter-species homologous recombination event took place between the two viruses (or their close ancestors or relatives) in the past. Genomics and bioinformatics analysis of simian adenovirus 19 confirms the need to establish a new adenovirus species.

- **Nicholas DOWNES** ESR, Kuopio University, Seppo Yla-Herttuala's team
  Title of oral presentation: ***Use of ncRNAs for cardiovascular disease therapy***

His presentation reintroduced the project: shRNA library and screen for the identification of novel small RNAs, whereby he outlined the most recent method developments and results for creating a library and screen of small promoter-targeted RNAs that can upregulate VEGF-A transcription. Nicholas also introduced two new projects on circular RNAs -modulating micro RNA activity and Differential gene expression in human cells transduced with AdV5 vectors. The second project outlined the use of vectorised and designed circRNAs for the use as miRNA sponges as a therapeutic tool to modulate miRNA activity. The latter project involved used high-throughput sequencing data from RNA-seq and GRO-seq to identify genes that are differentially expressed in cells transduced with AdV5 vectors in order to predict cell alterations that could arise from viral transduction and affect transgene expression.

- **Dragomira MAJHEN**, ER, Crucell, Leiden
  Title of oral presentation: ***AdV 26 basic biology***

AdV26 belongs to subgroup D and has short fiber of only 8 β-repeats. Recently it has been shown that AdV26 does not bind the Factor X at all; however receptors used by AdV26 are not very well defined. There are only a few reports regarding receptor usage, being rather controversial in conclusion. Accordingly, the main objective of the project Dragomira is involved in is to investigate in more details the infection pathway used by AdV26 & AdV26 based chimeras, namely: i) receptor usage, ii) binding and internalization and iii) intracellular traffic. She first did a loss of function study using siRNA approach. She showed efficient CAR, CD46 and αV integrin down-regulation in A549 cells after transfection with specific siRNA. Down-regulation of CAR slightly decreases, while down-regulating αV integrins significantly decreases transduction efficacy of Ad26. Surprisingly, CD46 silencing results in two fold increase of AdV26 transduction efficacy. While the internalization of Ad35, known to bind to CD46, provoked a significant decrease in the level of CD46 on the cell surface, the internalization of AdV26 had no influence on the CD46 level, indicating that the AdV26 internalization mechanism differs from the one used by AdV35.

• **Lukasz KURYK** ESR, ONCOS, Helsinki - Pekka Simula Team

Title of the poster presentation: ***Functionality and quantitative assays for human CD40 ligand coded by oncolytic adenovirus CGTG-401***

Lukasz's project aims towards the development of functionality and quantitative assays for human CD40 ligand coded by oncolytic adenovirus CGTG-401. Results obtained so far were presented as a poster. He showed the development of two in vitro methods for functionality and quantitative evaluation of CD40L produced by CGTG-401. Assays are based on HEK-Blue™ CD40L cells which serve to measure the bioactivity of CD40L through the secretion of embryonic alkaline phosphatase (SEAP) upon NF-κB activation following CD40 stimulation (qualitative and quantitative assay). In turn the second method-ELISA has been used for the quantitative measurement of human CD40L. Methods have been designed as a part of analytical assays to characterize and check bio-product before and during clinical research process for CGTG-401. HEK-BLUE assay showed ability to be used as an assay for biological evaluation for functionality of gene coding CD40L from CGTG-401. Both methods (ELISA & HEK-BLUE assays) presented in this work showed ability for quantitative evaluation for CD40L produced in vitro by CGTG-401.

• **Hugo CALDERÓN** ESR, PSIOXUS, Oxford – Kerry Fisher's team

Title of oral presentation: ***Elucidating the mechanism of action of a group B oncolytic adenovirus (ColoAd1)***

The objective of Hugo's project is to elucidate the mechanism of action of a group B oncolytic adenovirus (ColoAd1). ColoAd1 is a highly potent broad-spectrum anti-cancer therapeutic capable of destroying tumour cells at minute concentrations. This vector was created by directed mutagenesis and represents Ads from subgroup B-F pooled and passaged together on HT29 cell, but is predominantly derived from AdV11p. ColoAd1 shows 2-log increase in potency and relative selectivity compared to Ad11p (parental) and ONYX015 on colon cancer cells. Main objectives of this project are to study basic infection biology of the ColoAd1, virus host interaction and vectors immunity. So far Hugo's team they has indications that receptor cell surface expression might have a role for intake but replication might be dependent on other factors. They are also studying chemicals compounds which can influence viral replication, namely increase or decrease viral replication.

• **Jorien KOELEN** ESR, Oxford University – Len Seymour's team

Title of oral presentation: ***Effects of TNF and LTA on colorectal cancer and the tumour microenvironment***

Colorectal cancers (CRCs) are the third most prevalent cancers and third cause of cancer-related deaths. For patients with advanced CRC, median survival is only two years. This clearly indicates the need of improved therapy for advanced CRC patients.

The aim of Jorien's project is to investigate the usefulness of arming a novel oncolytic adenovirus, ColoAd1, with tumor necrosis factor α (TNF) and lymphotoxin α (LTA). ColoA1 is a chimeric adenovirus based serotype 3 and 11 and shows increased activity against human colorectal cancer cells. Both TNF and LTA, that are pro-inflammatory cytokines, have potent anticancer activities. However, the systemic use of TNF is limited, due to the severe side effects produced, as it is currently used in clinic for isolated limb perfusion. . Besides activation of the immune system, TNF and LTA have direct cytotoxic effects on some tumor cells and tumor-associated vasculature is highly sensitive to these cytokines. To study the effects of induction of TNF and LTA expression within a tumour, Jorien has set up a doxycycline-dependent system. Using this system, she can study the effects induction of TNF or LTA in established tumors has on tumor growth, immune infiltration and tumor-associated vasculature. Furthermore, this system will allow screening for synergy between TNF and other therapeutic modalities. Moreover, Jorien has recently cloned TNF and LTA into ColoAd1 with the aim of comparing wt ColoAd1 versus armed ColoAd1 and look for tumour growth, immune infiltration and effects on tumor-associated vasculature. Finally, she will look for synergy between ColoAd1 and chemo- and radiation therapy and other immune modulatory agents.

- **Estrella LOPEZ-GORDO** ESR, Glasgow University – Andrew Baker's team
  Title of poster presentation: ***Adenovirus tropism modification for targeted gene delivery***

In her project Estrella is studying the modifications of tropism of adenoviral vectors to improve the targeting of gene transfer, as these vectors are widely used as gene transfer vehicles. Despite AdV5 being the preferable serotype for gene therapy purposes as it has been fully characterized, it presents a natural tropism for the liver after intravascular delivery, due to its specific binding to coagulation factor X (FX), which also protects AdV5 from attack by the immune system. In this context, Estrella presented data indicating that that FX is not required for liver transduction in immune-compromised mice in contrast with wild type mice. Moreover, her data suggest that $\alpha V\beta 5,7$ integrins are involved in spleen transduction in immune-compromised mice. Also, in order to optimize adenoviral vectors for specific delivery to the kidney, Estrella develops strategies to re-target them by introducing a selective peptide that homes in the kidney.

- **Carlos Alberto FAJARDO** ESR, Catalan Institute of Oncology, Barcelona – Ramon Alemany's team
  Title of oral presentation: ***Arming oncolytic Adenoviruses to improve antitumor immunity***

Replication-competent adenoviruses have gained considerable attention as anticancer drugs, as they can be easily modified to generate oncolytic viruses which selectively replicate in tumor cells. Despite the potential of virotherapy, clinical trials with oncolytic adenoviruses (OAds) have identified the immune system as a limiting factor for their success in the clinics. In Carlos' laboratory, he and his colleagues are aiming at developing armed OAds with immunomodulatory genes which can both activate and direct the immune response towards the tumor, rather than to the OAds. The objective of Carlos' project is to arm oncolytic adenoviruses to improve antitumor

immunity. He has successfully generated an OAd encoding an immunomodulatory molecule which is expressed and secreted from infected cells. These molecules have been shown, by different immunological techniques, to exert ideal characteristics which might lead to the activation and direction of the immune response towards cancer cells. Further experiments are ongoing to validate the potential of this armed OAd in mouse cancer models.

- **Naresh CHANDRA** Umeå University – Niklas Arnberg's team

  Title of oral presentation: ***Adenovirus-glycan interactions: tropism and antivirals***

Naresh is involved in two projects, both of studying in more details biology of group D adenoviruses, especially Ad37, which causes ocular infection. The objective of first project is the identification and characterization of soluble components that regulate adenovirus tropism. In this project, he is involved in the investigation of host components (especially body fluid components) that regulate the tropism of Ad37. We know that major constituents of different body fluids are glycoproteins such as mucins and others. Thus, Naresh is investigating the role of secretory glycoproteins in Ad37 tropism. He hypothesized that it is the differential pattern of glycosylation in the secretory glycoproteins that may have an important role in the Ad37 tropism. In collaboration with the Norrlands Hospital, he has collected different body fluids (tear, saliva, nasal fluid and respiratory mucus) from healthy individuals. After quantification of body fluids, he has performed virus-binding assay with or without body fluids and found that respiratory and nasal fluids efficiently inhibited virus binding to the cells whereas tear fluid did not affect virus binding. In addition, he is also involved in the study of glycan binding specificity of Ad37 fiber knob. Thanks to Consortium for Functional Glycomics (CFG, USA), a platform that contains the large database of the glycan arrays that contains glycans to study of protein-glycan interactions. Now CFG have advanced arrays containing many more glycans (especially glycans that are sialylated and sulfated), Carlos could perform high performant analysis of glycan protein interactions. In collaboration with CFG, he performed glycan array screening with Ad37 fiber knobs, in order to investigate if there are additional glycans that may function as receptors for the Ad37. Results from this CFG glycan screening show several hits of Ad37 fiber knob interaction with sulfated glycans and these interaction data have been validated *in vitro* experiments. Further studies are under way on the significance of these interactions in more detail. In the second project, Naresh is involved in a collaborative project entitled antiviral drug development against Ad37 causing epidemic keratoconjunctivitis. Results of this research indicate that two Trisialic acid (TSA) compounds are efficient inhibitors of Ad37 binding and of the infection of human ocular cells

- **Nicole STICHLING** ESR, Zurich University – Silvio Hemmi's team

  Title of the poster presentation: ***Utilization of murine macrophages to study adenovirus entry into immune cells***

Nicole is interested in utilization of a particular macrophage system for studying adenovirus entry in immune cells and possibly subsequent immune responses. Objectives of her project are i) identification of viral receptors on the cell surface of macrophages, ii) characterization of

adenovirus entry pathway into macrophages and identification of cell mediators of the entry, iii) identification of the “sensors” for the incoming virus that triggers the innate immune response. For that she laid out the investigation on entry of human adenoviruses in MPI-cells, murine cell line resembles to alveolar macrophage at the actual place of AdV infection. She showed that MPI-2 cells bound adenovirus type 2 and 5, internalized, underwent fiber shedding and pVI exposure (always compared to A549 cells). Only the picture regarding nuclear targeting looked different between these two cell lines, which she is going to further investigate. Other prospective investigations will focus on a small compound screen to analyze the nuclear targeting phenotype and a working procedure for knockdown of cellular proteins, since that is still a bottleneck with these cells.

- **Karsten EICHHOLZ** ESR, CNRS-IGMM, Montpellier – Eric Kremer team

Title of the presentation: ***How do adenovirus immune complexes induce maturation of dendritic cells?***

Human adenovirus type 5 (Ad5) has a high sero-prevalence 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 applied. 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 infiltrate 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.

Karsten’s team recently showed that Ad5 opsonized with immunglobulins triggers several innate immune sensors in human DC and induce their maturation whereas the virus alone is much less potent. His aim is thereby to understand the interactions of the IC during the initial activation of DC. Karsten will investigate these interactions mainly in human primary cells to stay as close to the clinics as possible.

Currently, Karsten focuses on understanding the trafficking of the IC and Ad5 alone after they have been phagocytosed by the DC. He wants to know the fate of the capsid and viral DNA on its journey through endosomal compartments and if they separate at one point. Beyond that, there is increasing evidence 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 vesicle mature and can fuse with lysosomes to form autophagolysosomes and are thus also connected to endo-lysosomal trafficking and are involved in several processes in immune cells. It is therefore one of his objectives to understand the trafficking of the IC and the role of autophagy during the maturation of DC. To further dissect this interaction, biochemical assays, imaging and genomic approaches will be combined in this investigation.

- **Agnieszka LIPIEC** ER, BATAVIA Bioservices, Leiden – Menzo Havenga's team
  Title of the poster presentation: ***Towards a novel RCA assay***

In most applications of recombinant adenovirus (rAdV), replication of wild type vector particles is undesirable. Therefore, new generations of rAdV vectors have a deletion in E1 region, which renders these vectors replication-deficient. These rAdVs can be propagated in human HEK293 or HER911 cells, which bring in their genome the AdV E1 sequences (helper sequences) and are thus replication-competent for the rAdVs. During the propagation of the rAdV, however, homologous recombination may occur between rAdV genome and helper cell DNA giving rise to Replication Competent Adenovirus (RCA) particles . Appearance of RCA in rAdV batches is an unpredictable chance event . Because of such RCA particles produce serious side effects in patients when contaminate rAdV batches delivered in gene therapy protocols, testing for RCA in the clinical batches is required by all regulatory agencies and a maximal threshold of contamination has been fixed (by the FDA for instance) at < 1 RCA / 3 x 10^{10} particles of rAdV (VP). Therefore, *RCA assay* relates in general to the detection of RCA during the upstream production process of rAdV particles. Several methods to detect RCA particles are available, such as cell culture based assay, direct E1-specific PCR and infectivity PCR (that is the combination of cell culture assay and the PCR detection of replicated virus). Because the presently available methods are time consuming, laborious and the sensitivity of the assays is not sufficient in order to detect low amounts of RCA contaminants, a new, fast and reliable RCA assay needs to be developed. In Agnieszka's project they propose to improve the RCA assay, making it: (i) faster by using A549-cultured based assay developed on microcarriers - 250 or 500 ml spinner flask), (ii) more sensitive since it is able to detect less than 1 RCA out 3 x 10^{10} VP and (iii) cheaper, thanks to the use of standardized mini-devices that greatly reduce the quantity of reagents and tissue culture materials. For this purpose they performed experimental set-up and generated tools for pivotal experiments.

2. Practical workshop: *Upstream and Downstream Process Development*

This practical course took place in the excellent facilities at ***Batavia Bioservices*** (http://www.bataviabioservices.com) and it was brilliantly organized by Dr Joanna Lubelska. During the two afternoons demonstrations of techniques and experiments were carried out by the *ADVance* ESRs and ERs who acquired "hands-on" experience in Upstream and Downstream process development.. For most of the ESRs and ERs, in fact, this practical workshop was the first opportunity to get in touch with development of scalable, pharmaceutical grade mammalian cell lines and cell culture processes for the

production of all major classes of biopharmaceuticals including recombinant proteins, classical and vector based vaccines, monoclonal antibodies and gene therapy products.
The upstream process (USP) is defined as the entire process from early cell isolation and cultivation, culture expansion, final harvest until to cell banking, while the downstream bioprocess (DSP) refers to the part of the process where the cell mass from the upstream is processed to meet product purity and quality requirements.

The goal of the USP demonstration in the workshop was to prepare an adequate amount of a cell line producing IgGs (PER.C6 cells), that will subsequently be transferred to the DSP. Therefore, the experiment involved the whole culture process of PER.C6 cells: from thawing a frozen stock vial to the cell expansion in 1 L shakers flask in the appropriate growth medium. Cultures were passaged every 3-4 days by seeding them in fresh flasks and scale up was performed until enough cells were available for inoculation into 3 x 2.3 L ***Labfors*** bioreactors (Figure 1). The three bioreactors were inoculated on Thursday 19 Sep with 0.5 x 10^6 viable cells/mL in a total volume of 2.0 L medium and the cultures were maintained at 37°C, with 50 % dissolved oxygen, at pH 7.2 and stirred at a 125 rpm speed. By using this bioreactor, the ESRs and ERs were acquainted with the bioreactor cell culture method, i.e., bioreactor control parameters and performance. The ESRs and ERs performed sampling from the bioreactor, cell counting with different pieces of equipment (nucleo-counter and easy counter), as well as measuring glucose in the sample by using VITROS® DT60 II analyzer device that is routinely used as one of the control parameters. The ESRs and ERs were also introduced to the Good Manufacturing Procedures (GMP) of sterile handling in bio-safety cabinet.

Figure 1. *Labofors* benchtop bioreactor

The goal of a downstream process (DSP) was to purify from the medium components, cellular components and/or products, such as DNA, proteins membranes or antibodies. After a theoretical introduction of the concepts and methods of the DSP, the ESRs and ERs performed a practical work to purify the IgGs from the clarified material obtained from the cells harvested in USP (see above). The IgGs from the clarified harvest were captured by three different methods: affinity chromatography, anion exchange chromatography and cation exchanged chromatography. The capture step was performed either manually, in case of anion-exchange DEAE sepharose fast flow column, and carboxymethyl sepharose column in case of cation-exchange, or by using an automated AKTA[1] purification system. The capture step was followed by formulation step

[1] AKTA is automated purification system, name of the brand, it is the most widely used for protein purification (http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences/brands/akta/).

performed by size exclusion chromatography on Sephadex G25 resin, and/or dialysis by using 2 kD dialysis membrane.

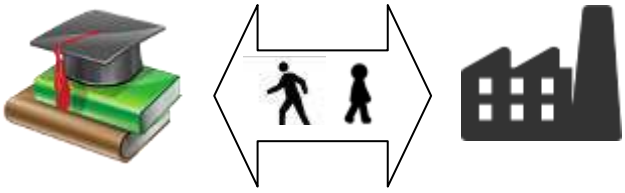
During this workshop, ESRs and ERs visited Crucell's main building where part of the laboratories and offices are situated. Born as a small SME in 1993, Crucell (http://www.crucell.com/) grew to become a global biopharmaceutical company dedicated to bringing meaningful innovation to global health. They do that by discovering, developing, manufacturing and marketing products that combat major threats to the health of people worldwide, especially infectious diseases. The driving force behind Crucell's success is innovation supported by a strong R&D pipeline. In particular they focus on the discovery and development of much-needed vaccines and antibodies for the prevention and treatment of infectious diseases. This has resulted in a broad pipeline of investigational products with the potential to revolutionize the fight against infectious diseases. The vaccine development is based on AdVac® technology which involves the use of novel adenoviral vectors, like Ad35 and Ad26, as vaccines for diseases caused by viruses, bacteria or parasites. Among others, using this versatile vaccine vector platform in combination with our PER.C6® manufacturing technology, they are working on the development of vaccines against tuberculosis, malaria, Ebola and Marburg, HIV, human papilloma virus (HPV) and respiratory syncytial virus (RSV). In 2010, 1,400 dedicated and skilled Crucell employees worldwide worked to bring significant benefit to the lives of people worldwide. In the same year alone, Crucell distributed more than 105 million doses of vaccines to people around the world, with the majority going to infants in developing countries, and invested €100.0 million in R&D. It was estimated that a Crucell vaccine was given to 190 individuals every minute during 2010. On February 22, 2011 Johnson & Johnson completed the tender offer for Crucell N.V. (Crucell) and declared the offer unconditional. As a result, Crucell now operate as the center for vaccines within the Johnson & Johnson pharmaceuticals group.

Figure 2: ESRs and ERs visiting Valerio building, at the CRUCELL Plant department for producing clinical trial material.

ESRs and ERs also got the opportunity to visit **CRUCELL**'s Process Technology Center (PTC) (figure 2). The PTC is Crucell's on-site pilot plant facility located in the Valerio Building.The plant's core unit is dedicated to the production activities of Clinical Trial Material by applying current Good Manufacturing Practice (GMP) guidelines. As a GMP facility, the main responsibility of the Pilot Plant is to produce Clinical Trial Material (CTM) Phase 1 and 2, virus seeds and cell banks. In addition to this, process development activities, Technology Transfer (TT) and the production of material for toxicity studies are performed. Both up-stream and down-stream processes are used at GMP level to produce CTM.

The Valerio facility, in which the PTC is housed, was constructed and completed in 2008 and is named after Dinko Valerio – a scientist and co-founder of Crucell. The PTC consists of 4 multipurpose production suites which are classified clean room areas supporting the required biosafety levels. As the process equipment is mobile, there is flexibility in the production capabilities for both upstream and downstream processing. In conjunction with these suites there is an in-house area for cleaning, assembly and sterilization operations.

3. EASCO session: *Switching career from/to academia and industry*



The fourth day of the Leiden meeting started with a session animated by EASCO. This session has been shared into three parts: (**3.1**) an introduction on the training objectives, (**3.2**) a seminar on the research settings and career opportunities in academia and industry, and (**3.3**) the presentation of all *ADVance* fellows on their perception of the quality of research and researchers and on their own skills and career.

3.1. Mauro MEZZINA (EASCO Manager - Chargé de mission CNRS, Paris) presented the plan of the session, he introduced the seminar of S. Constantinescu and explained why the presentation of the fellows. Mauro stressed the necessity of the reflection of the *ADVance* fellows on the concept of the quality of research and on their own qualities and skills. Mauro reminded us that the second third of the PhD tenure has already started for almost all ESRs and that the fellows' growth pathway is long, complex and sometime also difficult. He said that what the fellows need to learn with the EASCO courses is not science but behaviors. He said that, since behaviors that are deeply embedded in one's personality are difficult to change by simply thinking "*I want to change*". Thus, a co-operational process is necessary where fellows, PIs and EASCO teachers interact continuously along the overall *ADVance* tenure. In such triangular relationships, insight and feedback should circulate continuously to allow effective modification of behaviors. EASCO will make an effort to establish/implement this communication triangle, but the fellows must be an active element inside it and not merely passive receptors of concepts and information. Indeed, the ESRs and ERs will give with their presentations the feedback on their vision of research including the evaluation of their skills. When they suggest the topics to be developed in the future EASCO courses (see the table in the section **3.3.d**), their feedback will help to improve the quality of the

insight delivered not only by the EASCO trainers, but also by their PIs and mentors. Therefore, Mauro concluded that the identity of each individual ESR and ER who will talk in this session will be kept confidential of course, but the information that they deliver must be disclosed, to help teachers, Pis and mentors to perform better their educational role.

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3.2. Seminar *Research in academia and industry* Stefan CONSTANTINESCU (President EASCO, Member of Ludwig Institute for Cancer Research-Brussels Branch and Professor, Université Catholique de Louvain, Brussels). His seminar was divided in two parts.

Part 1: He started his talk with an historical reflection about industrial and academic research and that the interaction and exchange between the two becomes more and more important these days. In the same time the differences are now blurred, with significant overlap in the types of activities, the set-up of the groups and even publication policies. He further pointed out that the way of decision-making is fundamentally different. In academic research the fate of a project (successful grant applications that brings further funding) depends on peer-reviews, whereas in the industry managers decide based on what is financially the best for the company. While at the outset exploratory projects are unfolding in a similar way, except funding differs (seed money from academic institution or grant for academia, and research exploratory budget for the industry), the decision to expand the project, scale-up and valorize is critical for industry. The evolution of the project, if it has valorization potential, becomes a management responsibility with many units participating in the industrial set up in further development. Alternatively, highly successful projects, on a fundamental level, can be stopped in industry for business reasons.

Part 2: ***Career in academia and in industry.*** In the second part of the seminar, he described the importance of mobility for a career in both industry and academia and which skills are required to be successful in either direction. Thus, the ERs and ESRs were encouraged to reflect on their own skills and needs, which will contribute to find the right jobs in the future. Recently significant mobility has been seen at the top between industry and university settings, with several examples of scientists going from academia to top positions in industry and returning to academia. This trend is new and is also seen for small start-ups, especially for scientists in academia that start their own companies and are successful. The *ADVance* fellows were strongly encouraged to reflect on these fundamental aspects of their career and discuss collectively in order to have a clearer idea about their own future. Indeed, they have started this process/reflection and gave a short presentation on their perception of science and self-perception as a scientist (see below).

3.3. Presentations of the fellows The third part of the EASCO session was dedicated to the presentations of the ESRs and ERs' on themselves and their future career. The objective of this session was to give them the opportunity to reflect on their present and future professional situation, and to perform a collective exercise of communication on what they think about: **(a)** the quality of research and researchers; **(b)** their own skills; **(c)** their future career and **(d)** if *ADVance* produced a benefit to their skills and gave them a clearer idea on their career.

Without a doubt, this session was very successful. Despite the short time available (only 5 min/presentation), the fellows gave their presentations with motivation and enthusiasm, allowing also some time for a short discussion. We summarize in the following paragraphs the content of their presentations relating to the issues mentioned above. In this report we have grouped the information by issue, instead of presenting the summary of each single presentation, to avoid redundancy and also keep the confidentiality of the ideas and personal feeling of the fellows. We report the key words and key statements that were used most frequently or were most relevant in the presentations. The key words are in alphabetical order and not in function of their frequency with which they were used in the presentations.

(a) The **quality of research** was defined by a large majority of fellows with the following key words (in alphabetical order): "*applicability*", "*broad knowledge*", "*convergent results", "critic",* "*efficacy*", "*excellence*", "*hard work*", "*honesty*", "*importance to society*", "*innovation*", "*interdisciplinary approach*", "*international cooperation*", "*progress*", "*realistic*", "*relevance*", "*reproducibility*" and "*trustworthiness*".

↑ *from a slide of an ESR*

In the picture above we reproduce the slide of a fellow who showed that, when a research work is defined with some of the above words, the work can be published in *Nature* and *Science*. Other fellows used **methodological definitions** like: **(i)** "*the use of transparent and reproducible methods to produce evidence that is relevant to the questions being asked*", **(ii)** "*Minimizing errors within protocols, data collection and* analysis", **(iii)** "*clearly identify interferences, assumptions and other limitations in research*", or **(iv)**: "*research is simply…convincing analysis and interpretation of results*". Hence, these words and statements converge into the definition of research as activities well performed in order to understand phenomena, reproduce them experimentally and discover unknown phenomena and their mechanisms.

Other fellows outlined the ***rigorous methods*** that are necessary to produce new knowledge, especially during the developmental pathway that transforms research data into "*final products to be administered to humans*". The concept of *rigor* has been emphasized by all fellows based in industries. Indeed, on one hand, rigorous approaches and good practices are indispensable in translational research. On the other hand, strong management skills are required in researchers to achieve rapidly the *bench-to-clinic* pathway and allow the company to exploit commercially new bio-products.

Other fellows talked about ***ethics and integrity*** as an important aspect of the quality of research. In fact, if researchers discover new phenomena and produce new products to be spread through human population and the environment, ethical considerations must be taken into account such as the precautionary principle to assess the risks associated with the new products. Another fellow added the notion of the ***challenges*** of research for the economy and society, but reminded also the ***problems*** arising from the exploitation of science and technology (S&T). With this notion of *problems* the fellow refers to the **social responsibility** that researchers should have in being

aware of the importance to exploit research innovations in the respect of the ethics, environment and human rights.

Finally, one of the industry-based ESRs enriched the above cocktail of concepts by saying that science should have an "*impact on health, environment, food, industry… and on daily life of citizens*". In other words, we think that this ESR is absolutely right if he means that S&T makes sense as a source of wealth and social progress, only if it produces improvement of the quality of the daily life of each citizen.

In conclusion, all *ADVance* fellows have a very good global perception of the research. Not only is their perception in line with the standard international definition of research[2], but they also enriched this definition with several important concepts related to good policy and governance of research (see above) and to the quality of researchers (see below). Thus, the concept of quality of research is implemented with a human element that should be considered necessary when we talk about an activity made by humans. If the concept of excellence as **word-leading** in terms of originality, significance and rigor was not always developed explicitly by the fellows in their presentation, we think that it is certainly present in their mind.

(b) The following words and statements have been used for the **quality of researchers**: "*ability to make manageable project packages*", "*collaborations*", "*creativity*", "*critical thinking*", "*curiosity*", "*good writing and oral communication*", "*hard worker*", "*honesty*", "*independence*", "*literature search*", "*motivation*", "*techniques*". The fellows also said that a **good researcher** should be: *"adaptive*", "*ambitious*", "*constantly self-improving*", "*diligent"*, "*efficient and effective*", "*enthusiastic*", "*extroverted*", "*flexible*", "*independent*" and "*proactive*". Also, a good researcher should possess "*proneness to new techniques and languages*", "*mobility and an open mind*", "*ethical and integrity behaviors*" and he/she should possess "*critical examination and assessment of new and complex phenomena, issues and situations*". In their presentations, the ESRs and ERs were quite critical of their own qualities. For example, a fellow feels quite high motivation and good potential for research in herself, but she does not know really her skills and she knows better, rather, ***"what is still missing***" in her, or what needs to be developed with the experience. This perception of missing skills is also shared by other fellows, who said that they have to learn **more science and techniques** in disciplines related to their project. Their uncertainty on the skills needed to be a good researcher and/or their perception of missing skills could be overcome by constant co-operational pathway of analysis and reflection that the fellows carry out during their team-work and within their team. In fact, as one fellow said, "*the team-work is to share ideas… question yourself*

[2] "*Research and experimental development (R&D) comprise creative work undertaken on a systematic basis in order to increase the stock of knowledge, including knowledge of man, culture and society, and the use of this stock of knowledge to devise new applications*". Page 30 of the *Frascati Manual*/*OECD*, (ed. 2002). This is the official manual defining the framework of the research & development (http://www.tubitak.gov.tr/tubitak_content_files/BTYPD/kilavuzlar/Frascati.pdf).

and your opinion!". Another fellow said: "*As part of a large team, one has to be on his/her best regarding skills: ➢ if not, will be given opportunity to improve. ➢ Team members count on quality of your work*". Indeed, the role of the team is crucial and the responsibility of the PI is primary, since he/she manages the team and thus drives all interactions between the team members and the fellow. The importance of the PIs and mentors has been outlined by only one ESR, who mentioned *peoples that influenced her scientific path,* showing their picture in the slide. However, this does not mean that other fellows are unaware of the role of PIs and mentors in the process of skills transmission and their development. They know that the PI not only transmits scientific and technical skills, but is also viewed as a reference by the fellow, who integrates behavioral settings inherited from the PI and his/her environment. **Improving skills** is the core of the reflection of the ESRs and ERs and they want to interact more and better with their PI, as well as with other PIs during the *ADVance* meetings (as it has been said explicitly by one fellow, who noticed scarce fellow-PIs interaction in the previous meetings). Thus, the PIs should be aware of their crucial role in the co-operational pathway that determines the growth process of the researchers both scientific & complementary skills and behaviors.

(c) The perception of the **future career** of the ESRs and ERs is quite heterogeneous. We defined a first level of heterogeneity according to the level of clarity of the fellows' ideas, defining thus 2 groups described below. A second level of heterogeneity is based on the fellows' idea on their future kind of job, fields and sector, and we shared thus the fellows in each group in 3 subgroups.

(i) The 1st group is composed of 7 fellows, who seem to already have a **clear idea** about the kind of job, field and the sector in which they would like to develop their career. The 3 subgroups in this group could be:

• The 1st one with 2 ESRs, who feel that their career can be developed in **any sector and field** (research or other activities). One ESR plans her career in "*managing scientific-oriented projects within or outside academia".* Her presentation suggests that she has already a certain maturity enabling her to manage projects not only in research but also in other fields. The second ESR said to foresee the following scenario: "*First, a post doc in the industry, then shifting into the field of graphical designing/publishing or legislation/counseling sector".* Also this student seems already having skills and interests in research and beyond it as well. We think that both these ESRs have, in addition to a clear idea of their career, a clear perception also of the whole research system where they want to deal with, which evolves rapidly open to the global dimension.

• A 2nd subgroup of 3 fellows, who clearly have in mind to develop their career in the **industry**. Two of them foresee their career in "*the business area rather than in research departments"*. One wants to stay in the translational area and thinks that the industry is a better environment for that. The other one plans to, she said: "*create my own research group and, if possible, my own "small" business in the next 10 years*". The third fellow wants to find in this sector an "*internationally-*

oriented job, in a challenging environment that expands horizons, constantly exploring new ideas and increasing job satisfactions". These 3 fellows have convinced thus the audience that the industry is the best environment for translational research, business and personal satisfaction in the job.

• The 3rd subgroup of 2 ESRs, who said that they will certainly develop their career in **academia**. One fellow said that he wants "*to continue the research as a postdoctoral fellow and to become a good and innovative researcher or group leader"*. The other one said simply: "*Further education in academia"*, without any other comment. We think that both these ESRs have in their mind a clear academia-oriented career plan.

(ii) A 2nd group, composed of 7 fellows who still have **unclear idea** of their career, might be shared in 3 subgroups: fellows who have **no idea** at all yet, those who have awareness only of the **kind of job** they would like, and those considering the **academia as possible option**, but they are not sure at all on this.

• The 1st subgroup (no idea) concerns 3 ESRs: one said: "*Next…Postdoc – Industry or public science. Then….*" he showed the picture of the poster of the Spielberg's movie "*Back to the future*" in his slide. Another ESR said: "*Academia or industry? The **one-million-dollar question**. My answer so far: **???????**".* A third one: "*I would like to move forwards more in my PhD before I make decision".* It is clear that industry and academia are open options with the same weight for these ESRs and they need to progress more in their PhD work before maturing further their idea on their career.

• The 2nd subgroup (kind of job awareness) concerns 2 fellows: one of them said: "*Industry or academia?... Get a relatively **stable job**!*" and the other said: "*Having **my own research group and project and grants***". These fellows outline the important issue that the career plan cannot be dissociated from the goal to get a stable job and being independent doing their own research project and not for somebody else. This fellow put the finger on one of the major differences in managing research in EU compared to USA, where, for instance, the PhD students are owner of their project and they may bring it with them when they leave the lab after their thesis.

• The 3rd subgroup (academia as possible option) concerns 2 ESRs: one said: "*I am not entirely sure. Drawbacks for me in academia are: much career uncertainty and short-term contracts. Positive aspect is that I like academic research"*. The other one said: "*Well, for now I like this kind of research… to stay with students and working with them….I think it would be nice to work in academic area…However, I couldn't even say what would I like the most. But still I think that industry should be also tried to compare all the fields before making decision*".

The classification of the fellows according to the above groups and subgroups is obviously subjective, it corresponds perhaps to their feelings now, but it will evolve during the next months. Even if this classification is wrong, the fellows and the PIs might take it as a basis for discussion and further reflection.

(d) The **effect of ADVance**. Except for one fellow, who said that it is maybe too soon to gauge the effect of Advance on his skills, for all *ADVance* fellows their tenure produced or is producing a

significant impact on their skills. They acquired new **scientific competences** skills and technical expertise related to the fields of adenovirology, as well as **personal and complementary skills**.

Most skills mentioned in the previous chapter "b", were improved, thanks to their research work in the host laboratories, i.e. their daily team work and ability to interact with their PI and other members of the team. For some of them, their skills have also been enriched by interacting with fellows and PIs of the **private sector** in the *ADVance* consortium, outlining the role of these contacts in **improving the vision of their career**. For some fellows, their tenure actually improves more their perception and visibility of their professional future, than their perception of their scientific/technical skills. Also, the fellows working in companies have generally acquired more management skills (organization, time management, flexibility…) than scientific and technical competences. Despite the insufficient time to go deeper in discussing this issue, we think that this point merits being mentioned here (see below for additional information) and being matter of further discussions.

Indeed, the network improved their **communication skills** in learning new languages through exchanging with people in different societies and cultures. Furthermore, their research work and their participation in the Zürich and Leiden workshops this year improved their performance in scientific communication. Some of them have already had the opportunity to write scientific papers and/or to participate in international meetings; all have made the experience to present in *ADVance* meetings. The discussions with their mentors, PIs and guest senior scientists who participated in the workshops expanded the limits of their scientific vision and open them up to opportunities of scientific collaborations and to the perspective of **post-*ADVance* jobs**. Exchanging with *ADVance* partners expose them to a mixed academia-industry environment where the fellows discovered different research programs, approaches, working methods and visions of science, as well as alternative career pathways. As a fellow said clearly and very convincingly, *ADVance* is the chance *to have a profound reflection on the present status* of their early career stage, to make decision as to which direction to go.

We agree with the ESRs and ERs who say that it is too early to gauge their skills and the effect that *ADVance* tenure produced on their skills. We think in fact that it would probably be easier to discover what one would like to do in his/her career than to auto-gauge his/her own skills. The *ADVance* fellows seem to have made a progress in this context: just by starting their reflection before their presentation in Leiden, they started to have a **right perception** of themselves**.** As Mauro showed in his introduction (chapter **3.1**), when the ESRs and ERs have filled a questionnaire at the beginning of their stay, 13 out the 15 perceived themselves as be good or excellent researchers[4]. We think that their perception of themselves one year ago was good thanks to their successful scholarship, advices from previous advisors and their previous perception of research. Even in the absence of a survey with the same questionnaire now (one year later), we think that they still consider themselves good researchers, but they have now a more precise perception of their skills and research career and, more importantly, all exhibit a strong, clear motivation for research. They have a more realistic perception of the research and the skills necessary to perform high quality research, as indicated in the table below. We think that they are more aware now of the skills they need to acquire or improve than one year ago.

We shall do the best to satisfy such their training need in the future courses on complementary skills and, in particular, to establish the agenda of the course in Paris on March 2014 to cover the totality of the topics and subtopics according to their suggestions.

The table below shows the topics suggested by the ESRs and ERs. The title of the subtopics (in italic) are as they have indicated in their presentations (and not standard titles).

Topics/subtopics	Number of fellows having the preference	
	By topic	total
Personal development sessions - *upgrade the current knowledge related to career development*	1	3
- *how to design, priorities, career determinants,*	1	
- *personal coaching*	1	
Skills in scientific presentation - *Optimal slide content and ways to engage your audience and manage discussions*	5	15
- *How to sell a project to a crowd*	3	
- *Writing papers and grants applications, funding agencies*	7	
Job applications for future positions (for academia and industry) *Interviews, application forms, CV preparation…*	5	5
Management skills - *How to manage multiple players with a project in stress free and effective, leadership competences*	5	12
- *Project designing, priorities, budget, collaborations*	4	
- *The business and commercial aspects of research*	3	

4 We report here the part of the questionnaire where the fellows have evaluated their own researchers' skills. In questionnaire, they were asked to choose between "to be improved", "average", "good" and "excellent ". Among the 15 ESRs and ERs, only two choose the box "average" and one " excellent".

SELF EVALUATION OF THE SKILLS ***How your overall scientific and technical skills are?***

☐ excellent x	☐ good xxxxxxxxxxx	☐ average xx	☐ to be improved

4. Networking and socializing events

The meeting in Leiden was the second opportunity for the ESRs, ERs and PIs to meet altogether during the *ADVance* course. The good ambiance of the previous meeting in Zurich was carried over to this meeting and there were many occasions for scientific and also non-scientific discussions.

Wednesday 26th September

In the late afternoon ERs and ESRs went on a guided tour through Leiden's moved history. Rembrandt van Rijn, the famous Dutch painter, showed them round and told short and amusing anecdotes about the siege of Leiden by the Spanish and rise and fall of the city as a stronghold of fabric production in the Netherlands. Later the ERs and ESRs enjoyed a nice meal at the Restaurant Puur where they continued their talks and discussions.

Thursday 27th September

After the scientific talks of several guest speakers and the poster session of the ERs and ESRs, all participants gathered at the Restaurant Branderij to have dinner. A big surprise was following the starters as the captain of a small boat entered the restaurant and invited the whole group for a trip on the town canals. Seeing the dimly illuminated Leiden by night on the boat gave the background to exchange opinions and talk to different PIs. The tour was interrupted by stops at different restaurants to have the main dish and the dessert. This setup provided that the group could mix again and again and that there was the opportunity to meet everybody.

In conclusion, we would like to thank the organizers, lecturers and the people behind the scenes who contributed to the overall success of the event. We would also like to thank Nicole, Iva and

Estrella for sharing their pictures and all the ERs and ESRs for making the second *ADVance* training event so special.