

Research Programme of the Paul-Ehrlich-Institut

Full Version

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1. Preamble

The Paul-Ehrlich-Institut (PEI) is the descendent of the former "Institut für Serumforschung und Serumprüfung"¹ and the "Königliches Institut für experimentelle Therapie"², which were both headed by the Nobel Prize laureate Paul Ehrlich. From the very beginning, Paul Ehrlich and his co-workers performed research on both applied and basic scientific issues, such as the potency determination of antisera and the theory of antibody formation. Consequently, the "Gesetz über die Errichtung eines Bundesamtes für Sera und Impfstoffe" act of July 7, 1972, which established the PEI as the Federal Agency for Sera and Vaccines, defined research as integral part of the institute's remit.

The employees of the PEI are committed to make use of their knowledge and skills to assure and improve the quality, safety and efficacy of biomedicines by means of research and regulatory activities. We are dedicated to keep track of new developments in biomedicine with the objectives of providing patients with improved therapeutics and maintaining our state-of-the-art scientific knowledge in the field of biomedicine. The character of the PEI as a combined research centre and biomedicines agency is unique in Europe and resembles the concept followed by the U.S. Food and Drug Administration. The integration of research and regulatory activities enables chaperonage of biomedicines from development and regulation all the way to pharmacovigilance, which in turn directly supports our responsibilities as regulators and scientific advisors to politics and pharmaceutical industry. Hence, it is the pivotal fundament of our worldwide acceptance as a high-quality and reliable regulatory authority. On July 7, 2000, the "Wissenschaftsrat" (Scientific Council of the German Federal Government) stated that both activities are of high quality and complement each other. Encouraged by this judgment, the PEI's research programme describes aims and concepts to further strengthen and maintain its excellent position as a combined research centre and medicines agency. In this regard, the programme parallels the guiding principles³ and the strategic objectives of the institute.

¹ Institute for Serum Research and Serum Testing, 1896-1899 in Berlin

² Royal Institute for Experimental Therapy, since 1899 in Frankfurt am Main

³ to be found under the following web address: www.pei.de/guiding-principles

To assure the necessary impartiality, independence, and unbiasedness of a regulatory authority, the PEI takes strict measures with respect to medicinal products emanating from its research activities. This includes the exclusion of involved parties from respective licensing procedures as well the abdication of the PEI from international European licensing procedures regarding such medicinal products.

Research at the PEI is related to its legal responsibilities for biomedicines such as vaccines, antibodies and allergens, blood products, and gene as well as cell therapeutics. Biomedicines are highly complex medicinal products made from or containing biological substances, such as micro-organisms, viruses, tissues, cells, physiological fluids, antibodies, and antigens including allergens. Consequently, a number of biomedicinal products inherently carry the risk of infectious disease transmission. Moreover, these drugs continuously need to be assessed for pharmaceutical quality, safety and efficacy, and occasionally biomedicines can induce severe adverse effects (SAEs). New challenges are emerging regarding (i) the quality, safety and efficacy of biosimilars, (ii) the aetiology of certain adverse effects, and (iii) the rise of counterfeit biomedicines. Outstanding achievements addressing these challenges require focussing on selected domains, in which the PEI is leading or aims at reaching a leading position. To meet this claim, research at the PEI is performed in an atmosphere of scientific excellence:

- to assure and improve the quality, safety and efficacy of biomedicines and biosimilars
- to further the development, risk assessment and regulation of novel biomedicines
- to promote the PEI as a centre of excellence within the European network of regulatory authorities and control laboratories for medicinal products
- to provide health policy decision-makers, scientists, industry and public with expert advice on biomedicine-relevant issues
- to ensure excellent and up-to-date scientific expertise of staff as major basis of our international reputation
- to intensify international exchange of information, ideas and concepts
- to facilitate, where possible, the reduction or replacement of animal experiments in batch control and testing.

The research objectives of the PEI clearly differ from those of the other institutes affiliated to the German Federal Ministry of Health. The Robert Koch-Institut is the central German institution of surveying and preventing infectious diseases, why it is engaged in epidemiology

to detect, prevent and combat infectious diseases⁴. The Federal Institute for Drugs and Medical Devices⁵ is the German regulatory authority for chemical drugs and medical devices and performs research on pharmacologically active small molecules. In agreement with the research objectives, current and future research efforts at the PEI address the following four major research areas:

- quality, safety and efficacy of biomedicines
- experimental vaccines, therapies and diagnostics
- immune activation and evasion
- host interactions with pathogens and retroelements

⁴ further information at: <http://www.rki.de>

⁵ Bundesinstitut für Arzneimittel und Medizinprodukte; web address: <http://www.bfarm.de>

2. Quality, Safety and Efficacy of Biomedicines

Biomedicines and biosimilars are made from or contain biological substances. As a consequence, they inherently carry the risk of infectious disease transmission. Moreover, their biological origin requires continuous assessment of consistency. The approval of biomedicines requires highly sensitive tests to minimize the infection risk and appropriate potency assays as well as other analysis systems to judge pharmaceutical quality, safety and efficacy. It is expected that during the next years a number of novel biomedicinal product classes, such as gene and cell therapeutics, tissue-engineered products, conjugated antibodies and engineered antibody fragments, recombinant allergens and genetically modified live attenuated bacteria will be developed. These products require new test methods for their evaluation. Consequently, the PEI is engaged in keeping up with recent methodological, technological and diagnostic innovations in the field of biomedicines. In addition, important research topics of the PEI are the development, evaluation and improvement of novel test methods and technologies to prove or enhance the quality, safety and efficacy of biomedicines. Major objectives are to prevent transmission of infectious diseases, to minimize the risk adverse reactions and to verify the integrity and biological functions of biomedicines and biosimilars.

The PEI has developed a number of new test methods that subsequently have been implemented in the regular quality control of biomedicines. We support standardization of biomedicines by designing and providing biological reference preparations. New test methods and biological reference preparations are assessed under field conditions. To this end, the PEI arranges and participates in collaborative studies with international organizations such as the World Health Organization (WHO), the European Directorate for the Quality of Medicines (EDQM), and the European Centre for the Validation of Alternative Methods (ECVAM). These studies provide the scientific basis for the establishment of new, or the modification of existing, monographs of the European Pharmacopeia.

If adverse reactions to biomedicines are suspected, the PEI is responsible for the collection and analysis of respective data to possibly identify a direct correlation between the administration of a biomedicine and the induction of adverse reactions. If such a correlation can be substantiated, but the cause-effect relationship remains unclear, experimental research is needed in order to identify the underlying mechanisms. "Regulatory Science and Research" is a novel approach within this field, which targets the establishment of

translational links between biomedicines dossier assessment and related basic science projects.

2.1. Infection Risks of Biomedicines

Biomedicines inherently carry the risk of infectious disease transmission by viruses, bacteria, fungi, and parasites or transmissible spongiform encephalopathy (TSE) pathogens due to their nature, source and/or manufacture. To further minimize the infection risk of biomedicines, three complementary research approaches are used adopted at the PEI: (i) specific epidemiological studies, (ii) development of highly sensitive test systems, and (iii) the investigation of methods to inactivate infectious agents in biomedicines during manufacture.

Specific epidemiological information is required for the characterisation and evaluation of pathogen risks in the blood and tissue donor population. The PEI contributes to a few epidemiological studies (prevalence and incidence) on past (e.g. Human T-cell Leukaemia Virus Type I/II) and recent infections (e.g. Hepatitis G Virus) in donor populations. These studies enable risk assessment with respect to blood and other source materials used for the production of biomedicines. The respective studies are often performed in close cooperation with the Robert Koch-Institut and focus on common pathogens as well as on potentially (re)emerging and less common infectious agents, such as West Nile Virus.

The development of novel and highly sensitive (molecular) test systems for the detection of trace contamination of source material with infectious agents can significantly reduce the infection risk of biomedicines. In this regard the PEI develops highly specific and sensitive test systems and comparatively evaluates existing assays. Standardisation of molecular test systems is supported by generating WHO International Standards, which is one of the tasks of the PEI as a WHO Collaborating Centre for Quality Assurance of Blood Products and in-vitro Diagnostic Devices. Besides carrying out research on the transmission of common viruses and bacteria, the PEI also focuses on projects addressing the potential contamination of biomedicines with other agents such as TSE pathogens or the potential contamination of attenuated live virus vaccines with wild-type viruses or revertant pathogens. TSE pathogens are believed to be devoid of RNA and DNA and to consist of an “infectious” prion protein that upon peripheral infection causes neuronal degeneration. Currently, we are also investigating the minimal infectious dose of a TSE pathogen upon oral uptake in a primate model. In the same model, transmission of prion disease from one individual to another via blood or blood-derived products is being investigated at the PEI.

Safety of biomedicines can be maximised by inactivation or removal processes, which eliminate infectious agents during the production of biomedicines. Hence, the development and validation of improved elimination methods (e.g. for physically-resistant non-enveloped viruses such as Hepatitis A Virus and parvoviruses) is a further research topic at the PEI. A new challenge in this field is the inactivation of pathogens in cellular blood components, where the PEI currently focuses on the reduction of transfusion-transmitted bacterial infection. This work is accompanied by the development of novel principles in rapid detection of bacteria and fungi.

The microbial safety of Advanced Therapy Medicinal Products (ATMPs; gene therapy, somatic cell therapy and tissue engineered medicinal products) represents a new challenge. Here, the sterility of the human source material can often not be guaranteed. In addition, a technology that enables sterilization of the final product is currently not available. Furthermore, the shelf life of ATMPs is often only a few hours, which rules out the use of classical sterility test sometimes taking a week or longer. Consequently, the PEI is engaged in the development of novel methods for reliable and rapid sterility testing of ATMPs.

2.2. Safety and Efficacy of Biomedicines

Certain biomedicines carry further specific risks including cancer induction, specific quality/efficacy problems, and the triggering of acute systemic drug reactions as well as other adverse effects. This applies e.g. for certain gene and cell therapy products, blood products, antibodies, recombinant allergen therapeutics, seasonal vaccines and recombinant live attenuated bacteria. Some of their specific risks are mainly estimated on the basis of theoretical assumptions, since clinical experience is scarce. However, today the efficacy of biomedicines or the specific risk factors associated with their use can and should be evaluated using appropriate cell culture and suitable animal models, respectively.

Examples of research efforts at the PEI regarding the potential efficacy and the risks of gene and cell therapy are the investigation of (i) cancer induction by gene therapies with retroviral vectors, and (ii) the undesirable mobilisation of pre-existing retroelements or functional endogenous retroviruses. One example of respective studies at the PEI is the investigation of SIV-PBj (originating from a specific strain of the simian immunodeficiency virus) and HIV 1 derived lentiviral vectors due to their potential use for gene transfer into cell cycle-arrested mammalian cells.

Any denaturation during manufacture of blood-based biomedicines or the undesirable activation of proenzymes, such as coagulation factors, can cause major problems in patients. Highly sensitive assays and appropriate in-vitro models are needed in order to identify potential risk factors of thrombotic complications. To extend our knowledge in this field, we constantly are working on novel and appropriate ultra sensitive analytical techniques. Furthermore, we pursue the hypothesis that at least the thrombin generation test may in the future be applicable not only for the evaluation of biomedicines, but also for the diagnosis of inherited or acquired thromboembolic risks. Potency assays for recombinant products need to be optimized in order to evaluate the linkage between labelled content and clinical efficacy. An example is a modified recombinant factor VIII product causing discrepant values in potency assays according to different regulations in the USA and Europe. In this regard, the PEI is working on a novel test system for this molecule to provide a candidate harmonised assay maintaining the relation to the activity of the recombinant product to the natural factor VIII. For the examination of the quality of recombinant human proteins, especially for use in pre-licensing evaluation and post-marketing surveillance, we focus on the application of a rapid and convenient method analysing N-linked oligosaccharide and sialic acid. New drug product classes are ahead like fusion proteins, conjugated antibodies, or engineered antibody fragments, requiring the future development of appropriate test methods for their evaluation.

A major safety issue of allergen products is the presence of soluble and potentially anaphylactic proteins in adsorbed allergen preparations. To address this issue, innovative in-vitro test methods for the quantification of allergens, the verification of preparations, and the determination of allergenicity are developed at the PEI.

For most of the recently approved influenza vaccines PEI experts have acted as (co)rapporteurs or peer reviewers in the European centralised licensing procedure. The institute is responsible for batch release control of influenza vaccines and the standardisation of serological assays to validate the potency of these vaccines using tests that correlate with vaccine efficacy. Production and quality control of seasonal influenza vaccines are subject to time restrictions that impede efficacy examination in human trials. Instead, the efficacy of a novel influenza vaccine is confirmed on the basis of clearly defined serological parameters measured by the hemagglutination inhibition (HI) or the Single Radial Hemolysis (SRH) assay. However, the results obtained are variable. To address this issue, we have initiated studies on the reduction of variability with the aim to finally establish a standardized assay protocol using specific material (e.g. horse red blood cells, standard reagents). These studies

are conducted in close cooperation with the European Directorate for the Quality of Medicines (EDQM).

About 50% of the products currently being evaluated at the PEI are (monoclonal) antibodies. An increasing number of these products have been identified as potentially bearing the inherent risk of inducing SAEs. A dramatic example is the phase I clinical trial incident with the superagonistic anti-CD28 monoclonal antibody TGN1412 in March 2006, where a cytokine release syndrome was elicited in six healthy volunteers. The facts that preclinical tests in non-human primates had no predictive value and drug contamination or protocol irregularities could be excluded clearly indicate the need for more appropriate and predictive preclinical in-vivo models. Consequently, we are currently establishing and evaluating humanized mouse models with respect to their use as preclinical human equivalence models.

Frequently, novel immunotherapy strategies for allergy and allergen products are developed with the aim to exhibit increased efficacy by (i) including novel adjuvants (e.g. bacterial components, virus-like particles, extracts from immuno-active organisms), (ii) using novel delivery systems (e.g. microencapsulation, new administration routes) or (iii) applying DNA vaccination strategies or viral vectors for antigen expression. Research on the modulation of the immune system by these substances and routes targets the optimisation of the intended effects and the assessment of the safety profile of these products.

Live attenuated bacteria are used as prophylactic vaccines in human and veterinary medical practice. However, currently used strains have often been established empirically. Today, technologies are available that enable a rational design of live bacterial vaccines and vector systems. The use of such genetically modified bacteria as biomedicines requires scrutiny of their quality, safety and efficacy profiles. To gain broad acceptance of recombinant live attenuated bacteria as veterinary vaccines, the DIVA (Differentiating Infected from Vaccinated Animals) concept has to be implemented. In addition to vaccines, bacteria-derived medicines also target the treatment of cancer, gene deficiencies, autoimmunity and allergy. In this overall context, the PEI aims at developing and standardizing adequate testing methods, which can be used to monitor the quality, safety and efficacy of recombinant bacteria to be used as biomedicines.

Another key issue in the field of safety and efficacy testing is the replacement, reduction and refinement of animal tests (3 R concept). This concept has been further strengthened by demands defined by the European Pharmacopoeia in accordance with the "European

Convention on the Protection of Animals Used for Experimental and Other Scientific Purposes (1986)". In the field of human bacterial vaccines we identified and targeted key measures to fulfil the 3 R concept without reducing the high quality of control standards. Further examples of respective research activities at the PEI are the development of an in-vitro toxicity assay for tetanus vaccines replacing animal-based safety tests, and research on the refinement of the potency test for rabies vaccines aimed at reducing the number of animals required. A further objective is the identification of inflammatory activities of biomedicines in animal tests. Here, we focus on the development of alternative immunoassays and nucleic acid amplification techniques. In the last decade, this work of the PEI was honoured with half a dozen of animal protection awards such as the "Hessian Animal Protection Research Award (2008)".

2.3. Therapeutic Safety of Human Stem Cells

Haematopoietic stem cells (HSCs) promise a readily accessible source for the development of advanced therapy products. They can be obtained either from (i) bone marrow, (ii) cord blood or (iii) peripheral blood and are frequently used for the reconstitution of haematopoiesis. Cord blood can be banked, but so far its application was mainly confined to children owing to the limited yield of stem cells obtained from cord blood. Therefore, extensive efforts are made to achieve an ex-vivo expansion and long-term culture of stem cells. The respective studies include specific induction of differentiation and gene transfer. In order to control the safety and efficacy of such promising but not easily controllable manipulations, research at the PEI targets the development of new methods for studying the impact of various processing steps on the composition, viability, ex-vivo expansion and differentiation of cord blood HSCs.

Maintenance of stem cells in a multipotent, pluripotent or embryonic-like state for a high number of cell divisions may trigger genetic instability resulting from endogenous retroelement activity (see below). Therefore, one goal of research at the PEI in this field is to determine the ramifications of the activity of autonomous Long INterspersed Elements 1 (LINE-1) on the biosafety of therapeutic stem cells by examining retrotransposition activity in these cells and its consequences for the genomic stability of stem cells.

2.4. Pharmaceutical Quality of Therapeutic Proteins

Beside impurities related to processing, product-related impurities of recombinant therapeutic proteins (e.g. protein aggregates and modifications or degradation products) can cause reduced therapeutic efficacy and might represent potential risk factors for unwanted immunological reactions. Hence, research in this field at the PEI targets the development and

establishment of highly sensitive biochemical (e.g., capillary electrophoresis, analytical HPLC, mass spectrometry etc.) and immunological methods (e.g. discriminative ELISAs, functional in-vitro assays) to assess the quality, safety and efficacy of recombinant therapeutic proteins. Studies are focusing on the impact of posttranslational modifications (e.g. glycosylation), artefact formation and structure (e.g. protein folding, aggregates) regarding the immunogenicity and antigenicity of protein therapeutics.

The pharmaceutical quality of medicines depends on the content, integrity and identity of the active ingredient as well as the physicochemical characteristics of the medicinal product (e.g. due to formulation aspects). The structural and functional integrity of biomedicines can be affected by the purification process or by post-translational modifications such as glycosylation, and cleavage of the peptide chain. This may lead to alteration of potency, stability or immunogenicity. Thus, the development of routine test systems for the assessment of the glycosylation patterns of therapeutic proteins from eukaryotic expression systems, for example, is one major research objective of the PEI within this field. Modern polysaccharide vaccines are often coupled to proteins in order to enhance their immunogenicity. Quality surveillance of these complex products is hampered by the fact that currently a variety of non-standardized test methods is used. Therefore, we are also focussing on the development of adequate and standardized methods for quality surveillance of vaccines. Recombinant allergens are evaluated as new reference compounds for the standardization and batch control of allergen extracts in a joint research activity funded by the European Directorate for the Quality of Medicines. Most therapeutic allergen products are subjected to different kinds of modifications including adsorption, chemical or physical modification. This can result in a dramatic loss of the IgE antibody binding capacity of these products. Hence, new methods (e.g. novel and highly sensitive Mediator-Release-Tests) for the analysis and evaluation of the effects of such modifications on the allergenic activity as well as in-vitro tests for the quantification of allergen molecules in such products are required.

Due to the inherent variability of biomedicines, the development of generic biosimilars raises the question, to which extent variations are acceptable especially with respect to the definition of products of comparable quality, safety and efficacy. Further research is required to define the variability of approved products in order to select criteria that justify regulatory decisions. In addition, the correlation of quality parameters to non-clinical testing needs has to be further examined in order to identify specific and sensitive parameters defining a product.

2.5. Adverse Effects to Biomedicines

Immunogenicity is defined as the capacity of a molecule to provoke an immune response in an organism. In a number of biomedicines, such as vaccines or specific immunotherapeutics consisting of proteins or allergens, immunogenicity represents the intended effect. However, for other therapeutic proteins, such as blood-derived medicinal products, the most common and obvious undesirable reaction following their administration is the formation of anti-drug antibodies by the immune system possibly leading to a loss of efficacy and/or severe adverse effects (SAEs).

Biological and biotechnological medicines carry the risk of inducing SAEs including acute systemic drug reactions. Consequently, reports on SAEs to biomedicines are collected and analyzed at the PEI. These data are used to constantly readjust our safety research activities and approval procedures regarding SAEs. New methods are required to adequately analyse the causality of SAEs to biomedicines. These methods might have to significantly differ from the established methods for chemical drugs due to:

- the variability of biomedicinal products, which impacts their safety profile
- the limited knowledge on the immunological effects of novel biomedicines
- the specific immunological properties of excipients and residuals
- the limited size of the target population (varying from only a few patients worldwide for orphan medicines up to major parts of the population for e.g. vaccines)

Experiences from dossier assessment clearly indicate a lack of systematic measurement and evaluation of unwanted immunogenicity prior to and after marketing authorisation. The provision of appropriate scientific information and regulatory recommendations by the PEI will further promote the clinical safety of biomedicines. To enable the design of new methods for causality analysis and risk assessment, the PEI is currently managing an inventory of the existing guidance and regulations. This will enable the proposal of standardised criteria based on clinical plausibility, epidemiological studies and biological or experimental data. In this regard, the PEI is currently also involved in a project funded by the European Centre for Disease Prevention and Control to provide guidance concerning causality assessment of adverse events following vaccination.

The PEI also targets the scientific elucidation of specific SAEs to certain biomedicines. In this regard, we are currently investigating why the superagonistic anti-CD28 monoclonal antibody TGN1412 induced a cytokine release syndrome in humans but not in non-human primates.

To this end, we study the functional basis of the cytokine release syndrome post TGN1412 application. A better understanding of the underlying mechanisms inducing the cytokine release will help to improve the predictive value of preclinical tests and to established novel test systems for the side effects of therapeutic monoclonal antibodies.

Many therapeutic proteins are administered by the i.v. route and cause acute infusion reactions for which the reasons are not well understood. The interaction of drug, endothelium and blood is the focus of active investigations in order to judge the risk for humans in clinical trials and employ preventative measures.

2.6. Critical Factors in the Development and Approval of Biomedicines

Evaluation and authorisation processes for biomedicines are challenged by a number of critical factors including methodological aspects of the pivotal clinical trial, the optimal timing of non-clinical and/or pharmaceutical/biological testing during the development programme, and the choice of the appropriate preclinical model. Important concerns regarding successful marketing applications for biomedicines still persist despite the continuous improvement of European regulatory standards. To support regulatory assessors, industry and academia in addressing these problems, a novel research initiative at the PEI focuses on studying the critical scientific factors in biomedicinal product development towards marketing authorisation making use of the institute's experience as regulatory authority being responsible for the marketing authorisation of biomedicines. In the respective studies, cross-product data analyses are performed to identify critical factors in the development of biomedicinal products to facilitate successful marketing authorisation application. In this context, previous regulatory decisions on biomedicines are systematically evaluated. Dossiers are analysed and cross-compared ("regulatory data-mining") with respect to e.g. the comparability exercise following manufacturing changes or the systematic evaluation of unexpected immunogenicity of biomedicines. A further focus in this field is the influence of recent discoveries in biomedicinal product development on the design and mode of the respective studies, evaluations and authorisation processes. In addition, these studies will help to discover critical characteristics or properties of biomedicines, which require assessment in experimental studies.

3. Experimental Vaccines, Therapies and Diagnostics

The PEI is committed to assure and improve the allocation of excellent biomedicines, why we are dedicated to keep track of new developments in this field. State-of-the art scientific knowledge on novel biomedicines is a prerequisite for excellent achievements in scientific advice and regulatory procedures. Consequently, we track recent methodological and technological innovations, and are engaged in performing research on novel strategies in biomedicine. Novel gene transfer and biomedical cell therapy products, new vaccination approaches and advanced diagnostics are based on the direct implementation of innovative methods and technologies into biomedicine. Approaches based on molecular biology promise major progress in the therapy (and prophylaxis) of rare congenital disorders as well as common diseases, such as cardio-vascular diseases, infections, allergy and cancer. Moreover, the introduction of molecular biology and proteomics into diagnosis enables refined analysis of the underlying cause of disease. In order to improve the therapeutic and/or protective effects as well as the safety and diagnostic properties of molecular biology-based biomedicines, the PEI currently aims at (i) optimizing gene transfer as well as viral vaccination vectors suitable for the treatment of malignant tumours, viral infections and allergy, (ii) evaluating novel cell-based therapeutics (iii) developing optimized biomedicines for the immunotherapy and diagnosis of allergies on the basis of recombinant allergens, and (iv) engineering novel attenuated live bacterial vaccines and vector systems.

3.1. Viral Vectors for Gene Transfer and Tumour Therapy

Viral vectors promise improved gene therapeutic approaches with minimised risks of treatment. Therefore, the PEI is engaged in the exploration and optimization of different types of viral vector systems, which are intended to be used for (i) gene transfer into cells of the human haematopoietic system, (ii) tumour therapy and (iii) genetic vaccination.

Lentiviral and oncoretroviral vectors are frequently used for the genetic modification of the haematopoietic system with respect to the treatment of inherited diseases. To overcome current safety and efficacy limitations we are engineering lentiviral vectors that enable the transduction of quiescent cells and restrict gene transfer to selected target cell populations. For example, novel lentiviral vectors have been developed allowing gene transfer into unstimulated primary monocytes, which cannot be achieved with conventional vectors. The exact molecular mechanisms underlying this particular capacity are currently investigated. Moreover, it is tested if lentivirally transduced monocytes are capable of being differentiated into myeloid dendritic cells using protocols established in clinical trials. These cells are the

major antigen presenting cells, why their targeted manipulation might disclose new therapeutic options (e.g. with respect to the specific and efficient presentation of tumour antigens). The suitability of these vectors for human gene therapy by modification of monocytes is currently tested at the PEI in a cancer immunotherapy approach and for the treatment of Chronic Granulomatous Disease. In doing so, we also analyse the risk of insertional mutagenesis induced by these vectors.

In addition, a novel system of lentiviral vectors allowing targeted cell entry has recently been established. Lentiviral vectors are pseudotyped with paramyxovirus glycoproteins that are modified to display either polypeptide ligands or cell-specific single-chain antibodies, both of which are directed against specific cell surface receptors. In this context, vectors are being developed that specifically target lymphocyte subpopulations, haematopoietic stem cells, tumour cells or neuronal cell subtypes. Combining gene transfer into quiescent cells with targeting envelopes promises realisation of efficient and selective in-vivo gene transfer into cells of the haematopoietic system. To address the problem of insertional mutagenesis, functional human autonomous Long INterspersed Elements 1 (LINE-1) retrotransposons are being explored for their potential of site-specific gene integration into the human genome.

Oncolytic viruses or tumour targeting viral vectors that conditionally replicate in cancer cells represent an attractive novel class of agents for the therapy of solid tumours. Therefore, at the PEI replication-competent vectors are engineered, which derive from attenuated strains of the measles virus or murine leukaemia virus (MLV). These vectors are designed to only spread to tumour tissue. Proteolytic activation of envelope glycoproteins of measles virus and MLV is essential for cell entry. Consequently, one approach to restrict vector spreading at the level of cell entry is making it dependent on matrix metalloproteases (MMPs), which are selectively active in tumours. Suitable MMP substrate peptides are being identified by screening retroviral protease substrate libraries of human tumour cells. Besides measles virus and MLV, a large number of DNA and RNA viruses exert oncolytic activity. Consequently, we are currently also testing vaccinia viruses as potential anti-tumoural agents. The deletion of defined genes in the genome of oncolytic viruses, e.g. anti-apoptotic genes, is anticipated to enable the engineering of tumour-specific oncolytic agents. Arming oncolytic viruses with additional anti-tumoural genes, such as sh/miRNA expression cassettes, is also part of the PEI's research efforts within this field.

3.2. Attenuated Live Bacterial Vaccines and Vector Systems

Today, a number of different bacteria are known to deliver nucleic acids to host cells. Therefore, bacteria are now also suggested as promising vectors delivering therapeutic genes, DNA vaccines and therapeutic RNA molecules. A multitude of applications for recombinant live bacterial vaccines or therapeutics has been investigated in animal models and a few of such experimental biomedicines have already been tested in clinical studies. However, at present safety concerns and efficacy problems often restrain the introduction of live recombinant bacteria into human or veterinary medical practice.

To make use of the therapeutic and prophylactic potential of attenuated live bacteria, the PEI is engaged in the development of new bacterial vector strains suited for the efficient and stable delivery of heterologous vaccine antigens or therapeutic molecules. The attenuation by gene deletion allows nowadays the establishment of safe and defined strains. In addition, these strains can be marked and therefore comply with the DIVA concept. In order to establish efficacious multivalent bacterial vaccines, we focus on novel expression strategies for heterologous antigens. One example is the so called "in-vivo remote control" of bacterial vectors by the use of in-vivo inducible promoters, which enables a regulated expression of heterologous genes in a temporal and quantitative manner. A further research focus in this field at the PEI targets the development of improved bacterial vectors for the delivery of DNA vaccines. Here we investigate the use of inducible suicide modules that promote an efficient DNA transfer from bacteria into host cells.

3.3. Novel Vaccination Approaches to Combat Modern Epidemics

Besides their use in gene therapy, viral vectors can also be used for novel vaccination approaches, which promise major progress in the prevention and control of infectious as well as intrinsic diseases. To date, vaccination has been shown to be most effective in the prevention of important infectious diseases, such as smallpox, poliomyelitis, neonatal tetanus, diphtheria, pertussis, influenza and measles. However, the development of novel vaccines for a number of remaining or (re)emerging diseases is a challenging task. This includes vaccines for (i) infectious agents such as novel pandemic influenza virus strains and HIV as well as some important bacterial and metazoan pathogens, (ii) tumour diseases and (iii) diseases correlating with an imbalanced immune status such as autoimmune diseases and allergic disorders.

The risk of an influenza pandemic requires proactive countermeasures. Accordingly, federal research institutes are working together in a coordinated approach to develop strategies for the prevention and control of a future pandemic. While the Friedrich-Loeffler-Institut investigates the spread and pathogenicity of influenza in birds serving as vectors for the reassortment of avian and human subtypes and the Robert Koch-Institut performs epidemiological studies on human influenza, the task of the Paul-Ehrlich-Institut is research towards the development of (pre)pandemic influenza vaccines. Finally, our studies will be extended to assess candidate pandemic influenza vaccines with respect to the H5N1 and H9N2 strains. To support the development of novel influenza vaccine, the PEI investigates the protection from infection and disease elicited by a vector-based expression of influenza virus genes. Another strategy pursued is the generation of influenza reassortants with optimised growth properties by reverse genetics. The ferret is the most appropriate nonclinical model in this field, since these animals develop signs of illness highly comparable to those seen in infected humans and allows conventional animal testing of vaccine efficacy. The respective experiments will be performed in our recently established BSL3+ unit.

Viral vectors are an attractive tool for the simultaneous delivery of immunogens and adjuvant stimuli to the host immune system. Among them, measles and vaccinia viruses represent potent live virus vaccines with a well established safety profile in humans. To generate multivalent prophylactic or therapeutic vaccines, these viruses have been genetically modified by inserting the genetic information for specific antigens of interest into the viral genomes. At the PEI, e.g. a measles virus has been engineered for the vaccination against Simian Immunodeficiency Virus (SIV) infections, which serves as model for HIV vaccines. Future studies at the PEI will also address the potential of measles virus vectors for therapeutic vaccination against self antigens such as tumour markers.

As a novel antiviral strategy in HIV-infections, we are pursuing a dual function strategy by combining a vector vaccine with an in-vivo gene transfer approach. We utilize a highly attenuated poxvirus, the Modified Vaccinia Virus Ankara (MVA), which has been actively developed as a third-generation smallpox vaccine and has a high potential as vector vaccine due to its strong immunological potency and good safety profile. To protect T-cells from infection with HIV, a genetically modified MVA has been engineered. This dual function MVA vaccination vector delivers HIV-1 immunogens and concomitantly enables in-vivo delivery of therapeutic genes into target cells. Besides HIV-1 proteins the vector also encodes for a fully functional lentiviral vector genome delivering heterologous genes with HIV inhibition capacity.

After proof of principle, this approach can be extended to further applications of in-vivo gene transfer.

Attenuated poxviruses present prominent features as vaccine candidate, i.e. biological and clinical safety, large packaging capacity for recombinant DNA, precise virus-specific control of target gene expression, high level of immunogenicity, lack of persistence or genomic integration in the host, and ease of vector and vaccine production. Hence, we aim at developing MVA vaccine candidate vectors for pandemic/epidemic infectious diseases such as influenza and AIDS. We investigate the potential of modifying the MVA genome in order to improve the immunogenic characteristics of the vector and, hence, enhance the vaccine efficacy.

Non-clinical testing of vaccines is usually performed using mice models. However, more suitable animal models and the appropriate selection of experimental protocols are the basis for accurate prediction of the protection by a vaccine. Therefore, we have e.g. established a mouse model to test safety and efficacy of MVA-based smallpox and vector vaccines using an ISO-CAGE Biosafety-Station.

3.4. Cell-Based Therapeutic Approaches

Latest developments in stem cell research raise hope that cells required for novel advanced therapeutic purposes can be generated from multipotent human somatic stem cells such as haematopoietic and mesenchymal stem cells (HSC and MSC, respectively). HSC and MSC are especially interesting, since they can be differentiated into various different cell types. Consequently, research within this field at the PEI targets the elucidation of the underlying molecular mechanism of differentiation. One primary goal is the identification and guided modulation of critical signalling pathways involved in the differentiation program of human HSCs into immunological effector cells such as myeloid dendritic cells (mDC). We already showed that the proliferation and differentiation of HSCs induced by physiological stimuli, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin-4, result in a distinct and temporary activation of different intracellular signalling pathways. To further elucidate the precise signalling events involved in the specific differentiation processes of stem cells is of crucial importance, since it will enable surveillance of the differentiation process, but also to control it. With this knowledge we will ultimately be able to define the conditions necessary for the reprogramming of already differentiated cells.

The current experimental immune-cell-based therapeutic approach pursued at the PEI is the treatment of cancer with (modified) immune cells. Several murine tumour models, including models for malignant melanoma, fibrosarcoma and lung carcinoma, have been introduced at the PEI to study cell-based approaches for the treatment of malignant diseases. These animal models are also used to investigate the dependence of tumour surveillance on cytokine responses, such as type I interferon and we could recently prove that the growth kinetics of the mouse malignant melanoma B16 is largely enhanced in mice devoid of a functional type I interferon receptor (IFNAR). Moreover, tumours expressing a neo-antigen leading to tumour rejection in wild-type mice continued to grow in IFNAR knock-out mice. As a consequence, we are currently studying the underlying mechanisms of the type I interferon dependency of tumour surveillance. In future, it will be of special interest to investigate cell-based immunization approaches with the aim to overcome tumour-specific tolerance in IFNAR knock-out mice.

3.5. Novel Strategies for the Specific Immunotherapy of Allergy

Certain IgE-mediated allergies can be treated by specific immunotherapy (SIT) applying small but increasing amounts of allergen extract at regular intervals to increase clinical tolerance. However, SIT is often inconvenient for the patients due to a long treatment period with many hospital visits. Moreover, SIT is currently not available for the treatment of all IgE-mediated allergies (e.g. food allergies) or has limited or unproven efficacy. In addition, it is accompanied by a relatively high risk of adverse reactions, which may partly be due to the use of poorly defined and non-standardised allergen extracts. Novel vaccination strategies target the standardised use of recombinant allergens or modified allergens with improved immunological properties instead of allergen extracts. These novel treatment options simultaneously aim at higher efficacy and reduced severe adverse effects (SAEs).

Research in this field at the PEI targets e.g. the use of novel “hypoallergenic molecules”. These molecules will enable the administration of higher amounts of the therapeutic agent having a lower risk of adverse reactions, which should result in an improvement of the therapeutic outcome. Currently, a few hypoallergenic therapeutics are under clinical investigation. In addition, fusion proteins of (hypo)allergens with either functional peptides, allergen fragments or T-cell epitopes provide promising novel candidates for therapeutic allergy vaccines, since they aim at enhancement of antigenicity of the therapeutic agent. Moreover, we evaluate experimental allergy vaccines with novel adjuvants (e.g. toll-like receptor ligands derived from bacteria), which have been designed to successfully suppress

or prevent the allergic immune response by inducing regulatory T cells and/or a TH-1 biased immune response without a high risk of SAEs.

Novel immunotherapy strategies also include the evaluation of novel routes of antigen administration such as the intranodal or the non-invasive transcutaneous immunization and the evaluation of DNA vaccines or viral vectors in allergy treatment. In this context, studies at the PEI aim e.g. at the evaluation of allergen immunotherapy by the application of a recombinant Modified Virus Ankara for the transfer of allergen cDNA into host cells. Future approaches will focus on the specific targeting of antigen presenting cells (APCs) by cell-specific or conditional expression of therapeutic allergens in-vivo, for example, by the use of APC-specific inducible promoters.

In addition to therapeutic approaches for the secondary prevention of allergic diseases, the development and evaluation of prophylactic vaccines for the primary prevention of allergy is another focus of our research activities. As an important tool, mouse models will be further developed to study safety and efficacy issues (see below). Characterization of vaccine candidates using cellular in-vitro assays or wild type and knock out mice will contribute to the elucidation of the mechanism as well as the optimization of SITs.

3.6. Component-Resolved Diagnosis in Allergy

The currently available serological assays to determine sensitisation use allergen extracts and are not capable of differentiating between patients suffering from clinical (food) allergy and sensitized individuals cross-reacting without clinical relevance. Recombinant allergens may contribute to solve this problem, particularly if they were used in advanced in-vitro tests, which are, however, not yet commonly applied in allergy diagnosis. Recombinant allergens and native allergens generated, purified and characterised e.g. at the PEI represent a growing collection of well-defined allergenic proteins from several allergenic sources such as pollen (e.g. birch and grass pollen) or foods (e.g. hazelnut, soybean, celery, carrot, apple and peanut). Their use in a novel diagnostic approach called “component-resolved diagnosis” promises improved sensitivity of serological diagnosis and refined information on the allergic status of the patients. Accordingly, respective research efforts at the PEI clearly demonstrated enhanced sensitivity of recombinant allergens if compared to allergen extracts and highlighted the significant differences in sensitization patterns to allergenic proteins between allergic patients from northern and southern Europe. Moreover, the use of recombinant allergens enabled differentiation between sensitization against “mild” and “aggressive” food allergens. Our current research efforts within this field target the

introduction of functional assays based on the in-vitro activation of basophilic cell lines. To improve the correlation between in-vitro test results and the clinical situation, novel avidity assays are developed utilizing recombinant allergens including the evaluation of the potential of protein microchips.

4. Immune Activation and Evasion

Research at the PEI contributes to the development of potent vaccines as well as other prophylactic and therapeutic biomedicines targeting the immune system to combat infectious diseases, allergies and tumours. To disclose novel therapeutic options, detailed knowledge on the pathways used by pathogens to interact with the host immune system is desirable. Pathogen infections usually trigger a multitude of signals recognised by the host immune system to induce innate and adaptive immune responses. The reactions of the immune system are counterbalanced by a range of mechanisms triggered by the pathogen to suppress, circumvent and manipulate the host defence system. A detailed understanding of these immune activation and evasion mechanisms will eventually allow the design of novel vaccination strategies as well as other therapeutic immunological measures meeting the highest safety and efficacy standards.

4.1 Early Immune Activation

Early immune activation is the initial immune response, which ensures survival after infection until the pathogen-specific adaptive immune response comes in. In addition, early immune activation was shown to shape adaptive immunity and to be indispensable for the regular function of the whole immune response. The PEI is engaged in studying the molecular basis of early immune activation, since detailed knowledge of the underlying mechanisms promises the discovery of new immunotherapeutic strategies including the development of novel adjuvant stimuli used for vaccination or tumour therapy.

Regarding the early immune activation after virus infection, type I interferon (IFN- α/β) responses are of particular interest, since they are amongst the first immune reactions induced. Moreover, IFN- α/β mediates the linkage between innate and adaptive immunity. IFN- α/β is a very potent inhibitor of virus replication and spread. Recently, the PEI has contributed to the identification of a new subset of dendritic cells (DCs) termed “natural interferon producing cells” or “plasmacytoid dendritic cells” (pDCs). These cells were shown to produce large amounts of IFN- α/β after virus infection. We are currently studying the molecular basis of IFN- α/β induction in pDCs upon virus infection. Additionally, we analyse if and how other DC subsets can be triggered to produce IFN- α/β . These studies will help to elucidate how negative-strand RNA viruses (such as vesicular stomatitis virus) and DNA viruses (such as herpes simplex virus and vaccinia virus) influence the early activation of the host immune system.

Furthermore, we are studying the influence of IFN- α/β on the modulation of specific immunity. For this purpose, a transgenic mouse model has been developed at the PEI, which now enables the tissue-specific and cell-type-specific ablation of the type I IFN receptor. This model is used for the analysis of the direct impact of IFN- α/β on lymphocytes in the context of anti-viral humoral and cellular immunity. Together, we aim at gaining in depth insight in the pleiotropic effects of IFN- α/β with respect to autoimmunity, malignancy, and virus infection.

4.2 Viral Immune Evasion

The development of novel safe and effective vaccines against pathogens requires detailed knowledge of the fundamental mechanisms of pathogenicity. Most, if not all, viruses evolved effective immune evasion strategies that systematically modulate key components of innate and adaptive immunity. Large DNA viruses, such as poxviruses, encode for a variety of different proteins that specifically manipulate the function of critical factors/messengers of the host immune system (e.g. interferons, interferon response proteins, interleukins, chemokines, complement proteins, and the apoptotic cascade). Although many viral immune evasion factors have been identified in-vitro and/or in-silico, current knowledge on the function of these viral immune modulators is still limited. Consequently, the specific function of viral immune modulators within the molecular life cycle is being investigated at the PEI. In this context, we are currently studying their influence on innate or antigen-specific host responses that are elicited upon infection. Research strategies are based on targeted genetic engineering or random mutagenesis of viral genomes. This approach will enable the determination of the function of selected viral genes and the identification of novel viral genes with immune modulation potential. Detailed knowledge on viral immune modulators will help to elucidate the viral mechanisms involved in the induction of innate and adaptive immunity and to discover novel intervention strategies.

Another strategy to decipher the function of viral proteins in the modulation of cellular immune responses is the in-vivo examination of virus variants expressing mutants of viral proteins. This approach enables to examine the impact of these viral proteins on the host's antiviral barriers. In this context, we are investigating infections with mousepox virus variants, which provide a potent animal model appropriately mimicking the pathogenesis of human smallpox disease. This model also enables to test the design and efficacy of new poxvirus specific vaccines and therapeutics. Such medicines are urgently required due to (re)emerging zoonotic poxviral infections and the modern risk of bioterroristic attacks.

4.3 Molecular Basis of Allergenicity

To date, properties making a usually harmless protein an allergen are poorly defined. Identification of the characteristic features determining allergenicity is of major importance, since it will help to understand allergy in more detail and enable the design of novel treatment options. Most proteins have antigenic properties, while allergenicity seems to be restricted to a few protein families only. Our research on allergen identification and characterization has contributed significantly to this field. To decipher the molecular basis of allergy, the respective studies at the PEI address the stability of allergenic proteins, the detection of their T- and B-cell epitopes, and the characterisation of the biochemical (e.g. enzymatic activity) as well as pharmacodynamic properties (e.g. resorption, distribution and elimination in-vivo) of these molecules.

Moreover, we investigate the influence of processing on the allergenicity of food proteins. Therefore, we developed and optimized suitable in-vitro allergenicity assays, e.g. basophil activation, T-cell proliferation and other cellular tests, to investigate the allergenic potency of proteins and peptides. In addition to the properties defined by the protein itself, the allergenicity of a given source material depends on the amount of the allergens to which the patients are exposed. Therefore, the quantification of allergens in a potentially allergenic source (e.g. pollen extract, native or processed foods) with respect to the individual threshold level in eliciting a clinical reaction is important for allergy assessment and risk minimization. Consequently, we work on novel high-sensitivity and high-resolution assays using quantitative PCR and ELISA techniques as well as mass spectrometry methods.

4.4 Preclinical Allergy Models

The development of new strategies for the treatment of human allergy requires proof of safety and efficacy. Unfortunately, prediction of efficiency in humans is still hampered by the lack of appropriate pre-clinical models. First mouse asthma models involving the induction of IgE expression exist, but only a few very preliminary mouse models regarding clinical food allergy and the treatment of life-threatening food allergy have been introduced to date. Therefore, the development and validation of such mouse models used for the optimization and testing of food allergy vaccines as well for the investigation of food allergy mechanisms are an important research topic of the PEI. Despite the fact that, depending on the genetic background, previous studies revealed significant differences in the immune responses and sensitivity of sensitized mice, we have been able to establish new mouse models for specific food allergies. These models involve oral immunization regimes and reflect the immune

response in an allergic subject as well as the clinical reaction. Currently, these models are characterized with respect to murine immune responses at the epitope level and the clinical readout. Using these mouse models we also perform immunotherapy studies with novel allergen vaccines. These studies will help to considerably increase the efficiency of existing specific immunotherapies (SITs). Moreover, these models are extremely important for the development and evaluation of new therapeutic strategies for the treatment of allergic diseases, which currently cannot be treated by SIT (e.g. food allergy).

5. Host Interactions with Pathogens and Retroelements

A number of pathogen/host interactions have evolved in a way that allows a balanced co-existence for a certain period of time. Nevertheless, these pathogens can eventually still induce cancer, cytotoxicity or cell degeneration. In this regard, an increasing number of human diseases have been connected to long-term infections with pathogenic organisms and “genetic parasites” that can evoke cellular dysfunctions by influencing signal transduction pathways or causing genetic instability. Knowledge on these pathways is the basis of new therapeutic strategies and the fundament of appropriate risk assessment regarding certain biomedical therapeutics. Therefore, the PEI is engaged in defining the molecular interactions between such pathogens (including viruses, retroelements, bacteria, and prions) and the host cell focusing on the analysis of the direct molecular interactions between pathogen and host cells.

5.1 Impact and Control of Retroelement Activity

Approximately 42% of the human genome originate from the activity of mobile genetic elements termed retroelements, which multiply within the host genome by reverse transcription of the coding RNA and genomic reintegration of the resulting cDNA. The human genome harbours two subgroups of retroelements, (i) the predominant non-long terminal repeat (non-LTR) retrotransposons and (ii) the LTR-retrotransposons or endogenous retroviruses (ERVs). Retroelements can act as parasitic or symbiotic Mendelian genes of the host, either causing genetic disorders and cancer or contributing to gene function. Consequently, evaluating the impact of retroelement activity on genetic, tumourigenic and physiological processes is e.g. required for the assessment of risks inflicted on a patient by stem cell and tissue therapies by a possible therapy-related activation of retroelements.

Human non-LTR retrotransposons account for $\geq 34\%$ of the genome. They comprise autonomous Long INterspersed Elements 1 (LINE-1) and non-autonomous elements such as Short INterspersed Elements (SINEs) and SVAs (SINE/VNTR/Alu) as well as processed pseudogenes. Mobilization of all non-LTR retroelements is mediated by the LINE-1 encoded protein machinery. LINE-1-mediated retrotransposition impacts the stability as well as the shape of the host genome. It can be deleterious to the host cell, and has the potential to cause genetic disorders and cancer. The presence of retroelement-encoded proteins was shown to affect the differentiation and proliferation status of cells. Moreover, retroelement-encoded promoters can regulate the expression of adjacent host genes. Non-LTR

retrotransposons are expressed and mobilized in the germline as well as in a variety of stem cells, while their activity is restrained in the majority of differentiated somatic cells.

Due to the potential of adult and embryonic stem cells as therapeutic agents in a variety of human diseases (see 2.3), retroelement research at the PEI aims at elucidating host-encoded strategies to curb the proliferation of transposable elements in order to limit the negative effects of retrotransposition. To this end, we target the identification of host-encoded factors that interfere with the LINE-1-encoded protein machinery and influence LINE-1-mediated retrotransposition activity. Currently, we are focussing on the elucidation of the mechanism of intracellular defence against LINE-1 retrotransposition by members of the APOBEC3 protein family and on the influence of host-encoded double-strand break repair factors on LINE-1 retrotransposition. Our studies are performed with inducible and marked LINE-1 reporter elements in tissue culture as well as transgenic mouse models. These experiments will help to identify specific cell types, tissues and developmental stages that support LINE-1 expression and retrotransposition causing genetic disorders and tumour development

Human ERVs (HERVs) cover approximately 8% of the human genome. While HERV-K gene expression is generally repressed in adult somatic tissues, reactivation of HERV-K proviruses is known for several types of cancer. We investigate the diagnostic and therapeutic value of the immune responses to HERV-K gene products expressed by tumours. To this aim, we analyze the mechanisms of transcriptional activation of HERV-K elements during tumourigenesis as well as mechanisms responsible for HERV activation after HIV infection.

ERVs of animal species, such as porcine endogenous retroviruses (PERV), represent a potential risk in xenogeneic cell therapy and xenotransplantation, since treated patients could potentially be infected. Also, transmission of the viruses to third parties including relatives and health care workers may result in a risk of spreading such infections to the wider public. Consequently, we are currently focusing on the establishment of methods and models to identify possible PERV infection risks. We are investigating and characterizing the presence of functional xenotropic (PERV-A, PERV-B) and ecotropic (PERV-C) viruses in different pig strains as well as in genetically modified pigs. For risk evaluation, their potential to recombine and form more virulent variants will be tested. Moreover, the retrotransposition potential of integrated proviral elements as well as recombination events between animal and human ERVs is investigated. In this context, host cell mechanisms possibly influencing infections of human/primate cells are also being evaluated. By taking advantage of an existing non-human

primate model, we e.g. will examine the suggested role of the cellular prion protein in the control of activated ERVs.

Understanding the role and impact of retroelements on physiological, genetic and tumourigenic processes will provide a profound basis for risk assessment with respect to potential retroelement activation interfering with stem cell therapies and tissue therapeutics.

5.2 Viral Pathogenicity and Intervention Strategies

The genetic endowment determines the replication efficiency and pathogenicity of influenza virus strains and hence the evolution of novel pandemic subtypes. Consequently, an important research branch in the field of viral pathogenicity at the PEI currently targets the identification of the determinants that govern the hazardous nature of the H9N2 influenza virus strain using cell culture and animal models. For this purpose, reassortants are produced, which contain specific genes of the H9N2 strain in the background of mouse pathogenic acceptor strains, such as PR8 and WSN. This approach enables precise and individual monitoring of the specific effects of each H9N2 gene. To identify the impact of genetic predisposition on infection and disease susceptibility, we will investigate the pathogenicity of the reassortants in different inbred mouse strains. Moreover, these reassortants will be used for the selection of optimised vaccine strains on the basis of phenotypic attenuation and growth characteristics in different culture systems. Protection rates achieved with model vaccines produced from different reassortants will be investigated in a mouse model. The potential of these vaccines to elicit cross-protection to heterologous strains will be examined.

5.3 Pathogen-Induced Modulation of Signal Transduction

Increasing evidence proves that dysregulation and imbalance of cellular signal transduction pathways plays an important role in a variety of severe human pathogen-related diseases, such as infections with bacteria, viruses and prions. It is strongly suggested that the underlying mechanisms might also be responsible for the development of sequelae such as certain tumours and a number of degenerative diseases. Using *Helicobacter pylori* (*H. pylori*) as our current model organism, we investigate the molecular interaction between a human pathogen and the host cellular signal transduction pathways. This interaction is believed to be strongly associated with pathogenesis and the respective sequelae, i.e. gastric inflammation and cancer. Currently, we are analyzing pathogen-affected signalling molecules, especially (non)-receptor tyrosine kinases and their cellular substrates in epithelial cells. Moreover, we study the role of the dysregulation and imbalance of the cellular signal transduction pathways

in pathogen-induced cell migration, apoptosis, proliferation and virulence. We further aim at identifying and characterizing presently unknown pathogenic factors involved in these mechanisms. Together, our studies might help to develop completely novel intervention strategies in bacterial pathogenesis.

Human and Simian Immunodeficiency Viruses (HIV and SIV, respectively) share important genetic and biologic features. However, it is currently unknown why HIV-1 induces a pathogenic disease whereas certain SIV strains such as SIV_{agm3} lead to productive but non-pathogenic infections. Consequently, research at the PEI in this field aims at elucidating virus life cycles, virus-host interactions and molecular mechanism of disease progression. Our main focus is the identification and functional analysis of the molecular interactions between viral factors and cellular signalling pathways. SIV_{smmPBj} (PBj), an acutely pathogenic virus isolated from pig-tailed macaques, is used to investigate virus-host-cell-interactions especially during early phases of acute infection. One approach in these studies is e.g. the investigation of early intracellular signalling events induced by lentiviral envelope binding to host-cell receptors. To further decipher molecular viral components capable to modulate cellular signal pathways, we are also studying the interaction of viruses with the host cell (phospho)proteome. These studies should show how influencing the intracellular signalling facilitates viral replication. In turn, this should disclose novel intervention strategies to prevent lethal pathogenesis.

5.4 Modelling of Host-Pathogen Interaction

Experimental research at the PEI is complemented by interdisciplinary research in systems biology and expert biostatistics. Large numbers of elaborated empirical data are available on long-term infections with pathogens enabling the evaluation of biological systems with mathematical and in-silico models. Research in this field at the PEI covers the analysis of epidemics, pathogen (co-)evolution and biological network dynamics. A current systems biology research focus of the PEI investigates host pathogen interactions. We especially aim at elucidating the intra-host evolution of the human immunodeficiency virus. Consequently, we are designing in-silico models of pathogen evolution, which consider the variable environment due to the host's immune response and the therapeutic measure. Methods and tools derived from these studies will also be applicable to other research areas of the PEI, such as the evolution of influenza within the host population. Besides viral persistence or phylogeny, we are also interested in the evaluation of epidemic spreading and the analysis of the potential impact of (pan)epidemic events on scarce resources such as blood supplies.

6. Outlook - Research Areas

Research at the PEI is conducted to support and strengthen the institute's profile as a European centre of excellence for the evaluation of biomedicines, a competent German policy advisory authority, and an internationally recognized life science research institute. In accordance with its legal responsibilities and the "Konzept einer modernen Ressortforschung"⁶ of the German Government, the PEI targets to enhance its proactive research efforts especially regarding new emerging diseases. Viral and microbial safety of biomedicines as well as the development of vaccines/immunotherapies against infectious and intrinsic diseases are important tasks of the PEI. Risks of biomedicines may have to be minimized by suitable new regulatory requirements. To always keep up with new technologies and novel scientific concepts, we track recent developments and challenges in the field of biomedicines, and continuously establish and validate new assays and test methods.

The authorization of clinical trials is a challenging task of the PEI because biomedicines are increasingly complex and target sensitive molecular and physiological mechanisms. Moreover, some biomedicines, such as monoclonal antibodies, trigger species-specific potent immunological mechanisms requiring appropriate and predictive preclinical in-vivo models for their investigation. The choice of a relevant and suitable animal model may provide a safeguard to minimize adverse reactions in early clinical trials. As a consequence, one of the important future research topics of the PEI targets the evaluation of humanized mouse models for non-clinical proof-of-concept and safety testing of biomedicines. Humanized mice are transgenic immunodeficient mice (animals without B and T cells and often also with drastically reduced NK-cell function), which are reconstituted with human cells (CD34⁺ blood stem cells or PBMCs), by which they harbour human immune cells and mimic certain aspects of the human immune system. The PEI will focus on the investigation of new and established humanized mouse models for the testing of monoclonal antibodies as well as gene and cell therapy products and vaccines. Moreover, humanized mouse models will be used for in-vivo studies on cytokine responses upon immune stimulation and the impact of cytokines on adaptive immunity. A further aim is the establishment of humanized mouse models as new and more appropriate allergy models.

⁶ "Concept of a Modern Federal Research", to be found under the following web address:
http://www.bbr.bund.de/cln_005/nn_340398/BBSR/DE/Bundesinstitut/KonzeptRessortforschung,templateId=raw,property=publicationFile.pdf/KonzeptRessortforschung.pdf

Synthetic Biology comprises the design of novel features and functions at all levels of the hierarchy of biological structures to develop standardised components with defined functions. This includes the construction of artificial proteins, metabolic pathways, and genetic networks, or even the (re)programming of whole cells. In doing so, interdisciplinary Synthetic Biology integrates genetic engineering, comparative and functional genomics, bioinformatics and systems biology findings. In the future, a number of biomedicines could be produced by Synthetic Biology approaches. This especially applies for vaccines, gene and cell therapeutics, and recombinant biomedicines. Today, Advanced Therapy Medicinal Products based on Synthetic Biology are under development, and introductions into clinical trials as well as applications for marketing authorisation are expected. Accordingly, a number of research projects of the PEI already address the Synthetic Biology approach. Examples include the development of virus reassortants for the combat of infectious diseases, the design of synthetic viral vectors for gene transfer and tumour therapy, and the development of artificial hypoallergenic proteins for the treatment of allergy. Future activities at the PEI within this field target the modification of the (retro)viral envelope to improve directed cell entry of viral vectors. Such engineering of (retro)viral surface proteins will significantly contribute to the safety and efficiency of gene transfer technology. This work will not only focus on the targeted gene transfer to any human cell type of interest, but also on triggering specific cell signals. These efforts will enable specific influencing of cell physiology through targeting the respective cell surface receptor. Another future Synthetic Biology topic of the PEI is the rational design of attenuated life bacteria as safe and effective carrier systems for vaccines or therapeutic molecules. In order to increase the safety and efficacy profile of such vectors we will be engaged in the stepwise optimisation of conventional strains. To this end, conventional bacterial vectors will progressively be optimised by decreasing their genetic variability, virulence factors and metabolic load to finally obtain defined and safe vectors. The respective future research activities of the PEI in the field of System Biology also aim at developing sensitive assay systems to evaluate novel vector technologies including cell-based assays as well as in-vivo analyses in humanized mouse models.

To establish a “proteomics platform” a quadrupole time-of-flight mass spectrometer has been set up at the PEI. In the future, the PEI aims at implementing and developing cutting edge methods and technologies for the micro-characterisation of proteins and peptides. This includes the investigation of the impact of posttranslational protein modifications (i.e. any enzymatic or chemical modification of the amino acid side-chains after polypeptide synthesis) on the safety and efficacy of biomedicinal products. The respective methods and technologies will also support future activities of the PEI regarding the relatively new challenge of

counterfeit biomedicines, i.e. falsified medicine preparations of obscure origin and inappropriate quality, requiring highly sensitive and specific methods to distinguish counterfeit from original.

Based on previous and current research efforts to decipher the immunobiology of allergens, the PEI aims at further extending these activities to study the patho-mechanisms underlying the development of allergy with a special emphasis on food allergy. These studies will also address the still unknown mechanisms of clinical tolerance induction in allergy.

A current major research focus of the PEI with respect to the reduction, refinement or replacement of animal experiments is the development of in-vitro approaches for the testing of veterinary vaccines. A number of conventional animal test methods have yet to be replaced by in-vitro assays. In the future, the PEI aims at investigating the suitability of novel complex tissue culture systems as novel in-vitro models for the evaluation of vaccine induced immune responses.

7. Research Infrastructure

Following the recommendations of the "Wissenschaftsrat" (German Council of Science and Humanities) dated 07/07/2000⁷ and addressing the challenges, which arise from the structural changes of the national and international science system, the PEI continuously refines its research infrastructure. According to the recommendations of the German Research Foundation, the PEI has adopted guidelines to assure good scientific practice (i.e. "Principles of Scientific Work and Conduct at the Paul-Ehrlich-Institut"). All scientific personnel, in particular the scientists involved in experimental research, are obliged to follow these guidelines and to perform research in compliance with the recommendations of the international commission on professional self-regulation in science (cf. German Research Foundation). The PEI is an equal opportunities employer, meaning that in recruitment, selection, education and assessment of students and staff it is only considered if the individual meets the requirements of programme or post.

7.1. Scientific Advisory Board

Scientific evaluation of the research performance and infrastructure of the PEI is supervised by an independent Scientific Advisory Board. Its fifteen members (including a chairman) are appointed for four years by the President of the PEI in consultation with the Federal Ministry of Health. Its members are leading figures of the national and international scientific communities. They are renowned for outstanding expertise in clinical research/practice and/or basic science within areas relevant to the PEI's work. The Board meets twice a year, and provides expert advice and guidance with respect to research orientation, research performance, and future strategic research direction. Therefore, research group leaders present selected projects to discuss scientific progress and future objectives. Furthermore, general issues related to the research structure and activities of the institute are discussed, especially with respect to the annual evaluation. The Scientific Advisory Board provides guidance in updating the PEI's Research Programme. New versions of this programme are implemented by the President of the PEI in consultation with the Federal Ministry of Health.

7.2. Research Groups and Funding

Research at the Paul-Ehrlich-Institut is carried out by scientists and technical assistants as members of research groups⁸. These research groups are lead by the President or the respective head of division. Within the divisions, section heads may establish additional research groups in agreement with the respective division head. In addition, independent

⁷ to be found under the following web address: www.wissenschaftsrat.de/texte/4595-00.pdf

⁸ further information is to be found under the following web address: www.pei.de/research

junior research groups reporting to the President have been established at the PEI. The research groups of the President of the PEI, the division heads and the section heads are directed by experienced scientists. These groups perform research at an internationally competitive level covering the key research areas of the PEI. The reputation and expertise gained from research activities have major impact on the success, competitiveness and competence in medicinal product regulation and official medicines control laboratory activities of the PEI. Interdisciplinary collaborations of the research groups are encouraged to enable significant synergistic effects and a continuous exchange of ideas. Division heads and research group leaders are encouraged to maintain close relationship with scientists of universities and other research institutions and to lecture at universities. Group leaders without a university lecturer qualification (Privatdozent) are supported by the PEI in their efforts to acquire this qualification.

Junior research groups have been implemented to establish new research areas within the scope of the PEI. These groups are funded by the institute and set up for a period of five years. They offer highly qualified junior scientists an attractive career opportunity and the chance to develop an internationally recognized research profile. Funding includes the positions of the group leader, a PhD student and a technical assistant laboratory technician. In addition to the groups funded by the PEI, junior research groups can also be implemented on the basis of available grants from science foundations. Proposals for new research areas to be covered by junior research groups can be made by the Research Office and the internal Research Working Group. Final decisions are made by the President of the PEI in consultation with the Scientific Advisory Board.

In addition, each year, three internal PhD positions are awarded to projects in line with the Research Programme of the PEI following a competitive call for applications. Applications are submitted to the Research Office following rules that have been defined in detail by the internal Research Working Group. All proposals are evaluated in a peer review process according to the internal organisational ordinance "Funding of Internal PhD Positions". A priority list according to the peer review decision is submitted to the President for final decision. Research group leaders who are awarded an internal PhD position are obliged to present an annual progress report during meetings of the internal Research Working Group.

The "Concept of Modern Federal Research" by the German Government underlines the importance of external funding for the scientific excellence of federal research organizations. In line with this concept, research projects at the PEI are predominantly financed by external

funding and the salary of the majority of scientists conducting research at the PEI is financed by external grants. Internal funds of the PEI are primarily used to provide the infrastructure including laboratory space, equipment and, to a limited extent, consumables. However, positions for scientists and technicians performing research related to important regulatory issues of biomedicines may be financed by the PEI. External grant proposals may be submitted by every postdoctoral researcher at the PEI in consultation with the responsible supervisor (i.e. the President or the division head) and after initial examination by the Research Office and approval by the President. Details of the internal processes related to the submission of grant applications are described in the internal organisational ordinance "Applying for Grants".

7.3. Research Management

Further development and maintenance measures of the research infrastructure are managed by the Research Manager and its deputy (the Head of the Research Office) in cooperation with the internal Research Working Group and in consultation with the President of the PEI. In particular, the following tasks are assigned to the Research Manager and the Head of the Research Office on behalf of the President:

- to support and advice scientists e.g. with respect to grant application, research project management, and student training
- to monitor external grant proposals
- to coordinate review processes with respect to internal funding or awarding
- to organise the regular updating of the Research Programme
- to coordinate the development of education programmes
- to organise the acquisition/processing of data with respect to research evaluations
- to maintain research data bases
- to assume the cooperation with the Research Managers of the German Federal Ministry of Health and other institutions
- to support the "Arbeitsgemeinschaft der Ressortforschungseinrichtungen"⁹
- to assume the communication with executives and politicians with respect to research related or research management inquiries
- to coordinate the activities of the internal Research Working Group
- to supervise the Research Office (Head of the Research Office and secretary).
- to maintain a research information site on the institute's Intranet

⁹ Working Group of the German Federal Research Institutes; further information is to be found under the following web address: <http://www.ressortforschung.de/de/aktuelles/index.htm>

- to supervise Material Transfer Agreements (MTAs) governing the transfer of materials between the PEI and other organisations or private individuals (a non-negotiable standard MTA with minimum demands has been implemented by the President in consultation with the legal department and the internal Research Working Group).

The internal Research Working Group consists of permanent voting members (i.e. the division heads or their representatives, the Research Manager and the Head of the Research Office) as well as temporary voting members, which are appointed for a period of three years (i.e. one scientist per division nominated by the division heads, the representatives of the President's research groups and the junior research groups, and two PhD student representatives). The internal Research Working Group meets in working sessions, which are open to all scientific employees of the PEI as guests. Aims of the periodic meetings of the internal Research Working Group are assistance in the development of new research management strategies, of research infrastructure activities, information exchange between the Research Groups and initiation of interdisciplinary research projects. In particular, the following tasks are assigned to the internal Research Working Group:

- the development of the research infrastructure
- the management of a budget, which serves to finance research infrastructure activities, such as a bonus system and other performance-related allocations
- the allocation of internal research funds
- the development of new interdisciplinary project ideas
- the organization of the evaluation of the internal fellowship applications
- the evaluation of research activities
- the elaboration of proposals for new junior research groups
- the development of education programmes for young scientists.

7.4. Education Programmes

At the PEI students carry out their thesis work to achieve a Masters, Diploma, Medical Doctor (MD), Doctor of Veterinary Medicine (DVM) or PhD degree. Students working on research projects at the PEI are asked to discuss the project subject with both, the respective supervisor of the PEI and the responsible representative of the university faculty they are associated with. Students provide a brief project outline at the beginning of their work, which is submitted to the Research Office and, in the case of a PhD or DVM project, to the thesis committee. After completion of their project, the students are asked to present their final results at a Research Plenum seminar and to provide copies of their written thesis. Following

the term of an individual grant of the German Research Foundation, PhD and DVM thesis at the PEI are in general completed in three years.

A "Postgraduate Training & Education Programme in Biomedical Research (PEP-BIOMED)" has been developed and will be implemented¹⁰. PEP-BIOMED is intended to allocate a structured training programme and to provide an internationally competitive education curriculum. It outlines a three year training agenda for PhD and DVM students. Nevertheless, students working on their masters, diploma or MD thesis at the PEI are also invited to participate in the activities of the programme. The programme was designed following national as well as international recommendations¹¹. It is an attractive, target-oriented and multi-faceted basic life science training programme within the scope of the institution. A major aim of the programme is the education of scientists to be prepared for a future carrier in academic research, drug development and/or medicinal products regulations at universities, research institutes, medicines agencies or industry.

Each PhD student at the PEI performs a specific research project based on a defined scientific hypothesis. PEP-BIOMED provides assistance for students and supervisors to carry out successful research projects. To reach the goals of the programme, standards are set for supervisors, students and the PEI. The programme's hallmarks are as follows:

- PhD candidates are supported by a thesis committee, which (i) oversees the project plan, (ii) discusses project progress, (iii) promotes possible publications, and (iv) prepares the candidate for thesis defence
- PhD candidates, postdoctoral fellows and supervisors give oral presentations on the progress of their research projects at the Research Plenum seminars
- PhD candidates visit a triennial lecture series
- PhD candidates participate in special training courses and workshops
- supervisors arrange periodic Journal Clubs, Lab-Meetings, and One-on-One Meetings
- the programme demands and credits social activities of the PhD candidates
- successful participation of the PhD candidates is measured by the Credit Point System of PEP-BIOMED (to achieve a minimum of 300 credit points).

¹⁰ further information at: www.pei.de/pep-biomed

¹¹ i.e. recommendations of the "Commission for Professional Self-regulation in Science" of the German Research Foundation, dated 19/12/1997, further information at: www.dfg.de/aktuelles_presse/reden_stellungnahmen/download/empfehlung_wiss_praxis_0198.pdf; recommendations for postgraduate training of the German Council of Science and Humanities, dated 15/11/2002, www.wissenschaftsrat.de/texte/5459-02.pdf; objectives of the Bologna and Lisbon processes, http://www.bologna-bergen2005.no/Docs/00-Main_doc/050520_Bergen_Communique.pdf

8. Outlook - Research Infrastructure

The following central services and major equipments are available at the PEI:

- animal facility
- antibody characterization equipment
- FACS & cell sorter
- fluorescence & electron microscopy
- protein biochemistry and proteomics equipment
- real-time PCR, Northern & Southern blotting

The current list specifies major equipment and responsible contact persons capable to supervise usage. The PEI aims at developing advanced research platforms, which specifically combine the respective state-of-the-art equipment, facilities and methods to be available for all research groups and divisions. Each platform will be organized by a responsible person and will bring together all scientists having the expertise needed for the platform. Examples for research platforms to be developed at the PEI are:

- cell-sorting and FACS analysis
- protein biochemistry and proteomics including advanced mass spectrometry
- monoclonal antibody production & antibody characterization
- morphology & histology (histochemistry, fluorescence & electron microscopy)
- molecular biology (quantitative PCR methods, molecular cloning, expression systems, gene silencing and Northern & Southern blotting)
- biostatistics and biomathematics

The growing demand for the implementation of interdisciplinary and interregional research projects as well as the intended future collaboration in conjunction with PEP-BIOMED call for an extension of cooperation and the completion of Cooperation Agreements with further universities (such as the Johannes Gutenberg University Mainz, the University of Darmstadt and the Justus Liebig University Giessen).

Subscription rates for scientific journals have increased disproportionately. Changes in the copyright law have hampered access to and distribution of scientific articles. However, research projects are primarily financed by public money, and the scientists working on these projects write the respective manuscripts and conduct the necessary review processes free of charge. Hence, different international initiatives have been initiated to enable true open access to scientific publications. At the PEI, a project group has been appointed with the aim to implement an Internet-linked electronic repository for the scientific publications of PEI

scientists. For the future, the repository strategy promises less dependence from commercial publishers. The repository will also significantly improve the visibility of the PEI and its scientists.

9. Cooperation

The aim of the work of the Paul-Ehrlich-Institut is to guarantee the quality, safety and efficacy of biomedicines, according to the latest standards prevailing in science and technology, and thus to contribute to the availability of biomedicines with a positive benefit/risk assessment. To reach these goals and to perform research at the highest possible level, the Paul-Ehrlich-Institut seeks strategic partnerships with experts from federal research institutes and universities as well as other research or health organisations. Currently, the PEI is holding cooperation agreements with the Johann Wolfgang Goethe University at Frankfurt/Main and the Research Center Borstel Cooperation agreements are aimed at improving the trans- and interdisciplinary collaboration and the exploitation of synergy effects. Cooperation agreements with universities are the basis of the mutual exploitation of resources, concerted development of major research topics, organization of seminars and courses, and education of graduate students. The cooperation with clinical partners enables access to human material for studies on the quality, safety and efficacy of biomedicines. Thematically focused cooperation networks with national and international experts from different institutions are established and aim at achieving pivotal synergy effects. Under certain circumstances, cooperation with industrial partners is effective and necessary, for example if studying the standardization or the preclinical safety of biomedicines. As a federal regulatory authority the Paul-Ehrlich-Institut is bound to avoid conflict of interests, why cooperation with industry is often impossible.

10. Selected Project Cooperations of the Paul-Ehrlich-Institut

Cooperations of the PEI			
Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
Quality, Safety & Efficacy of Biomedicines (QSEB)	EDQM Project: "Recombinant major allergens (biological reference preparations & assay methods)"	Joint research activity to develop new allergen standards and assays on the basis of purified recombinant allergens	EDQM, Academic Medical Center Amsterdam, University of Salzburg, ISS Rome, Allergopharma, ALK-Abello, & Stallergenes
QSEB	German Research Foundation Transregio Project: "Xenotransplantation of tissues such as pancreatic islets and organs such as hearts and kidneys"	Tackle the acute vascular and the cellular rejection response to xenotransplants using genetically modified pigs (transgenic and/or knock-out) that do not transmit porcine pathogens to the recipient. Cooperation enables the PEI access to animal material	University of Bonn, Ludwig-Maximilian University Munich, Hannover Medical School, Friedrich-Loeffler-Institut Institute of Farm Animal Genetics (Neustadt), Life Sciences Centre (Weihenstephan), Robert Koch-Institut, & Diabetes Institute for Immunology and Transplantation (University of Minnesota, USA)
QSEB	Development of serological assays for potency testing of diphtheria, tetanus and pertussis vaccines	Collaborative study among European Authorities	European Directorate for Quality of Medicines Straßburg & numerous European Official Member States Control Authorities

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
QSEB	Evaluation of nuclear magnetic resonance spectrometry as a tool for the batch consistency control of polysaccharide vaccines	Multistep collaborative study (PEI project leader)	European Directorate for Quality of Medicines Straßburg, University of Würzburg, National Institute for Biological Standards and Controls (London, U.K), & Medical Products Agency (Uppsala, Sweden)
QSEB	Development of WHO bacteria references for cellular blood components and cell-based medicinal products	International cooperative study with clinical, governmental and industrial partners	Coordinating group: National Blood Service (UK), Australian Red Cross Blood Service, WHO, American Type Culture Collection (USA), & Sanquin Blood Supply Foundation (The Netherlands) Worldwide partners from universities, industry, blood centres, & regulatory authorities
QSEB	In-vivo calibration of the monocyte activation test	Clinical study	University Hospital Essen
QSEB	Detection of non-endotoxin pyrogens by the monocyte activation test	Cooperative study with industrial partners	Members of the German Association of Research-Based Pharmaceutical Companies such as Roche & Sanofi-Aventis

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
QSEB	Physical stability of human parvovirus B19 genotype 3	Cooperative study with an industrial partner: the PEI performs experiments to characterise the physical stability of a recently discovered virus genotype	Talecris, USA
QSEB	Evaluation of a genotype panel for parvovirus B19	Cooperation to evaluate different parvovirus B19 genotypes in comparison to the WHO International Standard	34 laboratories worldwide (clinical, qc, and blood/plasma testing laboratories, & kit manufacturers)
QSEB	Design and Production of a WHO Reference Panel covering all HBV-genotypes	Worldwide WHO Collaborative studies to evaluate different HBV-genotypes with respect to HBV-DNA and HBsAg	10 laboratories worldwide for sourcing materials; 30 laboratories worldwide for the evaluation
QSEB	Design and Production of the 1st International Standard for HDV-RNA	Collaborative study evaluating different HDV candidate materials	Hepatology Institute in Ankara University for sourcing materials; 30 laboratories worldwide for the evaluation
QSEB	EU-Project “Biological agents: strengthening the adequate response to deliberate releases by the establishment of a framework European-wide (BIOSAFE)	Cooperation between federal institutions: the PEI contributes information on poxviruses, influenza viruses and henipaviruses. The other partners work with other agents	3 partners across Europe
QSEB	EDQM-Project: Collaborative study for the validation of a serological potency assay for inactivated rabies vaccines for veterinary	To further validate and prove the transferability of an alternative test method	EDQM & several European OMCLs and MAHs

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
QSEB	Quantitation of AEV-antigen in avian vaccines; validation of a PCR method for the testing of extraneous agents	Cooperative study were the different partners work together in virus purification, production of antibodies, ELISA validation studies, the development of specific and sensitive PCR protocols	Lohmann Animal Health (Cuxhaven) & Institute of Virology and Immunoprophylaxis (Switzerland), EDQM
QSEB	Humanized mice	Establishment and analysis of different mouse models for their use as human equivalent models for preclinical testing of biomedicines and novel therapy concepts	University of Mainz
Experimental Vaccines, Therapies & Diagnostics (EVTD)	German Research Foundation: "Recombinant modified vaccinia virus Ankara and heat-killed listeria as novel vaccines for the prevention of allergies"	Cooperation on mouse models of food allergy -clinical and immunological readout	Charite Hospital Berlin
EVTD	EU Integrated project: "The prevalence, costs & basis of food allergy across Europe (EuroPrevall)"	Cooperation between clinical and molecular allergists. Role of PEI: management of serum bank, contribution to unique food allergen library, development of novel diagnostics	> 60 partners from across and outside the EU including major allergy hospitals (University of Zurich, Lodz, Madrid, Strasbourg, Utrecht, Vilnius, Sofia, Prague, Rejkjavik and other), & molecular allergists (e.g. Medical University of Vienna, Phadia AB)

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
EVTD	Clinical network to support research in molecular allergology	Acquire biological samples from subjects with confirmed inhalant or food allergy to evaluate new concepts of molecular diagnosis and experimental therapies	University Hospitals of Zurich, Mainz, Erlangen, Berlin, Leipzig, Milan (IT), Hospital in Castellon (ES), & Sagamihara (JP)
EVTD	Network of expert laboratories to support research in molecular allergology	Develop synergisms regarding the availability of allergens and methods for allergen characterisation including experimental approaches currently not available at PEI	University of Salzburg (physico-chemical characterisation), Medicinal University of Vienna and Swiss Institute for Allergy and Asthma Research (human T cell assays and exchange of allergens), Phadia AB and VBC Genomics (high throughput diagnostic testing), University of Erlangen (plant biotechnology), & University of Gießen (phytopathology)

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
EVTD	EU-Network of Excellence: "Safety and efficacy of viral vector mediated gene therapy (Clinigene)"	Access to patient material, establishing standards for quality and preclinical aspects of gene therapy products, on site exchange with manufacturers, clinical trial sponsors, ethical experts	29 European partners from academia, patient and ethical organisations, & industry including the Helmholtz Zentrum für Infektionsforschung (Braunschweig), the Royal Holloway University of London, the Ecole Normale Supérieure de Cachan (Paris), the Instituto de Biologia Experimental e Tecnologica (Lisbon), Genethon (Paris), & BioReliance (Glasgow)
EVTD	Priority programme funded by the German Research Foundation: "Mechanisms of gene vector entry and persistence"	To address mechanisms of viral vector cell entry, gene insertion into the cellular genome and insertional oncogenesis. The PEI investigates B cell targeted lentiviral vectors and the mechanisms of gene transfer into resting cells of the haematopoietic system	28 German partners from academia including the Hannover Medical School, the DKFZ, the Max-Delbrück-Zentrum (Berlin), the University of Heidelberg, the Medical School of Cologne, & the Georg-Speyer-Haus (Frankfurt)
EVTD	Assessment of lentiviral vectors for medical applications targeting the central nervous system	Strategic cooperation with experts in neurobiology to extend expertise in neuronal cell and brain tissue cultivation as well as animal handling	Partners from academia including the Veterinärmedizinische Universität Wien, the University of Heidelberg, & the Max-Planck-Institut for Brain Research

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
EVTD	Integrated EU-Project: "Persisting transgenesis (PERSIST)"	World-leading experts on lentiviral vectors cooperate to assessing and improve gene transfer vector systems with respect to safety and efficiency in. The PEI contributes by its expertise in engineering the surface of viral vectors	> 20 European partners from academia and industry including the University San Raffaele in Milano (coordinator), the INSERM (Lyon), the Hannover Medical School, the Erasmus University Medical Centre (Netherlands), the ETH Zurich, & EUFETS AG (Idar-Oberstein)
EVTD	BMBF funded collaborative project: "Oncolytic measles viruses for the treatment of hepatocellular cancer"	Access to biopsy material from cancer patients, exchange on this novel type of therapeutic approach, establishing preclinical standards in cooperation with clinical experts	University Hospital of Tübingen
EVTD	Cooperation within the graduate study program "GK1172 Biologicals"	Integrated education of PhD students focussing on biomedical products. Students from the PEI participate in the programme and receive advice from internal and external experts regarding their PhD project	> 20 partners from various institutes of the Goethe-University Frankfurt & the Georg-Speyer-Haus

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
EVTD	Immediate Research Program Influenza of the Federal Government: "Tenacity of influenza viruses (human and animal pathogens)"	Cooperation between federal research institutions: the Friedrich-Loeffler-Institut investigates avian influenza; the Robert-Koch-Institut develops DNA/vector vaccines that can be combined with MVA vaccines developed by the PEI	2 German federal research institutes
EVTD	BMBF funded collaborative project: "Comparing analysis of the highly attenuated vaccinia virus MVA and the conventional vaccinia virus Elstree in order to examine the suitability of MVA as 3 rd generation vaccine against smallpox"	Cooperation with a partner that is specialised in examining conventional smallpox vaccine, whereas the PEI is specialised in examining MVA vaccines	Israel Institute of Biological Research (IIBR)
EVTD	EU-Project "A combined pox-virus/lentiviral vector system to treat HIV infection. Immunization and direct in-vivo gene transfer in T lymphocytes (POX-GENE)"	The PEI oversees the vector constructions; other partners establish models for in-vitro and in-vivo testing	4 European partners from universities, federal institutions, & non-government organisations
EVTD	EU-Project: "Optimised and novel oncolytic adenoviruses and pox viruses in the treatment of cancer: Virotherapy combined with molecular chemotherapy (THERADPOX)"	The PEI task contributes to the development of poxvirus-based oncolytic viruses; other partners develop adenovirus vectors	9 European partners from universities, federal institutions, & private companies

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
EVTD	Project funded by the German José Carreras Leukemia-Foundation: „Modified Vaccinia Ankara in adoptive immunotherapy of cytomegalovirus infection”	The PEI designs vector vaccines that are then tested by the clinical partner	Julius-Maximilians University Würzburg
EVTD	BMG funded consortium on HIV resistance monitoring	The task of the PEI task is the longitudinal analysis of the HIV co-receptor usage of HIV strains, derived from patients. The other partners determine HIV resistances	4 partners in Germany
Host Interactions with Pathogens & Retroelements (HIPR)	Structural & functional characterization of the composite primate-specific SVA retrotransposon	Collaboration of molecular biologists & population geneticists to elucidate the current activity & mutagenic potential of SVA retrotransposons	Louisiana State University (Baton Rouge, LA, USA)& University of Klausenburg (Romania)
HIPR	Assessment of LINE1-mediated retrotransposition activity & its impact on the genomic stability of embryonic stem (ES) cells as well as iPS cells	Access to a variety of ES & iPS cell lines from primates & humans as well as to progeny cells of defined differentiation stages; to establish a transgenic mouse model for the evaluation of retrotransposition activity during embryonic development & in ES cells in-vivo; to identify LINE1-encoded protein machinery in tissues and cell types during early embryonic development.	Hannover Medical School, REBIRTH-Centre for Regenerative Medicine (Hannover), TWINCORE-Centre for Experimental and Clinical Infection Research (Hannover), & Universitätsklinikum Essen

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
HIPR	Host-encoded factors regulating LINE1-mediated retrotransposition in human cells	Network to generate a variety of gene -, mutant- & shRNA-expression constructs specific for <i>Double Strand-Break Repair</i> factors, p53 and APOBEC3 proteins with the aim to determine their function in LINE1-mediated retrotransposition	Heinrich-Pette Institute for Experimental Virology and Immunology (Hamburg), Heinrich-Heine-University (Düsseldorf), Johns-Hopkins-University (Baltimore, MD, USA), & Max-Delbrück-Center (Berlin)
HIPR	Studying virus evolution with bioinformatics approaches	Exchange of expertise in modelling, phylogenetic inference and data analysis	Genomics and Health Advanced Centre for Research in Public Health (Conselleria de Sanitat, Generalitat de Valencia)
HIPR	BMBF-Project: "Influenza-Fluresearchnet: genetic susceptibility of the host towards influenza infections – examination of low pathogenic avian influenza viruses"	Cooperation inter alia with the Helmholtz Centre for Infection Research (HZI), which examines the genetic basis of the susceptibility of various mouse strains, whereas the PEI examines the virulence features of influenza viruses	11 German partners from universities, clinics, federal research institutions & private companies
HIPR	In-silico analysis of <i>Helicobacter pylori</i> proteins	Joint Research Activity and exchange of expertise	JWG Universität Frankfurt am Main
HIPR	Signal transduction pathways in <i>Helicobacter pylori</i> infected cells	Joint Research Activity and exchange of expertise	University College (Dublin, Ireland)

Cooperations of the PEI

Research Area	Experimental Topic	Concept of Cooperation	Partners of the PEI
HIPR	Transgenic mice	Development of novel transgenic mouse models and exchange of transgenic mice to study specific T cell responses and cellular pathways of immune activation	> 10 partners including the Veterinärmedizinische Universität Wien, University of Zurich, Osaka University, & Helmholtz Zentrum für Infektionsforschung
Immune Activation & Evasion (IAE)	EU-Project: "Host immune activation optimised vaccinia virus vectors for vaccine development", (MVECTOR)	The cooperation aims at characterising and possibly improving MVA vaccines. The partners study complementary approaches	3 European partners from university & federal research institutions
IAE	Role of type I interferons	Cooperation to assess the role of type I interferons in viral infections, tumorigenesis, and immune modulation	University Hospital of Freiburg, Centro de Biología Molecular Severo Ochoa Madrid, Washington University (St. Louis, USA), & University of Oxford (UK)