TRANSVAC FINAL REPORT

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1 Final publishable summary report

1.1 Executive summary

TRANSVAC is a collaborative infrastructure FP7 project coordinated by Dr Odile Leroy at European Vaccine Initiative. During the last four years of operations, TRANSVAC has demonstrated the feasibility of integrating vaccine research and development activities in Europe. Vaccine development platforms offering services to excellent public and private European research groups are established.

The project aims at accelerating the development of promising European vaccine candidates by bridging the gap between academic research and clinical trials. Together with its 13 initial partners, and its 4 affiliated partners, TRANSVAC addresses the fragmentation of expertise and facilities in vaccine research within three activities: 1) research by improving the use of assays, adjuvants, animal models, standardised reagents, microarrays and protein expression in relation to the development of experimental vaccines, 2) networking by providing training in vaccine development, harmonising assays, and harmonising microarrays, and 3) services by providing researchers with free access to: adjuvant formulation, animal models, microarray analyses, and assays/standards.

Free access to all TRANSVAC services including adjuvants, animal models, reference reagents, and global analysis platforms have been provided to **29 high potential vaccine projects** , selected through a two-step peer review process, first on scientific excellence, and then on feasibility and impact. Since July 2012, TRANSVAC is also providing five additional infrastructures that may be accessed on a paid basis. A complete list of the TRANSVAC services is available at www.transvac.org.

To ensure that the services delivered by the partners are applying the state of the art in the field and the most innovative technologies or methodologies, TRANSVAC included significant research and networking components which support the development of new services or the improvement of existing services.

TRANSVAC activities led to the rationale development of novel vaccine formulations, reference standards, as well as new vaccine candidates (included the compilation of an Investigational Medicinal Product Dossier (IMPD) for the clinical trial application for two vaccine formulations). To ensure the quality of assays to address vaccine specifications, 13 characterised malaria and tuberculosis antigens are available to external users. TRANSVAC is providing cell bank of five new cell lines developed for improved growth of adenoviral vectors. Evaluation of vaccine candidates in different animal models (mice, humanised (HIS) mice, guinea pigs, pigs and non-human primates) combined with global analyses enabled further understanding of the mechanisms of action and harmonisation and standardisation of assays have provided critical data for decision making of choice of formulation, route of administration, vaccination dosages and schedule. University of Lausanne is providing users with formulation studies, i.e. combination of one antigen with one adjuvant. Up to date 47 formulation studies have been delivered through TRANSVAC. SOPs for immuno-assays for clinical trials are available and validated.

Research for biomarkers includes an intensive comparative analysis of the Affymetrix and Agilent microarray data as well as Illumina RNA sequencing data of a BCG vaccination Clinical Trial (CT) phase Ia study, harmonisation of Standard Operating Procedures (SOPs) for sample, microarray and data analyses, as well as transcriptome mapping of samples from the HIV CT in collaboration with the EuroNeut-41 project.

TRANSVAC organised two modular courses in vaccine development, each attended by 15 selected external participants. Ten workshops have also been held on subjects as diverse as statistical analyses, animal models, global analyses, and discussion to strengthen collaborations between European vaccine Research and Development (R&D) groups, assay developers, and vaccine producers.

TRANSVAC experience has confirmed high need for a sustainable infrastructure for vaccine R&D. Representatives of vaccine manufacturers, biotech companies, academic research, regulatory authorities, policy makers, and funding agencies have developed a roadmap analysing needs and gaps in European vaccine R&D infrastructure.

1.2 A summary description of project context and objectives

Vaccination is generally accepted as the most cost-effective intervention in controlling infectious diseases caused by viruses, bacteria and parasites. Immunisation with efficacious vaccines offers not only protection against disease for the individual but also, if immunisation programmes are effective, herd immunity which is of paramount public health importance for society. It has, many times, been demonstrated that the gains in terms of economic growth following successful immunisation programmes far exceeds society's investments. This achievement holds in affluent as well as in resource-constrained parts of the world.

Immunisation against infectious diseases is based on the dogma that when an individual is infected with a pathogen, and survives, then immunological memory can provide this individual with immunity against a subsequent challenge with the same pathogen. New vaccines are being developed against infectious diseases of major public health importance and almost every vaccine in development is facing the same kind of challenges and infrastructure shortcomings. At the research level, scientists are distinctly aware that a rational, evidence-based approach is required in order to achieve the goal of licensing any highly efficacious vaccine. However, it is a common occurrence that for most of the vaccines currently in development there are still essential, critical gaps in knowledge that are required to support evidence-based decision-making e.g. an incomplete understanding of the mechanisms of infection and disease or an incomplete understanding of mechanisms of immunity. Along the development pathway, a systematic, evidence-based approach for prioritising vaccine candidates will expedite the progression of promising vaccine candidates and promote greater confidence among scientists and funders that investments are focused on the best candidates.

Scientists strengths relating to basic science and preclinical expertise, is very often not extended into other fields such as chemistry, manufacturing, and regulatory control issues. As an example, academic researchers very often do not have access to the protein characterisation, formulation, and process development capabilities necessary to assess whether formulations can be economically manufactured in a good manufacturing practice environment. Furthermore, no single partner currently involved in European academic vaccine development has the required capacity to manufacture and formulate the wide range of vaccine candidates being developed. Establishing an accessible process development centre or a collaboration of research organisations that could be managed as a single entity would address this challenge.[1]

This approach would most likely represent an infrastructure of academic, commercial or contractual, and government institutions. The infrastructure should support rapid sharing of information, help define scope and objectives for any formulation or process development project pursued, and advise on integrating process development and formulation with the larger vaccine development process. Appropriate governance and transparency will be required for this approach to gain widespread acceptance among scientists and funding agencies.

These challenges are well recognized and experienced by all vaccine development groups irrespective of which pathogen they are dealing with, and can broadly be grouped as follows:

1) Insufficient numbers of trained scientists able to lead vaccine development.
2) Lack of access to technology platforms e.g. synthetic peptides, recombinant proteins, vectored delivery systems, virus-like particles, GMP development.
3) Difficulties with and lack of access to adjuvants and immune modulators.
4) Lack access to certain know-how and facilities, such as lyophilisation and formulation.
5) Inadequacy in quality and regulatory compliance assessment.

Unarguably, the fragmentation of expertise and facilities has slowed down, and in some instances distinctly impeded, development and subsequent validation of promising vaccine candidates.

Although a knowledge base already exists within Europe spanning different disease types, there is currently very limited horizontal coordination between vaccine R&D groups working on different diseases. Formation of cooperation between leading experts in each field associated with vaccine development would bring

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[1] Global HIV Vaccine Entreprise, Product Development and Manufacturing, Scientific Strategic Plan. http://www.hivvaccineenterprise.org/plan/3.aspx
Malaria Vaccine Technology Roadmap, Priorities, p.4.
http://www.malariavaccineroadmap.net/pdfs/Malaria_Vaccine_TRM_Final.pdf

innovative and harmonised approaches enabling the efficient development of efficacious and affordable vaccines for Europe and the rest of the world.

A. Rationale

To address these challenges, the European vaccine community needs to establish a collaborative infrastructure based on shared vision and goals.

The European Vaccine Initiative (EVI) and the Tuberculosis Vaccine Initiative (TBVI) have each demonstrated significant success in creating the essential know-how and infrastructures required to enable the development, production and initial clinical assessment of new candidate vaccines. In order to exploit the momentum and significant scientific, as well as managerial, expertise generated by EVI and TBVI to enhance and strengthen European vaccine development, these successes must be synergised and used to significantly improve European vaccine development. A consortium, managed by EVI, in which all stake holders commit to a common goal, i.e. to share research infrastructures and know-how on vaccine development will significantly boost European efforts in vaccine development. Thus, the consortium will comprise of major European stakeholders, considered to be leaders in their own fields of expertise. Each stakeholder will significantly enhance the value of what EVI and TBVI have already established by committing knowledge, resources and expertise to a collective long-term strategy.

This consortium will be the European driving force for a new era in the rapid development of accessible and efficacious vaccines, and membership will be open to all interested parties who are capable of contributing to key elements of the strategy in a synergistic fashion.

B. Overall goal and scientific and technological objectives

The overall objective is to accelerate the pharmaceutical and clinical development of promising vaccine candidates for public health use. This will be achieved by bridging the gap between academic research and early phase clinical development through carefully managing the advancement of promising vaccine candidates from preclinical animal experiments to proof-of-principle studies in humans.

The specific objectives are the creation of an enabling environment for:

1) Unrestricted exchange of experience, expertise and know-how between partners
    a. By setting up a training course on know-how for vaccine process development, expecting a total of 30 trainees during the duration of the project,
    b. By allowing researchers to access upstream knowledge on vaccine development good practices, and giving them access to technology platforms, expecting that a minimum of five candidate vaccines will benefit from optimisation of process development, validation of quality control assays, assessment of formulations, and rationale decision process.
2) Enhanced communication with other related projects through networking
    a. By setting up web-based working spaces,
    b. By implementing a communication strategy increasing the awareness of the worldwide community of vaccinologists on this European infrastructure as provider of client solution driven services, expecting to increase the rate of services over 10% for the whole duration of the project,
    c. By setting up “working groups” with representatives of the main European Institutions involved in vaccine development, of the European Vaccine Manufacturers Association, and of policy makers.
3) Transnational access to physical infrastructures and services owned by the project partners
    a. By delivering a minimum of 20 formulations of vaccines to be assessed pre-clinically,
    b. By delivering a minimum of three cell-lines and four purified proteins/antigens with the quality control procedures in order to facilitate the technology transfer to GMP manufacturers,
    c. By delivering pre-clinical testing in various animal species for a minimum of ten candidate vaccines for the whole duration of the project,
    d. By the development of pathogen specific reagents/standards for the three targeted diseases, tuberculosis, HIV, and malaria.
4) Joint execution of research and development activities to enhance and improve current infrastructures provided by the project by developing trans-diseases research programmes, in order to optimise the non-

disease specific knowledge, such as

a. Immuno-assays for pre-clinical assessment or quality control assessment, or

b. Analysis or physical-chemical characteristics of antigen/adjuvant formulations, in a regulatory perspective,

c. Proteomic/transcriptomic analysis.

5) Coordination of European vaccine research in synergy with on going global co-ordination efforts, by setting up accompanying measures for the creation of the European Vaccine Development Agency, which is expected to be set-up at the end of the current project, if the political support from European Member States and European Commission materialises.

TRANSVAC addresses the challenges mentioned above and manages solutions. TRANSVAC unites several groups and individuals to form a manageable single infrastructure capable of providing state of the art vaccine development facilities and expertise.

In meeting its objectives the project provides:

- Access to all the infrastructures owned by the network partners to internal and external organisations.
- User-friendly presentation of the different infrastructures as well as services available within the project via an electronic gateway.
- Reinforced coordination and interaction between the different facilities in the network by an Internet-based collaborative platform.
- Harmonised protocols and methodologies within the infrastructure.
- Jointly developed novel adjuvant formulations, protein production know-how, molecular tools, vaccine testing models, and functional assays.
- Upgraded infrastructures and services.
- A common vision for the future of vaccine development in terms of scientific and technology orientation.

In summary, TRANSVAC brings together Europe's leading vaccine researchers and pan-Union initiatives in an unprecedented collaboration to drive the pharmaceutical, clinical and regulatory development of efficacious, safe and affordable vaccines.

1.3 A description of the main S&T results/foregrounds

C. RTD activities

WP1: Adjuvant and vaccine formulation platform

The (prototype) antigens AMA1 and Antigen 85A (Ag85A) were selected from the EVI and TBVI portfolio's. AMA1 is available as a GMP product and Ag85A is available at GLP-quality with QC standards applied.
Three (prototype) adjuvants were selected for further investigation. Alhydrogel was selected as a reference adjuvant. Two promising novel adjuvants were selected for further evaluation: 1. A squalene in water emulsion (SWE), which is an oil in water emulsion similar to what is used for pandemic influenza vaccines. 2. A liposome-QS21 (Lip-QS21) formulation which shows some similarity with immune stimulating complexes (ISCOM). Thus 6 prototype vaccines are available for adjuvant benchmarking studies.

SOP's were drafted for the six planned formulations and stability data were generated for all formulations. The results from the stability studies confirmed that stable formulations can be prepared. Both AMA1 and Ag85A adsorbed to Alhydrogel to a satisfactory level (>95%), and the antigens remained stable when formulated with SWE or Lip-QS21. Further characterisation of the formulations revealed no abnormalities. The following characterisation tests were performed: Antigen adsorption (for Alum only), Particle size for SWE and Lip-QS21. Antigen characterisation (identity, integrity and stability), pH measurements were performed for all six formulations. The biochemical and biophysical characterisation has just been completed and the SOP's are available.
For selection of preferred formulations based on preliminary immunogenicity studies, TRANSVAC interfaced with Pharvat. Within the context of Pharvat (Platform for the Harmonisation of Vaccine Adjuvant Testing) a method has been proposed to harmonise adjuvant comparisons (mouse strain, immunisation schedule and route of immunisation). Using this method an experiment was performed in mice with the six prototype vaccine formulations.
Potency assays were developed for AMA1 formulated with a number of adjuvants. Potency assays are part of the antigen stability assessment and measure the capacity of the antigen to exert a biological effect, which in the case of AMA1 is an antibody response. A potency assay is now available for AMA1 formulations, and the potency assay set up can be adapted for other vaccine candidates.
MTA's for individual components in six prototype vaccines have been negotiated. The antigens are available from Lionex and BPRC and the adjuvants used can be obtained directly from VFL, UNIL.
The magnitude of immune responses following vaccination with six reference vaccines is described and correlations between T and B cell responses have been established. The antibody response induced by AMA1 formulations was shown to be functional (i.e. able to inhibit the in vitro growth of malaria parasites).
An Investigational Medicinal Product Dossier (IMPD) was compiled for AMA1-DiCo formulated with either Alhydrogel or SE-GLA. This dossier contains general information about the disease and vaccine products and it provides extensive information concerning the manufacturing process, the pharmaceutical development and product specifications of the Drug Substance and Drug Product. The dossier also includes a number of pre-clinical studies, these are Pharmacotoxicology as well as potency studies employing the potency assays. Product stability studies have shown that the lyophilised AMA1-DiCo vaccine candidate is stable.
Contractual agreements between BPRC, EVI, Inserm (Phase Ia) and CNRFP (Phase Ib) are now in place thus enabling the staggered Phase Ia / Ib clinical trial. Inserm will act as sponsor for the clinical trial. The CTP and IMPD have been submitted to the French regulatory authorities and final approval is currently being awaited. In the event the Phase I trial results show a good safety profile and good induction of parasite growth inhibitory antibodies to a variety of P. falciparum strains the product will progress towards Phase IIb studies as outlined in the clinical development plan. The agreements described facilitate the long-term development of 2 final AMA1-DiCo vaccine formulations.

The UOXF partner has made significant progress in the evaluation of adjuvants and vectors for prime-boost immunisation. A large series of adjuvants have been compared in mice both with ovalbumin and malaria blood-stage antigens as proteins (PPP regimes, i.e. three immunisations) and particularly promising adjuvants for antibody and T cell induction identified. These include CoVaccine HT and Matrix M and also some GLA plus stable emulsion formulations.
Following this the effect of adenoviral vectors priming before protein-adjuvant boosting was assessed (AP regimes, i.e. a single adenovirus followed by a single protein in adjuvant boost). These regimes were also strikingly immunogenic. However, an important novel finding was that the viral vector prime diminished the importance of a strong adjuvant for use with the protein boost. For example the widely used alum adjuvant could be used to achieve strong antibody responses after the adenoviral prime, even though alum in PPP regimes showed very modest antibody immunogenicity.
In an extension of this work in non-human primates adenoviral priming and boosting with the malarial protein AMA1 in adjuvant showed very impressive antibody immunogenicity, again using just a single adenoviral prime and a protein-adjuvant boost. In this study both alum and CoVaccine HT adjuvants were evaluated and a simian adenoviral vectors was found to be a suitable priming agent. This macaque study has led to plans for a clinical trial of vector prime-protein-boost regimes with P. falciparum AMA1.

WP2: Vaccine Preclinical Testing Platform

Studies performed in mice
Different validation systems for vaccine and anti-infective candidates based on strong readout systems for preclinical studies using conventional and advanced animal models were established. Animal models based on conventional mice have been developed in order to (i) characterise strength and functionality of vaccine adjuvant candidates and (ii) understand the role of different immune cells in elicitation of efficient immune responses. Furthermore, challenge models based on conventional mice have been established to investigate the efficacy of new vaccination strategies. Animal models of humanised mice have been developed and further optimised, i.e. generation of lymph nodes, increased cellular responses, increased reconstitution levels of uPA mice (HuHep model) with human hepatocytes and based on that efficient infection of HuHep mice with HBV and HCV. It is expected, that the obtained achievements will lead to increased predictability of vaccine and anti-infective candidate efficacy in the human system.
In depth investigations of host immune responses stimulated by different immune interventions in order to understand in more detail pathogen-host interactions and subsequently develop new vaccination strategies were performed. Different immunomodulators which allow evaluation of the impact of diverse immune cell subsets on achieving efficient immunity were identified and characterised in mice. For example, the role of various immune cells bridging the innate and adaptive immune systems and characterised in detail antigen-specific cell subsets by multi-parametric flow cytometric (FACS) analysis were identified. To this extent, multi-parametric FACS readout systems have been developed using 13-16 colour panels which allow differentiating and identifying T cell subpopulations by a unique combination of surface markers. Furthermore, the adjuvant candidates not only can be used to improve antigen-specific immune responses but also to modulate the stimulated immune responses for specific needs. Thus, partner 3 is now equipped with a pool of adjuvants to which vaccine developers can have access.

Studies performed in pigs
Guinea pigs are not only used as important models to evaluate vaccines against TB, but are also as advanced models for e.g. influenza research. Research is hampered by lack of validated reagents. In TRANSVAC project, more data on the expanded guinea pig microarray were obtained and several new methods have been developed for evaluation of immune responses in guinea pigs. Data on these new methods are currently further evaluated in projects outside the TRANSVAC consortium.
Pigs have been used in various biomedical research areas, but are now also more and more recognised as important models in infectious disease research. In the TRANSVAC consortium, partner 6 was responsible for the evaluation of immune responses after vaccination in neonatal and adolescent/adult pigs. Especially the possibility to study immune responses in neonatal and young animals with a sufficient window of opportunity with respect to the maturation of the immune

system makes pigs an important model to study immune responses to various infectious diseases and to vaccine candidates. During the project, partner 6 has been able to develop and validate various tools to enable the evaluation of the immune responses in neonatal, young and adult pigs. Most tools tested can now be used, but some were shown not to work well in pigs and need further optimisation. The developed techniques were used in further infectious disease research e.g influenza infections in young and adolescent pigs were studied. It was demonstrated that the course of disease and the pathology reflects the human situation and that a clear difference can be found between the age groups. Furthermore, several adjuvants for new influenza vaccines were tested in the pig. This makes the pig model an additional and relevant model for immunogenicity and efficacy studies.

Studies performed in non-human primates

In biomedical preclinical research, NHP as closest relatives to man comprise valuable tools with an anticipated high predictive validity for human responses to new therapies. Old-world monkey species and in particular rhesus macaques (Macaca mulatta) and cynomolgus macaques (Macaca fascicularis) are the most widely used NHP species for such biomedical research, including infectious disease studies and vaccine research and development. While primate species and in particular NHP rhesus and cynomolgus macaques can display different biological responses to for example infectious challenges with HIV, M.tuberculosis or malaria parasites, their innate immunological response patterns grossly appear similar. For the innate immune response, only minor differences were found between rhesus and cynos. A most remarkable observation is made with regard to the – unexpected – expression of IL12 in activated pDC in rhesus and in cynomolgus monkeys, where expression of this typical type-1 cytokine is not stimulated in human pDC. Apart from minor deviations, the general picture from comparing the capacity of cytokine and chemokine release from T lymphocytes from M.fascicularis versus M.mulatta PBMC indicates a (significantly) higher response level in cynos. At the same time, response levels appear higher in Chinese over Indian rhesus macaques, both by specific T cell stimulation via the T cell receptor as by non-specific T cell stimulation to address the potency to release cytokine/chemokine soluble factors using PMA/i. Furthermore, rhesus macaques of a Chinese breeding origin versus rhesus macaques of an Indian breeding origin, do display differential responses to live M.bovis BCG vaccination and subsequent M.tuberculosis strain Erdman infection.

WP3: Global Analyses Platform

SNP analysis in Indian and Chinese macaques and cynomolgus monkeys

Whole transcriptome SNP identification of Indian and Chinese macaques and cynomolgus monkeys was performed. Each individual sample was sequenced using Next Generation Sequencing (NGS) technology, Illumina Genetic Analyser (Illumina platform at the HZI). Species-specific identification of SNPs was done. Mapping of the raw Illumina data showed that only a fraction of the genome (0.09-11% of the chromosome) was covered by SNP analysis. This was expected as less than 5% of the primate genome codes for transcripts. 14,000-16,000 out of 30,000 transcripts could be detected by Illumina sequencing out of which coverage higher than 5 was used for SNP detection. There were 7,436 SNPs which were present only in all cynomolgus monkeys but not present in Indian and Chinese macaques. 731 of these mutations lead to a change in amino acid in the protein sequence. It was further observed that there were at least 2,569 SNPs that distinguish Indian and Chinese macaques from cynomolgus monkeys.

Transcriptome analysis of samples from an HIV clinical trial

The EC FP7-funded EuroNeut-41 project aims at developing new vaccines able to elicit neutralising antibodies that block entry of HIV into cells, at mucosal sites and in blood. These vaccine candidates are derived from the envelope of HIV. This envelope mediates viral entry and represents the only viral protein that is targeted by neutralising antibodies. The gp41 trans-membrane region plays a key role in virus entry and displays regions that are well preserved among sub-types. It is expected that antibodies directed against this protein will provide sterilising immunity against a broad range of viruses. The EuroNeut-41 project has received the ethical approval to use these samples and patient informed consent documents are available. An MTA has been signed between EuroNeut-41 and LIONEX so that LIONEX can also use the samples.

The primary objectives were to describe the safety of three EN41-FPA2 priming immunisations by nasal route followed by two EN41-FPA2 booster immunisations by intramuscular route up to 28 days after the final immunisation and to assess EN41-FPA-2 specific serum IgG responses by ELISA assay induced by the vaccine candidate up to 28 days after the final immunisation. Another important goal is to define genomic markers and study vaccine efficacy through the pathway study of regulated gene set which could facilitate the introduction of this vaccine into the clinic. Furthermore, gene expression study of clinical trial may pinpointing the potential role of mucosal priming as well as determine the specific B cell response of three EN41-FPA2 priming immunisations by nasal route followed by two EN41-FPA2 booster immunisations by intramuscular route at any time from first dose until last visit.
LIONEX performed Whole Transcriptome mapping of the blinded samples received from Surrey. We embarked on an exciting project and analysed all transcriptome data on the 166 samples successfully. Depending on the information available till now (individual code and visits number only) in this clinical trial, the Partek software (PGS) analysis results showed differences when comparing different visit groups. Results are presented as a list of genes ranked by significance level, and attention is focused on the top differentially expressed genes. Furthermore, pathway analysis and GO analysis were applied on Gene-expression profile. The next step will be to reanalyse and/or reinterpret the results once the decoding of samples has taken place.

Transcriptome analysis of samples from a BCG vaccination study in humans
In addition, analysis was also conducted on human CT RNA samples (provided by MPIIB), which were isolated from European (Caucasian) males, 25-35 years old, Bacillus Calmette-Guérin (BCG) vaccination study participants. For the analysis eight individuals, 4 reacting positively to purified protein derivative (PPD) and 4 individuals who did not react to PPD were used at four different time points vs. prior to immunisation, 29 days after immunisation, 57 days after immunisation and 180 days after immunisation. Cross comparisons of two groups (PPD negative and PPD positive) and different time points (naive, 29 days post vaccination, 57 days post vaccination and 180 days post vaccination) were done. From the expression analysis a clear distinction could be made between post-vaccinated PPD positive and PPD negative cohorts (Table 1.3.1).

Table 1.3.1: Number of genes differentially regulated in PPD negative and PPD positive groups.

Time Interval	PPD negative			PPD positive		
	Up	Down	Total	Up	Down	Total
Day 29	1	5	6	0	0	0
Day 29 & Day 57	0	0	0	0	1	1
Day 57	25	9	34	40	7	47
Day 180	5	3	8	267	6	273
Day 57 & Day 180	4	0	4	16	0	16

Transcriptome analysis of samples from a BCG vaccination study in non-human primates
Post-vaccination whole blood RNA samples (PaxGENE) relevant for transcriptome analysis were collected at BPRC along a BCG vaccination study in NHP rhesus spectrotypes of Indian and Chinese origin. The data analysis of this non-human primate M.bovis BCG vaccination study was completed and the results were verified for biological significance using gene ontology (GO) enrichments analysis. MPIIB identified clear differences of the two macaque spectrotypes and important biological processes as well as molecular functions of vaccine induced immunity in this pre-clinical study with high relevance to human vaccine testing. In adjunct to this analysis MPIIB compared two different CT studies of BCG vaccination done in Germany and South-Africa by VPM. In conclusion clear patterns of biomarkers were identified with high impact on vaccine development, but also crucial factors and basic recommendations for global transcriptomics measurements in vaccine testing.

Inter-platform comparison

Cross-platform utilisation of gene expression data may reduce the need for duplicate experiments and facilitate more extensive exchange of data within the research community. This requires a robust measure of comparability of results from different gene expression platforms. TRANSVAC WP3 applied three major microarray platforms (Affymetrix, Agilent and Illumina) in conjunction with real-life samples. A total of 77, 74 and 32 human RNA samples isolated from blood samples from a phase Ia CT with M. bovis BCG vaccination study were analysed by Affymetrix, Agilent and Illumina platforms, respectively. After critical specimen selection and samples processing all results from each platform were analysed. The primary issue addressed was the comparability of models constructed from different platforms. Then further evaluated was cross-platform consistency with regard to whether predictive signature features selected on one platform's datasets match the other platforms with comparable performance. Full bioinformatics analysis of data from Affymetrix, Agilent and Illumina platforms were performed using two software packages, Partek genomic suit (PGS) and Arraystar (DNASTAR). The analyses include QC of the raw data using different statistical analysis in Partek and Arraystar and Principal Components Analysis (PCA). Identification of differentially expressed gene sets was done using different comparison criteria. GO analyses of differentially expressed gene sets where functional groups (Molecular function, Cellular component and Biological process) are identified was performed. Pathway analysis also was applied for identifying pathways in which a given gene/product is involved (Independent Software package).
Sources of variation were identified for Microarray platforms after statistical analysis and batch effects removal using PGS. PCA (Partek software) analysis was also used to compare the gene expression signatures of PPD positive individuals with those of PPD negative. Differentially expressed genes were identified in PPD groups and time course (prior to immunisation and after immunisation) in all platforms. To obtain a broad picture of the behaviour of differentially expressed genes, Global functional analysis was performed by using a gene ontology analysis. Since many biological systems, gene expression levels measured across different time points during a given biological process provide more insights into the underlying system, pathway analysis of different comparisons was done within all platforms in the Partek pathway software using KEGG database.
To sum up, we performed a comparative analysis frame-work for most important gene profiling platforms, with understanding of the dynamic regulatory pathways among genes by using efficient analysis software. The framework implements a powerful integrated analysis that supports both understanding of the gene interactions over time and the understanding of gene function. All platforms tested are effective in detecting differential transcripts. Intraplatform reproducibility was generally high. Although the numbers of differential expression detection among microarray platforms was different, the overall pathways identified are preserved. Dynamic detection range was much greater for next-generation sequencing as compared to microarray analyses.

Harmonisation
Harmonisation of SOPS for sample analysis, microarray analysis and data analysis was performed using the three platforms. Finally, we found that using the protocols and kits used, Affymetrix platform and Partek Software package provides the most reliable and thorough analysis, highly suitable for identifying relevant biomarkers. Obviously, these data must be analysed together with *in vitro* assays and protection data.

WP4: Assay Identification; Qualification/Validation and Regulatory Guidance Platform

In WP4, together with UOXF and LSHTM NIBSC coordinated and took part on assay harmonisation of the three main technologies: an enzyme-linked immunospot (ELISpot) assay; an intracellular cytokine staining and flow cytometry (ICS) assay and an enzyme-linked immunosorbent (ELISA) assay, each designed to measure antigen-specific interferon gamma (IFNγ) expression; and identification of key operational specifications. This WP focussed on protocol transfer and assay establishment then subsequent assay harmonisation, performance and reproducibility within and between groups. Selected assay SOPs were assessed and optimised. Results from the final round of assay harmonisation and 3 other previous rounds were also sent and analysed by NIBSC's statistician and inter-laboratory variations were calculated for each assay. In general, the values of Coefficient of Variation (CV) between laboratories for these assays were

reduced to 40% - 75% with the finalised harmonised protocol when compared with the data using protocols from early rounds. The data showed robust reproducibility within the specialist laboratories and between operators. There were some issues that came to light regarding detail of protocols and SOPs, and technology transfer; and were refined during the studies. Regulatory considerations have been taken into account during the harmonisation process. Consensus SOPs for core aspects of these assays platform have been established, and are now published on the TRANSVAC website and are freely available to the scientific research community.

WP5: Process development and GMP

Production of recombinant proteins

Altogether 8 recombinant vaccine candidates (ESAT6, CFP10, 85A, 85B, 85C, PstS1, PstS3, and MSP-FusN) were produced and resulting batches passed in-depth quality control procedure. They are all antigens originating from Mycobacterium tuberculosis, the causative agent of tuberculosis, except MSP-FusN, which is a fusion product of three P. falciparum (causative agent of malaria) epitopes from three different proteins (MSP2 and a conserved Plasmodium protein of unknown function). The 8 recombinant proteins were produced as recombinant proteins in *E. coli*.

LIO was successful in producing all recombinant proteins in sufficient quantities of very high purity free of endotoxin. This was proven by detailed QC (SDS gel electrophoresis, Western blotting, host cell proteins, N-terminal sequencing, endotoxin measurement (LIO) and N-terminal sequencing and ESI MS done by partner HZI. Detailed documentation has been developed for each stage: 1. Master Cell Bank (MCB), 2. Upstream process (Biomass production), 3. Downstream process development with In-process QC, and 4. Final QC. All work was supervised by a scientist with experience in GMP manufacturing. Practically all documentation follows the near GMP guidelines. For each step a Master Batch record (MBR) was developed, which is a large document containing full details of all processes, chemicals, consumables, instruments. Most of the details of processes were integrated into the MBR. More than 30 detailed SOPs were developed. The filled MBR finally led to the Batch Record (BR) of the batch produced and stored at LIONEX. The Batch record contains full documentation and full report of the proteins produced under GMP compliant conditions.

Production of cell banks

The following cells banks were produced at UOXF:

- First cell bank: HEK293 (low passage) was produced a vial of passage 27 HEK 293 cells received from Microbix Biosystems Ltd.
- Second cell bank: 911 MCB1 was produced from human 911 cells (sisters of the PER C6 cell line) obtained from Prof Rob Hoeben at the Leiden University Medical Centre.
- Third cell bank: VERO (WHO) MCB1 was produced from a glass vial of VERO cells from ECACC Salisbury after authorisation of the WHO.
- Fourth cell bank: 293 ADH cell bank was produced from a CBF Working Cell Bank which, in turn, was prepared from the Bioreliance fully-tested Master Cell Bank.
- Fifth cell bank: Procell92 ADH cell bank was derived from a CBF Primary Freeze vial which had been adapted back to adherent culture (more useful for generating virus starting material) from the Okairos tested suspension Master Cell Bank.

These cell banks are suitable for partners for making pre GMP starting materials for vaccines. They have been externally tested for mycoplasma and are clean. They are not fully certified cGMP banks (this would require extensive external testing not covered by this collaboration agreement).

WP6: Production of recombinant vaccine candidates

Production of antigens

The 19 following antigens have been produced and were stored frozen in liquid state routinely. They are now available:

1. ESAT6
2. CFP10
3. 19 kDa
4. PncA
5. HSP70
6. HSP65

Antigen	Country of beneficiary institution
ESAT6 (Rv3875)	Brasil, Spain, Taiwan, UK, Argentina, France, Greece, Italy, Mexico, Belgium, USA, Egypt
CFP10 (Rv3874)	Brasil, Taiwan, UK, Argentina, France, Spain, Greece, Italy, Mexico, Belgium, USA, Egypt, Japan
CFP10+ESAT6	Germany, Greece, Belgium, Spain, UK
19 kDa (Rv3763)	Sweden, UK
PncA (Rv2043c)	Italy
HSP70 (Rv0350)	Japan, UK

7. Antigen 85B
8. Antigen 85C
9. AlaDH
10. Apa
11. MPB83
12. Rv0251c
13. Rv1636
14. PstS1
15. Antigen 85A
16. 16 kDa antigen
17. MSP-Fus
18. PstS3
19. p24

These antigens were offered for free and provided to researchers in 17 countries all over the world (see table above). They comprise important diagnostic markers such as ESAT6, CFP10, and PstS1, on which relevant research projects for drug and vaccine development often depend.

The antigens 1-16 listed above were further modified after the initial standard purification procedure, according to user specifications (e. g. the final formulation was changed, or the endotoxin contents were lowered to make the antigens suitable for cell culture assays).

Detailed characterisation of the purified vaccine candidates and other biomarkers was done by N-terminal protein sequencing, MALDI, MS-MS and peptide mapping (HZI-PROQC). On demand, HZI-PROQC offers analyses by mass spectrometry (ESI-QqTOF-MS, MALDI-TOF/TOF-MS), nuclear magnetic resonance spectroscopy, X-ray crystallography, light scattering, protein sequencing, and electron microscopy or N-terminal protein sequencing by automated Edman degradation.

Development of single antigen specific ELISA tests has been performed at the LIOPROVAC facility. Highly purified recombinant antigens from mycobacteria proteins from LIOPROVAC have been immobilised to develop enzyme immuno sorbent based assays containing ready-to-use reagents. Components of the assays are antigen coated microtiter plates, sample diluent buffer, wash buffer concentrate, conjugate solution, substrate solution, standards and stop solution. All solutions are ready-to-use except the wash buffer concentrate. Development steps necessary for establishing the immobilisation on coated surfaces were: selection of suitable raw materials, establishment of coating method, optimisation of the antigen concentration in the coating solution, conjugate concentration and standard curve establishing. As soon as all concentrations of reagents were optimised, documents and SOP were prepared, which allowed production of test kits under controlled conditions. Cut off values have been determined by measuring a panel of defined samples and shelf life was determined by performing stability studies.

Using the immobilised proteins on coted surfaces the following test kits have been produced:

- Antigen 85A and antigen 85B (IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to Antigen 85A and Antigen 85B)
- CFP10 IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to Rv3874)
- ESAT6 IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to ESAT6)
- hspX IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to Rv2031c)
- MPT64 IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to Rv1980c)
- PstS1 IgG, IgA and IgM Single antigen ELISA (detection of human IgG, IgA or IgM antibodies to Rv0934)

Production of cell lines

Five new cells lines better suited for the growth of adenoviral vectors are available for use. These cell lines have been developed in-house at UOXF for improved growth of adenoviral vectors. They are all derived from HEK 293 cells and contain an expression cassette for tetracycline repressor (TetR) protein that reduces or ablates expression of the transgene during adenoviral vector growth. CODOP contains a codon optimised tetR whereas Reg1 TetR expression cassette contains a

regulatory sequence which allows continued expression of the tetracycline repressor protein during adenovirus late expression. The other cell lines (target1, Reg1-target and Reg2) combine different regulatory sequences with a nuclear target sequence. Three of these cell lines repress transgene expression 1.5-3 fold better than commercially available T-Rex cells and produces higher virus yields. Transgene expression in Reg1-target cells was shown to be lower than in all other cell lines. However, it was generated using a commercial system so is currently being re-generated to GMP standards. HEK293 cells banked under GMP (WP5) will be used as the starting material.
There are discussions ongoing with several potential users who may wish to have access to these cell lines.

WP7: Standards Provision Pipeline for the Stable Assay and Reference Standard Platform
In WP7, several reference reagents were identified for immunoassays used in clinical trial studies and pathogen specific reagents for vaccine research, assay development and calibration of internal references. A batch each of positive and negative lyophilised human PBMCs with CD4+ IFNγ+ were produced (about 4500 vials in total). These flow cytometry standards were characterised and they represent different levels of interferon gamma (IFN-γ) expression as well as establishing expression levels for other cytokines of interest, such as tumour necrosis factor alpha (TNF-α), interleukin 2 and 10 (IL-2 and IL-10). Two batches (about 500 vials each) of lyophilised mycobacterial Antigen 85a were produced with optimised formulation in the presence of two different stabilisers, albumin or trehalose.
A specific subset of Malaria reactive serum samples were source from endemic countries in Africa. These samples were pooled and lyophilised as a candidate reference standard. This preparation (about 5000 ampoules) was also evaluated in an international collaborative study to be established as international standard. Coordination of international collaborative study, data analysis, stability studies, characterisation and establishment of robust specifications for the Anti-malaria (*Plasmodium falciparum*) human serum standard was completed. Biological Standard report was submitted to WHO Expert Committee on Biological Standardisation (ECBS) for approval. The WHO ECBS could not make the decision this year and the decision has been deferred to the next WHO ECBS meeting in October 2014. This reference standard is ready for distribution and will be available as an NIBSC reference reagent until it is accepted as WHO International standard of anti-malaria (Plasmodium falciparum) human serum.
A development of a non-cell based ELISPOT reference standard (in conjunction with Aston University) that can mimic human PBMC and be used as a positive control in an ELISPOT assay. The optimisation of formulation was completed. The results obtained have shown that scale up (from 4 to 40 ml in volume) within the laboratory setting can be achieved without a significant impact in vesicle size or entrapment of marker. It has demonstrated the proof-of-concept that liposomes encapsulating cytokine IFNγ can be freeze-dried and rehydrated to form spots on ELISPOT wells with a thermo-trigger release mechanism.

A. Networking activities

WP8: Modular course on concepts in vaccine development
Overall 30 participants working in vaccine development were trained on numerous aspects of vaccine development and provided with a thorough overview of the full vaccine development process, during two one-week courses organised at UNIL. All participants were asked to fill in the Questionnaire at the end of the courses to evaluate the training. Briefly, the rating was very similar for both courses. 73% of the students have rated the course as “excellent” (highest score out of five), the rest with “good” (the second best score out of five). Besides the scoring, participants have expressed feedback (written on the questionnaires) acknowledging their learning experience and the opportunity of networking with experts in the vaccine field. Some comments are listed below:
“The course offered an immense amount of valuable knowledge to me”; “I got to realise how complex it is to develop a vaccine that can successfully reach the market”; “The training has provided me with a network of people that I may consult for advice in the future”; “Delighted to hear such speakers and would like to thank TRANSVAC for the opportunity to attend”; “Extraordinary experience in terms of understanding on how this field works”; “The course has been a great source of theoretical and practical information”; “I have really learned many things that will be directly applicable to my job tasks”; “It was an exciting and eye-opening experience.”;

"Important was all the contacts that I made with key players in the field"; "I would absolutely recommend this course to anybody working in the vaccine field."
In addition, months after the TRANSVAC courses, the outcome of the training can still be tracked back. For example, the VFL is contacted on a regular basis by previous participants who are asking for news on future training courses. Furthermore, several contacts between participants and course speakers were mediated and collaborations were started based on the initial contact during the TRANSVAC courses.
In summary, the number of applications received for the two courses as well as the positive feedback and follow-up highlight the success of the two TRANSVAC training courses and stress the demand of such courses within the European vaccine community.

WP9: Vaccine Preclinical Testing Platform
In total four, instead of three, workshops have been organised during the TRANSVAC program. All workshops were widely advertised through different media, including the TRANSVAC website, other related EU-projects such as NADIR, EUPRIM-NET2 and NewTBVAC, and various organisations such as the Dutch Society for Animal Experimentation, and the Dutch Vaccines Group. The first workshop was held at the CVI. Lelystad with the title "From bench to trench? Necessity of animal models in preclinical vaccine research: required evidence for efficacy and safety". In this workshop, attended by 24 participants from different countries, lectures were given by representatives from regulatory authorities, industry and academia. The various approaches by the different partners were discussed. This provided a clear understanding on differences in approach by academia versus industry and regulatory authorities. The second workshop was held at HPA (now Public Health England), Porton Down UK and focused completely on statistical evaluation of results and how to obtain the most reliable data with a minimum number of animals. The title was: "Linear Mixed Effects Models" and included besides presentations a hands-on training on more individual basis. One of the main conclusions is that in studies with repeated measurement, which is often the case in vaccine studies, analyses using statistical means such as Linear Mixed Effect Models are very useful. Due to the specifics of the statistics, the effects of vaccines on different parameters can be determined much more precise with these methods compared to classical analysis. Because of the strength of these statistics, the number of animals can be kept limited (Reduction) without losing the reliability. Because of the importance of statistics in animal models a second workshop was organised on statistics in Amsterdam in June 2013 "Data reduction methods for analysis of animal studies with small experimental groups and multiple parameter measurements". Due to the hands-on training, the number of participants was limited to 7 scientists. Case reports were sued to explain how to proceed with data analysis. The final workshop was a dedicated workshop regarding pigs as model for vaccine research. The workshop was held at the CVI in Lelystad and included speakers from 8 different countries in Europe and Canada. The total number of participants was 55 (62 registrations). Topics on various aspects in the use of pigs for vaccine research, including immunological and genomic tools and methods, and specific disease models, such as TB and influenza were presented by highly recognised international experts in this field. This overview on the current status of pig models was highly informative for those directly involved in animal studies with this species, but also for those who are more involved in vaccine design and looking for the most suitable model for their investigational product. A summary of this day will be published in a meeting report that will be published in Vaccine.

WP10: Global Analyses Platform
A workshop on global analyses strategic planning issues was organised by MPIIB and held in Berlin on 7 October 2010. SOPs for sample collection and preparation were prepared and sent to the TRANSVAC partners in November 2010. Sample requirements and processing procedures have been implemented by the different partners, as established by the standardisation and harmonisation of the protocols of this WP.

A workshop on harmonisation and standardisation of (sample taking for) global platforms and on data management and bioinformatics was organised, with a view to compare data between different platforms and technologies. The objectives of the workshop were to bring together experts in the fields of transcriptomic profiling and vaccine development/testing to discuss standardisation and harmonisation issues to monitor host responses in preclinical and clinical vaccine studies. The one

day workshop organised by TBVI, with the support of EVI, took place on 6 March 2013 in Heidelberg and involved participation from other networks focusing on TB, HIV or Malaria vaccine development including NEWTBVAC, ADITEC, SYSMALVAC, and BIOVACSAFE. Main conclusions of the workshop were that Global Transcriptomics Platforms are rarely standardised for use in preclinical and clinical studies. Coordinated efforts should continue to harmonise the experimental set up of these studies, as well as the establishment of internal standards and controls. This effort will ensure comparability, efficiency and feasibility of the global analyses performed on preclinical and clinical data sets.

B. Transnational access (TNA)

WP11: Access to Adjuvant

Within the scope of Work Package 11, UNIL was requested to provide a total of 47 access units. One access unit is defined as one formulation study, wherein one formulation is the combination of one "antigen" with one "adjuvant". A formulation study investigates physic-chemical compatibility and stability of a given antigen-adjuvant combination. In total, access was granted to six applications submitted by six different scientists from six research facilities in four different countries (France, The Netherlands, United Kingdom and Ireland). The results generated through the different projects are presented below.

a) Evaluation of adjuvant formulation in the induction of mucosal immune responses against HIV after subcutaneous or intramuscular vaccination with p24 antigen encoated on PLA particles (MUCOSTIM, 9 access units).

The project aimed to evaluate nine different innovative antigen-adjuvant combinations (formulations) of the p24 HIV antigen encoated onto PLA particles (antigen) with a series of UNIL-VFL adjuvants. All formulations were studied for their compatibility and stability over a period of one week (up to 37°C) and checked for different parameters. Additionally, recommendations on good practices of formulation were given based on the results. The final study report from the Vaccine Formulation Laboratory was sent to the user Dr Stéphane Paul who reviewed it, and fully accepted it without modifications. Discussions on the publication of these results and continuing collaboration are currently ongoing between Dr Paul and UNIL.

b) Development of potent mucosal vaccines by combining an effective targeting agent with immunostiumlatory particulate adjuvants (TARGET-PART-QS21, 5 access units).

The project consisted in the study of five different formulations of H1N1 split influenza (antigen) with UNIL-VFL adjuvants. All TARGET-PART-QS21 formulations were studied for their compatibility and stability over a period of one week (up to 37°C) and checked for different parameters. Notably, potential major aggregation (size > 1000 nm) and potential changes in particle sizes were carefully studied. In addition, hemolysis testing of all formulations was investigated. Recommendations on good practices of formulation were provided within the study report. The final study report from the Vaccine Formulation Laboratory was sent to the user Dr Karen Misstear who reviewed and accepted it without modifications. Discussions on the publication of these results and continuing collaboration are currently ongoing between Dr Misstear and UNIL

c) Formulation of model vaccines for the PHARVAT adjuvant harmonisation platform (rAMA1_VAC, 9 access units).

The project rAMA1_VAC consisted in the study of nine different formulations of three reference "antigens" (AMA1, Ag85A and HBsAg) with three different UNIL-VFL "adjuvants" (aluminum hydroxide, squalene in water emulsion (SWE) and QS21-liposomes). Aluminium hydroxide based rAMA1_VAC formulations were studied for potential flocculation and antigen integrity up to one week at 25°C. The QS21-liposomal rAMA1_VAC formulations were assessed for compatibility and stability up to 24 hours at 25°C (particle size, antigen integrity, and pH). The SWE-based formulations were assessed for compatibility and stability to one week at 25°C by analysis of pH, zeta potential, particle size and antigen integrity. Recommendations on good practices of formulation were provided within the study report. The final study report was sent to the user Dr Edmond Remarque who fully accepted the report without modifications. Discussions on the publication of these results and continuing collaboration are currently ongoing between Dr Remarque and UNIL.

d) Combination (vector-adjuvant-protein) vaccine against malaria (COMBIVAX, 5 access units).

The COMBIVAX project aimed to characterise the short-term stability and compatibility of five distinct formulations consisting of the combination of one malaria antigen (R21) with five adjuvants

from UNIL. After preparation, formulations were incubated at 25°C for two hours and thereafter at 4°C, 25°C or 37°C for 24 hours or one week. Four COMBIVAX formulations were compatible and stable over a period of one week (4°C - 37°C) in terms of particles size, PDI, zeta potential, pH and antigen integrity by Western blot. As only exception, the pH of one of the COMBIVAX formulations dropped down after one week at 37°C. The fifth COMBIVAX formulation was compatible and stable only up to 24 hours in view zeta potential analysis. No flocculation of R21 aluminium formulation was observed. The pH remained constant at ~ 7.4. R21 adsorption appeared to be much higher than 75%; no R21 was detected after desorption procedure with 100 mM phosphate buffer. Further studies need to investigate this issue. In summary, as good practices of formulation, the four more stable COMBIVAX formulations should be used between two and eight hours after preparation at 25°C.

e) Improving the stability of DDA liposomes to deliver lipid antigens (LipTBvaccine, 8 access units).

The goal of this project was to improve the stability of the cationic DDA/TDB liposomes (based on dimethyl dioctadecyl ammonium bromide and trehalose dibehenate) loaded with two Mycobaterium tuberculosis (TB) lipid antigens: diacylated sulfoglycolipids (Ac2SGL) and phosphatidyl-myo-inositol mannosides (PIMs). The first step of this work consisted in a pilot study where the stability of two formulations (DDA/TDB and DDA/TDB + PIM + SGL) produced with five different liposomes preparation methods and two buffers were investigated. The stability of all formulations was studied for one week and checked for different parameters. The results of this work showed that the best buffer and liposome production methods, in term of liposomes stability, were: Tris-Trehalose buffer, film hydration, extrusion and freeze drying. Therefore these settings were selected for the main study, and the subsequent production of eight liposome formulations. All formulations produced with extrusion and film hydration were stored at 4°C and studied for their stability over a period of eight weeks. After this period of time all formulations showed major aggregation (size > 1000 nm) compared to the size measured at week 0. In conclusion, the methods tested to produce DDA/TDB liposomes mixed PIM were not efficient at improving the liposome stability and none of the formulation tested were stable after eight weeks storage at 4 °C. Further studies will be necessary to optimise the methods investigated in the context of this project.

f) From mice to appropriate model of human intradermal immunisation: efficacy and signature of the TLR5 agonist flagellin in Sus scrofa pigs (FLAGTRIAL, 14 access units).

The aim of the study was to investigate the relevance of the intradermal route for human vaccination with an antigen combined to the flagellin adjuvant. For this purpose two studies using two different antigen models were tested in two animal models.

The first study investigates the stability of several formulations based on an OVA antigen mixed with an extended list of adjuvants.

The formulations were prepared at UNIL and checked for different parameters. The results showed that formulations #0 (OVA alone) and #1 (OVA + flagellin) were less stable after 30 days of incubation at 4 and 25°C than the other formulations containing flagellin and additional adjuvants in term of PS and ZP, which demonstrate the advantage of formulating the flagellin with a delivery system. However at 37°C the formulation #4 (CL + OVA) and #8 (MPL + OVA) showed an increase of the PS and ZP, superior to 10% of their initial value at day 0, making the formulation not suitable for further use. All the formulations were shipped to Lille and tested in vitro for bioactivity. Currently, analysis of formulation efficacy by intradermal route is conducted in vivo in a mice model through the study of OVA-specific T cell and antibody responses.

In the second study a H1N1 influenza antigen (cGMP grade) was selected and provided with no additional costs by UNIL to the User of the service. The stability of flagellin formulation was investigated and compared to the reference formulation containing SWE. All Flagellin-H1N1 formulations were assessed for compatibility and stability up to 1 week at 37°C (PS, ZP, SRID, and pH). The data showed that the stability is similar to the refernce SWE-based formulations.

In summary, all formulations tested in this study showed compatibility and these results allowed the further investigations on the flagellin formulations in complementary platforms. Their potency to increase systemic immune response via the intradermal route in a pig model was investigated in a challenge study conducted at CVI. The determination of which flagellin dose was effective was analysed via the skin transcriptional response by microarray analysis, conducted in the Agilent microarrays platform of MPIIB.

WP12: Access to vaccine preclinical testing platform

The objective of the animal models transnational access (TNA) is that external groups can test their experimental vaccines/antigens in mouse, pig, and non-human primate models. In total, access was granted to six applications submitted by six different scientists from six research facilities in six different countries (Spain, France, Belgium, Switzerland, Denmark and Ireland). The results of the projects granted by the different WP12 infrastructures are presented below.

BPRC

Evaluation of the immunogenicity and tolerability of an antigenic formulation in CD4 T-cell inducing CAF01 and CD4/CD8 T-cell inducing CAF05 adjuvant upon simultaneous muco-parenteral compared to traditional parenteral delivery in rhesus macaques (CD4/8 responses by CAF, 20 access units).

In this study, Macaca fascicularis (cynomolgus macaques) were used to evaluate tolerability (safety) and adjuvanticity / immunogenicity of SSI's proprietary cationic adjuvant formulations CAF01 and CAF09. In a factorial design comprising four treatment groups of N=5 subjects each, both adjuvant formulations were compared and administered either by (conventional) parenteral or by combined simultaneous muco-parenteral delivery. The proprietary hybrid fusion protein H56 of mycobacterial antigens Ag85B, ESAT6 and Rv2660c was used as a prototype antigen to allow antigen specific cellular and humoral immune readouts as measures of adjuvant potency.

After priming, all four vaccine groups received two times a homologous intramuscular booster injection at four-weekly intervals. Readout of adaptive immunity was warranted up until 12 weeks after the final injection by co-formulation of the subunit vaccine candidate H56, a recombinant fusion of mycobacterial Antigen85B, ESAT6 and Rv2660.

Together, the data supported the safety and adjuvanticity of CAF01 and CAF09 by both parenteral and mucosal (muco-parenteral) administration in a primate host, with the latter adjuvant demonstrating superior activation and with enhanced mobilisation of CD8+ T cells upon antigen-specific recall stimulation.

DH-PHE (former HPA-CEPR)

Immunogenicity of an HIV/AIDS vaccine candidate lacking vaccinia virus gene C6L in non-human primates (C6LVAC, 2 access units)

A study has been conducted in non-human primates to define the advantage of MVA vectors as HIV vaccine candidates. The MVA–B and MVA-B deltaC6L HIV/AIDS vaccine candidates were well tolerated in non-human primates with no adverse clinical or behavioural reactions detected. HIV-1-specific CD4+ and CD8+ T cell immune responses elicited by MVA-B and MVA-B deltaC6L vaccination groups against Env, Gag and Gag-Pol-Nef peptide pools were comparable. Both vaccination groups elicited high levels of IgG antibodies against gp120, which are boosted after the second dose of the vaccine, and then are maintained over time. Additional analyses by ELISA, neutralisation, antibodies and ICS will confirm the advantage of one vector over the other and role of the viral gene C6L on HIV immune responses.

The aim of the study was the assessment of the safety/tolerability and immunogenicity of a vaccine therapy in a rhesus macaque model. MVA-B is a Modified Vaccinia Virus Ankara (MVA)-based HIV/AIDS vaccine candidate vaccine expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B that has been shown to induce a good immunogenicity profile against HIV-1 in a phase I clinical trial in humans. An MVA-B recombinant lacking the immunomodulatory vaccinia gene C6L (termed MVA-B deltaC6L) that blocks IFN-beta has been shown to significantly enhance the immunogenicity against HIV-1 in a mouse model, compared to parental MVA-B. The aim of this study was to further characterise the immunogenicity of MVA-B deltaC6L in macaques and compare it with the immunogenicity induced by MVA-B. More specifically, the user wanted to analyse the cellular and humoral HIV-1-specific immune responses elicited by both vaccines and to identify any differences in the magnitude, breadth, polyfunctionality and memory CD4+ and CD8+ T-cell responses to HIV-1 antigens, as well as the levels of antibodies against Env induced by both HIV-1 vaccines. The study comprised two groups of four animals (outbred rhesus macaques [Macaca mulatta] of Indian origin). At weeks 0, 4 and 8, the four macaques in group A were immunised with MVA-B in the upper arm by intramuscular route, (i.m.), and the four macaques in group B will receive MVA-B deltaC6L i.m., in the upper arm,. At week 14, all animals in groups A and B were immunised with the A27/gp120 subunit vaccine by the i.m. route in to the upper arm.

Clinical parameters were monitored and blood samples collected at two to four weekly intervals from all animals throughout the 20 week study. The study supported the tolerability and immunogenicity of both the MVA-B and MVA-B deltaC6L in macaques.

CVI

a) Pre-clinical Testing of Influenza Vaccine Incorporated in Dissolvable ImmuPatch Microneedle Arrays (ImmuPatch, 431 access units).

Immunisation programmes are currently limited by cost and logistic issues. Use of a simpler, needle-free, self-administered vaccine delivery device and stabilised vaccine that eliminates cold chain would have a significant positive impact on the cost and success of immunisation programmes. ImmuPatch is a platform silicon and dissolvable microneedle technology for pain-free, self-administered, transcutaneous vaccine delivery. Microneedles are micron-scale needles that penetrate the outermost, impermeable skin layer, creating temporary conduits for vaccine administration. The group of A. Moore developed novel methods of producing 'dissolvable microneedle' (DMN) patches. Live virus and protein antigen stability in DMN under accelerated stability conditions, virus infection in ex vivo skin and murine immunogenicity was already demonstrated. The objective of this study was to determine the potency and efficacy of Dissolvable ImmuPatch-mediated influenza virus vaccination in a relevant large animal model, namely pigs, compared to conventional liquid vaccine administered by needle-and-syringe intramuscularly (IM). Pigs are industry-recognised models of transdermal delivery. This study is a critical milestone for the commercial and clinical development and will underpin future clinical development and partnering with vaccine manufacturers. We successfully demonstrated that suitably sized ImmuPatch patches (i) could be fabricated, (ii) can be delivered to the host institute and (iii) administered to pigs without detectable local adverse events. Sampling of blood according to the study plan was successfully achieved. Monitoring of the post-immunisation immune responses is currently being conducted. In a second part, all animals were challenged with influenza virus. The challenge procedure went according to plan. The challenge strain chosen matched one of the strains used in the vaccine. This was the first time that ID-L used this influenza virus strain, so there was a risk of using a previously untested virus strain in pigs. The level of protective efficacy and virus-induced lung pathology that was achieved is currently being analysed.

b) Increasing the protective efficacy of M. bovis BCG vaccine by combined vaccination with plasmid DNA encoding the mycolyl-transferase Ag85A (Rv3804c) (BCG-p85A-combo, 302 access units).

The only vaccine currently available to prevent TB is the live, attenuated M. bovis Bacille Calmette Guérin (BCG) vaccine. BCG is one of the most widely administered vaccines and in 2000 covered 86% of the world population. BCG vaccination protects children against TB meningitis and against disseminated, miliary disease, but confers a variable protection (ranging from 0% to 80%) against pulmonary TB in adults and has been found to be of variable efficacy in a number of clinical trials. There is a clear need for a more efficacious TB vaccine for both prophylactic use (preventing the infection) and post-exposure use (people already infected with TB).

The aim of this study was to test the immunogenicity in pigs of the combination of the existing BCG vaccine with plasmid DNA encoding the mycolyl-transferase AG85 antigen expressed during different stages of the infection. This combination can lead to the induction of stronger protective CD4+ and CD8+ T cell responses, the latter being particularly important for the control of latent tuberculosis. This proof of concept could be extended to other antigens, preferentially expressed by Mtb during latency, such as the Dormancy (DosR) regulon encoded antigens. Also, the feasibility and efficacy of DNA vaccination in combination with *in vivo* electroporation in pigs was demonstrated.

c) From mice to appropriate model of human intradermal immunisation: efficacy and signature of the TLR5 agonist flagellin in Sus scrofa pigs (FLAGTRIAL, 562 access units).

The aim of this study was to analyse the adjuvant efficacy of the TLR5 agonist flagellin. Two animal experiments in pigs were performed in which the TLR5-stimulating activity, the immunogenicity and the protection of flagellin adjuvants in combination with seasonal influenza vaccine were tested.

In a first experiment the early local transcriptome response of skin after intradermal injection with different doses of flagellin was addressed. The dose-effect relationship between the quantity of

intradermally administered flagellin in regard to transcriptome expression analysed by microarray and PCR was compared and the doses determined for the main study.
In a second experiment pigs were vaccinated with H1N1pandemic (flu virus from 2009) adjuvanted with flagellin and effects were compared to pigs either vaccinated with H1N1 pandemic alone or in combination with a known adjuvant. Next to the animal study also serological and virological analyses were performed at CVI. All raw data and results were forwarded to the applicant and discussed. A publication on the results of the study is currently in process.

HZI
Evaluation of the immunogenicity and functionality of a glycoprotein vaccine against Streptococcus pneumoniae serotype X (GVXN Pn4-EPA_1, 1 access unit).
The humoral and cellular responses to a novel *S. pneumoniae* serotype X capsular polysaccharide conjugate vaccine (SPXe-EPA) produced by innovative proprietary technology was performed in C3H mice. The polysaccharide was conjugated to a detoxified *Pseudomonas aeruginosa* exotoxin A (EPA). A non-adjuvanted version of the vaccine was compared with different formulations, adjuvants and administration strategies. In addition, the immunogenicity of the novel vaccine was also compared to that of Prevenar 13, a vaccine which is already in the market. This experiment served as a proof of concept for the subsequent fast development of pneumo conjugates not contained in current vaccines. The co-administration of *S. pneumoniae* serotype X capsular polysaccharide conjugate vaccine (SPXe-EPA) with the adjuvant c-di-AMP by subcutaneous or intranasal route to C3H mice resulted in the elicitation of significantly higher serum EPA and CPX-specific IgG titers than in controls. The induction of local immune responses was shown by the production of EPA and CPX-specific secretory IgA in samples derived from different mucosal territories of mice vaccinated by i.n. route. Strong cellular immune responses were only observed against EPA in splenocytes derived from mice vaccinated with SPXe-EPA co-administered with c-di-AMP by i.n. route. The ratio of EPA or SPXe-EPA-specific antibodies and the secreted cytokine profiles by *in vitro* re-stimulated splenocytes suggested that a balanced Th1/Th2/Th17 response pattern is promoted by c-di-AMP given by both s.c. and i.n. route, whereas Prevenar 13 induced a strong Th2 response shown by IL-4 secretion. When C3H mice were immunised with SPXe-EPA and c-di-AMP by the i.n. route, enhanced proliferation was also observed. These results indicate that c-di-AMP exhibits a high potential as adjuvant for the development of mucosal *S. pneumoniae* serotype X vaccines, in particular when cellular immunity is needed.

WP13: Access to global analyses platform
The global analyses platform provided access to Affymetrix and Agilent microarrays and Illumina deep sequencing platforms for analysing samples received from external groups. In total, access was granted to 14 applications submitted by 12 different scientists from 10 research facilities.

The MPIIB provided access to their Agilent microarray platform. In total, 14 units of access were provided to 3 different users from 2 countries (France and Spain). All projects were completed successfully.
The results of the projects selected for the Agilent TNA service were:
a) Transcriptional analysis of flagellin-mediated adjuvant activity: defining predictive biomarkers of efficacy in blood and skin samples (FLAGMARK, 2 access units).
The present study sought to identify the antigen presenting cells involved in adjuvant activity following the intranasal administration of the Toll-like receptor 5 (TLR5) agonist flagellin. We used molecular profiling to show that cytokine/chemokine and dendritic cell maturation pathways are surrogate signatures for flagellin activation in the lung. We found that flagellin signalling enhanced their maturation and migration to the lymph nodes. In particular, CD11b+ migratory dendritic cells were essential for induction of a CD4+ T-cell response. The functional activation of dendritic cells was independent of direct signalling via TLR5. Our results demonstrated that migratory CD11b+ dendritic cells are essential components in flagellin's mucosal adjuvant effect. Furthermore, the observation that flagellin's adjuvant activity can be dissociated from inflammatory cell recruitment opens up new perspectives for vaccine improvement (ref. submitted manuscript). We are currently asessing whether this mode of action can operate by the intradermal route by comparing skin and lung as well as blood transcriptional and cellular responses with regards to the various routes of administration.

b) Transcriptional signatures of clinical trial phase I subjects vaccinated with BCG and the novel live-attenuated tuberculosis vaccine MTBVAC (MTBVAC-TS, 9 access units).
Since this is an ongoing CT, in accordance with the strict rules for entire CT phase I, all samples were completely blinded. Hence unprocessed raw data were to be delivered to the requestor for further evaluation and in-depth analyses by an independent third party.
c) From mice to appropriate model of human intradermal immunisation: efficacy and signature of the TLR5 agonist flagellin in Sus scrofa pigs (FLAGTRIAL, 1 access unit).
The TLR5 agonist flagellin seems to have similar induction kinetics between skin and blood as already identified in the murine model. A potential adjuvant activity of TLR5 was identified and seems to be at least partially comparable to oil-in-water adjuvant. As soon as the experiments of pig vaccination and challenge with flu H1N1will be finalized, we will define a signature of efficacy on the basis of the global transcriptomics measurement and identify whether the mode of action of the boosting effect of TLR5 agonist as adjuvant.
d) Transcriptional analysis of BPZE1, an attenuated Bordetella pertussis vaccine strain, genetically engineered by detoxifying pertussis toxin (PTX), removing tracheal cytotoxin (TCT), and dermonecrotic toxin (DNT) and the anti-inflammatory vaccine properties against influenza virus-induced mortality / lethal pneumococcal infection (BPZE1-Immuntranscript, 3 access units).
MPIIB measured by global transcriptomics analyses clear kinetic differences between BPZE1 vaccination and B. pertussis infection. Specific signatures of biological processes and molecular functions of the anti-inflammatory properties of BPZE1 are expected to be identified, which should help to understand the prevention of the cytokine storm by BPZE1 vaccination and leading to protect mice against lethal heterologous flu and S. pneumonia disease.

UREG provided access to their Affymetrix microarrays. In total, 13.5 units of access were provided to 5 different users from 5 countries (Spain, Poland, Denmark, France, and the United Kingdom). All projects were finished successfully, and the data quality was generally very high.
The results of the projects selected for the Affymetrix TNA service were:
a) A human malaria challenge on non-immune adults was carried out in two different phases to establish optimal dosing to safely infect healthy individuals with P. falciparum sporozoites (PfSPZ) via intramuscular injection (BACHMI01_VacGeneExp, 2 access units).
Controlled human malarial infection studies are a powerful tool to assess the efficacy of novel malaria vaccines. A human malaria challenge on non-immune adults was carried out in two different phases to establish optimal dosing to safely infect healthy individuals with P. falciparum sporozoites (PfSPZ) via intramuscular injection. The first 18 volunteers received three different inoculation volumes of PfSPZ to test the effect of volume on the infective rate. In the second phase, 12 out of 18 volunteers received two different concentrations of PfSPZ via intramuscular injection to test if infectivity increases with injection dose and 6 volunteers received PfSPZ by intravenous injection. In total, 24 samples from the first phase of the clinical trial and 72 samples from the second phase of the clinical trial were analysed by microarrays. The results show that the responses at the day of detection of parasites in blood cluster together. Although there is no obvious clustering by dose or route of inoculation, a number of differentially regulated genes one day after the SPZ inoculation and later liver stage/beginning of blood stage could be identified. To date no responses in blood have been detected for these stages of the infection. This will provide critical information and a broader analysis of the immunological responses elicited by P. falciparum primo-infection. It is expected that the data set will help to elucidate how innate immune responses may be modulated in different stages of infection and after different doses of parasites. An additional goal of the study is to define how the innate responses during the challenge determine the acquired immune responses one month and three months after the challenge. Overall, it is anticipated that very useful immunological information for other studies of whole parasite vaccines under development will be obtained, such as Irradiated sporozoites (Seder, RA., et al. Science 2013) or CVac (Combination of chemoprophylaxis with chloroquine and controlled human malaria infections, Roestenberg, M., et al. N Engl J Med, 2009). The identification of biomarkers detected by this process could have an impact on disease treatment. It may help direct therapeutic interventions that target and kill malaria parasites prior to the onset of inflammatory or pathological processes that lead to illness and even death.
b) A cell-line based transcriptome study in the context of melanoma vaccine research (TRANSMELVAX, 1 access unit).

Therapeutic gene modified melanoma vaccine (AGI-101H) is composed of two human melanoma cell lines which are referred to as Mich-1 and Mich-2. These cell lines were modified with the designer cytokine cDNA – Hyper-IL-6 (H6). Before administration to patients, the cells are gamma-irradiated with a sterilising dose. It is already known that H6 secreted by vaccine cells acts in an autocrine fashion and changes their phenotype by increasing or decreasing expression of some important genes. Moreover, gamma-irradiation has similar effects. The aim of the project was to analyse the gene expression profile in wild Mich-1 and -2 cells, cells modified with H6 and irradiated vaccine cells. Two sets of 16 RNA samples (total 32 RNA samples) were sent to UREG. Global expression profiles obtained from the first set of samples indicate that IL-6 pathway members, such as IL6, IL-6R, JAK2 and JAK3 are upregulated in Mich cells modified with designer cytokine H6. In contrast, Nicotinate and Nicotinamide metabolism and Glutathione pathways are enriched in unmodified Mich cells. Results of the analysis of the second set of samples were obtained in the same manner as the first set to enhance statistical significance, and to select mRNA candidates for further studies. Obtained data from both experimental sets can be correlated with the immune response of patients treated with the AGI-101H vaccine and will help to refine cancer vaccines. Results of the study will be published.

c) A head-to-head comparative strategy was used to compare the potency of two adjuvant formulations – CAF01 and CAF09 – in non-human primates upon two different administration regimes: either by parenteral or by combined muco-parenteral administration (Transc-resp-prof-by-CAF, 5 access units).

The specific objective of the project was to compare the gene expression in the vaccinated primates in order to correlate with translational immunological and clinical readouts and therefore better understand the mechanism of action. Whole blood RNA collections for 20 primates were established at baseline 4 weeks prior to primary vaccination and at week 0, and post-prime at weeks 2, 4, 6, 10, 12 and 20. This gives 160 whole-blood samples which were shipped to Regensburg. All Affymetrix global analyses were done and the full data set was sent to the requestor. The data set is quite comprehensive, and it is the hope of the user that the gene profiling will enable them to get an overview on the immunological mechanisms of action of the CAF01 and CAF09 adjuvants as well as any potential safety concerns related to their use. In mice, the two adjuvants have shown differential modes of action that will allow most optimal orchestration of the various components of humoral and/or cellular immunity towards (safer and) more efficacious vaccination regimes in general. Furthermore the comparison of immune responses obtained by the two different administration strategies will be of great interest for vaccinology in general, as it may help to obtain a vaccination strategy for future subunit vaccines in which both mucosal and systemic immunity is obtained. The ability to compare the gene expression in primates vaccinated with CAF01 and CAF09 using different vaccination regimes will make it more accurately possible to investigate the correlation to data obtained in mice and validate translational immunological and clinical readouts. Furthermore, the data set will be useful in order to better understand the mechanisms of action of the two adjuvants and to obtain important knowledge for the further development of CAF01 and CAF09.

d) A study aimed at understanding the immunosuppressive tumour environment for induction of anti-tumour immunity using TLR7/8 agonists, in the context of ovarian carcinoma (OvarianCarcinoma&TLR7, 2 access units).

The long-term aim of this study is to develop a vaccine for the treatment of ovarian carcinoma (OC) based on antibody-mediated antigen delivery to dendritic cells (DC) *in vivo*. Monocyte-derived DCs from healthy donors were generated and were treated over night with the TLR7 agonist R848 in the presence or absence of ascites from OC patients. To investigate the immunosuppressive role of IL-10 and PGE2, ascites depleted of these factors was used in comparison to mock-treated ascites. A total of 48 RNA samples were provided which were derived from 4x independent experiments. The monocyte-derived DCs were from 3 different healthy donors and were treated with the ascites fluid from the same two patients. The DCs from one donor were treated in addition with the ascites fluid from two additional OC patients. What became obvious from the first analyses of the data set was that there is a very high donor variation in the gene expression profile of untreated monocyte-derived DC and R848-treated monocyte-derived DC. It is currently unclear why there is such a big difference in the baseline gene expression of the monocyte-derived DCs and why donors respond differently to R848. However, this has implications for the further analysis of the data and it may become necessary to look at IL-10 and PGE2-dependent suppression of TLR7/8-mediated DC

activation for each donor separately. At least there are two data sets for each donor using two different ascites fluids - however, there is only one sample for untreated cells and one sample for the R848-treated cells and we may have to generate 1-2 further data sets using the same donors.

e) The transcriptional response to vaccination with live attenuated B. pertussis was measured, as part of an evaluation of a novel Pertussis vaccination strategy in newborn children (BP-VAC transcriptome, 3 access units).

Pertussis is still a major public health issue worldwide. The investigator's aim is to develop a new vaccine to be administered at birth in order to provide early protection and prevent severe forms of the disease in newborns. An attenuated B. pertussis strain, named BPZE1, has been engineered by genetically altering or removing three toxins, pertussis toxin, tracheal cytotoxin, and dermonecrotic toxin. BPZE1 is non-pathogenic in mouse models, yet able to colonise the mouse respiratory tract and to quickly protect against B. pertussis challenge via TLR-4 signalling. A phase-I trial has shown that BPZE1 is safe in humans, yet able to colonise the nasopharynx and to induce immune responses in all colonised subjects. However, not all subjects were colonised. This project aimed at establishing the transcriptomes of colonise versus non-colonise subjects and compare them to those of placebo controls in order to establish transcriptomic profiles early and later during BPZE1 administration. RNA extracted from 7 colonised subjects, from 7 non-colonised but vaccinated subjects, and from 7 placebo controls at all time points for which PBMC were collected (i. e. 2 to 6 weeks before BPZE1/Placebo administration, on the day of vaccination and at 1, 2, and 4 weeks, and 5-6 months after vaccination) were sent to Regensburg (a total of 96 samples). The Affymetrix microarray data were generated and it is expected that the data will be of the utmost importance for the requestor to investigate the early and sustained immune responses in BPZE1-vaccinated subjects, and that it will lead to major publications in the field. In general, the data provided in this contract will be very useful to compare the human model with the mouse model, for which similar transcriptomic approaches have been carried out in parallel.

The HZI provided access to their Illumina deep sequencing platform. In total, 146 units of access were provided to 5 different users from 4 countries (Austria, Croatia, Czech Republic, and the United Kingdom). All projects were finished successfully.

The results of the projects selected for the Illumina TNA service were:

a) A single tetravalent live attenuated recombinant Measles Virus Vaccine against DENGUE and CHIKUNGUNYA (MVDVAX, 10 access units).

This study is focused on a vaccine vector system to create new vaccines against emerging infectious diseases including Dengue Virus and Chikungunya Virus. The vector is based on the live attenuated measles vaccine, which can be used as backbone to express large (up to 5kb) heterologous antigens. Attenuation is achieved by genetically adapting viruses for replication in a different host species, to lose their pathogenic potential while remaining their antigenic potential. However, in very rare instances, attenuated viruses can accumulate mutations or recombine with pathogenic viral strains carrying a potential safety risk. In addition, contamination with adventitious viruses from cell lines used and/or animal sera and biologics used in cell cultures cannot be ruled out. The measles vector can be loaded with large inserts, which could affect virus replication, lead to transcription of subgenomic RNAs, increase mutation rates, and theoretically even allow recombination with other viruses. Vaccine manufacturing requires the amplification of the vaccine virus strains over several passages on mammalian cells. To exclude any of these events we collected samples at various steps throughout manufacturing process development and manufacturing of a clinical vaccine lot00000 for detailed sequence analysis by deep sequencing. This sequencing method is a highly sensitive method to detect virus sequence changes, quasi species, and adventitious viruses to help maintain the safety of the live-attenuated vaccine. TruSeq RNA libraries of the six samples were prepared and sequenced on the MiSeq sequencer (Illumina) with 250 bp paired end. Alignment, comparative analysis and variant detection were done at the HZI. The results were made available as raw multiple HQ FASTQ files, as sequence alignment format (.sam) and variant call sheets (*.xls). A very detailed analysis of the data is currently performed by Themis Bioscience GmbH. However, a very important preliminary finding is that the sequence in a vaccine lot that is currently tested for the first time in humans was stable over 10 passages. Thus, the Masles Virus vector is a highly stable technology that can be implemented for a number of applications including other infectious disease models or cancer therapy. The sequence analysis of the additional vaccine candidates is currently ongoing.

b) The influence of viral population diversity on safety and immunogenicity of licensed mumps vaccine strain (MUMPSVAC, 36 access units).

The objectives of this project were:

- Comparison of viral populations' structures in L-Zagreb samples differing in neurovirulence levels, with a goal to identify genetic neuroattenuation markers.
- Genetic analysis, immunogenicity evaluation and neurovirulence potential assessment.
- Development of a new, optimally neuroattenuated L-Zagreb master seed, equally immunogenic as the current one.

Reference samples for new L-Zagreb master seed and working seed were developed. Libraries from all samples were prepared at the HZI. The samples were sequenced on the Illumina GA IIx sequencer with 110 bp paired end. For verifying the results of variant call, amplification of selected samples was done and sequencing of the PCR product was done with an ABI sequencer. Alignment, comparative analysis, variant detection and additionally analysis involving population diversity were done at the HZI. The results were made available for the user as multiple HQ FASTQ, as sequence alignment format (SAM/BAM), variant call sheets (*.xls) and electropherograms (.abi). By combining deep-sequencing results with newborn rat-based neurovirulence testing, precise population characteristics that L-Zagreb strain must have in order to be optimally neuroattenuated were defined. Besides acceptable neuroattenuation level, vaccine immunogenicity testing also gave good results. The user is now working on the preparation of a new L-Zagreb master seed.

c) The discovery of candidate 'dual' antigens for vaccine development by deep sequencing of Ixodes ricinus salivary and midgut transcriptomes (DUALVACANTIRICINUS, 60 access units).

Recent increases in Ixodes ricinus abundance and the infection prevalence of ticks with Borrelia burgdorferi s.l., the etiologic agent of Lyme disease, have led to an increased exposure of humans to tick bites and Borrelia. I. ricinus saliva accelerates the proliferation of B. burgdorferi in the skin and blood of infected mice, but the constituents accounting for this effect are currently poorly described due to the limited genomic information for this tick. The aim of the project was to apply deep transcriptome sequencing to study the tick salivary transcript repertoire as a function of time of feeding and developmental stage (nymph, adult). We will also explore the I. ricinus midgut transcriptome aiming to discover 'dual antigens' from this tick, i.e. antigens that are 'exposed' (found in tick salivary glands and thus in contact with the vertebrate host) and 'concealed' (found in tick midgut and thus playing a role in tick physiology).

The HZI received 11 RNA samples and 8 libraries. Libraries for RNAseq were prepared from 11 RNA samples. All the libraries were sequenced on the Illumina GA IIx sequencer. The user-provided libraries were sequenced with 120 bp single reads, small RNA libraries were sequenced with 36 bp single reads and the RNA libraries that HZI constructed for the user were sequenced with 75 bp paired end. The results were made available as multiple HQ FASTQ files. The dynamic expression of 17000 transcription units in tick salivary glands and midguts -within the first couple of days of tick attachment to the host- was revealed. The bioinformatic analysis of the assembly/contig annotation has been completed. The Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GANP00000000. The version described in this report is the first version, GANP01000000 (Subid: SUB334716, Bioproject: PRJNA217984). Two manuscripts are under preparation with anticipated publication within 2014. The produced knowledge might pioneer the immediate discovery of candidate tick antigens for anti-tick vaccine development with an overall aim to 'poison' a tick upon its attachment to the host -via an antibody mediated mechanism.

d) Tuberculosis RNA sequencing for the discovery of potential biomarkers of disease and protection in bovine TB and their potential use as model for TB in humans (RNA seq in bovine TB, 30 access units).

The objectives of this project were:

1. To develop correlates of protection and infection in bovine TB.
2. To contribute to the rational design of improved vaccines and diagnostics against M. bovis.
3. To gain a basic understanding of the nature of immune responses to mycobacteria and the metabolic pathways involved in the development of these responses.
4. To inform research and development in human TB.

Samples from the differently protected and non-protected animals were identified and 30 RNA samples were sent to HZI. Library preparation and sequencing of the libraries was done on the Illumina HiSeq 2500 with 50 bp single reads. Mapping and expression analysis of the raw data was

done at the HZI. Multiple HQ FASTQ files, expression values and comparative analysis were provided to the user and are currently being analysed.

e) Pilot study to deep sequence HIV breakthrough infections that have occurred during HIV vaccine trials conducted by the TaMoVac consortium and partner institutions (HIV_vaccine_breakthrough, 40 access units).

The HZI received 14 samples (11 DNA samples and 3 ss cDNA). Library preparation and sequencing on the Illumina MiSeq sequencer with 250 bp paired was done. The results were provided to the user as HQ FASTQ files. The complete analysis should be completed by the end of Q4 2013/Q1 2014.

WP14: Access to stable assay and reference standards platform

The objective of WP14 is to provide a platform for provision of characterised and stable reference reagents prepared and characterised in WP7 for immunoassays used in clinical trials; and pathogen specific reagents for research, assay development and calibration of internal references. In total, access was granted to three applications submitted by three different scientists from three research facilities in two different countries (United Kingdom and Denmark).

a) Evaluation of the immunogenicity and tolerability of an antigenic formulation in CD4 T-cell inducing CAF01 and CD4/CD8 T-cell inducing CAF09 adjuvant upon nasal or intramuscular delivery in rhesus macaques (CD4-8 resp by CAF, 44 access units).

In continuation of the work done under WP12, the objective was to improve the data quality of the ELISPOT and ICS protocols by use of relevant reference reagents. The ICS reference is useful for longitudinal studies and for comparing multiple studies. The ELISPOT reference requires "in life" use to help qualify the reagent as being fit for purpose as well as contribute to the dataset surrounding the reference reagents. The expected outcome of the study is improved assay data analysis, improved capacity to compare and contrast different studies in the NHP platform, improved qualification of assay protocols and outcomes, and improved sensitivity and specificity. The expected impact is improved data analysis capability, better understanding of the NHP platform for immunogenicity studies.

b) HIV-1 Envelopes Associated with MTCT (HEAM, 20 access units).

The aim was to study viral envelopes generated from mothers and infant pairs where viral transmission has occurred or not. By comparing such differences the user will be able to map which genotypic differences in viral envelopes that undergo transmission can associate with phenotypes, and specifically neutralisation. The project tested generated viruses for neutralisation with a number of neutralising antibodies obtained. The user utilised a well-established HIV-1 pseudo-typed neutralisation assay using the Tzm-bl cell lines and standard protocols. A number of reference Abs were used to investigate the viral envelopes undergoing HIV-1 MTCT and to improve the design of immunogens aimed at inducing potent neutralising antibodies targeting such viruses. The results from this research will translate into a better understanding of the viral envelopes undergoing HIV-1 MTCT and the improved design of immunogens aimed at inducing potent neutralising Abs aimed at targeting such viruses. The data generated may impact on vaccine strategies aimed at either inducing strong neutralising antibody responses or passive immunisation in HIV-1 pregnant women.

c) Standardisation of CMI assessment by flow cytometry for the development of Adeno-based TB vaccine candidate (CMIforTBAd-vacc, 100 access units).

A clinical trial study is assessing AERAS-402/Crucell Ad35 which comprises a replication-deficient adenovirus serotype 35 (Ad35) that serves as a viral vector—a virus modified to deliver TB genetic material—for DNA-expressing TB antigens 85A, 85B, and 10.4. Adenoviruses can be potent inducers of CD8 T-cell responses, which are considered an important component of immunity to TB and many other infections. The clinical trial is ongoing and peripheral blood lymphocyte samples from the trial participants have been frozen and stored for subsequent assessment of immune responses to the vaccine. For each ICS study (every time the protocol is run on clinical samples) samples of the positive and negative control reference reagents will be included and treated as far as possible exactly the same as the clinical materials. The ICS reference reagents (both positive and negative reference reagents) requested will be used to monitor the performance of the primary assay utilized for assessment of the immunological responses to the candidate TB vaccine, which is a multicolour intracellular flow cytometry staining for cytokine expression. The access to the ICS reference reagents will enable improved comparability and analysis of data

obtained from these cells through the life of the study. This will also ensure improved quality management of the assay process itself through use of the stable reference reagents. The data derived from this study (clinical immunological data, including the use of the ICS reference reagent) will be used to inform clinical trial outcome, and subsequent clinical development plans.

WP15: Project Coordination and Management

Roadmap

In collaboration with European vaccine stakeholders, TRANSVAC Consortium prepared a roadmap aimed at securing sustainable vaccine development infrastructures in Europe. Using a bottom-up approach, the needs and priorities regarding European vaccine R&D were identified through a series of stakeholder consultations and workshops, and translated into a proposal of establishment of a European Vaccine Research & Development Infrastructure (EVRI). European vaccine stakeholders present at the final "validation" Workshop in June 2013 in Brussels, were very supportive to the project of the establishment of EVRI as presented in the roadmap, and many of them expressed interest to join the initiative. It has been acknowledged that EVRI replies on numerous needs of European vaccine developers and has a potential to accelerate the development of new or improved vaccines, and shares the vision of the European research agenda Horizon 2020.

Consortium management tasks and achievements

EVI successfully submitted three amendments to the Description of Work, as well as the three periodic reports, a mid-term-review report and a final report. The MT prepared the different templates and distributed guidelines and instructions to the Consortium members.

Throughout the duration of the project, the MT has been monitoring the project progress by various means, mainly by telephone calls and discussions in meetings and discussions with the work package leaders (WPL) at several international vaccine conferences attended by EVI MT.

More details on the dissemination activities and exploitation of results are given in section 1.4.

Ethical Reviews:

All studies involving animal or human samples have been done in compliance with the relevant Ethics Review/Screening Requirements, as described below:

- BPRC: Experimental work at BPRC and involving NHP was executed in accordance with Dutch law and European directives on animal experiments. Study plan(s) including animal handling were submitted and granted ethical approval by the independent local ethical committee (called 'DEC' for 'Dier Experiment Commissie') prior to the start of the experiments. These ethical approval documents are kept in the BPRC archives and are at disposable to the Coordinator or EC upon request.

For random NHP blood sampling (WP2, task 2.4): advice DEC655, d.d. 1 November 2010.

For BCG vaccination/rhesus spectrotype comparison study (WP2, task 2.4): advice DEC671, d.d. 23 May 2011 (amendments approved on 16 April 2012 and 15 November 2012).

For (TNA) CAFVAC adjuvant comparison study (WP12): advice DEC685, d.d. 13 December 2011 (amendment approved on 20 July 2012).

As a means of ethical reduction by (control) group sharing, the standard BCG treatment group (N=6 animals) and the non-vaccinated controls (N=6 animals) of Chinese type rhesus macaques under WP2 TRANSVAC RTD were shared with another simultaneous experiment, which was executed under the Transnational Access (TNA) instrument PRIMOCID2 of the infrastructure project called EUPRIM-Net-2, under the EC FP7 programme, grant number 262443. Treatment and sampling in the two experiments were synchronised accordingly (The *in vivo* part of these studies ran from July 2012 up until March 2013).

- PHE: All animal experiments were subject to ethical review and approved according to the UK law on animal experimentation. The numbers of the ethical approvals are 30/2704 and 30/2993.
- CVI: All animal experiments were subject to ethical review and approved according to the Dutch law on animal experimentation; documentation is filed under the following CVI approval nrs.: 2010148.c; 2012039.b; 2012113.c; 2012146.b; 2013017.c; 2013075.c.
- HZI: All the experiments performed by the HZI in the context of this project were approved by an independent ethics commission established by the state of Lower Saxony, according to the animal welfare laws of Germany. These permissions are filed under the numbers: AZ: 33.42502/07-04.01;

AZ: 33.42502/07-08.05; AZ: 33.42502/07-05.05; AZ: 33.42502/07-02.03; AZ: 33.42502/07-06.03; AZ: 33.42502/07-08.05; AZ: 33.42502/07-05.05; AZ: 33.42502-084/06.

- LIONEX: The EuroNeut-41 project has received the ethical approval to use the samples (Approved by Health Research Authority, EudraCT number: 2010-023693-39) and patient informed consent documents are available. A Material Transfer Agreement (MTA) has been signed between EuroNeut-41 (Sanofi Pasteur) and LIONEX so that LIONEX can also use the samples.

1.4 The potential impact and the main dissemination activities and exploitation of results

1.4.1 Potential impact

Services

The TRANSVAC services had a very strong impact on both the research activities performed by the selected European users, as well as on the infrastructures of the service providers. The individual impacts on each of the involved infrastructures are detailed below.

- BPRC: Transnational access activities have provided relevant information on the safety and immunogenicity of novel adjuvant modalities in a primate host, which will support further clinical development strategies. Moreover, the TNA activities have allowed BPRC to build further upon its expertise on infectious disease modelling and vaccine (immunogenicity) evaluations, and have allowed leveraging into a more generic approach of preclinical vaccine and adjuvant assessment. From the number of applications and expressed interest it may be concluded that there is substantial demand for (independent) expert NHP facilities for preclinical vaccine research.
  TRANSVAC has facilitated multi-disciplinary approaches and the integration of immunological and clinical vaccine analysis, global transcriptomics, assay/protocol harmonisation and novel assay development, all towards accellerated and more rationalised vaccine R&D. Altogether, TRANSVAC has provided for a most optimal exposure of the NHP models, strengthened BPRC's adjuvant research activities and increased BPRC's visibility as a vaccinology expertise centre.
- Department of Health, PHE: The Health Protection Agency at Porton Down (now Public Health England) provided pre-clinical testing systems to the TRANSVAC project. It is essential to make sure that new vaccines are safe and effective before they are tested in humans and the pre-clinical models at HPA were able to provide information which allowed a University in Spain to choose the best vaccine to take forward to further development. In this way, the HPA, through TRANSVAC was able to help European researchers in their efforts to find new and better vaccines to protect humans against important diseases. In turn, the HPA benefited from the financial contribution of TRANSVAC to help maintain the capability to perform this important work, but more significantly, the HPA gained a wider experience and knowledge of vaccine research from being a part of the TRANSVAC project which strengthened the capabilities of the institution.
- CVI: The core business of CVI in the past was research and diagnostics in the field of veterinary medicine and agricultural science. Through the activities offered during transnational access services, complementary and new information was generated by the use of pig models compared to information from mouse models. Next to this information about working mechanisms of vaccines, immunological methods and expertise were established to be used in the pig, which enables CVI to more actively contribute to question around human vaccine development. The intense collaboration and the demonstration of added value of this farm animal species for vaccine development have resulted in new projects and submissions of two KP7-EU research proposals.
- Department of Health, MHRA-NIBSC: NIBSC provided the service of Stable Assay and Reference Standard Platform (WP14) as part of the TRANSVAC consortium/ project. The use of centralised reference reagents and standards is essential for vaccine research and development, especially in assay development and validation, and to allow cross-comparison of experimental results among research centres and laboratories in clinical studies. Through TRANSVAC transnational access, this service was made available to European researchers to facilitate their work in new vaccines development, including preclinical and clinical studies.
- UNIL: Aim of the WP11 was to support the vaccine community with access to vaccine adjuvants and formulation. The project has thereby addressed the gaps in research projects of users who have the goal to support development of future vaccines within Europe and ultimately contributed to global vaccine product development. Briefly, and on top of providing adjuvant systems to Users of the adjuvant platform, the VFL at UNIL has provided know-how required by the users and helped them to acquire best practices and expertise in the field of adjuvants and

vaccine formulation. The project has also helped the users to further develop themselves in form of academic publications (numerous are in preparation), networks, and acquisition of new projects outside of the scope of TRANSVAC.
The Vaccine Formulation Laboratory has benefited from the project by new collaborations and back-and forth exchange with the users. Thus, the transnational access has gone way beyond the simple provision of adjuvants, but has established long lasting collaborations within the European vaccine community.

- MPIIB: Global transcriptome analyses provide an excellent basis for the identification and definition of biomarkers with relevance during infection, diagnosis and therapy. TRANSVAC has contributed to MPIIB's research towards definition of generic biosignatures that allow assessment of vaccine efficacy. The lack of knowledge about the mechanisms of protection during vaccination still is a major hurdle to improve and accelerate development of novel vaccines and vaccination strategies against diseases, including neglected diseases. MPIIB's activities' results are important to improve explorative studies of candidate vaccines in their early phase of development and for immune monitoring.
- HZI: NGS and/or analysis were provided to external parties selected from TRANSVAC. This infrastructure was very useful for vaccine research in Europe since only very large institutions in a limited number of European countries have access to these technologies. The HZI animal validation platform has provided access to the Swiss company GlycoVaxyn to evaluate the immunogenicity of a glycoprotein vaccine candidate against Streptococcus pneumoniae serotype XX. The results generated will be extremely useful to guide further preclinical development of conjugate vaccines.
- UREG: The Center of Excellence for Fluorescent Bioanalytics (KFB) provided expression profiling using the Affymetrix microarray platform. All TNA projects were finished successfully, and the data was handed over to the access users. Preliminary results indicate that exciting new insights will be gained from the microarray results which will lead to many publications in the coming months and years. In these publications, the invaluable financial and intellectual contribution of the TRANSVAC Transnational Access program will be duly acknowledged.

Integrated approach
Three granted users have made use of different TRANSVAC services and are really good examples of the integrated approach of the project. These users have successfully accomplished the following projects:

- “CD4/8 responses by CAF” (P. Andersen and D. Christensen, Statens Serum Institut (SSI), Copenhagen, Denmark):
  The aim of this project was to assess the role of different adjuvants for inducing both CD4 and CD8 responses for a multistage TB-vaccine. The comparison of the immunogenicity was done by performing studies in Cynomolgus macaques (BPRC), in order to determine whether or not Poly I:C and/or mucosal boosting is necessary in order to obtain a strong CD8, to compare the adjuvants CAF01 vs. CAF09 and the route of administration parenteral vs. Muco-parenteral, and to get preclinical safety data. The users then got granted access to reference reagents (MHRA-NIBSC) with the aim to improve assay data analysis, improve the capacity to compare and contrast different studies in the NHP platform, and to further the processes of qualifying the assay protocols. Finally, a genome-wide transcriptome analysis was performed using Affymetrix microarrays (UREG), in order to document the differential effects of the two adjuvant formulations in a NHP model and to better understand the mechanism of action of the adjuvants. In summary, the TRANSVAC services had an impact on important knowledge on the immunogenicity in more human-like species, the effect of the route of administration, and the safety of novel vaccine adjuvants. These important results generated through the TRANSVAC services will make a strong case in the regulatory dossier (IMPD) for the application to a Phase I clinical trial.
- “FLAGTRIAL” (J.C. Sirard, INSERM, Lille, France):
  The aim of this project was to investigate the relevance of the intradermal route for human vaccination with an antigen combined to the Flagellin adjuvant. The user got first granted access to formulations (UNIL) which were tested *in vivo* in a mice model by the user

himself. The potency of the formulations to increase systemic immune response via the intradermal route in a pig model (Sus scrofa pigs) was investigated in a challenge study conducted at CVI. Next to the animal study also serological and virological analyses were performed at CVI. The dose-effect relationship between the quantities of intradermally administered flagellin in regard to transcriptome expression was analysed with the Agilent microarrays platform (MPIIB). Overall, this study of an appropriate model of human intradermal immunisation would not have been possible in the facilities of the applicant and the overarching TRANSVAC TNA activities on transcriptome analysis, *in vivo* study and immunological assessment were essential for the success of this study.

- "BPZE1-Immuntranscript" and "BPVACtranscriptome" (C. Locht, INSERM, Lille, France): The aim of the first project was to study the immunogenicity of the novel pertussis vaccine BPZE1, a live attenuated nasal vaccine, by performing transcriptional analysis in a mouse model, using Agilent microarrays (MPIIB). The study was then pursued and transcriptional analysis of human subjects (from a phase I trial) nasally vaccinated with BPZE1 was performed using Affymetrix microarrays (UREG) in both responders, non-responders, and in the placebo group. Both microarray platforms had a high impact on the study in order to help identifying the different biological processes and molecular functions of the vaccines, as well as understanding the vaccination kinetics and challenge experiments. The data provided to the users will be very useful to compare the human model with the mouse model, for which similar transcriptomic approaches have been carried out in parallel. The results will have a high impact in the development of a new pertussis vaccine, to be administered at birth in order to provide early protection and prevent severe forms of the disease in newborns.

Integration of Interested Parties providing new services

Due to the high interest of service providers in being a TRANSVAC partner, TRANSVAC launched in Q1 2012 in order to add Interested Parties to the TRANSVAC project. It was decided to integrate Interested Parties that are offering paid services which are not covered under TRANSVAC in order to sustain vaccine research infrastructure in Europe. Since September 2012, the five newly included services are:

- The GMP Pilot Production Plant Service from the Vaccinology Department of the National Institute for Public Health and the Environment (RIVM, The Netherlands).
- The Vaccine Development and Production Service from the Animal Cell Technology Unit of the Institute of Experimental and Technological Biology (IBET, Portugal).
- The MultiBac Platform Service from the European Molecular Biology Laboratory (EMBL, France).
- The Protein and Peptide Chemistry Facility Service from the Department of Biochemistry of the University of Lausanne (UNIL, Switzerland).
- The Reverse Transcription Multiplex Ligation-Dependent Probe Amplification (RT-MLPA) Assay Service from the Department of Infectious Diseases of the Leiden University Medical Centre (LUMC, The Netherlands).

The services provided by the Interested Parties have been integrated in the advertising plan and the TNA call in order for them to extend their network and make their services available on a paid basis to the scientific community. Interested users can get more information on the paid services on the TRANSVAC website and can directly liaise with the service providers until new funding capacity is available within TRANSVAC. An "advisory scientific review process" was also offered to the applicants who wanted to get advice on their project, objectives, and the methodology used. This review process was organised in parallel to the review of the applications to the free-of-charge services. No user requested this free-of-charge review process, probably due to the burden of the forms which had to be filled and the timelines of the process. This "advisory scientific review process" will be designed differently in the future if paid services continue to be provided to users.

Evaluation of the services and call procedures

After the last 1304-10 call, two surveys were performed in order to evaluate the impact of the TRANSVAC TNA on vaccine research and development in Europe, to evaluate the actual needs in

services, to judge the quality of the services provided, and to improve the advertisement, application forms, review process, etc.
The first questionnaire was directed to all applicants of the TRANSVAC TNA calls (rejected and selected applicants) while the second one was directed to the TRANSVAC Consortium members (including the service providers) as well as SAC and USP members. In summary, very positive feedback was received as well as useful suggestions in order to improve the TNA in future projects.

Research and development

Process Development and Adjuvants

Malaria is estimated to kill almost a million people each year, most of these being young children in Africa. Although a huge investment in malaria control is reducing disease incidence, many of the current control measures have a limited life span so that new tools will be required very soon. A malaria vaccine would offer a major boost to these efforts but a vaccine would probably need to outperform current control measures to be cost-effective to deploy.
UOXF is investigating the possibility that combining the two leading approaches to malaria vaccine development could provide a high efficacy cost-effective product: a viral-vectored pre-erythrocytic malaria prime-boost regimen combined with an adjuvanted blood stage malaria protein vaccine. The TRANSVAC project has enabled UOXF to formulate the protein component of their vaccine with a clinically applicable adjuvant and they are now closer to having a new combination vaccine ready to start clinical development and assessment in a phase I/IIa sporozoite challenge study. Improving the only vaccine that has shown efficacy correlated with CD8 T cell induction in humans (Ad- and MVA-ME.TRAP) by partnering it with a potent protein antigen and novel adjuvants has an importance beyond malaria. Vaccines that work via CD8+ T cell immunity would be important for diseases such as HIV, HCV, influenza and many forms of cancer. Some of these diseases will require strong antibody as well as cellular immunity to protect, and the work proposed here on how two major vaccine technologies that induce complementary types of immunity can be successfully combined could be of fundamental importance to vaccinology.
Cell banks of HEK 293 cells, 911 MCB1 cells, Vero (WHO) MCB1 cells, 293 ADH cells, and Procell92S ADH cells have been manufactured by the University of Oxford. These cell banks are not fully certified cGMP banks (this would have required extensive external testing that was not funded by this grant) but they are available to partners for use for making pre-GMP starting materials for vaccines (providing partners have MTAs in place with the originators of the cell lines).

Around two billion people, or one third of the world's population, are estimated to be infected with the Mycobacterium tuberculosis and are at risk of developing the disease. Bacille Calmette-Guérin (BCG), the only available TB vaccine, is administered to over 100 million babies every year and effective in preventing severe forms of TB in children. However, its efficacy against pulmonary TB – the most common form of TB worldwide – is poor or variable and new vaccines are urgently needed. Within TRANSVAC, the project partners have benefited from TBVI's partners and links have been established between TBVI's TB vaccine R&D associated partners and service providers for TB vaccine candidate evaluations and development. R&D activities focussed on TB have been performed within the TRANSVAC project and were focussed on the establishment of reference reagents/standards, adjuvants and vaccine candidates. Moreover, VPM's extensive experience of project management and its guidance and advice on aspects related to accredited cGMP laboratories were used in the validation programme of the qualification of TB-related assays.
NIBSC-MHRA has benefited from the financial support of TRANSVAC to generate a few more new reference reagents/ standards (including flow cytometry reference human PBMC positive and negative standards, purified Ag85A (TB antigen), and anti-malaria (Plasmodium falciparum) human serum standard) which are continuously made available to European researchers during and after the life of the TRANSVAC project, for use in vaccine research and development. Importantly, a Biological Standard report for the Anti-malaria (*Plasmodium falciparum*) human serum standard was submitted to WHO Expert Committee on Biological Standardisation (ECBS) for approval. This reference standard is ready for distribution and will be available as an NIBSC reference reagent until it is accepted as WHO International standard of anti-malaria (Plasmodium falciparum) human serum.

In addition, the scientists from NIBSC-MHRA have gained a wider experience and knowledge of vaccine research and development from being a part of the TRANSVAC project which strengthened the capabilities of the institute.
The adjuvants activities performed in TRANSVAC (WP1) have generated SOPs and prototype vaccines for preclinical assessments and regulatory support, and will provide guidance to vaccine developers.
LIONEX has produced and provided vaccine candidates relevant to TB and Malaria to European as well as other researchers from several countries engaged in vaccine development. This activity shall continue even after TRANSVAC is completed. Such activities do have a strong societal impact since these contribute to solving the major health problems caused by TB and Malaria. LIONEX has benefited strongly from TRANSVAC by expanding its areas of research whereby the LIONEX scientists have established further international contacts and exchange of scientific information. A major benefit has also been in the area of learning and finally mastering the GMP documentation and process development. Now, LIONEX is in an excellent position to be engaged in activities concerning near GMP vaccine research.

Finally, TRANSVAC took part in HIV R&D activities through LIONEX, which provided expression analysis from blood samples from an HIV clinical trial (in collaboration with the FP7-funded EuroNeut-41 project), as described in the *Global Analysis* section below.

Animal studies
It is expected that the development and improvement of the conventional and advanced animal models will: 1) facilitate the screening, selection and prioritisation of different vaccine candidates/formulations, 2) lead to better characterisation of the robustness, intrinsic value and functionality of vaccine technologies, such as adjuvant candidates and antigen delivery systems, 3) advance the understanding of the role of different immune cells in elicitation of protective immune responses following vaccination, 4) allow investigation of the efficacy of new vaccination strategies, and 5) lead to a more reliable predictability of vaccine candidate efficacy in the human system. Furthermore, the advancement of our multi-parametric flow cytometric readout systems will allow collecting a wealth of data which in turn will facilitate the evaluation of the impact of diverse immune cell subsets on achieving efficient immunity.
NHP model activities and the comprehensive demonstration of differential immune response profiles amongst different primate host populations (cynomolgus, rhesus, rhesus spectrotypes and human responses) will impact on preclinical NHP study design and contribute to optimal selection and stratification, which in turn will have beneficial effects on refinement and reduction of the use of NHP in vaccine research.
TRANSVAC created an excellent opportunity to link the experience and knowledge in immunology and physiology of farm animal species and veterinary vaccine development with needs and gaps in human vaccine development. The research activity at CVI in regard to influenza vaccines and especially the use of very young animals as models for vaccination in baby and toddler age offer new opportunities to address efficacy and safety of human, but also of veterinary vaccines. The developed new networks through collaborations in research and transnational service activities, for example by addressing the pandemic H1N1 influenza model have also stimulated an awareness and possible future collaborations on zoonotic, one-health issues.

Global Analysis
Based on microarray analyses and NGS, HZI, MPIIB and LIONEX have provided expression analysis from blood samples from an HIV clinical trial (in collaboration with the FP7 funded EuroNeut-41 project), and from transcriptomes of cells from BCG vaccinated and non-vaccinated groups obtained at different time intervals. Expression analysis generated from samples obtained from these vaccine trials should provide the basis for identification of biomarkers of protective immunity.
The SNP analysis of rhesus and cynomolgus monkeys yields information on species-specific response patterns relating to (cell-mediated) immunity which can have a high impact on other studies using these animal models, and when planning pre-clinical studies in NHP.

Harmonisation

TRANSVAC has facilitated links between TB and malaria assay harmonisation communities.
Three institutes (LSHTM, UOXF and NIBSC) have performed, in WP4, a series of experimental comparisons designed to optimise and ultimately harmonise protocols for three assays designed to assess the T-cell immune responses induced by vaccination. The 3-centre approach has allowed the determination of factors that are essential to address in protocol design when considering the transfer of assay technology from a specialist lab to a less experienced one. Three optimised assay SOPs have been produced and a manuscript describing the major contributors to variability in assay performance is in preparation.
LSHTM has also participated in proficiency panels for aspects of ELISpot and intracellular cytokine staining (ICS) T-cell assay performance, run by the Cancer Immunotherapy Consortium. This has allowed LSHTM to increase their experience of assay performance in the context of a much larger panel of laboratories than was possible within TRANSVAC and the knowledge gained regarding such things as common flow cytometry gating strategies and ELISpot reader settings has fed back into the TRANSVAC effort.
In order to monitor the effectiveness of the knowledge gained from the optimisation of assays, changes to protocols determined via the TRANSVAC harmonisation process have been incorporated into the studies of the infants' immune responses to BCG in the UK. For example, TRANSVAC has been responsible for the optimisation of the LSHTM approach to gating of flow cytometry data derived from these infant studies. Also, the sharing of ICS assay protocols with UOXF during TRANSVAC has resulted in a much improved detection of cytokine responses in infant cells following BCG vaccination. The experience of the improved performance of assays for infant T-cell responses has therefore directly impacted upon the success of the TRANSVAC project. Finally, with a view to optimisation of additional assays for T-cell cytokine responses, LSTMH has been able to monitor the performance of the multiplex cytokine detection assay performed on the Luminex platform. TRANSVAC has allowed an assessment of the robustness of this assay as compared to the single cytokine ELISA assay that it may now replace due to its increased potential for the detection of cytokine biosignatures.
Overall, these studies have generated very valuable insights into these core assays for the assessment of vaccine immunogenicity that will contribute to the development of more robust harmonised assays for use in vaccine studies in the future, and with the potential for further cross-disease harmonisation in future consortia. The standardisation of these assays, together with data on reproducibility and robustness across different laboratories, is indeed essential as these assays are used to compare between different vaccine candidates for TB, malaria and HIV. The work which has been conducted in WP4 is thus of important use to groups developing vaccines for TB, malaria and HIV.

Knowledge sharing and networking
The www.transvac.eu website and the TRANSVAC roadmap (described below) will be the main source of information regarding the results generated through TRANSVAC. They will contribute to strengthen vaccinologist networks by providing SOPs, standard reagents, cell lines and antigens generated in TRANSVAC to the research community. Moreover, the services provided by TRANSVAC will continue to be listed on the TRANSVAC website and will be accessible to researchers in academia and SMEs on a paid basis.

Courses
The two TRANSVAC training courses on "Practical approaches to vaccine development" have contributed to increase excellence of European vaccine researchers. By training extensively 30 people working in vaccine development in various institutions and countries, knowledge was distributed throughout Europe. Feedback from the participants gives evidence that the knowledge obtained during the courses was further transferred to their colleagues. Participants as well as other people from their institutions can apply the know-how from the courses directly in their daily work. Furthermore, the courses gave the unique opportunity of strengthening existing links and building new networks within the vaccine community. This was of great value not only for the participants, but also for the speakers and for the VFL at UNIL. Several new partnerships and collaborations (between speakers and VFL, speakers and participants, participants and participants, participants and VFL) have been discussed since the courses. Some of them have already started and more are to be expected in the near future. Besides the networking, the TRANSVAC courses reinforced the

position of the Vaccine Formulation Laboratory in the coordination and management of advanced courses in vaccinology in Europe. TRANSVAC will continue to advert on its website any further advanced course in vaccinology organised for European participants.

Workshops
Ten workshops have also held on subjects as diverse as statistical analyses, animal models, global analyses, and discussion to strengthen collaborations between European vaccine Research and Development (R&D) groups, assay developers, and vaccine producers. These successful workshops helped scientists in their daily research activities and strengthened their networks.

Networking
Networking activities have taken place between the partners which work together on different projects, e.g. for the global analysis studies of vaccine studies involving animal models as well as formulation and/or reference reagents. TBVI was also able to bring in TRANSVAC a number of its associated partners with specific expertise, like for example the standardised preclinical animal models for vaccine evaluations. Moreover, TRANSVAC partners have also exchanged, disseminated and promoted each other's activities through their websites and through mutual participation in annual and project meetings. TRANSVAC has thus facilitated and strengthened the interactions between its partners and additional stakeholders in vaccinology.
In order to joint efforts for the development of a future program dedicated to the delivery of vaccine R&D services, a Memorandum of Understanding was signed on 1st October 2013 with the Sclavo Association, the coordinator of the FP7-funded ADITEC program. Discussions have taken place between the TRANSVAC and ADITEC coordinators to avoid duplication and to share information.
A preliminary proposal of setting up of an integrated European Infrastructure for vaccine R&D, co-signed by TRANSVAC and European Advanced Translational Research InfraStructure in Medicine (EATRIS), have been submitted to EC (DG Research Innovation: Unit B3), in October 2012 in a reply to a consultation on possible topics for future activities for integrating activities and opening national research infrastructures. The proposal has been classified by EC as topic with high potential and with merit for further Horizon 2020 actions (results published in March 2013).

Discussion with EATRIS have led to their involvement in the development of the roadmap. Because of the legal status of EATRIS was pending, preliminary negociations have taken place in order to set up a closer collaboration. EVI being an EEIG could not be a partner of EATRIS. A memorandum of understanding has been drafted and should be endorsed by the governance of both organisations.

Roadmap for a European Vaccine Research and Development Infrastructure
In collaboration with European vaccine stakeholders, TRANSVAC Consortium prepared a roadmap aimed at securing sustainable vaccine development infrastructures in Europe. Using a bottom-up approach, the needs and priorities regarding European vaccine R&D were identified through a series of stakeholder consultations and workshops, and translated into a proposal of establishment of a European Vaccine Research & Development Infrastructure (EVRI).
The advanced draft of a roadmap was presented and discussed at TRANSVAC annual meeting in March 2013, and then circulated for comments and validation amongst European stakeholders. Overall, the feedback received from the consultation process was very positive. In addition, a detailed questionnaire comprised in this first round of consultation process confirmed high priority level, as perceived by European vaccine stakeholders, for activities and services proposed.
Finally the proposal for a European Infrastructure for vaccine R&D has been discussed and validated during a workshop held in June 2013, by 70 representatives of vaccine developers and manufacturers (academic researchers, biotech companies, large vaccine development companies, PDPs and other European vaccine-related projects), regulatory authorities, international and national policy makers, and funding agencies. European vaccine stakeholders present at the Workshop were very supportive to the project of the establishment of EVRI as presented in the roadmap, and many of them expressed interest to join the initiative. It has been acknowledged that EVRI replies on numerous needs of European vaccine developers and has a potential to accelerate the development of new or improved vaccines, and shares the vision of the European research agenda Horizon 2020.

The roadmap is now finalised and will be available in December 2013 on the TRANSVAC website. Paper versions of the roadmap will be sent to relevant governmental bodies. The roadmap provides national and European policymakers and funders with valuable outline on current situation, and with a blueprint for the establishment of a European Vaccine R&D Infrastructure (EVRI) which is expected to fill the gaps and foster innovation in vaccine R&D, maximising public health and socio-economic impacts.

1.4.2 Dissemination activities

The publications and other dissemination activities are listed in section 2. The most recent results of the RTD activities will be disseminated by aiming at publication in international peer-reviewed journals. Manuscripts will be drafted in the coming months and dissemination will be an activity exceeding beyond the lifetime of the programme.

Within the last four years, the TRANSVAC project dissemination activities were almost always connected with the advertisement of the TRANSVAC call for TNA. A great variety of actions have been undertaken by the MT in order to advertise and publicise the TNA activities of the project (as detailed in Table A.2). These include: targeted mailings (including National Contact Points Technology Transfer and International Grants Offices), publication of the advert on specific websites, inclusion of the ad in newsletters by relevant institutions, presentation of the project and its TNA activities at scientific conferences and congresses as well as with official authorities; drafting and distributing a press release, as well as placing the advertisement in relevant scientific journals. The project partners have activated and contacted their own networks and spread information on TRANSVAC via those. They mentioned the support of the project and the EC in their scientific publications. The TRANSVAC website (www.transvac.org) is the project's main presentation. The project has also produced flyers, a project summary sheet, posters and a standard project presentation in order to advertise on-going activities. The project also received great visibility during the stakeholder meetings and the different workshops organised via TRANSVAC.

Although valorisation results from the Consortium are difficult to assess at this time, TRANSVAC will help partners to protect and exploit their results as well as helping them to translate them into potential industrial products. For instance the on-going production of cell lines for the production of vaccines at the UOXF is an example which should enable further implementation of technology and materials developed by the consortium. Furthermore, standards which are developed within the consortium will contribute to national or international standards and be maintained by the NIBSC. Antigens and other recombinant proteins from the LIO facility developed according to user requirements will continue to be available for external users engaged in research activities concerning vaccine development. Finally, the huge efforts put in the development of formulations at the UNIL will be pursued to help researchers to further develop vaccines.

1.4 List of beneficiaries and project website

Beneficiary number	Beneficiary name	Beneficiary short name	Country	Contact persons
1	European Vaccine Initiative	EVI	Germany	Odile Leroy odile.leroy@euvaccine.eu
2	Biomedical Primate Research Centre	BPRC	The Netherlands	Ed Remarque remarque@bprc.nl
3	Helmholtz Zentrum für Infektionsforschung GmbH	HZI	Germany	Carlos Guzman cag@helmholtz-hzi.de
4	Vakzine Projekt Management GmbH	VPM	Germany	Leander Grode grode@vakzine-manager.de
5	LIONEX GmbH	LIONEX	Germany	Mahavir Singh info@LIONEX.de
8	Max Planck Society / Max Planck Institute for Infection Biology	MPIIB	Germany	Stefan Kaufmann kaufmann@mpiib-berlin.mpg.de
9	University of Regensburg	UREG	Germany	Thomas Stempfl thomas.stempfl@exfor.uni-regensburg.de
11	London School of Hygiene and Tropical Medicine	LSHTM	United Kingdom	Hazel Dockrell hazel.dockrell@lshtm.ac.uk
12	University of Oxford	UOXF	United Kingdom	Adrian Hill adrian.hill@ndm.ox.ac.uk
13	University of Lausanne	UNIL	Switzerland	Nicolas Collin nicolas.collin@unil.ch
14	Tuberculosis Vaccine Initiative	TBVI	The Netherlands	Jelle Thole Jelle.Thole@tbvi.eu
15	Stichting Dienst Landbouwkundig Onderzoek	CVI	The Netherlands	Jan Langermans langermans@bprc.nl
16	Department of Health	DH	United Kingdom	Mei Mei Ho mei.ho@nibsc.org

For more information about the TRANSVAC project, please visit the website www.transvac.org or contact transvacinfo@euvaccine.eu.

2 Use and dissemination of the foreground

2.1 Section A - Public

Table A.1 List of all scientific (peer reviewed) publications relating to the foreground of the project.

LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers[2] (if available)**	**Is/Will open access[3] provided to this publication?**
1	TRANSVAC Research Infrastructure – Results and Lessons Learned from the European Network of Vaccine Research and Development	M. Geels	Vaccine	To be submitted in December 2013	Elsevier					yes
2	TRANSVAC workshop on standardisation and harmonisation of analysis platforms for HIV, TB and malaria vaccines. 'How can big data help?'	C. Dutruel	Vaccine	To be submitted in December 2013	Elsevier					yes
3	A Roadmap for a European Vaccine Research and Development Infrastructure	S. Jungbluth	Vaccine	To be submitted in December 2013	Elsevier					yes

[2] A permanent identifier should be a persistent link to the published version (full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).
[3] Open Access is defined as free of charge access for anyone via the internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers[2] (if available)**	**Is/Will open access[3] provided to this publication?**
4	TRANSVAC: European Network of Vaccine Research and Development	O. Leroy	Human Vaccines and Immunotherapeutics	7(7)	Landes Bioscience	DE	2011	715	DOI: 10.4161/hv.7.7.17072	yes
5	The Vaccine Formulation Laboratory: A platform for access to adjuvants	N. Collin	Vaccine	29(S1)	Elsevier	CH	2011	A37-A39	http://dx.doi.org/10.1016/j.vaccine.2011.04.125	yes
6	Bis-(3',5')-cyclic dimeric adenosine monophosphate: Strong Th1/Th2/Th17 promoting mucosal adjuvant,	T. Ebensen	Vaccine	29 (32)	Elsevier	NL	2011	5210-5220	http://www.sciencedirect.com/science/article/pii/S0264410X11007365	yes
7	Cyclic di-nucleotides: new era for small molecules as adjuvants	R. Libanova	Microbial Biotechnology	5(2)	Wiley-Blackwell	USA	2012	168-176	http://onlinelibrary.wiley.com/doi/10.1111/j.1751-7915.2011.00306.x/abstract	yes
8	Vaccines: from Empirical Development to Rational Design	C. Rueckert	PLoS Pathogens	8(11)	PLOS	USA	2012	1-7	http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003001	yes

LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers[2] (if available)**	**Is/Will open access[3] provided to this publication?**
9	Vaccine adjuvants: key tools for innovative vaccine design	P. Riese	Curr Top Med Chem.	13(20)	Bentham Science	NL	2013	2562-80	http://www.ncbi.nlm.nih.gov/pubmed/24066891	no
10	Enabling biomarkers for tuberculosis control	J. Maertzdorf	Int J Tuberc Lung Dis	16(9)	The Union	DE	2012	1140-8	http://dx.doi.org/10.5588/ijtld.12.0246	yes
11	Safety and immunogenicity of the recombinant BCG vaccine VPM1002 against tuberculosis in a phase I open-label randomized clinical trial	L. Grode	Vaccine	31	Elsevier	DE	2013	1340-48	http://dx.doi.org/10.1016/j.vaccine.2012.12.053	yes
12	Targeted nasal vaccination provides antibody independent protection against Staphylococcus aureus.	K. Misstear, E.C. Lavelle	The Journal of Infectious Diseases	In press	Oxford Journals	IE	2013	In press	Not yet available	yes
13	Comparison of three assays measuring cell mediated immunity across three different laboratories and standardisation of important laboratory parameters.	NIBSC, UOXF and LSHTM		To be submitted early 2014						

Table A.2 List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities**[4]	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience**[5]	**Size of audience**	**Countries addressed**
1	Presentation at Conference	C. Dutruel	*TRANSVAC: European Network of Vaccine Research and Development* presented at 7th Vaccine and ISV Congress	27-29 October 2013	Barcelona, Spain	Scientific community, Industry	~300	Global
2	Round table discussion	O. Leroy	*TRANSVAC Roadmap* discussed at 14th World Vaccine Congress	16-17 October 2013	Lille, France	Scientific community, Industry	~300	Global
3	Presentation at Conference	F. Verreck	*Overview of the rhesus spectrotype comparison (WP2) NHP in vivo study results on TB disease susceptibility and BCG protective efficacy* presented at AERAS Workshop on 'NHP TB Model Development'	3 October 2013	Rockville, USA	Scientific community, Industry	~100	Global
4	Presentation at Conference	S.Weißman	*Novel candidate adjuvants for tailoring immune responses*	25-27 September 2013	Copenhagen, Denmark	Scientific Community	86	Global
5	Workshop	J. Langermans	*The pig as model in human vaccine development*	20 September 2013	CVI, Lelystad, The Netherlands	Scientific community, Industry	55	Global
6	Presentation at Conference	H. Dockrell	*What would make a good biomarker for susceptibility of protection against TB?* presented at Conference/Meeting – "TB Or Not TB"	19 September 2013	Borstel, Germany	Research	100	EU
7	Websites	NIBSC, LSHTM, UoX	Human PBMC cytokine enzyme-linked immunosorbent (ELISA) assay protocol to measure antigen-specific IFNg expression	July 2013	-	Scientific community, Industry		UK

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
8	Websites	NIBSC, LSHTM, UoX	Human PBMC enzyme-linked immunospot (ELISpot) assay protocol to measure antigen-specific IFNg expression	July 2013	-	Scientific community, Industry		UK
9	Websites	NIBSC, LSHTM, UoX	Human PBMC ntracellular cytokine staining and flow cytometry (ICS) assay protocol to measure antigen-specific IFNg expression	July 2013	-	Scientific community, Industry		UK
10	Thesis	S. Bibi (Aston University)	*The formulation of artificial reference standards for use within the ELISPOT assay*	June 2013	Birmingham, UK	Scientific community		Global
11	Workshop	O. Leroy	TRANSVAC Stakeholder Meeting	20 June 2013	Brussels, Belgium	Scientific community, Industry, Policy makers, Regulatory authorities	70	Global
12	Exhibition/ Presentation	MPIIB	Open doors day	8 June 2013	Berlin, Germany	Civil Society, Media	1,500	DE
13	Workshop	N. Stockhofe	*Data reduction methods for analyses of animal studies with small experimental groups and multiple parameter measurements*	6 June 2013	Amsterdam, The Netherlands	Scientific community, Industry	13	UK, NL, DE
14	Presentation at Conference	C. Barnier-Quer	*Basic and Clinical Research on Administration Routes of Vaccines* presented at Corevac Meeting, Institut Pasteur	30 May 2013	Paris, France	Scientific community	150	FR
15	Presentation at Conference	F. Verreck	*Overview of major findings of the WP12 TNA NHP Study with SSI* presented at 9th Elsinore Meeting in Infection Immunity on 'Translating Breakthroughs in Immunology into Vaccines of Global Importance'	29– 31 May 2013	Helsingør, Denmark	Scientific community	~300	EU

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
16	Presentation at Conference	N. Collin	*Addressing the challenge of access to adjuvants* presented at the Modern Vaccine Adjuvants Formulation meeting (MVAF)	15-17 May 2013	Lausanne, Switzerland	Scientific community	100	Global
17	Presentation at Conference	M. Geels	*TRANSVAC: European Network of Vaccine Research and Development* presented at 3rd Malaria Vaccines for the World Conference	22-24 April 2013	Lausanne, Switzerland	Scientific community	~300	Global
18	Presentation at Conference	P. Dubois	*Formulation development at the Vaccine Formulation Laboratory* presented at Canadian Adjuvant Workshop	26 March 2013	Ottawa, Canada	Scientific community, Industry, Policy makers	50	CA
19	Presentation at Conference	S. Smith	*Cytokine expression profile of Mycobacterium tuberculosis PPD-specific CD4+ T-cells that are detectable 3 and 12 months following BCG vaccination of UK infants* presented at TB Vaccines Global Forum	24 March 2013	Cape Town, South Africa	Scientific community	200	Global
20	Course	O. Leroy	*Practical approaches to vaccine development*	11-15 March 2013	Lausanne, Switzerland	Scientific community	15	EU
21	Workshop	J. Thole	*TRANSVAC Workshop on Global Analysis Platforms for HIV, TB and Malaria*	6 March 2013	Heidelberg, Germany	Scientific community	24	DE, CH, NL, UK, IT, USA
22	Conference	S. Jungbluth	*TRANSVAC: European Network of Vaccine Research and Development* presented at GSK Vaccines Business Development Forum	1 March 2013	Brussels, Belgium	Industry	40	Global
23	Presentation at Conference	N. Collin	*The Vaccine Formulation Laboratory: a platform to facilitate access to vaccine adjuvants & formulation services* presented at EVI 15th Anniversary Symposium	26 February 2013	Heidelberg, Germany	Scientific community	60	EU

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
24	Presentation at Symposium	O. Leroy	*Developments in vaccines for infectious disease* presented at symposium "Emerging and re-emerging infectious diseases: how prepared are we for the global threat?."	2 March 2013	Munich. Germany	Scientific community	50	EU
25	Flyer	O. Leroy	*TRANSVAC New vaccines faster* Updated version of the flyer included the achievements until January 2013	February 2013		Global		Global
26	Presentation at Conference	F. Verreck	*Overview of major findings of the WP12 TNA NHP Study with SSI* presented at the NEWTBVAC Annual Meeting (FP7 Consortium)	29 January – 1 February 2013	Les Diablerets, Switzerland	Scientific community	50	EU
27	Presentation at Conference	N. Collin	*Adjuvant: Formulation work, training and tools* presented at EVI Rendez-Vous 2012	6 December 2012	Heidelberg, Germany	Scientific community	90	EU
28	Press release		*The Transnational Access Network TRANSVAC integrates New Interested Parties* via AlphaGalileo	November 2012	AlphaGalileo	Global		Global
29	Press release		*The Transnational Access Network TRANSVAC integrates New Interested Parties* on EVI website	November 2012	EVI website	Global		Global
30	Press release		*EC FP7 TRANSVAC: New Vaccines Faster – now with MultiBac!* on Complexinc.eu website	November 2012	Complexinc.eu	Global		Global
31	Presentation at Conference	H. Dockrell	*Can biomarkers help us control TB?* presented at research seminar at Imperial College	29 October 2012	London, UK	Scientific community	50	UK
32	Presentation at Conference	O. Leroy	*Possible Implications of a Convention for R&D in the European Context* presented at World Health Summit (session "Do We Need a Global Convention for Research & Development?")	21 – 24 October 2012	Berlin, Germany	Scientific community, Industry, Policy makers	~300	Global
33	Presentation at Conference	H. Dockrell	*Clinical biomarkers – research studies and vaccine trials* presented at W.H.O. Meeting - Potency Testing Of New TB Vaccines	2 October 2012	Geneva, Switzerland	Scientific community, Policy makers	30	EU

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
34	Newsletter		*Advert of TNA calls* in Nationalen Kontaktstelle Lebenswissenschaften Newsletter	October 2012		Scientific community, Industry		DE
35	Presentation at Conference	S. Kaufmann	*Bridging Health Gaps with Vaccines* presented at The World Health Summit at Charité Hospital	Oct 2012	Berlin, Germany	All, focus on policy makers and media	1,000	Global
36	Presentation at Conference	J. Heeney	*TRANSVAC and its role for One Health* presented at World Vaccine Congress	17-18 October 2012	Lyon, France	Scientific community, Industry	~300	Global
37	Presentation at Conference	H. Dockrell	*Can biomarkers help us eradicate TB?* presented at 1st Symposium TB Watch	27 September 2012	Barcelona, Spain	Scientific community	100	ES
38	Course	O. Leroy	*Practical approaches to vaccine development*	3-6 September 2012	Lausanne, Switzerland	Scientific community	15	EU
39	Session and Presentation at Conference	O. Leroy	*Session: Tomorrow's vaccines today and presentation: Vaccine Research Infrastructures* at Euroscience Open Forum 2012	11-15 July 2012	Dublin, Ireland	Scientific community, Industry	~300	Global
40	Presentation at Conference	N. Collin	*TRANSVAC Adjuvant Programme & Vaccine Formulation Services at UNIL* presented at Meeting in Vaccine Adjuvants and Delivery Systems	6 July 2012	Copenhagen, Denmark	Scientific community	100	Global
41	Newsletter		*Advert of TNA calls* in National Contact Point Spain Website and Newsletter	July 2012		Scientific community, Industry		ES
42	Website		*Advert of TNA calls* in National Contact Point Spain Website and Newsletter	July 2012		Scientific community, Industry		ES

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
43	Web application		*Advert of TNA calls* in Centro para el Desarrollo Tecnológico Industrial Tweeter alert	July 2012		Scientific community, Industry		ES
44	Web application		*Advert of TNA calls* in National Contact Point Health Lithuania Tweeter alert	July 2012		Scientific community, Industry		LT
45	Website		*Advert of TNA calls* on SMEs go Health / Fit for Health website	July 2012		Scientific community, Industry		Global
46	Workshop	O. Leroy	TRANSVAC Stakeholder Meeting	13 June 2012	Brussels, Belgium	Scientific community, Industry, Policy makers, Regulatory authorities	46	EU
47	Article published in the popular press	M. Kotsyfakis	*Bio-tick-nology* published in The Parliament Magazine, 351, p.79	June 2012	The Parliament Magazine	Civil society		Global
48	Presentation at Conference	H. Dockrell	*Towards correlates of protection for TB vaccine trials* presented at Conference TB Vaccines for the World	24 May 2012	Orlando, USA	Scientific community	300	Global
49	Poster	R. Thoegersen	*TRANSVAC: European Network of Vaccine Research and Development* presented at 8th Annual BioMalPar Conference	May 2012	Heidelberg, Germany	Scientific community	~300	Global
50	Website		*Advert of TNA calls* on Cordis Infrastructure News website	April 2012		Scientific community, Industry		Global
51	Web application		*Advert of TNA calls* in Centro para el Desarrollo Tecnológico Industrial Tweeter alert	April 2012		Scientific community, Industry		EU

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
52	Web application		*Advert of TNA calls* in National Contact Point Health Lithuania Tweeter alert	April 2012		Scientific community, Industry		LT
53	Website		*Advert of TNA calls* on SMEs go Health / Fit for Health website	April 2012		Scientific community, Industry		Global
54	Presentation at Conference	H. Dockrell	Biomarkers of protection and of response to therapy presented at TB Centre Meeting at LSHTM	26 March 2012	London, UK	Scientific community	80	UK
55	Presentation at Conference	M. Geels	*TRANSVAC: European Network of Vaccine Research and Development* presented at Meeting of the German Societies for Parasitology/Tropical Medicine and International Health	14-17 March 2012	Heidelberg, Germany	Scientific community	~300	Global
56	Presentation at Conference	M. Geels	*How is the European Vaccine Initiative addressing the global need for new vaccines?* presented at Phacilitate Vaccine Forum 2012, 9th North American Vaccine Forum	30 January - 1 February 2012	Washington, United States of America	Scientific community, Industry, Policy makers	~300	Global
57	Presentation at Conference	M. Geels	*From technical development of Malaria Vaccines to other diseases of poverty vaccines* presented at Vaccines Europe 2011	30 November - 1 December 2011	Brussels, Belgium	Scientific community, Industry	~300	Global
58	Presentation at Conference	N. Collin	*Enhancing workforce capacity in Developing Countries through training* presented at WHO workshop on enhancing the global workforce for vaccine manufacturing	30 November - 2 December 2011	Cape Town, South Africa	Scientific community, Policy makers	200	World
59	Presentation at Conference	S. Kaufmann, H-J. Mollenkopf	*Transcriptomics of M. tuberculosis Infected Cells: Cytochalasin D experiments* (incl. Services description of MPIIB CF Microarrays) presented at NIH TBSysBio and 3rd Annual Systems Biology Programmatic and SBWG Meeting	5-10 November 2011	Seattle, U.S.A	Scientific community	50	Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
60	Workshop	S. Sharpe	*Linear mixed effects models*	31 October & 1 November 2011	HPA, Porton Down, UK	Scientific community	15	UK, NL
61	Presentation at Conference	M. Geels	*How to strengthen the vaccine research infrastructure in Europe? The role of TRANSVAC* (http://youtu.be/Nl8Kab9aIjI) presented at World Vaccine Congress	10-13 October 2011	Lyon, France	Scientific community, Industry	~300	Global
62	Workshop	O. Leroy	TRANSVAC Stakeholder Meeting	7 October 2011	Brussels, Belgium	Scientific community, Industry	40	EU
63	Conference	Charité Hospital	The World Health Summit	October 2011	Berlin	All, focus on policy makers and media	1,000	Global
64	Newsletter		*Advert of TNA calls* in EFIS (the European Federation of Immunological Societies) Newsletter	July 2011		Scientific community, Industry		Global
65	Newsletter		*Advert of TNA calls* in National Contact Point Germany Newsletter	July 2011		Scientific community, Industry		DE
66	Round table discussion	O. Leroy	*The translation of basic research in microbiology into its clinical application and the ways how the European community funding structures can support such activities* discussed at 4th Congress of the Federation of European Microbiological Societies (FEMS)	26-30 June 2011	Geneva, Switzerland	Scientific community	~300	Global
67	Press release		*Advert of TNA calls* on Cordis Infrastructure News website	June 2011		Global		Global
68	Website		*Advert of TNA calls* on NCP Health Taiwan	June 2011		Scientific community, Industry		TW

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
69	Website		*Advert of TNA calls* on NCP Health Malta	June 2011		Scientific community, Industry		MT
70	Website		*Advert of TNA calls* on Provaxs Uni Gent	June 2011		Scientific community, Industry		BE
71	Newsletter		*Advert of TNA calls* in National Contact Point Czech Republic Newsletter	June 2011		Scientific community, Industry		CZ
72	Newsletter		*Advert of TNA calls* in National Contact Point Greece Newsletter	June 2011		Scientific community, Industry		GR
73	Newsletter		*Advert of TNA calls* in University of Hannover Grants Newsletter	June 2011		Scientific community, Industry		DE
74	Newsletter		*Advert of TNA calls* in University of Braunschweig Grants Newsletter	June 2011		Scientific community, Industry		DE
75	Newsletter		*Advert of TNA calls* in Universtiy of Duisburg-Essen Grants Newsletter	June 2011		Scientific community, Industry		DE
76	Newsletter		*Advert of TNA calls* in Jagiellonian University Krakow Grants Newsletter	June 2011		Scientific community, Industry		PL
77	Exhibition/ Presentation	MPIIB	Open doors day	28 May 2011	Berlin, Germany	Civil Society, Media	1,500	DE
78	Poster	M. Geels	*TRANSVAC: European Network of Vaccine Research and Development* presented at 7th Annual BioMalPar Conference: Biology and Pathology of the Malaria Parasite	16-18 May 2011	Heidelberg, Germany	Scientific community	~300	Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
79	Workshop	J. Langermans	*From bench to trench? Necessity of animal models in pre-clinical vaccine research: required evidence for efficacy and safety*	12 May 2011	CVI, Lelystad, The Netherlands	Scientific community, Industry	26	UK, DE, NL
80	Newsletter		*Advert of TNA calls* in Immunology Newsletter by EFIS	May 2011		Scientific community, Industry		Global
81	Press release		*TRANSVAC: The European Commission's new initiative to boost vaccine innovation* on EVI website	May 2011	EVI website	Global		Global
82	Press release		*TRANSVAC European Network of Vaccine Research and Development* on website of the UniversitätsKlinikum Heidelberg	April 2011		Scientific community		DE
83	Newsletter		*Advert of TNA calls* in National Contact Point Germany Newsletter	April 2011		Scientific community, Industry		DE
84	Newsletter		*Advert of TNA calls* in National Contact Point Austria Newsletter	April 2011		Scientific community, Industry		AU
85	Newsletter		*Advert of TNA calls* in EFIS (the European Federation of Immunological Societies) Newsletter	April 2011		Scientific community, Industry		Global
86	Website		*Advert of TNA calls* on PLoS Pathogens	April 2011		Scientific community, Industry		Global
87	Website		*Advert of TNA calls* on PLoS Neglected Diseases	April 2011		Scientific community, Industry		Global
88	Website		*Advert of TNA calls* on CIBERES Website	April 2011		Scientific community, Industry		Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
89	Website		*Advert of TNA calls* on Dutch Immunology website	April 2011		Scientific community, Industry		NL
90	Website		*Advert of TNA calls* on EC Infrastructures website	April 2011		Scientific community, Industry		Global
91	Website		*Advert of TNA calls* on EC Innovation and Research News website	April 2011		Scientific community, Industry		Global
92	Website		*Advert of TNA calls* on Innovation Union Facebook website	April 2011		Scientific community, Industry		Global
93	Website		*Advert of TNA calls* on Norwegian Forum for Global Health website	April 2011		Scientific community, Industry		NO
94	Poster	M. Geels	*TRANSVAC: European Network of Vaccine Research and Development* presented at EC conference, Innovations in Health Care	30-31 March 2011	Brussels, Belgium	Scientific community, Industry, Policy makers	~300	Global
95	Meeting	O. Leroy	*TRANSVAC: European Network of Vaccine Research and Development* discussed at German Federal Ministry of Education and Research	22 March 2011	Bonn, Germany	Policy makers	10	DE
96	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at Vaccines against AIDS, TB & Malaria Symposium	15 March 2011	Paris, France	Scientific community, Industry	~300	Global
97	Presentation at Conference	O. Leroy	*TRANSVAC horizontal vaccine approach* presented at TBVI Symposium: Future avenues for TB vaccine development	1 February 2011	Les Diablerets, Switzerland	Scientific community	50	Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
98	Meeting	O. Leroy	*TRANSVAC: European Network of Vaccine Research and Development* discussed at German Federal Ministry of Education and Research	28 January 2011	Berlin, Germany	Policy makers	3	DE
99	Meeting	O. Leroy	*TRANSVAC: European Network of Vaccine Research and Development* discussed at CEOs of Drugs for Neglected Diseases (DNDi) and Millennium Foundation	9 December 2010	Geneva, Switzerland	Policy makers	2	Global
100	Meeting	O. Leroy	*TRANSVAC: European Network of Vaccine Research and Development* discussed at Meeting of European Parliament Working Group on Innovation, Public Health and Neglected Diseases	18 Nov 2010	Brussels, Belgium	Policy makers	~20	Global
101	Conferences, Workshops, Reports	S. Kaufmann, H-J. Mollenkopf	*Transcriptomics of Host from M. tuberculosis Infected Cells* (incl. Introduction of MPIIB CF Microarrays) presented at 2nd Annual Systems Biology Programmatic and SBWG Meeting	7-9 November 2010	Boston, USA	Scientific community	>500	Global
102	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at Modern Vaccines/Adjuvants Formulation (MVAF): Impact on Future Development	13-15 October 2010	Cannes, France	Scientific community, Industry	~300	Global
103	Workshop	O. Leroy	TRANSVAC Stakeholder Meeting	12 October 2010	Brussels, Belgium	Scientific community, Industry	50	EU
104	Workshop	S. Kaufmann	*Global analyses strategic planning issues*	7 October 2010	Berlin, Germany	Scientific community	11	DE, NL, UK
105	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at 4th Vaccine and ISV Annual Global Congress	3-5 October 2010	Vienna, Austria	Scientific community, Industry	~300	Global
106	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at OECD Workshop Better Health through Innovation in Biomedicine	28 September 2010	Berlin, Germany	Scientific community, Industry	~300	Global
107	Flyer	O. Leroy	*TRANSVAC New vaccines faster* (available through whole project duration)	July 2010		Global		Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
108	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at New vaccines for global health	15 June 2010	London, UK	Scientific community, Industry	~300	Global
109	Poster	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at 7th world congress on vaccines	26-28 May 2010	Berlin, Germany	Scientific community, Industry	~300	Global
110	Press release		*TRANSVAC - New Vaccines Faster* on EVI website	May 2010	EVI website	Global		Global
111	Fact sheet	O. Leroy	*TRANSVAC European Network of Vaccine Research and Development* (available through whole project duration)	May 2010		Global		Global
112	Presentation at Conference	R. Ventura	*TRANSVAC: European Network of Vaccine Research and Development* presented at ECRI2010: Sixth European Conference on Research Infrastructures	23-24 March 2010	Barcelona, Spain	Scientific community, Industry, Policy makers	~300	Global
113	Conferences, Workshops, Reports	S. Kaufmann, H-J. Mollenkopf	*TB Vaccine and Biomarker Research at the MPI for Infection Biology* presented at Keystone Meeting and 5th Annual GCGH Grantee Meeting and EDCTP meeting	21-24 October 2009	Arusha, Tanzania	Scientific community	>500	Global
114	Website		*Advert of TNA calls* on EVI website	Before each call		Scientific community, Industry		Global
115	Website		*Advert of TNA calls* on TRANSVAC website	Before each call		Scientific community, Industry		Global
116	Website		*Advert of TNA calls* on INYVAX website	Before each call		Scientific community, Industry		Global
117	Website		*Advert of TNA calls* on PHARVAT website	Before each call		Scientific community, Industry		Global

LIST OF DISSEMINATION ACTIVITIES								
No.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
118	Website		*Advert of TNA calls* on OPTIMALVAC website	Before each call		Scientific community, Industry		Global
119	Website		*Advert of TNA calls* on TBVI website	Before each call		Scientific community, Industry		Global
120	Website		*Advert of TNA calls* on UNIL website	Before each call		Scientific community, Industry		Global
121	Website		*Advert of TNA calls* on HZI website	Before each call		Scientific community, Industry		Global
122	Web application		*Advert of TNA calls* on Linked-In (Vaccine related groups, e.g. Vaccine Knowledge Network, Global Public Health)	Before each call		Scientific community, Industry		Global

2.2 Section B - Confidential

Part B1

Table B.1 List of applications for patents, trademarks, registered designs, etc.

LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[6]:	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Application reference(s) (e.g. EP123456)**	**Subject or title of application**	**Applicant (s) (as on the application)**
NA					

Part B2

Table B.2 Exploitable foreground

Type of Exploitable Foreground[7]	**Description of exploitable foreground**	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application[8]**	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Exploitation of results through EU policies	Roadmap document	NO	Not applicable	Roadmap	M72.1.1 –Research and experimental development on biotechnology M75 –Veterinary activities P85.4 –Higher education			TRANSVAC Consortium

[6] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

[7] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[8] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

Type of Exploitable Foreground[7]	**Description of exploitable foreground**	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application**[8]	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Exploitation of R&D results via standards	HEK 293 cells: Cell line for production of recombinant adenoviral vectored vaccines	NO	Not applicable	Cell line	M72.1.1 –Research and experimental development on biotechnology	On request	To be discussed with cell line owner: conditions vary	Microbix, Mississauga, Canada
Exploitation of R&D results via standards	911 MCB1 cells: Cell line for production of recombinant adenoviral vectored vaccines, with reduced risk of replication competent virus generation	NO	Not applicable	Cell line	M72.1.1 –Research and experimental development on biotechnology	On request	To be discussed with cell line owner: conditions vary	R Hoeben, Leiden, The Netherlands
Exploitation of R&D results via standards	293 ADH cells: Cell line for production of recombinant adenoviral vectored vaccines with adherent phenotype	NO	Not applicable	Cell line	M72.1.1 –Research and experimental development on biotechnology	On request	To be discussed with cell line owner: conditions vary	Microbix, Mississauga, Canada

Type of Exploitable Foreground[7]	**Description of exploitable foreground**	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application**[8]	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Exploitation of R&D results via standards	Procell92S cells: Cell line for production of recombinant adenoviral vectored vaccines with tetracyclien sensitive to regulate transgene expression during manufacture	NO	Not applicable	Cell line	M72.1.1 –Research and experimental development on biotechnology	On request	To be discussed with cell line owner: conditions vary	Okairos, Rome, Italy
Commercial exploitation	Expression clones and Know-How on antigen purification: 85 (TB) 85B (TB) Cfp10 (TB) Esat6 (TB) MSPFusN (Malaria) PstS1 (TB) PstS3 (TB) p24 (HIV) 16kDA and 19kDa	YES	31.12.2023	Manufacturing Process	M72.1.1 –Research and experimental development on biotechnology	5 years	In preparation	LIONEX

Exploitable foreground 1: Roadmap document "Towards a sustainable European Vaccine Research and Development Infrastructure" – to be published on TRANSVAC website in December 2013

Its purpose

Document identifies and highlight gaps and current and future needs in EU vaccine R&D pertaining to research infrastructures, and provides a blueprint for the establishment of a sustainable infrastructure for vaccine R&D in Europe.

How the foreground might be exploited, when and by whom

Publically available, it will provide vaccine stakeholders and national and European policy makers and funders with a snapshot of current situation and with a reference document for the development of a European Vaccine Research & Development Infrastructure (EVRI). EVRI will be launched in three phases: (i) Preparatory 2013-2014; (ii) Implementation 2015-2016, and (iii) Operational Phase 2016/17. EVI wants to submit the TRANSVAC2 project to the Horizon2020 call in order to work on the preparatory and implementation phases. EVRI has been identified as a "Topic with high potential and with merit for further Horizon 2020 actions" at the EC infrastructures consultation in October 2012 (proposal co-signed by TRANSVAC, EATRIS and ADITEC).

IPR exploitable measures taken or intended

None.

Further research necessary, if any

None.

Potential/expected impact (quantify where possible)

The expected impact is large for the development of EVRI.

Exploitable foreground 2: HEK 293 cell line

Its purpose

Vaccine biomanufacture.

How the foreground might be exploited, when and by whom

Companies developing viral vectored vaccines especially adenovectors.

IPR exploitable measures taken or intended

None.

Further research necessary, if any

Further release assays required before cell line can be used for clinical biomanufacture.

Potential/expected impact (quantify where possible)

Facilitation of vaccine development.

Exploitable foreground 3: 911 MCB1 cells

Its purpose

Vaccine biomanufacture.

How the foreground might be exploited, when and by whom

Companies developing viral vectored vaccines especially adenovectors.

IPR exploitable measures taken or intended

None.

Further research necessary, if any
Further release assays required before cell line can be used for clinical biomanufacture.

Potential/expected impact (quantify where possible)
Facilitation of vaccine development.

Exploitable foreground 4: 293 ADH cells
Its purpose
Vaccine biomanufacture.

How the foreground might be exploited, when and by whom
Companies developing viral vectored vaccines especially adenovectors.

IPR exploitable measures taken or intended
None.

Further research necessary, if any
Further release assays required before cell line can be used for clinical biomanufacture.

Potential/expected impact (quantify where possible)
Facilitation of vaccine development.

Exploitable foreground 5: Procell 92S cell line
Its purpose
Vaccine biomanufacture.

How the foreground might be exploited, when and by whom
Companies developing viral vectored vaccines especially adenovectors.

IPR exploitable measures taken or intended
None.

Further research necessary, if any
Further release assays required before cell line can be used for clinical biomanufacture.

Potential/expected impact (quantify where possible)
Facilitation of vaccine development.

Exploitable foreground 6: Expression clones and Know-How on antigen purification
Its purpose
Commercial use.

How the foreground might be exploited, when and by whom
Sale or licensing of antigens medical use.

IPR exploitable measures taken or intended
Diagnostic test development in progress; GMP foreseen.

Further research necessary, if any
GMP manufacturing if to be used for vaccine development.

Potential/expected impact (quantify where possible)
Could lead to 10% increase in revenue for LIONEX.

3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	FP7-INFRASTRUCTURES-2008-1-228403
Title of Project:	European Network of Vaccine Research and Development
Name and Title of Coordinator:	Dr. Odile Leroy

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***Yes***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	✓
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	✓
• Did the project involve Human genetic material?	✓
• Did the project involve Human biological samples?	✓
• Did the project involve Human data collection?	✓
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	✓
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	✓
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	✓
• Were those animals transgenic small laboratory animals?	✓
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	✓
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	

DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	2	5
Work package leaders	3	14
Experienced researchers (i.e. PhD holders)	21	28
PhD Students	7	3
Other	37	17

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**9**
Of which, indicate the number of men:	4

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

✓	Yes
○	No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
✓	Design and implement an equal opportunity policy	○	○	○	○	●
✓	Set targets to achieve a gender balance in the workforce	○	○	○	○	●
❑	Organise conferences and workshops on gender	○	○	○	○	○
✓	Actions to improve work-life balance	○	○	○	○	●
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

- ● Yes- please specify
  Modular courses (WP8): selection of candidates with the same score made on the basis of the EVI gender-policy, giving priority to women.
  No clinical research was conducted within TRANSVAC.
- ○ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

- ● Yes- please specify
  Open doors days of the MPIIB 2011 and 2013.
  Work with undergraduate students at LIONEX.
  Phd students were invited to present their work through posters at the EVI Rendez-vous 2011 meeting.
- ○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

- ● Yes- please specify
  TRANSVAC website (limited to standardisation of procedures and protocols)
- ○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

- ○ Main discipline[9]: 1.5
- ○ Associated disciplines[9]: 3.1, 5.3, 4.2

G Engaging with Civil society and policy makers

[9] Insert number from list below (Frascati Manual).

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	● Yes ○ No	

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ● Yes- in determining what research should be performed
- ● Yes - in implementing the research
- ● Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	● Yes ○ No	

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ● Yes- in framing the research agenda
- ● Yes - in implementing the research agenda (through representation of scientific advisory committee)
- ● Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ● Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ● Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	✓
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	✓
		Foreign and Security Policy		Space	
Education, Training, Youth	✓	Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ● National level
- ● European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**9**
To how many of these is open access[10] provided?	**8**
How many of these are published in open access journals?	**8**
How many of these are published in open repositories?	**9**
To how many of these is open access not provided?	**1**
Please check all applicable reasons for not providing open access:	
● publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ● no suitable open access journal available ● no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[11]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

●	Increase in employment, or	●	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	●	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

[10] Open Access is defined as free of charge access for anyone via Internet.
[11] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE* = *one person working fulltime for a year*) jobs:

Difficult to estimate / not possible to quantify

Indicate figure:
13.75

3.5 FTE for MPIIB
2 FTE for LIONEX
0.75 for LSTMH
2 for MHRA
0.5 for TBVI
2.5 FTE for UNIL
2.5 for EVI

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

● Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

● Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

●	Press Release	●	Coverage in specialist press
❑	Media briefing	●	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
●	Brochures /posters / flyers	●	Website for the general public / internet
❑	DVD /Film /Multimedia	●	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	●	English
●	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]