IMI1 Final Project Report
Public Summary

Project Acronym: BioVacSafe

Project Title: Biomarkers for Enhanced Vaccine Safety

Grant Agreement: 115308
Project Duration: 01/03/2012 – 28/02/2018

1. Executive summary

The executive summary will be made publically available, and therefore should not include information deemed as confidential by the consortium. It should be concise (preferably no more than 40 pages), comprehensive and should capture the updates for the last reporting period as well as the overall outputs of the project and its impact. It shall at least cover the following items:

1.1. Project rationale and overall objectives of the project

BioVacSafe is a consortium of vaccine manufacturers together with academic, public, and SME (Small and Medium Sized Entities) participants, organized into focused workpackages that generated and disseminated knowledge and tools to benchmark licensed vaccine reactogenicity, and created practical and generalizable guidelines and techniques to enhance immunosafety of novel vaccines from pre- development to post-marketing surveillance. It aimed to develop predictive biomarkers, models of inflammation and autoimmunity, and clinical events classification through a series of clinical trials and population-based studies with parallel pre-clinical models. The consortium had capability in transcriptomics, genotyping, proteomics, metabolomics and data mining, with depth and capacity to discover, validate and distribute novel biomarkers. A full range of ex vivo and in vivo small animal models of inflammation and biomarker discovery, with advanced immunology and imaging were available.

1.2. Overall deliverables of the project

BioVacSafe safely and efficiently conducted clinical studies of vaccine immunosafety for biomarker discovery from small intensive trials to large-scale studies of adult, paediatric and other populations, enrolled clinical cohorts (with globalization capability, autoimmune, chronic, inflammatory and infectious disease groups), conducted large-scale genotyping and sequencing, and developed safely accessible central databases for online analyses of large datasets. It synthesized outputs to generate and disseminate classifications, guidelines, reference standards for vaccine development, and inform EFPIA activities. The work packages interoperated to maximize discovery – validation – application – re-discovery cycles, to deliver project goals on time, within budget, while enhancing interactions with other closely integrated actors such as US Food and Drugs Administration, European Medicines Agency.

1.3. Summary of progress versus plan since last period

In WP1, the extraction of WB RNA and analysis of Agilent chip transcriptomics was completed for all clinical vaccine trials by MPIIB, except for an externally conducted rBCG trial for which clinical specimens were not provided by the collaborators in time. Integration with clinical adverse events data was completed and biomarkers of reactogenicity identified. A Digital Archive of over 2000 clinical samples for RNA seqencing after immunisation in the inpatient vaccine trials has been completed by UGent and was uploaded to the public domain – thereby preserving these unique time course samples which otherwise would have to have been destroyed for ethical and financial reasons. Together with the Agilent chip samples the richly annotated public domain data (Umbrella BioProject PRJNA515032 identifies all BIOVACSAFE transcriptomics in public domain: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA515032) are a resource for future safety

biomarker benchmarking and research. Clinical and linked laboratory data have also been uploaded to public domain repository. In WP2 transcriptomics was completed in the cross-species models of biomarkers after immunisation, and integrated with human panels. Additional tasks and deliverables funded by DFC from GSK and Sanofi Pasteur have been completed and analysed in WPs 1, 2, 7 including human infectious influenza challenge and human natural pneumococcal infection models, and primate yellow fever infection / immunisation.

1.4. Significant achievements since last report &

1.5. Scientific and technical results/foregrounds of the project

Sections 1.4 and 1.5 are taken together as the most significant scientific and technical results/foregrounds of the project were achieved in the final period

- An Umbrella BioProject PRJNA515032 has been created that identifies all BIOVACSAFE transcriptomics in public domain: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA515032. This allows scientists to quickly find these raw data that could be used in their own analyses.

- Digital Archive of over 2000 clinical samples for RNAseq after immunisation in the inpatient vaccine trials has been uploaded by UGent to the public domain thereby preserving these unique time course samples which otherwise would have to have been destroyed for ethical and financial reasons. These data can be mined by other researchers to complement their own work to identify new drugs, vaccines or safety markers. The digital archive is accessible at:
    - https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?study=SRP162500
    - https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?study=SRP162414
    - https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?study=SRP162411

- Preparation of all transcriptomics data with rich annotation with reactogenicity data uploaded to public domain repository under the Umbrella BioProject. Available to all researchers.

- Integration of results of transcriptomics for intensive inpatient trials using 5 vaccines and placebo; follow-on large outpatient trials with Fluad and Boostrix; live VSV vaccine trials in USA; pneumococcal and controlled infectious challenge influenza infection; and Boostrix immunisation of pregnant women in WP1 have been integrated with clinical data. These unique data allow scientists to compare responses to vaccines across a wide range of population groups for many different types of vaccines, to help identify safety profiles and risk factors, also as part of personalised medicine.

- Immune activation in muscle and draining lymph nodes of human vaccinees characterised by positron emission tomography using labelled glucose or cell specific ligands as biomarker of reactogenicity. This provides a potential clinical tool to quickly identify vaccine preparations that may be poorly tolerated without exposing many persons to them in larger trials.

- Relation between early innate immune events and local and general adverse events and humoral and cellular immune responses induced by the administration of an adjuvanted trivalent influenza vaccine to human participants compared with those induced by a natural infection following a challenge of healthy volunteers with influenza A/H1N1. This calibrates the responses seen after

vaccination which are actually rather trivial and not unsafe, with the far more significant activation of the immune responses by the actual infections that vaccines prevent. Also allowing us to identify common markers between infections and the vaccines that prevent them to assist with designing better vaccines.

- Integration of murine and human transcriptomics data of responses of pregnant females to immunisation with DTP vaccines completed. By comparing animal models with human responses we can design better animal models that better predict the responses of humans and ideally requiring fewer animals. Also potentially eliminating animals from vaccine testing to some extent.

- Analysis completed of Stamaril immunisation and Yellow fever infection study with wild-type Asibi strain completed in non-human primates. Integration with human clinical and reactogenicity data completed and in preparation for publication. Yellow fever vaccine is in short supply but of great public health importance. It very rarely may cause a serious complication in susceptible humans. By better understanding the pathological outcomes of the actual Yellow Fever virus infection and comparing it with the vaccine virus we can better understand why the vaccine may rarely cause serious disease.

- The molecular signatures of Varilrix in Cynomolgus macaques was completed and compared with in depth transcriptomics analysis combined with immunological read outs of Varilrix in mice and humans. Varilrix is an effective vaccine against shingles and chickenpox but as a live vaccine we need to understand more about how it affects humans to avoid potential adverse effects. These results allow us to compare human and animal responses to design better animal models to predict adverse effects.

- Mice at different ages were infected with influenza strain H1N1 intranasally. Biomarkers were assessed in the serum and lungs. Humans respond differently to influenza infection at different ages, and this model helps us to understand the immune mechanisms behind the age-related responses to this important infection for which no highly effective vaccine is available for the elderly and infants.

- Human blood samples were collected from patients with suspected pneumococcal infection. Next-Generation Sequencing (NGS) technique was employed to obtain transcriptomic data from 20518 human genes in order to highlight an immune signature against Streptococcus pneumoniae. By understanding the adverse responses to actual infection we can better calibrate our models of the responses to vaccines against those infections, to identify significant reactogenicity from actually harmless responses to vaccines.

- To discover sequence variants in the genome that associate with immune mediated adverse events (AE) and with autoimmune disease (AID) genome wide association scans (GWAS) were completed in Icelandic individuals. These data help us to identify genetic predisposition to rare complications from vaccination such as autoimmune disease.

- In genome wide association scans (GWAS) no common or rare sequence variants in the genome were found to show significant association with smallpox vaccination side effects (Smallpox AE) of other rarer immune mediated AEs; Severe outcome of influenza, Narcolepsy, or Guillain-Barré

syndrome. These data help us to quantify the genetic component of adverse reactions to vaccines to better understand the mechanisms of vaccine safety and develop personalised medicine.

- Samples collected were used for the development, verification and validation of the SLE-key® rule-out test for a definitive rule-out of a diagnosis of systemic lupus erythematosus (SLE). The test uses the proprietary iCHIP® micro-array technology platform. This test offers rapid screening for this significant rheumatological disease, as well as a tool to potentially detect rare adverse responses to vaccines.

- Samples collected from children before and after DT vaccination who did and did not develop fever following vaccination were tested on IAL microarrays, and a single feature – GLP-2 IgM – showed a difference between the groups (a role has been reported for GLP-1 in fever reduction). High fever after some vaccines was associated with convulsions in the past and therefore the mechanisms associated with fever after vaccination have been explored to identify possible predictors that could be used in the screening and testing of new vaccines.

- The prevalence of autoimmune disease among men and women in Iceland, and role of inflammation during infection was reported by deCODE. Data on the background of naturally occurring diseases is crucial to evaluate and detect any increase in these diseases as a result of introducing a new vaccine.

- A birth cohort was used to analyse the associations between routine childhood immunizations and the risk of wheeze and eczema (atopic dermatitis) and , with no associated increased risk identified. Some evidence that childhood immunizations with MMR and rotavirus may affect the risk of atopy during childhood in this setting. The impact of routine childhood vaccines and common immune-mediated diseases such as allergies os of great importance, and these preliminary data provide reassurance that within this study no increased risk was observed with these common childhood vaccines.

- A platform and an underlying metadata framework to support data management for BioVacSafe project's data was completed and activated. This allowed standardisation of clinical and laboratory data which maximises the ability to compare the diverse data outputs across all the research in this consortium. This maximises the chance of detecting signals of vaccine safety. Data that cannot be compared is often a major weakness of large projects with many laboratories contributing.

- A bioinformatics web tool to analyse data particularly metabolome generated from specific trials within the consortium, and a web tool to analyse transcriptome data using mock data sets have been developed and integrated to the platform. This allows other scientists to easily interrogate the masses of data generated for their own research, across the internet.

- A review and evaluation of existing standards and definitions was completed to establish consensus definitions in collaboration with stakeholders, including public participation through webinars. A Vaccines Therapeutic Area User Guide v1.1 was published by CDSIC and made available in the public domain. This is part of a global effort to ensure standardisation of data collection so that rare events can be identified by assembling huge datasets efficiently from many sources.

- Samples from subjects receiving a single dose of Vesicular stomatitis virus (VSV) expressing the HIV gag protein (VSV-HIVgag) vaccine were provided by HIV Vaccine Trial Network (HVTN) and analysed for whole blood transcriptomics. This vaccine vector has been hugely successful as an Ebola vaccine but was associated with significant side effects in preliminary trials. It acts as a good positive control to calibrate other responses and to identify mechanisms involved in the adverse outcomes observed. The BIOVACSAFE consortium was spared the costs of organising clinical trials, being gifted these samples to analyse on out standardised platform.

- A pilot study successfully developed a high resolution flow cytometry-based method for detection of plasma-derived extracellular vesicles (EV), able to detect MHCII-containing EVs in plasma. EVs are thought to play an important role in regulating immune responses, including to vaccines. However, their role remains elusive and this model begins to unravel their involvement in responses to commonly used licensed vaccines.

1.6. Potential impact and main dissemination activities and exploitation of results

The project scientific/technical outputs will contribute to the overall IMI objectives:

- to provide socio-economic benefits for European citizens,
- to contribute to the health of European citizens,
- to increase the competitiveness of Europe and help to establish Europe as the most attractive place for biopharmaceutical research and development

by increasing the knowledge base of biomarkers – clinical and pre-clinical – that may predict vaccine immunosafety. This knowledge base may be developed further by researchers and product development actors in the field of immunisation. For example new models of systems biology responses to vaccines and vaccine components have been developed across multiple animal species in a series of head to head comparisons, including in many cases direct comparison with humans. As well as the data from these experiments the protocols and methodologies are made available so that future researchers within the industry and academia can more rapidly advance knowledge of vaccine safety using clinical and pre-clinical models. Specific software tools to display and analyse the multiparametric data generated have been made available on the web to all researchers. Novel human in vivo techniques such as biopsy of local tissue, radionucleotide scanning have been pioneered and methodology shared widely, to encourage more experimental medicine approaches and reduction of use of animals. CDISC have developed broad definitions to assist with structuring and reporting vaccine safety and systems biology data.

The project outputs have the potential to be rapidly and broadly spread and taken up within the scientific/industrial community and healthcare professionals due to the rapid upload of complete and richly annotated data to public domain online repositories, and though presentations a meetings and in publications. The close interactions of the industry with all data generation and analysis has ensured that the companies have gained knowledge of novel methods to assess and predict vaccine safety that can be applied internally with novel vaccine candidate development.

1.7. Lessons learned and further opportunities for research

The strong interaction between the public and private members of the consortium took time to develop over the course of several years. Greater involvement of private actors in the detailed planning of the description of work may have expedited this. The greatest returns were achieved in the final years when collaborations between partners really strengthened, and two project extensions greatly capitalised on these enhanced working relationships. Future projects should endeavour to build on developed inter-actor associations and reinforce / expand them.

The ability to use DFC to fine-tune and redirect tasks based on outputs was extremely valuable. The strong support the project received from IMI JU to make regular amendments to the DOW was essential and hugely contributed to exponential increase in project outcomes as the project progressed, together with continuing relevance is a rapidly developing field of knowledge.

Bioinformatics-intensive projects should greatly prioritise financial resources to directly support staff capable of undertaking data analysis, statistics and bioinformatics – very much higher proportions of total budget should flow to this rather than clinical, pre-clinical or laboratory tasks.

Discovery activities should be curtailed after an initial project phase to allow time for complex and time consuming bioinformatics analysis within the project timeframe; or funders should acknowledge that outputs may not appear until after the project completes. Publications are likely to be even more delayed due to the long timelines in data analysis and reporting – dissemination assessments that are confined to the project timeframe or within a few years after will be highly misleading of the eventual impact of the project.

Ways to finance the preservation of clinical samples collected during the project but not included in primary analyses needs to be urgently identified, especially for discovery projects that may generate pilot or training sample collections. Otherwise unique collections risk being destroyed with associated loss of financial investment by the public funding.