Introducing the project

FastVac is a consortium of seven public health institutes and a university from seven European countries. The consortium was started in April 2010 and was co-funded by the members of the FastVac Consortium and the Health Programme of the European Union. The consortium held a final meeting in April 2014. The consortium was organized in nine work packages. The six core work packages (WP), individually and collectively aimed to accelerate key steps in the vaccine discovery and the development process. Three horizontal work packages provided the managerial support to the project.

FastVac’s remit was to produce ‘a comprehensive set of predictive rules that will enable accelerated development, evaluation, production and release of emergency vaccines’. To this end, work by the consortium was directed towards carrying out an extensive systematic literature review (SLR) of the scientific record by text mining to identify predictors of vaccine success or failure (work package 4).

Experts from work package 5 to 8 assisted work package 4, in four expert groups by building and testing technical elements for the SLR, e.g. ontology development, formulation of questions, standard document collection, expert evaluation. The output of the SLR by text mining contribute to a review on correlates of protection (COPs). The principles of this review were applied to rationally design two candidate vaccines to a specific pathogen (work package 5). The candidate vaccines were tested for the induction of the relevant COPs in an animal model available within the consortium (work package 8). In work package 6 partners contributed to the project by developing a fast track within the consortium (work package 8). In work package 6 partners contributed to the project by developing a fast track navigation tool (FastVac prospector), the main achievements and outputs of the work packages are summarised on the next page and were shared and discussed with various stakeholders during the final meeting.

The FastVac consortium has conducted two separate SLRs that focus on two parallel pathways within the vaccine development process:

- SLR1 deals with the design and testing of candidate vaccines based on probable mechanisms of protective immunity and predicted correlates of protection (COPs). Knowledge of protective immune mechanisms and identification of potential COPs is required to design and test candidate vaccines and can facilitate their licensure and release in the absence of full clinical trials.

- SLR2 deals with the design the manufacturing process and the use of appropriate process analytical technology to optimise vaccine quality and yield. These are essential requirements for rational vaccine scale-up.

The FastVac Project Consortium consists of:

- Intravacc, NL
- National Institute for Public Health & the Environment, NL
- Public Health England, UK
- Statens Serum Institute, DK
- Cantacuzino Institute, RO
- National Centre for Epidemiology, HU
- Statens Serum Institute, DK
- Public Health England, UK
- Norwegian Institute of Public Health, NO
- National Institute for Public Health & the Environment - RIVM

www.fastvac.eu - corinne.touwes@intravacc.nl - June 2014
A GENERIC FRAMEWORK FOR FAST PRODUCTION AND EVALUATION OF EMERGENCY VACCINES

SUMMARY: PART II OF II

Main achievements and outputs and how to access them

Work package 4
The key objective for FastVac work package 4 was to systematically review the literature about successful and failed vaccines and from this, make evidence-based predictions about how to optimise vaccine design and accelerate the testing, manufacture and release of vaccines. To achieve this objective, FastVac needed to review an evidence base that spans immunology, vaccinology and process technology and draws information from a wide range of viral and bacterial diseases and across a selection of vaccine platforms. It was estimated that over 10 million documents would need to be reviewed which could not be done manually using conventional systematic review approaches. FastVac therefore had to develop a novel approach. The chosen approach uses automated text-mining to carry out high-throughput processing and annotation of documents and a bespoke user interface, the FastVac Prospector, to retrieve and analyse relevant documents; together they constitute the FastVac vaccinology decision support platform.

• The FastVac ontology will be made available as an open access resource and will be published
• Worked examples using the FastVac Prospector and FastVac Prospector SLR platforms will be published. The systems are available for evaluation on request: corine.kruiswijk@intravacc.nl
• The annotated SRL1 and SLR2 Mímir indices may be available on request: corine.kruiswijk@intravacc.nl

Work package 5
Work package 5 partners have contributed in building and testing technical elements necessary to perform systematic literature reviews (SLR) based on automated text mining. Work package 5 has demonstrated that the established text mining approach can be used to generate systematic knowledge about protective antigens and immune responses (including COPs) necessary for rational vaccine design. By using the automated SLR approach, supported by manual review processes, work package 5 has deduced vaccine candidates for two selected model organisms: influenza and coxiella. Two principally different experimental influenza vaccines were successfully manufactured (work package 5) and tested in animal models for immunogenicity and protective efficacy (work package 8). The work in work package 5 has demonstrated that the established text-mining system used in combination with FastVac Prospector can serve as a valuable decision support platform for efficient evidence-based design of vaccines against a variety of emerging diseases.

Work package 6
Work package 6 partners have developed a fast track scenario for vaccine process development, scale up and technology transfer. Regarding process development, an optimized process for the production of influenza vaccine on eggs has been developed. Thereafter the process was successfully transferred to another partner, where the process was implemented at a larger scale without operational deviations and the product met the pre-set specifications. Next to this example for influenza vaccine production, in addition a fast track scenario was developed for optimisation of medium for bacterial growth, based on the principles of Design of Experiment.

Work package 7
Work package 7 partners have applied Process Analytical Technology to different vaccine platforms to aid product release and showed that better product monitoring of vaccines can support faster process development as well as faster release of intermediate products. For example, T(EM) and DLS coupled to (A)SEC were developed to characterise influenza virus and NIR was used to analyse liposomes containingCAF09 adjuvant. Also, in-process monitoring of antigen production was shown to indeed support a faster release of intermediate product.

Work package 8
Work package 8 partners have tested work package 5 candidate influenza vaccines and optimised delivery route and/or formulation of vaccines against varicella zoster and tuberculosis. Furthermore, based on the automated SLR and existing consortium data on interspecies extrapolation, work package 8 partners have found that animals can indeed be used as suitable animal models and saving valuable time in the initial phase of vaccine development which will even be more relevant in case of new pandemics.
The key objective for FastVac work package 4 was to systematically review the literature about successful and failed vaccines and from this, make evidence-based predictions about how to optimise vaccine design and accelerate the testing, manufacture and release of vaccines.

To achieve this objective, FastVac needed to review an evidence base that spans immunology, vaccinology and process technology and draws information from a wide range of viral and bacterial diseases and across a selection of vaccine platforms. It was estimated that over 10 million documents would need to be reviewed which could not be done manually using conventional systematic review approaches. FastVac therefore had to develop a novel approach. The chosen approach uses automated text-mining to carry out high-throughput processing and annotation of documents and a bespoke user interface, the FastVac Prospector, to retrieve and analyse relevant documents; together they constitute the FastVac vaccinology decision support platform.

The FastVac vaccinology platform uses semantic and relational annotations rather than single keywords to retrieve, in a single search, relevant documents from a vast amount of literature that covers 128 viral and bacterial pathogens of public health significance (the ‘FastVac pathogens’) and includes a wide range of vaccine platforms, from classical inactivated pathogen vaccine induces cell mediated immunity and whether it does so and relationships to be identified, for example what type of associations can be reviewed by the user. This enables trends between different sets of variables, e.g. vaccine platforms and approaches to more experimental systems such as synthetic range of vaccine platforms, from classical inactivated pathogen to more experimental systems such as synthetic vaccines.

The FastVac reviews

FastVac has conducted 2 separate reviews (SLRs) that focus on 2 parallel pathways within the vaccine development process: SLR1 deals with the design and testing of candidate vaccines based on probable mechanisms of protective immunity and predicted correlates of protection (COPs). Knowledge of protective immune mechanisms and identification of potential COPs is required to design and test candidate vaccines and can facilitate their licensure and release in the absence of full clinical trials. SLR2 deals with the design the manufacturing process and the use of appropriate process analytical technology to optimise vaccine quality and yield. These are essential requirements for rational vaccine scale-up.

The process followed for each review is summarised in figure 1. A large corpus of documents (abstracts) was extracted from online databases (PubMed, the Cochrane library and IFI CLAIMS Global Patent database) using very broad search terms. These documents were processed and annotated using a purpose built text-mining application that uses the open source General Architecture for Text Engineering (GATE) platform. The resulting index of annotated documents was loaded into the FastVac Prospector interface and queries were used to retrieve sets of relevant documents. Within the retrieved documents, associations and trends were identified using visualisation tools built into FastVac Prospector.
Key components of the FastVac vaccinology platform

It has taken approximately 3 years of effort to develop and evaluate the process described above, first for SLR1 and then for SLR2. Key components that had to be developed and refined included:

The FastVac Ontology

The key terminology relating to vaccinology and immunology that would need to be annotated in the document corpus was collected into a purpose-built ontology of structured terms (entities). The FastVac ontology encompasses terms about pathogens, diseases, immunology and vaccine manufacture (Table 1). At its most complex, parts of the FastVac Ontology contain nine hierarchical levels of terminology, allowing either very broad or extremely specific analyses of the data to be undertaken.

FastVac text-mining application

A text mining application was developed that splits each document in the corpus into its structural components (sentences, words, punctuation etc) and annotates (1) entities according to the FastVac ontology, and (2) key relations e.g. ‘protective’ according to bespoke term lists. This process produces an index of annotated documents.

FastVac questions and query algorithms

Questions were designed to direct the retrieval of relevant documents from the annotated index. SLR1: 15 questions (Q1-15; Table 2) addressed key decision points along the vaccine design and testing pathway (Figure 2); Q18 & Q19 were designed to identify suitable adjuvants for particular pathogens / vaccine platforms (Table 2). Beneath each tier 1 question, 2 further tiers of ‘child’ questions were designed, of increasing specificity (not shown).

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**Table 1: The top two hierarchical levels of terms in the FastVac ontology.**

<table>
<thead>
<tr>
<th>Top class</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active immunity</td>
<td>Artificial active immunity</td>
</tr>
<tr>
<td>Adverse response</td>
<td>Adverse event</td>
</tr>
<tr>
<td>Animals and models</td>
<td>Animal model</td>
</tr>
<tr>
<td>Antigen</td>
<td>Passive immunity</td>
</tr>
<tr>
<td>Disease</td>
<td>Process and testing</td>
</tr>
<tr>
<td>Disease pathogenesis</td>
<td>Manufacturing process</td>
</tr>
<tr>
<td>Immune response</td>
<td>Adjuvant response</td>
</tr>
<tr>
<td>Immunisation protocol</td>
<td>Vaccine valency</td>
</tr>
<tr>
<td>Immunisation route</td>
<td>Vaccine vector</td>
</tr>
<tr>
<td>Immunisation schedule</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2: FastVac Questions**

1. For the FastVac pathogens, what antigens are protective or associated with disease amelioration or control of infection?
2. For the FastVac pathogens, what antigens are known not to be protective or show no association with disease amelioration or control of infection?
3. For the FastVac pathogens, what immune responses are associated with protection, disease amelioration or control of infection?
4. For the FastVac pathogens, what immune responses are associated with protection, disease amelioration or control of infection?
5. For the FastVac pathogens, what immune responses are known not to be associated with protection, disease amelioration or control of infection?
6. Is immune-mediated disease exacerbation or an adverse immune response associated with any of the FastVac pathogens?
7. What are the known correlates of protection for the FastVac pathogens?
8. What studies/assays/tests are used to measure immune protection/COPs for the FastVac pathogens?
9. What vaccines protect against the FastVac pathogens or the diseases they cause?
10. What vaccines do not protect against the FastVac pathogens or the diseases they cause?
11. What vaccines elicit a humoral immune response against the FastVac pathogens or the diseases they cause?
12. What vaccines elicit a cell-mediated immune response against the FastVac pathogens or the diseases they cause?
13. For the FastVac pathogens or the diseases they cause, what vaccines are reactogenic or associated with disease exacerbation or an adverse response?
14. For the FastVac pathogens or the diseases they cause, what animals or animal models have been used to test vaccines or immunisation protocols?
15. For the FastVac pathogens or the diseases they cause, what are the features of the disease pathogenesis process?
16. For vaccines against the FastVac pathogens or the diseases they cause, what adjuvants enhance the immunogenicity/immune response or the protective efficacy of the vaccine?
17. For vaccines against the FastVac pathogens or the diseases they cause, what adjuvants do not enhance the immunogenicity/immune response or the protective efficacy of the vaccine?
AIDS designed (Table 3).

The association matrix or the association chart contains the requisite combination of entities and relations to answer each FastVac question.

Table 3: The tier 1 and tier 2 questions designed for FastVac SLR2. For Q1 and Q2, additional tier 3 questions divide the results into vaccines and biopharmaceuticals (not shown).

<table>
<thead>
<tr>
<th>No.</th>
<th>Tier 1 Question</th>
<th>No.</th>
<th>Tier 2 Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>For vaccines and other biopharmaceuticals, what analytical methods are used to determine product quality?</td>
<td>1.1</td>
<td>For vaccines and other biopharmaceuticals, what functional analytical methods are used to determine product quality?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td>For vaccines and other biopharmaceuticals, what non-functional analytical methods are used to determine product quality?</td>
</tr>
<tr>
<td>2</td>
<td>For vaccines and other biopharmaceuticals, what manufacturing processes influence product quality?</td>
<td>2.1</td>
<td>For vaccines and other biopharmaceuticals, what manufacturing processes influence product purity?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2</td>
<td>For vaccines and other biopharmaceuticals, what manufacturing processes influence product stability?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3</td>
<td>For vaccines and other biopharmaceuticals, what manufacturing processes influence product potency?</td>
</tr>
<tr>
<td>3</td>
<td>For vaccines and other biopharmaceuticals, what manufacturing processes influence product yield?</td>
<td>3.1</td>
<td>For vaccines, what manufacturing processes influence product yield?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2</td>
<td>For other biopharmaceuticals, what manufacturing processes influence product yield?</td>
</tr>
</tbody>
</table>

FastVac Prospector

The index of annotated documents and the query algorithms were incorporated into FastVac Prospector, designed for FastVac by the University of Sheffield. This provides a password-protected online user interface which allows researchers to retrieve relevant documents from the indexed corpus and create visual representations of the results to help them make evidence-based decisions.

For a set of retrieved documents, FastVac Prospector allows particular sets of annotated terms to be selected. Associations between terms in different sets can then be visualised using one of 2 views: the association matrix or the association chart (Figure 3). To compare many terms the association matrix is most suitable.

For SLR2, 3 questions were designed to direct the retrieval of relevant documents from the annotated index. Beneath each tier 1 question, 1 or 2 further tiers of ‘child’ questions were designed (Table 3).

For all questions, corresponding query algorithms (Mímir queries) were developed to retrieve documents which contained the requisite combination of entities and relations to answer each FastVac question.

Figure 3: Animal models used to test successful DNA vaccines against Mycobacteriaceae. Two-dimensional association matrix (A) produced using FastVac Prospector to analyse the documents retrieved by Q9 indicating numbers of PubMed abstracts in which types of vaccine platform (x axis) co-occur with named bacterial pathogens (y axis; grouped in families for visualisation). Colour intensity of association matrix cells uses a relative colour scale based on the number of documents used to create the matrix. Documents selected for this visualisation were required to mention a vaccine against a relevant pathogen which produced a protective effect. Documents in which ‘Mycobacteriaceae’ co-occurred with ‘Nucleic acid vaccine’ where used in an association chart (B), showing the different species used in those studies. The width of each arc gives a relative indication of how many documents contain both terms joined by that arc.
AIDS in preclinical testing for selected exemplar pathogens.

As proof of concept, FastVac has used the platform to identify pathogens or the genera and families to which they belong. Making decisions for any of the 128 annotated 'FastVac support platform for developing new vaccines. It can be used to FastVac has produced a dynamic and interactive decision support platform for developing new vaccines. It can be used to make decisions for any of the 128 annotated ‘FastVac pathogens’ or the genera and families to which they belong. As proof of concept, FastVac has used the platform to identify mechanisms of protection, define theoretical vaccine candidates and predict potential correlates of protection for use in preclinical testing for selected exemplar pathogens. In brief:

- For model pathogens (N. meningitidis and hepatitis B virus) where the protective immune response has been well-characterised, the FastVac platform correctly identified the protective mechanism.

Proof-of-concept and beta testing

FastVac has produced a dynamic and interactive decision support platform for developing new vaccines. It can be used to make decisions for any of the 128 annotated ‘FastVac pathogens’ or the genera and families to which they belong. As proof of concept, FastVac has used the platform to identify mechanisms of protection, define theoretical vaccine candidates and predict potential correlates of protection for use in preclinical testing for selected exemplar pathogens. In brief:

- For model pathogens (N. meningitidis and hepatitis B virus) where the protective immune response has been well-characterised, the FastVac platform correctly identified the protective mechanism.

Performance of the FastVac system

The FastVac text mining system was developed and refined through 2-3 iterations (Figure 1). Each iteration used a new set of evaluation standards selected by domain experts to positively answer the top-level text mining questions (positive standards) or mark them to be irrelevant (negative standards). Documents that were classified inappropriately by the system were examined manually to check for missing or erroneous annotations or defects in the query algorithms. After each evaluation round the text mining application, queries and ontology were improved.

The performance metrics used to evaluate the system were the standard text mining metrics of precision and recall. For SLR1, qualitative analysis by a panel of experts was also undertaken in the third round of evaluation. Question performance was optimised to prioritise high precision over high recall, in order to reduce the number of false positives and so minimise the ‘noise’ when the results were displayed visually.

Final performance scores for each review, averaged across all the top-level questions were:

- SLR1: 70% precision, 63% recall
- SLR2: 72% precision, 70% recall

Applications

The FastVac vaccinology decision support platform shows promise for the following applications:

- For ‘dynamic’ querying in the event of an outbreak of a re-emergent or newly-emerged pathogen e.g. by public health institutes, health organisations, SMEs and vaccine manufacturers.
- As an interface with the vaccine R&D for anyone embarking on developing a new vaccine against an existing pathogen e.g. universities, SMEs etc.
- To identify gaps in the knowledge base for research funders and vaccine developers (pathogens for which neither successful nor failed vaccines exist or for which there is no data on protective or non-protective immune responses).
- For regulators to identify if the vaccine development pathway taken by a vaccine developer is appropriate.

The outputs of FastVac and how to access them

- The FastVac ontology will be made available as an open access resource and will be published
- Working examples using the FastVac Prospector and FastVac Prospector SLR platforms will be published. The systems are available for evaluation on request: corine.kruiswijk@intravacc.nl
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- Medical University Plovdiv, BG
- Co-funded by the Health Programme of the European Union
- Co-funded by the members of the FastVac Consortium

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WORK PACKAGE 5

CANDIDATE PREDICTION AND COPs IDENTIFICATION: PART I OF II

WP5 has contributed to the FastVacc project by:

- Building and testing technical elements necessary to perform systematic literature reviews (SLR) based on text mining
- Demonstration that automated SLR can be used to generate and integrate key knowledge within vaccinology
- Designing model vaccines for influenza and coxliella based on automated SLR processes
- Manufacturing influenza model vaccines for testing in animal models
- Demonstration that SLR can be used as a decision support platform for fast and evidence based vaccine design

Establishment of automated Systematic Literature Reviews (SLR) for evidence based vaccine development:

Workpackage 5 partners have been involved in expert groups tasked to specify the pathogens studied by FastVacc, provide expert advice about correlates of protection (COPs), and collect animal model data. Most effort has been devoted to assist work package 4 in building the necessary ontology elements (immunology domains) for SLR, constructing query questions used in the text mining processes to address work package 5 milestones, and evaluating the performance of the text mining by reviewing documents in a web-based expert evaluation tool (EET).

Work package 5 addressed the milestones and deliverables by analyzing query questions 1 to 13 in the FastVacc Prospector (Figure 1).

Figure 1: Vaccine development pathway: The 5 key decision points in the vaccine design and testing process targeted by the FastVacc SLR1 questions (in blue). Work package 5 used Q1 - Q13 of the FastVacc prospector tool for analyses.

Figure 2: The 2D maps show the FastVacc prospector results for A) Q9 (Tier 1): What vaccines protect against the FastVacc pathogens or the diseases they cause? Co-cocurrence of influenza A types vs vaccine platform. B) Q1 (Tier 1): For the FastVacc pathogens, which antigens are protective or associated with disease amelioration or control of infection?: Co-cocurrence of influenza A types vs type of viral antigen.

The FastVacc Project Consortium consists of:

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- National Institute for Public Health & the Environment, NL
- Public Health England, UK
- Stadler Serum Institute, DK
- Carscuco Instituto, RO
- National Centre for Epidemiology, HU
- Norwegian Institute of Public Health, NO
- Medical University Plovdiv, BG

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SLR: Identification of protective antigens and immune responses for a spectrum of pathogens:

Based on the established text mining approach, work package 5 has carried out a systematic literature search to identify protective antigens, protective immune responses, and COPs for a range of viral and bacterial pathogens. Application of FastVac Prospector was used as a critical tool for visualising and analysing the results (Figure 2), enabling any single or group of FastVac pathogens to be selected for detailed study with respect to a variety of vaccinology related issues. This knowledge provided the basis for COP based vaccine design. Worked examples of principally different viral and bacterial pathogens have been presented in the project.

SLR: Designing of model vaccines based on COPs

Influenza and coxiella were chosen as two model pathogens for designing candidate vaccines according to COP based principles as deduced from literature review processes. Influenza was subsequently selected as the model disease for which two experimental vaccines were designed, manufactured at laboratory scale, and tested in pre-clinical studies (WP8). An SLR based prediction of model vaccines was performed by integrating information retrieved from the whole range of work package 5 related query questions involving protective antigens, protective immune responses, COPs, and vaccine platforms. By specifically focusing on the linkage between identified COPs and the known vaccine concepts that induce protective immune responses, relevant sets of criteria were developed for design of optimal influenza and coxiella vaccine candidates. Using these criteria, the following model vaccines for influenza were proposed for production and pre-clinical investigation in work package 8: Inactivated whole virus vaccines formulated with CAF adjuvants and polypeptide subunit vaccines based on conserved T cell epitopes (NP, M, PB) and B cell epitopes (M2e, HA2) directly coupled to a universal T helper cell epitope sequence (PADRE). Synthetic peptides were prepared by Fmoc-chemistry and checked for purity and integrity using UPLC-MS and MT mass spectrometry. Before use, peptides were mixed with IFA and the TLR2 ligand Pam3CysSK4 as adjuvant components.

Manufacturing influenza model vaccines for testing in animal models:

**Inactivated whole virus vaccine** was manufactured by using a hen egg derived virus source. Embryonated eggs were inoculated with the H3N2 strain HK-X31 and incubated for 3 days before the allantoic fluid was harvested. Virus was purified before inactivation with beta-propiolactone (BPL). HI titer, total protein, and ovalbumin content were determined according to standard procedures, and inactivation was verified by using MDCK cells. Inactivated virus was formulated with CAF01 or CAF09 as adjuvant.

**Subunit polypeptide vaccine** was designed based on conserved CD4+ and CD8+ T cell epitopes from the H1N1 and H5H1 viral proteins M1, NP, and PB1 combined with B cell epitopes (M2e and HA2) directly coupled to a universal T helper cell epitope sequence (PADRE). Synthetic peptides were prepared by Fmoc-chemistry and checked for purity and integrity using UPLC-MS and MT mass spectrometry. Before use, peptides were mixed with IFA and the TLR2 ligand Pam3CysSK4 as adjuvant components.

WP5: Output and main achievements:

Work package 5 partners have contributed in building and testing technical elements necessary to perform systematic literature reviews (SLR) based on automated text mining. Work package 5 has demonstrated that the established text mining approach can be used to generate systematic knowledge about protective antigens and immune responses (including COPs) necessary for rational vaccine design. By using the automated SLR approach, supported by manual review processes, work package 5 has deduced vaccine candidates for two selected model organisms: influenza and coxiella. Two principally different experimental influenza vaccines were successfully manufactured (work package 5) and tested in animal models for immunogenicity and protective efficacy (work package 8). The work in work package 5 has demonstrated that the established text-mining system used in combination with FastVac Prospector can serve as a valuable decision support platform for efficient evidence-based design of vaccines against a variety of emerging diseases.
Process development: the product

To describe fast track scenario’s for vaccine process development the target product profile needs to be well described. Within Fastvac this target is a prophylactic vaccine product against infectious diseases. To evoke a long lasting, broad immune response, modified pathogen like structures have been proven useful. In complexity and size these vaccine products are positioned between the small (nm), well-characterized, recombinant protein biopharmaceuticals and the large (µm) autologous cell-based regenerative medicine products. Recent guidelines (ICH Q11) give the choice to follow a “traditional route” for vaccine development or an “enhanced approach”, based upon scientific understanding of product and process. The traditional route is more empirical but still will have to yield a description of the process within certain specifications with a rationale that explains why these settings are chosen.

Process Development Scheme

Vaccine development from idea to market launch on average requires 12-15 years development time. In early stages of development some product is already required for research and it is important that the chosen expression system for this initial product is selected on the basis of a target product profile to prevent that an expression system is selected for immediate use that cannot support a robust large-scale production process later on.

has to be demonstrated to support process changes. For less defined products, first a small clinical phase I/II study should be planned after initial preclinical development, and this will need to be repeated after completion of the scale-up and process validation studies.

Methods supporting fast vaccine process development

There are a number of enabling technologies that can support faster process development for vaccines. These include the use of platform technology, high-throughput technology, and the use of disposables. In addition, the application of a Quality-by-Design approach, as feasible for well characterized vaccine products, may support faster development but will certainly provide better control.

Scale up and Process Validation

The scale up principles for vaccines are not different from general scale up strategies for biopharmaceuticals. The used strategies include I. avoiding real scale up by applying multiplication of small units, II. Rules of thumb for specific process steps, as maintaining column height for chromatography, III. Scale down based upon analysis from a large scale system used for a comparable product, and truly as last option IV. the iterative empirical route.

Fastvac experimental work

As part of the experimental work for work package 6 a revised process for the production of influenza vaccine on eggs has been developed at Intravacc and transferred to Cantacuzino, where the process was implemented at a larger scale. It was shown to run without operational deviations and importantly, the product met the pre-set specifications, as required for cGMP productions.
Good Manufacturing Practice requires predictability: in a set of documents a production process is described that, if executed within the pre-set specifications, will yield a product that meets the pre-set criteria for the product, ensuring safety and efficacy. Pharmaceutical products need to be approved for the market within the pre-set specifications, will yield a product that meets the pre-set criteria for the product, ensuring safety and efficacy. For this, it is required to have control systems in place to enable a transparent production performance evaluation. Thus, process development required to have control systems in place to enable a transparent production performance evaluation. This, process development and production runs are primarily governed by product quality properties and product yield is of secondary importance.

In the last decades many recombinant biopharmaceuticals have been launched on the market and regulatory requirements have evolved concomitantly. A Quality-by-Design (QbD) approach was described, starting with the description of the target product. Using assays that are appropriate for demonstrating the required quality characteristics of the target product, the production process is monitored. If this QbD is combined with on-line monitoring of the product quality, enabling faster release of intermediate product for the next process step, the approach also fits with Process Analytical Technology. With this development strategy, based upon scientific understanding, an optimal control – or predictability – is obtained for product and process.

Importantly, vaccines differ from biopharmaceuticals in several aspects, leading to the following challenges for the application of PAT to vaccine development:

1) Most vaccines are not well-characterized products and therefore it is difficult to apply QbD or PAT to the full extend. The knowledge on pathways involved in host-pathogen interaction has been increasing recently. However, practical applications of this knowledge has been proven difficult since currently it is not understood to which extent different pathways influence each other. In addition genetic and environmental factors will influence those interactions and thus the in vivo outcome.

2) The interpretation of PAT has inflated from on-line monitoring of product quality during the different steps of the production process to the on-line, or fast off-line, measurement of any process parameter. For many vaccines, batch release is based on potency assays that are often not robust, making it more difficult to correlate indirect signals to product potency. For Work package 7 it was evaluated if better product monitoring of vaccines can support faster process development as well as faster release of intermediate product.

Experiments performed within Work package 7 of the Fastvac project has been aimed at illustrating that it is worthwhile to increase monitoring of product quality properties to support vaccine process development. This was ranging from characterizing influenza virus with (T)EM and DLS coupled to (A)SEC to analyzing CAF09 adjuvant containing liposomes with NIR.

For Work package 7 it was evaluated if better product monitoring of vaccines can support faster process development as well as faster release of intermediate product.

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AIDS - Q15 of the FastVac proctor tool for analyses and tested COPs (WP5) pre-clinically.

Figure 1: Vaccine development pathway: The 5 key decision points in the vaccine design and testing process targeted by the FastVac SLR1 questions (in blue). Work package 8 used Q14 and Q15 of the FastVac prospector tool for analyses and tested COPs (WP5) pre-clinically.

WP8 has contributed to the FastVac project by:

- Building and testing technical elements necessary to perform systematic literature reviews (SLR) based on text mining.
- Demonstration that automated SLR can be used to generate and integrate key knowledge within vaccinology.
- Optimizing delivery route and formulation for preclinical influenza vaccines designed by work package 5.
- Demonstrating that correlates of protection (COPs) defined by automated SLR can accelerate vaccine testing.
- Demonstrating extrapolation of results between species.

Establishment of automated Systematic Literature Reviews (SLR) for evidence based vaccine development:

Work package 8 partners have been involved in expert groups tasked to specify the pathogens studied by FastVac, provide expert advice and collect animal model data. Most effort has been devoted to assisting work package 4 in building the necessary ontology elements (adjuvant and immunology domains) for SLR1, constructing query questions used in the text mining processes, and evaluating the performance of the text mining by reviewing documents in a web-based expert evaluation tool (EET) as well as providing standard documents to test the performance of SLR1. Work package 8 addressed the milestones and deliverables by analyzing query questions 1 to 13 in the FastVac Prospector and tested the use of COPs preclinically (Figure 1).

Choice of animal models for vaccine testing and optimization of route and formulation

The work package 5 candidate influenza vaccines were tested in mice and ferrets which were predicted to be good animal models for influenza by the SLR (Figure 2). The two vaccines were a universal poly-peptide and a whole-inactivated virus (WIV). They were administered to the animals by the intramuscular or intranasal route. Furthermore, the vaccines were formulated with selected adjuvants:

- Cationic Adjuvant Formulation (CAF) 01, CAF09, Incomplete Freund’s Adjuvant and/or Pam3CysSK4. For the WIV, the CAF09 adjuvant enhanced the efficacy of the vaccine leading to dose sparing and a broader immune response.

Figure 2: Choice of best animal model. (A) Association map generated in FastVac Prospector showing co-occurrences of documents describing different animal models (incl. humans) vs affected part of the respiratory system. The association was done on Pubmed articles on selected pathogens (Q15). Scale: white (no documents), yellow (low number of documents, Red (maximum number of documents). (B) Association chart showing the relative contribution of articles related to disease pathogenesis which describe influenza A (red) and either of the animal models: ferrets (blue), mice (orange) and/or guinea pigs (green).
AIDS - Q15 of the FastVac proctor tool for analyses and tested COPs (WP5) pre-clinically.

Use of COPs to accelerate preclinical vaccine testing

Assays for the COPs identified by the SLR (work package 5) were used to evaluate the candidate influenza vaccines (Figure 3). Thus, after vaccination, IgG, IgA, hemagglutination inhibition antibodies, CD4+ and CD8+ T-cell responses were analyzed by ELISA and flow cytometry in serum and blood samples. After two vaccinations the animals were challenged with influenza virus to address vaccine-induced protection. Most of the selected COPs correlated well with protection but interspecies correlations were hampered by the lack of reagents and assays available for the ferret model. The results support the principle of using an SLR-based approach for defining COPs to be used for preclinical evaluation of vaccines. A wider range of assays and pathogens is available in FastVac prospector (Figure 4 and 5).

Extrapolation between species

In case of a pandemic with limited time to run large clinical trials on the new vaccines it is imperative to know if results from animal studies can be extrapolated to humans. Thus, we have used new and existing data within the FastVac consortium to investigate if extrapolation between animal species can be demonstrated. Our data show that extrapolation is possible for a set of parameters but not for all. A major problem when attempting to extrapolate between species is the lack of comparable assays and reagents, e.g. in one study the most relevant biomarkers for protection against TB as determined in a human study, could not be addressed in the animal models.

WP8: Output and main achievements

Work package 8 partners have tested work package 5 candidate influenza vaccines and optimized delivery route and/or formulation of vaccines against varicella zoster and tuberculosis. Furthermore, based on the automated SLR and existing consortium data on interspecies extrapolation, work package 8 partners have found that animals can indeed be used as models for vaccine efficacy when substantial knowledge is available about the disease patterns in the these models to be certain that correlations with humans are valid. A text-mining based SLR approach is thus a valuable tool to scan vast amounts of literature for such correlations and for identifying suitable animal models and saving valuable time in the initial phase of vaccine development which will even be more revelant in case of new pandemics.
Figure 5: A wider range of assays are available for testing the protective efficacy of vaccines against viral pathogens for mice than for ferrets or humans. Association map showing co-occurrences for the indicated species and virus documents within Fastvac prospector query Q8.3 (main: What studies/assays/tests are used to measure immune protection/COPs in response to vaccination in Man?) and Q8.4 (mouse and ferret: What studies/assays/tests are used to measure immune protection/COPs in response to vaccination in animals?). Horizontal axis = assays, vertical axis = virus families. White = no documents, red = maximum number of documents for the indicated species. The total number of documents retrieved for viral pathogens is indicated in brackets for each species.