Summary Report UNISEC

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Executive Summary

What problem we had to solve

Influenza is one of the major respiratory infections in humans, with high societal and economic impact in Europe. The influenza virus may change antigenically from season to season in an unpredictable way. Current influenza vaccines protect only against virus strains antigenically similar to those included in the vaccine formulation and protect poorly if these strains do not match the circulating strains. A broadly protective, highly effective influenza vaccine is urgently needed to protect the growing and aging European population.

Who we are

UNISEC is a consortium of 11 partners from academia, public health institutes and vaccine industry from 7 European countries and from Israel. The consortium combines expertise in influenza virus and vaccine production, vaccine formulation, vaccine administration, pre-clinical animal models, immunological read-outs, clinical trial organization and execution, data management and data analysis.

What we aimed at

The primary objective of UNISEC was to compare different novel influenza vaccine concepts in order to identify, develop and clinically test the most promising leads for a universal influenza vaccine. The selected candidate vaccine(s) needed to (1) demonstrate broad protection against several influenza virus strains, (2) be technically suitable for development to a marketed product, and (3) in case of the most advanced vaccine candidates, be ready to enter Phase 3 clinical evaluation at the end of the funding period. To this end, we planned to define criteria to be fulfilled by a broadly reactive influenza vaccine, optimize vaccine production and stabilization, test various vaccine concepts in animal models, develop standardized and robust assays for evaluation of vaccine effects, generate knowledge about

immunological correlates of protection, and test the most promising concepts in clinical Phase 2 trials. We also envisaged to disseminate the knowledge generated through publications suitable for both a scientific and a non-scientific audience.

What we achieved

In discussion within the consortium and with the 'Innovative Task Force' and the "Vaccine Working Party" of the European Medicine Agency we prepared a white paper laying out the criteria a broad spectrum influenza vaccine should fulfill in terms of efficacy but also in terms of production, storage, safety and regulatory approval. We developed a toolbox consisting of well characterized virus and vaccine products, robust preclinical animal models (mouse, ferret, cotton rat, pig) for different influenza virus strains, validated standard operating procedures for a range of immunological assays, and sophisticated statistical procedures for data evaluation. Making use of the established animal models we identified three new highly promising influenza vaccine candidates for which clinical Phase 1 evaluation is now envisaged. We established a clinical trial network consisting of experienced and audited hospitals in Hungary and the Netherlands. We performed two clinical Phase 2B trials, each with approximately 200 participants. Both trials rendered highly promising results enabling organization of a Phase 3 trial for one of the candidate vaccines in Europe, to be started in the autumn of 2018. We initiated and organized regular meetings with other European consortia working on universal influenza vaccines in order to coordinate activities and pave the way for regulatory acceptance of new broadly reactive candidate influenza vaccines.

How the European population profits

A broadly protective influenza vaccine is urgently needed. The successful finalization of Phase 2 evaluation of two of the UNISEC candidate vaccines, Multimeric-001 and Flu-v, allows these vaccines to advance to Phase 3 evaluation. If this evaluation is successful the vaccines can be made available to the population within a time frame of about 3 years. Yet, the development will go on. With 12 PhD students having been working on the various projects, receiving additional training during regular meetings and lab exchanges, and building their own scientific networks, UNISEC delivers the next generation of influenza vaccine experts ready to take on the challenges of the future.

Summary description of project context and objectives

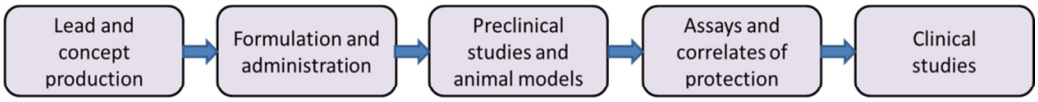
Influenza is probably the infectious disease with the greatest impact on the European population. Each year about 5-10% of the general population experiences an influenza infection which often goes along with serious illness requiring medical attendance; annually the infection takes a fatal course in 15-75000 Europeans. Many of the fatal cases are in the elderly, underlining that influenza is a severe problem for an aging population. Economic losses in the EU due to influenza, caused by absence from work and by health system attendance, were estimated to be about € 2.5 billion/year with a tendency to increase.

Vaccination is the most effective method to control influenza epidemics. The classical seasonal influenza vaccines as used in Europe, are produced by growing virus in eggs or cell cultures. The seasonal flu shot consists of a mix of three or four different viruses and provides short-lasting protection against the virus strains included in the vaccine. This strategy remained similar over the last 70 years. Unfortunately, influenza virus changes its antigenic makeup constantly and the vaccine therefore has to be adjusted every year. Even then it happens regularly that the viruses in the vaccine and the viruses causing infection in the population do not match. If that is the case the vaccine is poorly effective or not effective at all. To avoid this problem it is of great importance to develop 'universal' vaccines which can protect against a broad range of influenza virus strains.

UNISEC was one of 5 consortia selected by the EU in 2013 to work on the development of universal influenza vaccines. The main objective of UNISEC was to identify, develop and clinically test the most promising concept(s) for a universal influenza vaccine by comparing a number of different vaccine concepts. The vaccine concept(s) to be selected needed to offer adequate (universal) protection, but also needed to be technically suitable for further development to a marketable product.

To achieve this overall objective, UNISEC defined six sub-objectives:

1. To define a set of criteria concerning technical, immunological and market aspects along which universal influenza vaccine candidates can be evaluated.
2. To develop innovative formulations and production processes for stable influenza vaccines.
3. To compare various vaccine concepts in suitable animal models, either head-to-head or by using standardized experimental conditions in order to enable the selection of the most promising vaccine candidates for further evaluation.
4. To establish experimental conditions including standardized assays which allow the comparative evaluation of vaccine concepts in animal models and in clinical trials.
5. To evaluate promising vaccine concepts in (SME-driven) clinical trials.
6. To disseminate knowledge on UNISEC research during the several pre-registration stages of a universal influenza vaccine.



These objectives culminated in the UNISEC pipeline of vaccine development which comprises all steps from vaccine identification, production, formulation, and preclinical testing to clinical testing. In the context of the UNISEC pipeline we aimed at developing a toolbox consisting of standardized reagents, immunological assays, operating procedures, and conditions for preclinical vaccine evaluation and clinical trial studies which would allow the comparative evaluation of different candidate vaccines throughout every stage of development.

Description of main Scientific and Technological results / foreground

Pre-clinical studies

Side-by-side comparison studies were performed within the UNISEC consortium to provide pre-clinical animal model data on various vaccine regimens and formulations, which could be used in the development of a broadly protective universal influenza vaccine. The animal models tested were mouse, cotton rat, pig and ferrets. These were used to identify the most promising vaccine candidates and formulations.

Apart from animal studies, new preclinical model systems based on human immune cells have been developed by UNISEC partners which can provide insight how the vaccines interact with the human immune system. These model systems make use of blood cells (PBMCs) from human volunteers which are either cultured and exposed to vaccines *in vitro* or are injected into immune-deficient mice to generate so-called 'humanized mice' which mimic the human immune system *in vivo.*

Four vaccine concepts were evaluated in detail in preclinical studies. The first concept made use of the existing whole inactivated virus (WIV) influenza vaccine in combination with different adjuvants and delivered via intramuscular injection or via application into the nose. Two adjuvants were identified which were particularly potent in enhancing immune responses in the blood and in the upper respiratory tract and facilitated protection from infection by a broad range of viruses.

The second concept consisted of adeno associated virus (AAV) which was cripple and manipulated such that it induced the production influenza virus proteins. These AAV influenza-vaccine vectors generated by RKI are active for at least a year after application and produce vaccine within the vaccinated animals without detectable side-effects. Our studies

have shown that they are suitable to induce broadly reactive antibodies, both in the mouse and the ferret model.

The third concept, a DNA vaccine, was tested in a ferret and in a pig model. The results show that virus replication was restricted to the upper respiratory tract and viral shedding was reduced in vaccinated animals following homologous seasonal influenza challenge. This vaccine also protected ferrets against highly pathogenic H5N1 virus with vaccinated animals being 100% protected with regards to clinical symptoms and weight loss compared to unvaccinated animals. These results indicate a broad immune response against influenza viruses.

The fourth concept, a reverse peptide vaccine designed on basis of immune responses found in humans, has been formulated by the consortium partners and tested using the newly developed human PBMC assay. Our results indicate a significant immune response in human immune cells after *in vitro* exposure to the vaccine and the vaccine is now ready for *in vivo* testing in animal models.

Vaccines are traditionally formulated as solutions which are usually administered via intramuscular injection. Vaccines in dry powder from would have the advantage of being much less vulnerable to degradation and could be administered via inhalation. Therefore, techniques were developed to formulate the different UNISEC vaccine candidates as dry powder. In addition, it was demonstrated that these vaccine are suitable for delivery with a dry powder inhaler, the Twincer, which was developed by one of the UNISEC partners.

UNISEC product portfolio and developmental stage in 2018

Product	Product Details	Main Developer	Developmental stage
M-001 vaccine	Recombinant polypeptide	BiondVax	Ready for Phase 3
FLU-v vaccine	Conserved peptides	SEEK	Ready for Phase 3
DNA vaccine	6 DNA plasmids	SSI	Ready for Phase 1
AAV viral vector	NP / HA based	RKI	Ready for Phase 1
Reverse vaccine	Active peptides	hVIVO / RUG	Preclinical
Twincer	Pulmonary vaccine delivery system	RUG	Ready for Phase 1
CAF01/09 adjuvant	Cationic liposomes	SSI	Ready for Phase 1
CTA-3M2e-DD	M2e adjuvant	UGOT	Preclinical

Standardised Immune Assays

A universal influenza vaccine will most likely need to induce T cell immunity as this type of immune response targets conserved parts of the influenza virus in contrast to antibodies which mostly target highly variable parts. There are numerous approaches to measure T-cell responses to a stimulant, all have their advantages and disadvantages with regard to handling, expense, sensitivity and the amount of information acquired. For example, the multi-parametric intracellular cytokine staining assay selected for analysing the UNISEC trial

samples delivers a large amount of data concerning the nature of the response but is relatively expensive and labour-intensive. The IFN-γ Elispot is simpler, cheaper and potentially more sensitive, but is relatively limited in the breadth of information gained. The measurement of cytokine RNA by PCR has the potential advantage of sensitivity and can be used to measure responses in species for which the necessary anti-cytokine antibodies are not available, an important factor for animal models such as ferrets and cotton rats where these reagents are not readily available. Within the UNISEC project all these assays were employed and their potential was evaluated.

In addition to the 'core' assay for T-cell response described above, a suite of additional assays that can yield valuable information concerning the nature of the immune response to the different vaccines was developed and validated. Partners have established and standardized antibody- and T cell-based assays for evaluating immunogenicity of universal influenza vaccine candidates tested in our clinical trials): HAI assay, micro-neutralization assay, ELISA for IgG and IgM, IgG subclasses analysis (multiplex), and Elispot assay. These antibodies are considered the most potent for protective effector functions against virus infections, including influenza.

With respect to the clinical studies, the participating laboratories developed detailed standard operating protocols (SOPs) and other quality control (QC) documents for use in the handling, storing, and analyses of clinical samples. The laboratories underwent a comprehensive external evaluation of these established laboratory QC systems, including the SOPs. This resulted in the laboratories concerned acquiring GCLP compliance.

As the putative correlates of protection for vaccines designed to induce predominantly T-cell responses remain unknown, it was considered important to analyse the clinical samples using an approach that would maximise the information generated. To this end, a strategy for the multi-parametric FACS analysis of immune responses in the human immune system (PBMC cells isolated from vaccinated individuals) was developed in which cells stimulated with different immunogens are evaluated with regard to the induction of multiple intracellular cytokine responses in $CD4^+$ and $CD8^+$ T-cells.

Originally, our objective was to establish and define recommended techniques to analyse samples from clinical trial studies. As the project proceeded, it was decided not only to make recommendations but to also carry out all these recommended assays for the UNISEC clinical trials in a centralized way. Redistribution of funding within UNISEC, made possible by two amendments in the grant agreement, allowed these additional tasks to be performed at RKI and NIPH. High throughput Cell Mediated Immunity (CMI) assay analyses on clinical trial samples were performed. As a result, an interactive database of the immune responses induced by the vaccination of humans was generated, allowing a very detailed characterization and comparison of the responses.

This is valuable because regulatory authorities are awaiting such data to provide a basis for new criteria and guidelines for the new generation of universal influenza vaccines. These complex assays developed by UNISEC, as part of the complete toolbox, allow to get a detailed picture of the immune responses induced by vaccination. This should be of benefit to the efforts being made to identify correlates of protective immunity and mode of action for novel influenza vaccines, particularly those that are designed to predominantly induce T-cell responses i.e. CMI. Successful analysis of PBMC samples from the volunteers enrolled in the

SEEK and BiondVax Phase 2B clinical trials CMI confirmed that the developed assays are robust and suitable to be employed in context of medium scale clinical trials which was unprecedented, at least in influenza vaccine development. The assays are 'universal' and can be applied to any vaccine, as the only variable is the sequence of the peptides required to stimulate the PBMCs. The successful development and implementation of this assay is a major achievement of the UNISEC consortium.

UNISEC Trial Studies

In the context of the UNISEC project two vaccines, FLU-v (SEEK) and M-001 (BiondVax), were tested in separate Phase 2B trials. For each vaccine, different formulations and regimens were assessed. Both studies had similar study population characteristics (though regionally different), used common sample collection protocols and collection time-points, and all PBMCs were analyzed by flowcytometric analysis conducted centrally at the RKI using the above mentioned CMI assay. A total of 937 PBMC samples were analysed, requiring 11,504 individual stimulation cultures to be set up to yield 93,960 data points for statistical analysis.
The objective of the studies was to assess the differences in T-cell activation markers induced at short-term after vaccination with Flu-v or M-001 compared to placebo. In a sensitivity analysis we also determined the effects in only those subjects that were non-responders at baseline,that is after removing those subjects with pre-existing responses before vaccination.

Conclusions FLU-v

In summary, the results in this trial are encouraging. Adjuvanted FLU-v has been shown to be strongly immunogenic both in cellular and humoral responses. Higher injection site reactions were observed in the adjuvanted FLU-v arm compared to the non-adjuvanted FLU-v arm, these were mild and moderate in intensity in the majority of participants and severe in intensity in around 9% of participants. It is clear that in the adjuvanted FLU-v group the reactions observed are the result of the additive inflammatory effect caused by the adjuvant and the antigen, FLU-v. The data presented grants further development of a single dose of adjuvanted FLU-v in a Phase 3 setting with a larger cohort of vaccinated people where efficacy and safety can be explored in a real world setting.

Conclusions M-001

The results of this successful study demonstrate that M-001 induces the desired TH1 response, no response was found of TH2 cytokines that were also measured. Among the responders in the experimental group, CD4 cells secreted statistically significantly higher levels of IFN-gamma and IL-2 as well as some TNF-alpha as compared to the placebo control group. CD4 T cells have many roles in promoting antiviral immunity through multiple direct and indirect cellular interactions. It should be noted that in previous clinical trials, M-001 induced also significant CD8 responses which were not found in the current trial.
There was a dose related response and higher responses to the high dose vaccine (1.0mg) which will be used in future trials. No treatment-related Severe Adverse Events were observed, all adverse events were mild to moderate, mostly at the injection site and all adverse

events observed were transient. A clinical efficacy Phase 3 trial with a large sample size will translate the immunogenicity outcomes to efficacy. This study will start in autumn 2018.

Potential impact and main dissemination activities and exploitation results

Within the UNISEC consortium we have successfully completed a comparative Phase 2B clinical trial study of two different universal influenza vaccines. One of these products, the BiondVax M-001 universal influenza vaccine, will start a Phase 3 clinical trial study in Europe, starting in the autumn of 2018. Funding for this Phase 3 efficacy study has been provided by the European Investment Bank (EIB). The fact that BiondVax participated in the public UNISEC consortium, and the transparency of the results, has contributed to the EIB making this loan available to a small SME. The company SEEK also successfully completed the UNISEC Phase 2B clinical study. UNISEC has proved that small SME organizations like BiondVax and SEEK, each with less than 20 employees, can still develop a novel vaccine candidate and bring it through clinical development. UNISEC proves that consortia of European national health institutes, academic organizations and companies can work together to develop innovative products throughout the complete pipeline of product development.
The development of our cell-mediated immunity (CMI) assays and their routine application in the analyses of clinical trial samples allowed a complete insight in the T-and B cell responses in vaccinated individuals.
UNISEC has also developed a number of other universal influenza vaccine candidates throughout the preclinical stages. Three of these products successfully completed their pre-clinical testing and are now ready for clinical development. The DNA vaccine concept from SSI in Denmark showed broad protection against seasonal and highly pathogenic influenza strains in pig and ferret models. The AAV vector vaccine from RKI in Germany also generated broad immune responses in various animal models and the reverse peptide vaccine showed promising immune responses in our enhanced PBMC *in vitro* assays.
Standardised testing of different universal influenza concepts with the UNISEC toolbox throughout all stages of development allows a fast, transparent and cost-efficient analyses of different products.
Dissemination of all these results from the UNISEC consortium has not only been concentrated on scientific publications (34 so far), Ph.D. thesis projects (12) and scientific presentations (over 100 so far), but also resulted in two international patents, ten press releases, 20 TV interviews and national newspaper articles.. UNISEC has also organized meetings with international stakeholders like DG-SANTE, WHO, BARDA, NIH, and meetings with the Innovative Task Force and Vaccine Working Party of the European Medical Agency. UNISEC has collaborated extensively with other European consortia involved in universal influenza vaccines and has also participated in the development of new WHO guidelines for the next generations of influenza vaccines.
The results have been received positively by the different stakeholders in the field of universal influenza vaccines. The toolbox has proven to be an excellent instrument for the comparative evaluation of new vaccines. With all of the above in mind, we hope to continue our activities with future public funding.

Address of project public website and relevant contact details

Project Website: www.unisecconsortium.eu

Project Manager Dr. E.D.L. Schmidt
e.d.l.schmidt@umcg.nl / edlschmidt@outlook.com
+31 6 177 54 636
The Netherlands

Project Coordinator Prof. Dr. W.H Frijlink
h.w.frijlink@rug.nl
+31 503633285

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