ENOVA - European Network of Vaccine Adjuvants (COST Action CA16231)

2nd Workshop on Vaccine Adjuvants

Bench to Bedside – How to get your vaccine into clinical testing

Eigtveds Pakhus, Copenhagen, Denmark, January 21-22, 2019

Meeting report.

21 January 2019

Session 1: Plenary

Clinical development or not – when to take the decision. Dennis Christensen (Statens Serum Institute).

Dennis Christensen gave an overview on SSI adjuvant development and the important consideratinos of adjuvant development. SSI have four first-in-man trials with adjuvants:

1. AG85B -ESAT-6 in IC31
2. AG85B ESAT -6 in LTK63
3. Ag85B-ESAT-6 in CAF01
4. BCL-XL in CAF09b.

CAF01 currently in trial as follows: TB in phase 1, HIC in Phase 1 Malaria Phase 1, Chlamydia also in Phase 1. Vaccines for Flu, IPV and Group A strep all at preclinical stage. SSI have developed many adjuvants developed. In general, there are only a small number of adjuvants that are approved: MF59, AS01, AS03 and AS04. One of SSI's lead adjuvants, **CAF01,** induces Th1 central memory cells, induces stronger humoral immune response than aluminium hydroxide. It induces Th17 after parenteral immunisation (which is not achieved by other adjuvants to date) and consequently mediates fast recall on mucosal tissues. Effective further development of any adjuvant requires teaming up between Ag designer & an adjuvant developer.

Key Steps in the development of an adjuvant:

1. Describe the uniqueness of your product
2. Make a target product profile
3. Make a financial plan that can be committed to in a collaboration
4. Search for potential partners ideally at the same product development stag

A developer need to know their product. It is important to ask – "Is it ready ?" The following questions can act as a reality check:

- Can the adjuvant compete with or does it differ from freely accessible state of the art?
- Is the formulation stable (chemical stability / physical stability) Vaccines by their nature are not stable and stability should be studied for over a year.
- Can the adjuvant be produced at GMP quality: Scalable, reproducible, does production fit with standard equipment; is there a stable supplier of raw materials.
- Is the adjuvant antigen formulation procedure durable?

There can also be unforeseen circumstances which can cause dramatic delays, for example the development of CAF09 was delayed. Immunogenicity should be benchmarked with positive controls already tested in humans – humoral responses benchmarked against AlOH, MF59 and AS03; CMI responses AS01, IC31, CAF01. Also as many samples should be taken samples in small animals as are possible to get in humans, e.g. Blood, skin, mucosal responses etc. It is important to ask if the adjuvant has been tested in relevant animal models, because even humanised mice do not respond like humans. Can potential side

effects be reduced / eliminated by formulation? Finally, the issue of intellectual property was discussed. If there is no patent, there is no product. Filing patents is essential. Investors are needed for development, and they need a revenue in the end.

From Lab bench production to GMP – what is required. Lars Andreasen (Statens Serum Institute)

SSI have considerable experience in this area with 25 clinical trials to date up to and including Phase III and over 10 adjuvanted vaccine trials.

Steps from research to clinical trial:

- Research - leading to CMC development (cloning, process, analysis, formulation),
- GMP production
- GMP analysis (specification characterisation stability and GMP release)
- Clinical Toxicology, clinical design, clinical operations.

Documentation is critical from the minute the product leaves research.

Questions to consider: Is the expression system your own? Or is it GMP compliant? The process is important for production, know what you have and what was done. The developer needs control of analysis and formulation. When these are all clear – go to "real" GMP production. At the end of this stage, no more changes should take place in the product, the product must be fixed.

GMP production means documenting everything. GMP analysis needs to be developed in line with GMP production. Stability takes minimum of 2 years, because clinical trials take 2 years and you don't want to have to remake a batch half way through a study.

Safety must be demonstrated before clinical phase I, which means a repeated dose toxicological study in vivo is required. This should be supported by in vitro studies, if a relevant model can be found. These studies should be carried out at GLP level and supported by relevant pre-clinical studies (non-GLP). Again the importance of documentation was emphasised. "If you don't document, it hasn't happened". Vaccine used in toxicology should be the same vaccine as the one you take to clinical trial. It must be of the same quality, but doesn't need to be manufactured at full GMP.

If a CMO is doing manufacture, it is still the adjuvant developer's responsibility to make sure that everything happens correctly. Better documentation of research means better tech transfer.

Bottom line – know your vaccine. Understand GMP manufacturing, product spec (ID, impact of concentration, impurities, stability indicating assays, appearance, sterility, Endotoxin levels). For example, CAF01 contains some antibacterial properties, so they need special sterility indicating assays for CAF01 products. To prepare for clinical trial, one needs to know whether formulation can be done in advance or if you want to use on-site mixing.

Take home – be sure to plan for product development to GMP level. Consider time and resources, competences of CMO/ CROs. Know the vaccine you are putting into clinical: build in quality, which is much better than testing for quality. Finally, make sure the product fits with the idea of the clinical trial in terms of the dose titre, formulation and route of administration.

Session 2: Case-Studies.

Gliovac: Towards a therapeutic vaccine against Glioma. Virgil Schijns (Epitopoeitic Research Corporation).

Glioblastoma multiforme (GBM) survival is 12 to 18 months and is uniformly fatal. There is only one therapy Temozolomide for newly diagnosed GBM which results in only 2.5 mo increase in survival of combination over radiotherapy alone. However there is a tail in curve for Tem/XRT treatment.

Avastin which acts by blocking vascularisation is approved by the FDA for use in GBM patients in the US. It is not approved in EU because no increase in survival has been shown. ERC has developed GlioVac as therapeutic vaccine which has been approved for compassionate use in phase I trials. MRI data showed that the vaccine caused nice regression of tumour growth. New active substance – allogeneic and autologous tumour antigens. The process involves Treg depletion first with cyclophosphamide which reduces immunosuppressive immune cells in patients. GMCSF is used as an immunopotentiator. It has been shown to be very safe. Finally the antigens were isolated from the excised patient tumours and they make an autologous vaccine from this in ERC. They also consent the patients to be able to supply antigens to prepare vaccines for other patients. Both allogenic and autologous tumour antigens are then administered together to an individual patient. Each patient gets material from 3 allogeneic patients which results in a broad polyclonal immuno-specific response. He acknowledged that there is a theoretical risk of inflammation in the brain, but they haven't observed this in patients yet.

Radiated inactivated tumour cells and disintegrated/ lysed tumour antigens are administered. Each cycle takes 1 month and allogeneic and autologous antigens are administered on different days. The antigens are injected into the leg close to groin lymph nodes, which generates a strong polyclonal immune response. The improved survival in compassionate use patients was reported to be 10.6 months. All patients were still alive at 6 months (50% died in control group) and at 12 months they observed 40% survival with GlioVac, compared with only 10% without vaccine. The vaccine is now in Phase II clinical trial in US. Details available on ClinicalTrials.gov.

Inclusion criteria: Patients must have a CD4 lymphocyte count of >450 /ul.

Development pathway: The GMP production of antigens was built from scratch on campus with clean room. It is an orphan disease, so orphan Gliovac is an drug. Once have approval in phase II study, they will get marketing licence in parallel with phase III study

Paper in Vaccine. 2015;33(23):2690-6.

Clinical development of therapeutic Allergy Vaccines. Peter Andersen (ALK Abello)

Alk Abello develop sublingual allergy Immunotherapies. One of their main products is ACARIXAX, a dust mite vaccine in tablet form.

Over 1.5 million patients have been treated with ALK products, with a revenue of 2.9 B DKK. They are an immunotherapy company, but immunotherapy is not its main priority. Their sublingual product results in a 30% reduction in symptoms to grass, which is maintained after treatment stops. They also produce injectables due to country-specific formulations, e.g Germany prefers alum and France has a preference for drops. The following products are in the ALK Abello portfolio:

- GRAZAX: the tablet against grass allergy is approved in North America and Europe.
- House dust mite tablet approved since 2015 in Japan and US.
- Cedarcure in Japan for the prevention of allergic rhinitis.
- Ragweed vaccine available in North America since 2014.

There are five main tablet based products. The mode of action of these was reviewed by Shamji and Durham, J Allergy Clin Immunol (2017 140(6) 1485-1498). Patients show Th1-like responses; Treg responses and blocking antibodies are also stimulated. High levels of blocking antigens were reported. There are EU guidelines on allergen products and guidelines on the clinical development of products for specific

immunotherapies which are very short (13 pages). ACARIZAX targets the most prevalent allergen in the world (dust mite antigens). The antigens are quite conserved across the world and 50% of asthma patients are allergic to these. They used a simple mouse house dust mite model and showed that interleukin-5 and IL-13 and Der specific IgE were reduced. The regulatory authorities accepted this as part of the documentation.

A Phase II dose finding clinical trial was performed with 200 patients in each arm (MT-02). A pivotal Phase III study was performed also, with 1 year treatment and 6 month monitoring (MT-04). The phase II trial looked at corticosteroid use before and after treatment. They also monitored IgE in patients, and countering IgG4 which increases over time. The IgE blocking factor (measure ability of serum to block IgE) increased overtime up to 80 weeks. Even patients treated with the lowest dose, showed an increase in blocking factor. They have also performed a 5 year trial, where patients were treated for 3 years and followed up for 2 years. Antibodies and blocking factor increased for 3 years and gradually reduced when treatment stopped, showing the that it modifies the immune system in a manner which is somewhat sustained.

Challenges: changing clinical practises – tablets versus injectables

Biomarkers predictive of clinical effect

Improved efficacy with adjuvants?

Fewer administrations.

Clinical development of Veterinary vaccines/ adjuvants: Gregers Jungersen (DTU Dept of Health Technology).

There are different approaches for Vet vaccines. Prophylactic vaccinations are standard for human and companion animals. But it is different for livestock and endemic diseases. Good farmers don't need to vaccinate and don't need antibiotics, farmers with no control over the environment, need vaccines and use high levels of antibiotics. Emergency vaccinations are only needed in connection to outbreaks. One of the critical points is that vaccinations against diseases like swine fever are not carried out because one needs to be able to test and demonstrate that animal is infection free, and antibodies are used as markers of infection or exposure. DIVA vaccines, or marker vaccines, do not interfere with diagnostic tests as it is important to diagnose infected animals even among those that are vaccinated. The DIVA vaccine must be protective and there must be an accompanying DIVA diagnostic test. On another note, if one can argue that the vaccine can reduce antimicrobial use then that is something that regulatory authorities really like.

The EMA CVMP committee regulates medicinal products for vet use in EU and outside of EU they are informed by the VICH guidelines. The EU wants more and better vet vaccines and it is a priority area for the EMA. There currently is a joint EMA HMA action plan to facilitate more timely access to Vet vaccines. The process is rigorous and involves an Eligibility request followed by notification of intention to submit for Marketing Authorisation application (7 months before); confirmed at 2 to 3 months before CVMP appoints rapporteurs (2 to 3 months before MAA). There is a pre-submissions meeting, followed by submission and validation of the application. The Scientific evaluation takes up to 210 days for a CVMP scientific opinion. There is then an EC decision within 67 days of the CVMP opinion.

There are different pre-authorisation procedures for different countries and one can also get a national authorisation if only licencing for one country. In terms of adjuvant guidelines, a mechanism of action is required, as well as safety and other usual quality and safety requirements. The potency of each batch needs to be established. It should be noted that farmers tend to half or quarter the vaccine dose to save money. This cannot be recommended without efficacy studies, but on this note it is interesting that DTU have shown when the antigen level of tetanus toxoid is reduced, it stimulates a reduced antibody response without compromising the cell-mediated immune response.

Clinical development of prophylactic vaccines/ adjuvants. Morten Ruhwald (CMO SSI).

Much of their work focuses on TB and chlamydia vaccines. No vaccine is perfectly safe and no vaccine is perfectly effective. Also, one bad vaccine story can be detrimental for support to vaccines. There was a bad news story in Denmark regarding HPV in 2015 and took a long time to recover confidence in the HPV vaccine after that.

His presentation focussed on strategic planning for success and the critical steps, i.e. develop a target product profile (TPP), a clinical development strategy AND manufacturing and formulation strategy which comes together in the Exit Strategy. The TPP is a Blue print, informing clinical manufacturing targets.

Phase 1 includes adjuvant selection as aim, dose escalation and healthy volunteers (20 to 40).

Endpoints safety and immunogenicity. The key question is does it look safe at a low dose to high dose?

Phase 2: Dose schedule efficacy in the target population. One can select a high risk population for accelerated efficacy 100 to 500 patients. Choose the most susceptible people in target population.

Phase 3 effectiveness rare adverse events.

The importance of screening patients was described to ensure that they meet criteria. Once they are ramdomised across vaccine and placebo arms, they should be called in a clinical exam and bloods. It was recommended to call them at 3 days, 7 days and so on. There should be a safety visit clinic at 28 days and another vaccine treatment followed by more safety tests. In all, a lot of visits are needed with a lot of clinical exams.

By phase 3, the emphasis has moved from immunogenicity, to identifying adverse events. About 66% of vaccine trials move to phase 2, 33% move to phase 3, which means that 74% progress. If something goes wrong its back to the beginning.

The likelihood of approval is 16% in phase 1; likelihood in phase 2 is 24%, and 74% in phase 3 based on a large number of trials. The cost of trials is huge, for example the total cost was 5.5 M for small pox; $605 M for malaria (100,000 patients) and 1.5B for Ebola.

The safety profile must be well characterised in the clinical development plan. The vaccine must be well tolerated for prophylactic vaccines. The safety profile is less stringent for lethal diseases e.g. haemorrhagic fevers. All serious adverse events must be detailed and monitored and collected and figure out whether related to vaccine. Assay robustness is critical. Flow cytometry is immunologically detailed but there is a lot of drift in the assay, which means one may have to lower the bar immunologically. The Ab response is more robust and more reproducible, although it is not so relevant for TB immunology. Take home message:

- Know your TPP and review it often.
- Clinical dev plan and regulatory filing are always disease specific – get help from professionals
- Safety first and start saving up – its costly.
- There are no quick fixes

The story about aluminium hydroxide - Erik Lindblad (CRODA)

The history of aluminium hydroxide was described from 1931 to present day. Initial formulations were alum precipitated vaccines, but this further developed into aluminium adsorbed vaccines.

Mannhalter et al., looked at Ag uptake and antigen presentation.

In 1989 it was shown that Alum is a Th2 stimulator with strong inhibition of IL1 alpha, strong inhibition of anti-interferon and il4 antibody and strong inhibition of CD4, which was indiciative of a Th2 subpopulation in comparison with Freunds. In 2006 Tschopp discovered inflammasomes.

Day 2: 22nd January.
Session 6: Access and facilities

Transvac Services and Training - Hilde Depraetere (European Vaccine initiative)

Hilde Depraetere spoke about Transvac 2. There are 26 partners in 9 countries with H2020 for 5 years. There are three types of activities within Transvac: 1) transnational access; 2) joint research activities and 3) networking activities. Transvac supports vaccine related projects in preclinical phases of development. They support and accelerate vaccine related projects by providing access to the services and expertise contained within the Transvac infrastructure. The majority of services are available free of charge, and to obtain access an application is submitted. Technologies include cross platform screening and optimisation of protein expression in a range of expression platforms including insects, MVA, Pichia. One can also get access to formulations including liposomal or mucosal adjuvants. Analytical services include SPR, MS based proteome analysis; preclinical GLP production services; GMP production services (paid service) Structural biology (paid service). The overarching themes are:

1. Immuno-correlates of protection and systems biology: NGS, Mass spectrometry, metabolomics, cytokine analysis, transcriptome profiling; Mathematical modelling and MS imaging (paid)
2. Animal models ferrets, mice, farm animals; immunogenicity, efficacy and challenge studies in different animal models.
3. Regulatory support - human clinical trial support.

Applicants are encouraged to follow an integrated approach by applying for complementing services, e.g. one project has applied for 5 different services.

Eligibility - Must work in an EU member state and work in a different country than the TRANSVAC provider. Also one must openly disseminate results generated with support of TRANSVAC2. The deadline for the next services call is 15 April 2019

Another aspect is training in different aspects of vaccine dev. There are 14 different courses; each one given twice. Check out website (http://transvac.org/training.html#applyTraining). Many of the course also include accommodation for free. People were asked to contact Hilde if they want to be involved in a more sustainable infrastructure.

SSI service facilities - Anders Baek Vestermark (Statens Serum Institute)

SSI can help with product development from expression to downstream processing and analysis. Specifically, SSI can help with cloning and expression in *E. coli* and *L. lactis* and have two SSI-owned expression systems (*L. lactis* and *E. coli*). A typical developmental process was outlined: The first thing to do is removing any His-tag and perform codon optimisation to improve the yield. The downstream purification commences with depth filtration with removal of endotoxin and so on, typically, 2um filtration IEX and TFF. SSI can also be involved in formulation to stabilise and to adjuvate antigens. GMP production from 5L to 30 L bioreactors. Can manufacture pure GMP product. Usually three batches are prepared to demonstrate that it can be done consistently. GMP analysis for release of product for human trials: Appearance identity purity and impurities, potency quantity, general tests. All from guidelines.

Jenner Institute Service Contract Facilities - Anita Milicic (University of Oxford)

The Jenner Institute has a viral vector core facility (if used via Transvac the costs will be covered). They supply external academic and industrial collaborators across both human and veterinary vaccines, where there is no conflict of interest with the research carried out at the Jenner Institute. They facilitate the production of recombinant adenovirus and pox-virus vectors in adequate yields and with appropriate controls for preliminary animal studies. They also manufacture starting materials for GMP work (pre-GMP seed stock).

In addition there is a DNA/RNA extraction service. Clinical and preclinical samples can be processed for high quality RNA, DNA and protein extraction, using QIA symphony, at around €80 per hour. Agilent tape-station is used for the quality analysis of the extracted material.

VFI Service contract facilities - Roland Ventura (Vaccine Formulation Institute)

The Vaccine Formulation Institute (UK) is a not-for-profit organisation, which formed from the Vaccine Formulation Lab at the University of Lausanne. They provide adjuvants and formulation expertise to groups working in vaccine manufacturing, development and research. They work with a wide range of adjuvant activities, from formulation studies to process development and upscaling from lab-scale to GMP-compatible systems for adjuvants. The VFI can offer technology transfer to the clients' own site if needed. They can also provide access to adjuvants, adjuvant preparation equipment, and QC equipment. Alternatively, they can transport equipment to the client's own site if needed. Their expertise includes emulsions, microparticles, aluminium salts, liposomes etc. They also provide a wide range of adjuvant/formulation characterisation studies on a fee-for-service or collaborative basis. They can also provide training and services via GHVAP for Gates grantees, or to researchers through Transvac's free of charge services. The VFI avoids any conflict of interest by not developing their own vaccines.