ENOVA Deliverable 1: Report on the gaps in the development of adjuvants

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1. Introduction

The European expertise in the field of adjuvanted vaccines and vaccine formulations is extensive, however it is fragmented and dispersed over numerous research centres and pharmaceutical vaccine developer companies. One of the aims of the ENOVA project is to bring this knowledge together. The aim of deliverable 1 was to identify the gaps of knowledge in the adjuvant field, and for that purpose a round table discussion was organised during the 1st ENOVA Adjuvant Workshop in Lausanne on 26-27 March 2018.

2. Methodology

To be able to address the gaps, four round tables were organised in the following areas: Vaccine formulations, Therapeutic vaccines, Preclinical vaccine development and Clinical development/ Regulatory aspects. Two rounds of discussions were held in each field so that the participants could take part in at least 2 subjects. Each table had a list of previously recognised gaps in specific areas that were extracted from participants' slides and used as starting points of discussion.

3. Results

Gaps in the development of adjuvants: Formulation and Characterization

The following topics were used as a starting point for discussions and were prepared by the organising committee:

- Theoretical and practical know-how on formulations of delivery systems
- Tools to characterize the formulations (analytical competencies are often missing)
- Awareness and skills within the vaccine community to formulate and characterize adjuvants
- Biocompatible nanomaterials to be decorated with immunogenic carbohydrates and synthetic glycosylated antigens
- Nanoadjuvants that need to be tailored for particular antigens
- Selection of antigen for vaccine formulation: pathogen/model organism

Based on the discussion on formulation and characterisation, several gaps and recommendations have been identified.

Interactions between antigen and adjuvant should be further characterised to enable optimum vaccine safety and efficacy. Lack of knowledge on how to characterise antigen/adjuvant formulations was identified. It has been proposed to create guidelines/recommendations for antigen–adjuvant systems aiming to harmonise existing protocols. The protocols should include size, surface charge, pH, HLB, morphology, chemical properties, formulation stability, toxicity, etc.

The doses should be extrapolated based on route of administration (from animal to animal/human systems).

An overview on commercially available adjuvants, along with their route of administration, type of immune response, animal models and chemical properties should be reviewed and presented.

Gaps in the development of adjuvants: Immune response / preclinical

The following points were used as starting points of discussion and were prepared by the organising committee:

- How do you select adjuvants to tailor response?
  - Direct the response towards the desired Th1, Th2, Th3-type responses.
  - Induction of mucosal responses
  - CD8+ T cell inducing adjuvants
  - Adjuvant combinations to improve immune response in elderly
  - Adjuvants to boost activity of DNA (RNA) based vaccines
  - Veterinary adjuvants for antibody induction
- Elucidating the mechanism of action
  - What are the main immune signatures of different adjuvants
  - Depot function versus immuno-stimulatory function
  - Which immune pathways are triggered (PRR)?
  - Qualitative versus quantitative differences between different adjuvants
  - Bio-distribution studies via fluorescence labelling or imaging
  - Mode of action in different settings (in vitro and in vivo)
- Bioassays for classification of the stimulatory activity of adjuvants:
  - Harmonisation of assays
  - Introduction of reference materials
  - Validated screening assays for adjuvants
  - Standardized set of parameters to evaluate adjuvant activity
  - Panel of standardized adjuvants to allow benchmarking
- Animal models
  - In vivo models and antigens for testing immunogenicity of adjuvants
  - Access to test systems/animal models
  - Translation of knowledge to larger animals and humans
- Safety
  - Balance between immunogenicity and toxicity
  - Better knowledge of adjuvant side effects
  - Validation of adjuvant safety
  - Better understanding of animal models for predicting adjuvant safety and efficacy Recognize, manage and reduce adverse effects that can affect animal health and production after vaccination
  - Parameters to evaluate adjuvant safety / toxicity
- Translational
  - Difficulty to extrapolate preclinical data to human data
  - Development of systems vaccinology platforms allowing prediction of human performance based on in vitro data and experimental/preclinical tests
  - Sharing practical experience in using molecular genetics techniques and bioinformatics tools in vaccine adjuvant field

During the discussions on preclinical development of adjuvants it was recommended to prepare a detailed summary table on adjuvants used within ENOVA with following information:

- Type of adjuvant
- Species involved
- Type of antigen, mode of activation, way of derivation
- Dose
- Route of administration
- Mechanism of action
- Side effects
- Immune response measured (as effect of adjuvant)
- Animal details (strain, sex, age)
- Formulation of vaccine

To be able to fill the gaps between different groups and foster the individual research, sharing the knowledge and methodology is requested in several aspects. Firstly, the information on specific offers or requests on adjuvants should be made available (e.g. internal section on the website) to enable collaborations within ENOVA. It is suggested to collect information on any work related to translation between species and/or adjuvants specific for different species. In addition, transfer of knowledge is recommended for analytical method development and access to different facilities such as formulation, synthesis, in vitro/in vivo testing that are not available to some groups.

For new adjuvant system, there is always a potential toxicity issue when transferring from mouse models to humans. It is therefore recommended to look for adequate cell models for initial studies prior to preclinical phase, for better selection of potential adjuvants. It is suggested that the adjuvant response should be correlated based on retrospective correlation (in vitro/mouse/ human). Additionally, larger-animals should be considered as models in preclinical studies. Addition of a co-adjuvant should be considered for a better response. The activity of adjuvant in the resolution phase and absence of the normal immune response or tolerance can be a potential issue that should be addressed when testing new adjuvants. The last point was that knowledge on potential LPS contamination as well as impact of vaccine residues on environment should be considered early on.

Gaps in the development of adjuvants: Clinical development / Regulatory

The following points were used as starting points of discussion and were prepared by the organising committee:

- GMP adjuvants
  - Funding for scaling-up laboratory adjuvants to GMP
  - Availability of commercial GMP adjuvants (with "no strings attached"), effective in inducing robust B- and T-cell responses in animal models and humans
  - GMP grade and bulk production of TLR based adjuvants such as CpG ODN and/or dsRNA

- Clinical testing
  - Find a way to assess more frequently new adjuvants in clinics

- Regulatory
  - Overview of guidelines and regulations for human and veterinary vaccines/adjuvants

The discussions regarding the clinical development and regulatory aspects identified large gaps between the basic research and clinical development. The basic research groups have little understanding to regulatory requirements for a final product, which is to be used in clinical applications. The transition from preclinical to clinical phase represents a gap in the knowledge for GxP regulations related to production and facilities requirements. These gaps are a potential problem as some products developed in preclinical phase fail to fulfil the regulatory requirements later on. The action should therefore collect the guidelines and regulations and make them available on the web platform of the ENOVA project. Training possibilities with regulatory partners from ENOVA will be offered. Additionally, the availability of GMP adjuvants has been identified as an issue, as they are usually restricted to certain manufacturers. In this regard, securing funds for scaling up from laboratory to GMP have been recognised as a potential solution.

Regarding more specific clinical development gaps, some questions related to clinical trial design has been raised. There were concerns raised with regards to the optimal selection of cohorts, addressing different age, gender, ethnicity, metabolism, body temperature etc. and how these parameters translate into vaccine/adjuvant efficacy, and extent to which the latter can be compromised by inadequate design of the clinical trial.

Gaps in the development of adjuvants for therapeutic vaccines

The following points were used as starting points of discussion and were prepared by the organising committee:

- Knowledge on novel safe adjuvants for cancer immunotherapy
- Knowledge on use of adjuvants for therapeutic veterinary vaccines
- Tolerance-inducing adjuvants as potential component of therapeutic vaccine for antigen-specific immunotherapy
- Therapeutic vaccine adjuvants for T-cell induction with proteins and VLPs
- Adjuvants synthesized on site when co-injecting genes of enzymes with DNA vaccines
- Knowledge and skills for in situ applications of adjuvants (for local unspecific cancer immunotherapy)
- Beyond topical application: How can we deliver TLR agonists to the tumour site or the draining lymph nodes without causing overall systemic immune activation and the adverse responses associated with it?
- Understanding of adjuvant effect(s) of trauma, which becomes increasingly important with introduction of novel methods of immunisation/vaccination, such as electroporation or tattooing.

During the discussions, the ENOVA partners recognised the need for the creation of a databank for adjuvant used in therapeutic vaccines, which would include the mechanism of action, antigens tested and specific assays for that adjuvant system. Interactions between antigen and adjuvant formulations should be well characterised to enable optimum vaccine stability and efficacy. The participants recommended characterising the adjuvant types such as peptides, mRNA, proteins, cellular components, carbohydrates etc. and matching with the antigen of interest. With regards to the application, research is needed to identify the best route, targeting and cell homing for specific adjuvants. Also, it has been suggested that tests for immunogenicity and safety should be standardised with involvement of industry partners, proposing common standards for testing. The preclinical models for studying of adjuvants should be evaluated for efficacy and better animal models should be identified. The specific types of immune response needed are to be considered too.

Immune tolerance, such as at the injection site or in tumor microenvironment, has been recognised as an issue where further research is needed. In this respect, it would be beneficial to identify the adjuvants inducing tolerance, such as vitamin D, retinoic acid, etc.

Further research, which would allow overcoming the exhaustion of T cells and their activation in tumor microenvironment is needed. One interesting approach is introduction of telomerase reverse transcriptase that can rescue T cell proliferative capacity and specific reactivity.

Trauma has a significant immunomodulatory effect, affecting a broad array of immunological components. In combination with adjuvants this could lead to a greater reorganisation of the immune system. It is not known whether trauma has a tolerising or activating effects in combination with adjuvants. New adjuvant targets like Toll-like receptors (TLRs), which show good potential are not yet well researched and a number of questions remain to be answered for their application. Lastly, the participants have highlighted the difficulties in accessing adjuvants due to IP issues, cost etc. These specific recommendations were suggested to be reviewed in a scientific paper.

4. Conclusion

The ENOVA partners have identified the major gaps in different stages of adjuvant research for prophylactic and therapeutic vaccines. The participants suggested more interaction between the groups where they could learn from the experiences of others. The ENOVA meetings and web platform should provide an easier access to the methods and individual group expertise to researchers in need. Training opportunities in formulation technologies, regulatory and scientific knowledge have been requested and will be offered throughout the ENOVA project.

In conclusion, the overall output of the discussions and the future networking should provide the list of checkpoints for studies using adjuvants.