FRONT PAGE

PROJECT FINAL REPORT

Grant Agreement number: 280873

Project acronym: ADITEC

Project title: Advanced Immunization Technologies

Funding Scheme: Collaborative Project: Large-scale integrating project

Period covered: from 1/10/2011 to 30/09/2017

Name of the scientific representative of the project's co-ordinator[1], Title and Organisation:

Rino Rappuoli
Sclavo Vaccines Association
Via Fiorentina 1
53100 Siena (Italy)
Tel: 0039 0577
Fax:
E-mail: president@sclavo.org

Project website address: www.aditecproject.eu

[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

1. Executive summary

The High Impact Project ADITEC (Advanced Immunization Technologies), was launched in October 2011 to develop new vaccination strategies. Scientists from 42 partner institutions in 13 different European countries and USA joined forces in the ADITEC project. The scope of the project, was to accelerate the development of novel and powerful immunization technologies for the next generation of human vaccines. With a budget of about €30 million over 6 years, ADITEC has made significant advances in the development of novel immunisation technologies. The ADITEC Fact Sheet is reported as Figure 1.

Specific results, such as novel immunisation technologies, adjuvants, vectors and delivery systems, optimised formulations and vaccination methods for different age groups, all came together in a **toolbox of advanced technologies** enabling the best possible insight into fighting diseases. These technologies have been **tested head to head** in animal models, combined in prime-boost strategies, and their mechanism of action has been investigated both in pre-clinical and clinical studies.

ADITEC not only met the goal to develop new advanced technologies, but also took the leadership to make them broadly available, favoring the formation of a new, **collaborative ecosystem** where the European scientific and industrial sector act as an enterprise that is able to lead the innovation globally. ADITEC has successfully conducted and **completed 12 clinical studies** and is contributing to **international regulation and standards** for these novel technologies. Systems biology analysis have been applied to fully decipher the vaccine immune response.

Along with regularly setting up and running **European training programmes** ADITEC has also created synergies and cross-fertilization between research areas that have the potential to fill existing gaps and advance this knowledge well into the future. ADITEC has provided support, as project partners or users of the project technologies, to **19 SMEs** active in the vaccine field. To protect discoveries for future research, consortium members have taken out **7 patents**, with more in the pipeline. An ADITEC **Biobank** for samples collected in the project clinical trials has been created and samples made available to the international scientific community. To date, ADITEC has a track record of **253 scientific publications** in international peer-reviewed journals and over 20 submitted manuscripts (see publication list). An **ADITEC Research Topic** on Advanced Immunization Technologies has been produced in Frontiers (https://www.frontiersin.org/research-topics/5997/advanced-immunization-technologies-for-next-generation-vaccines). The ADITEC **Socio-Economic Impact** (http://www.aditecproject.eu/news-events/news/news-message/aditec-impact-report-september-2017-update.html) was analysed showing that ADITEC has produced over the double of the publications, conducted 2,6 times more clinical trials and supported 3 times more SMEs per million invested compared to FP7 projects in the area of vaccines for infectious diseases.

New immunization technologies developed by ADITEC, along with efficient management and governance, can effectively advance these innovations to the clinic and make a real difference for future health.

Fig. 1 ADITEC Fact Sheet

Project title:	Advanced Immunization Technologies (ADITEC)
Duration:	1 Oct 2011 – 30 Sept 2017
EC contribution:	29.98 million €
Partners:	42 partners from 13 EU countries and USA, 7 Affiliated Members
Industries:	11 SMEs and 3 Large Pharma
Clinical Trials:	12 Clinical Studies
Publications:	253 Publications, 1.740 Total Impact Factor, >4,800 Citations
Web-site:	www.aditecproject.eu

2. Summary description of project context and objectives

The science behind vaccines has become so sophisticated that no one laboratory can tackle modern vaccine science in isolation. Genomics, structure-based design and optimization of immunogens, small molecule adjuvants targeting specific receptors and systems biology, together with sophisticated assays to monitor the immune response have transformed the old field of vaccinology into one of the most dynamic of this century and are opening the door to fight those diseases that, so far, have been reluctant to vaccine development transforming the medicine of the future.

The scope of the ADITEC project was to produce the knowledge necessary to develop novel and powerful immunization technologies for the next generation of human vaccines. This goal required a multidisciplinary approach in which diverse but complementary scientific disciplines and technologies converge. Therefore, some of the most competitive European research groups from public institutions and biotechs have agreed to join forces in ADITEC, together with top US groups on systems biology and adjuvants to support this enterprise and accelerate development of the novel concept of vaccines and vaccination.

The working concept of ADITEC was to use systems biology and advanced immune assays to elucidate, at a highly sophisticated level, how effective vaccines stimulate the human immune system and to apply this information to the rational design of novel and highly targeted immunization technologies. The technologies applied consist of novel and highly specific adjuvants, a range of different antigen delivery systems and vaccine formulation and delivery modalities that will be used for design of novel immunisation strategies, targeted to different gender and age groups.

To reach this aim the project was structured around two major components:
i) human immune response to vaccination studied through latest generation methodologies and
ii) development of advanced immunization technologies.

These two components were strictly interlinked and each benefit from the activities of the other as shown in the schematic representation of the ADITEC project structure (Fig. 2).

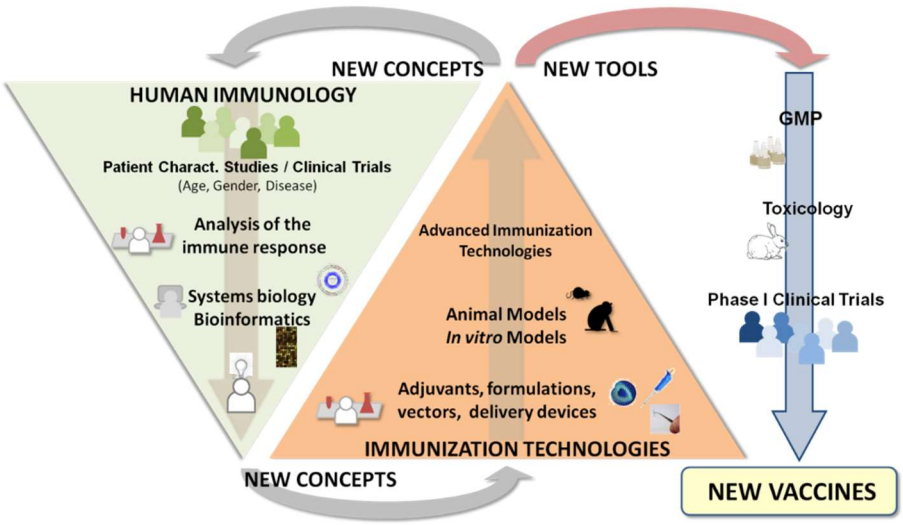


Fig. 2 Schematic representation of the basic concept of ADITEC project

The **Human Immunology** component utilized the full range of translational and clinical trial approaches to elucidate fundamental mechanisms of immune response to vaccination and identify generic approaches suitable for wider application to immunization. Patient Characterization Studies (PCS) and Phase 4 trials, using licensed vaccines and vaccine technologies, were conducted in which

physiology and efficacy of vaccines was explored. Specific attention was given to age, gender and genetic aspects and pathologic conditions. ***Systems biology approaches*** were employed to understand how effective vaccines stimulate the human immune system. In addition to the core antigens (influenza, tuberculosis, chlamydia), licensed vaccines were selected that offer advantages for the hypothesis to be tested, e.g. discordant adjuvants and matched antigens administered to healthy volunteers to profile the human immune response to different adjuvants. By using heterologous prime-boost schedules (nasal and injected flu vaccines), the possibility of inducing broadly reactive antibody and memory B and T cell responses was assessed. Novel routes of immunization were evaluated in humans such as mucosal delivery (i.e. sublingual using live influenza and killed cholera vaccines) and targeted lymph node immunization using vaccines licensed for injection into the leg, buttock and arm (e.g. Tick Borne Encephalitis vaccine). Human transcriptomic and immune response to enteric bacteria challenge elucidated the effect of immunization on inflammatory and immune response to infection and signature of protective immunity.

New concepts arising from human studies guided the development of **advanced immunization technologies** in terms of next generation adjuvants, vaccine vectors, formulations and delivery devices. Focus was on comparing vaccine formulations using common prototype antigens. Different routes of immunization and optimal heterologous prime-boost approaches were tested for improving the efficacy and safety of immunization. Candidates were selected in advanced animal models. From these pre-clinical studies, new concepts and new tools were generated. The new concepts were further validated in the human studies. The new tools generated new vaccine candidates that were advanced in phase I clinical trials. These trials were focused on a cutting-edge application of novel technology developed within the project that is a genuine advance or paradigm-change. Through this comprehensive unprecedented effort, ADITEC offered the unique opportunity of creating synergies and cross fertilization between different research areas, filling the existing knowledge gaps to enable the introduction of new effective and safe immunization technologies.

Focus on common antigens. In order to have a coordinated effort to answer the questions of the human immune response, few antigens to work with were selected. ***Influenza*** was used in pre-clinical and clinical studies, as: 1) there is a large unmet medical need requiring better vaccines and vaccination strategies; 2) different routine vaccines are available (live-attenuated, subunit with and without adjuvants), allowing study of immune responses with different formulations and in different populations (infants, elderly, adults, genetically defined populations); 3) different routes of immunization (intranasal, intradermal, intramuscular) are all available allowing the study of human immune response following different delivery mechanisms, routes and prime-boost; 4) the possibility exists to immunize with antigens for which the human population is already primed (routine seasonal vaccines) or antigens for which the population is naive (such as H5N1, H7, H9 etc). **TB** vaccines already in clinical studies, allowed system biology approaches to study human immune response to vaccination with improved BCGs. The recombinant protein H56 was used both as BCG booster and as vaccine formulation with adjuvant. In some human studies, **antigens were selected from outside this core** set where the available formulations licensed for human use offer an advantage to the hypothesis under test, such that the particular antigen is less important (e.g. when comparing discordant adjuvants, routes, prime-boost).

Many groups: leveraging other funding. The project brought together high quality European and US groups to answer the most relevant questions of human immunization. All the partners of the project had independent funds that contributed to the program, greatly increasing by co-funding the returns on the EU contribution. In addition, ADITEC partners leveraged over 17 million of additional funds in order to provide additional budget for innovative ideas and advance promising candidates to the clinic.

Matrix structure. To address these issues in a coordinated manner, ADITEC was organised in a matrix structure in which research themes and experimental approaches feed into each other (Fig. 3). Activities were closely integrated in a "horizontal" - thematic manner, as well as "vertical" manner based on the experimental approach. This means that technology, non-clinical and human immunology activities feed into each other in a reciprocal way to ensure advances in one area are explored and developed in another (For detailed description see "Overall strategy" page 29). Vertical and horizontal activities had a responsible coordinator to ensure tasks remain on track and integrated with the whole.

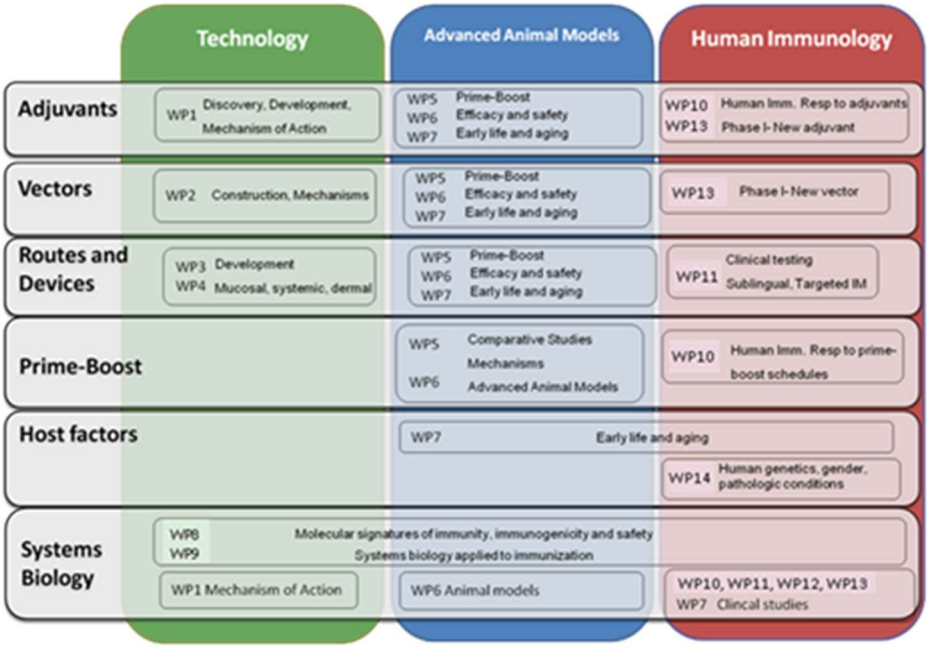


Fig. 3 Matrix structure of the ADITEC project

Scientific and Technical objectives of the proposed approach.

The Scientific and Technological Objectives of the project can be summarised in:

1. Development of advanced immunization technologies: adjuvants, vectors, formulations and delivery devices **(WP1, WP2, WP3)**
2. Selection of candidates, routes of immunization and prime-boost combinations in animal models **(WP4, WP5, WP6)**
3. Assessment of the impact of host factors in response to vaccination **(WP7, WP14)**
4. Development of concepts and tools from human immunization **(WP8-9, WP10-13)**:
    - Molecular signature of immunity and immunogenicity.
    - Systems biology of innate and adaptive immune response to vaccination
    - Profile of immune response to defined adjuvants and prime-boost strategies
    - Alternative routes of immunization
    - Transcriptomics and immune response: enteric infection versus prophylactic immunization
    - Phase I clinical trials of novel immunization technologies developed by the consortium
5. Development of concepts and tools to address regulatory and ethical issues posed by novel immunization technologies **(WP15).**
6. Creation of an internationally recognized training program for translational immunology and vaccinology **(WP16)**

3. Description of the main S&T results/foregrounds

ADITEC has made significant advances in the development **novel immunisation technologies**, adjuvants, vectors and delivery systems, formulations and vaccination methods optimised for different age groups. The project has developed a toolbox of novel immunization technologies with well profiled mechanisms of action. These have been **tested head to head in animal models**, combined in prime-boost strategies, and their mechanism of action has been investigated both in pre-clinical and clinical studies. ADITEC not only met the goal to develop new advanced technologies, but also took the leadership to make them broadly available, favoring the formation of a new, collaborative ecosystem where the European scientific and industrial sector act as an enterprise that is able to lead the innovation globally (Fig. 4).

In six years, the project has successfully conducted and fully completed **12 clinical studies** and is contributing to international regulation and standards for these novel technologies. Along with regularly setting up and running **European training programmes** ADITEC has also created synergies and cross-fertilization between research areas that have the potential to fill existing gaps and advance this knowledge well into the future. ADITEC has provided **support to 19 SMEs** both as partners or trough funding of innovative research projects selected through 5 open calls. To protect discoveries for future research, consortium members have taken out **seven patents**, with more in the pipeline.

Through the project's continual success and achievement, **253 scientific publications** in international peer-reviewed journals have been produced to date (see publication list) and over 20 are submitted for publication. An **ADITEC Research Topic** on Advanced Immunization Technologies has been produced in Frontiers Immunology open access journal (https://www.frontiersin.org/research-topics/5997/advanced-immunization-technologies-for-next-generation-vaccines). The ADITEC **Socio-Economic Impact** (http://www.aditecproject.eu/news-events/news/news-message/aditec-impact-report-september-2017-update.html) was analyses showing that ADITEC has produced over the double of the publications, conducted 2,6 times more clinical trials and supported 3 times more SMEs per million invested compared to FP7 projects in the area of vaccines for infectious diseases.
New technologies, along with efficient management and governance, can effectively advance these innovations to the clinic and make a real difference for future health.

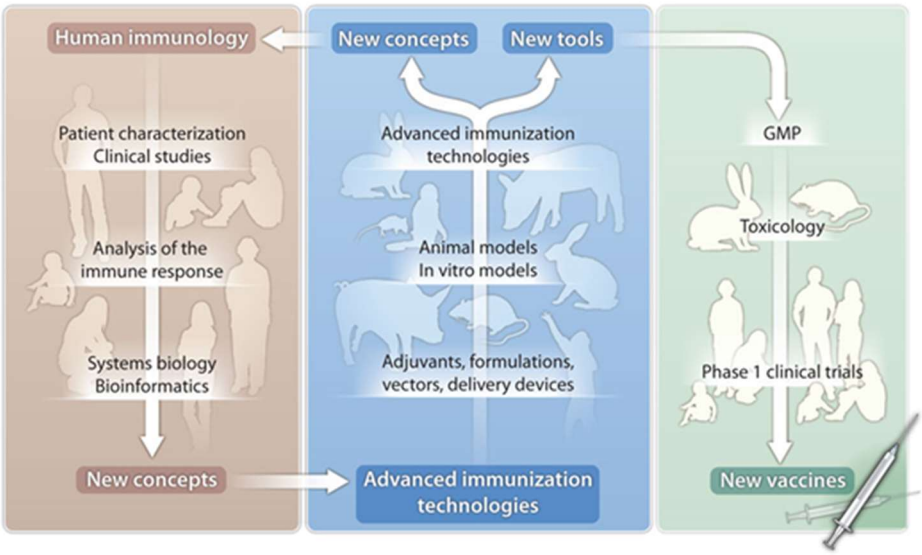


Figure 4. ADITEC Strategy. Medaglini D. and Rappuoli R. Science Translational Medicine 2012

The **main achievements obtained in the 3 subprojects are summarised below**:

Sub-project 1: Advanced Immunization Technologies

The objective of ADITEC Subproject 1 was the development of advanced immunization technologies: adjuvants, vectors, formulations and delivery devices (WP1"Adjuvants", WP2 "Vectors", WP3 "Formulations") (Fig.5). Main achievements of this subproject include the development of adjuvants and vectors, their comparative testing in animal models and systems biology analysis allowing profiling, prioritisation and design of better immunization technologies.

By using common prototype antigens, standardizing the immunoassays and using centralized animal testing for challenge studies, it was possible to make side-by-side comparisons and compare the potential of vaccine delivery systems for different diseases.

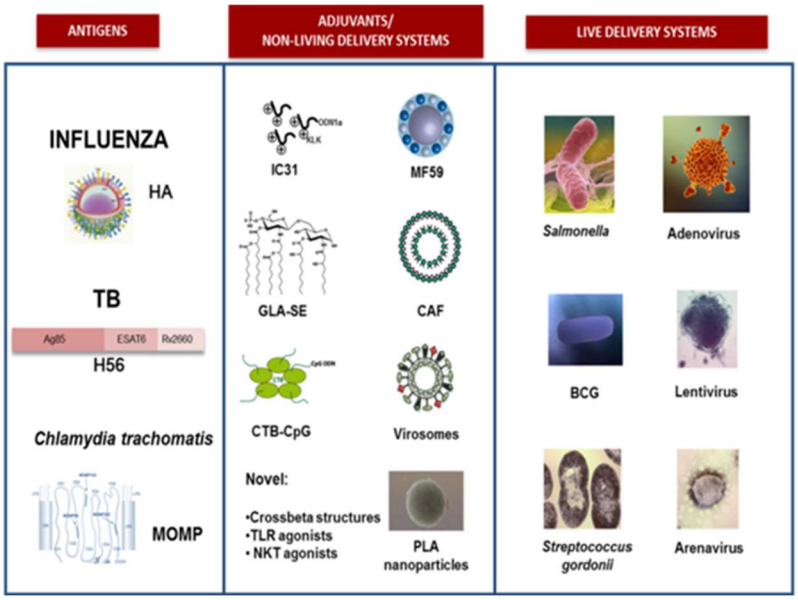


Figure 5 ADITEC Immunization Technologies

Vaccine Adjuvants (WP1). Several **first generation adjuvants (IC31, GLA-SE, CAF01, MF59)** with different modes of action have been tested in comparative studies (Fig. 6). ADITEC succeeded, for the first time, to conduct head-to-head comparison of some of the most promising clinically tested adjuvants as pertain to their immunogenicity, ability to induce protective immunity and systems biology analysis (whole genome transcriptomics) in mice using standardized experimental approaches. This is a major undertaking taking into account that so far research on adjuvants has been fragmented and intellectual property issues hampered comparative evaluation of clinically tested adjuvants.

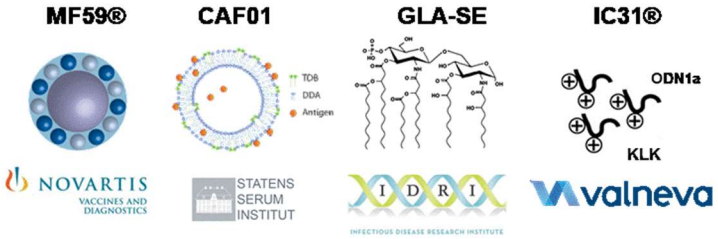


Fig. 6. First generation adjuvants testes head to head

The results of the comparative immunogenicity and systems biology analyses of the ADITEC 1st generation adjuvants were published in 2 articles in Nature- Scientific Reports (Olafsdottir T. *et al*. Sci. Report 2016; Knudsen N. *et al*. Sci. Report 2016). Different human vaccine adjuvants promote distinct antigen-independent immunological signatures tailored to different pathogens. The mode of action of different first generation adjuvants has also been studied by systems biology analysis and untargeted metabolomics analysis. Integration of the two datasets (transcriptomics and metabolomics), suggests pathways that could potentially be used as a metabolics biomarker of adjuvanticity. First generation adjuvants such as CAF01 have been advanced to phase I clinical trials in ADITEC. A series of 2nd and 3rd generation adjuvants with improved/tailored potency and safety were developed. **Second generation adjuvants** with improved potency include CAF09, TLR7 agonists, Nod ligands, glycosidic iNK cell agonists, crossbeta antigens and vaccibody DNA. Comparative analysis of first and second generation ADITEC adjuvants based on prototype antigens has been conducted. Some adjuvants like CAF09 have been tested by various immunization strategies to improve the CD8 immune response. A large scale comparative study using a subunit Influenza A/California/2009 (H1N1) vaccine antigen has been performed in order to rank 2nd generation adjuvants based on the same comparative analysis performed with the 1st generation adjuvants. Development **of third generation adjuvants** include improved versions of the CAF platform in novel formulations aimed to improve the humoral response. Novel versions of mutated cholera toxin, SIPR immunomodulators and biodegradable co- polymers based on PLA were developed and innovative tools for their toxicological evaluation employed. Several activities were also carried out, for the identification of transcript profile of CAF01 and GLA-SE in neonate mice (important given the unique adjuvant property of CAF01 in neonate mice not shared by other ADITEC 1st generation adjuvants), molecular signatures of GLA in healthy volunteers, as well as transcript profile of the second generation ADITEC adjuvants iParticle and i-Particle-NOD1 in mice. All in all, WP1 has succeeded to meet all assigned deliverables and milestones, and in addition carried out new tasks related to the overarching objectives of WP1 adjuvant.

Recombinant viral and bacterial vectors (WP2) expressing ADITEC prototype antigens. The general objective was the development of efficient and safe recombinant live attenuated microorganisms as immunization vectors. Attenuated viruses or bacteria, genetically engineered to express heterologous antigens, represent potential vectors for the delivery of immunogens to the immune system. These live attenuated vectors present the advantage that simple modes of inoculation, such as oral or intranasal immunization, can induce protective immunity subsequent to a single dose. WP2 was organized around three main tasks: (i) the characterization of the immunogenicity profile of these vectors, expressing the same ADITEC prototype antigens, (ii) the improvement of the safety and immunogenicity of these vectors, based in particular on the innate correlates identified in this WP and (iii) the identification of the immunological mechanisms underlying the immune characteristics of these vectors, particularly at the level of innate immunity. ADITEC members have constructed vaccines based on Lentivirus (IDLV), rBCG, Salmonella outer membrane vesicles, Adenovector vaccines, *Streptococcus gordonii* and cDNA constructs. These constructs insert either the H56 antigen, MOMP or HA which allow for direct comparison of immunogenicity and profile. For some of the vectors such as Adeno from OKARIOS or OMV from ABERA several of the target antigens were expressed. Detailed dissection of immune stimulation and innate activation was conducted for many of the vaccines.

A safer 2nd generation Salmonella outer membrane vesicle (OMV) vaccine was constructed by deleting the msbB or lpxL1 gene respectively. The resulting deletion strains express LPS containing penta-acylated lipid A, which is less toxic compared to wild type LPS, thereby reducing the reactogenicity of OMVs but retaining their immunogenicity. The detoxified OMVs were much better tolerated by the mice. Both Neisseria and Salmonella OMVs, displaying Hbp-H56 at the surface were tested in ADITECs call for immunogenicity and protective efficacy. In combination with a BCG prime, the Neisseria OMVs induced a potent antigen-specific immune response. However, none of the vaccines were able to further increase the protection mounted by BCG. Salmonella OMVs, displaying Hbp-MOMP or Hbp-MOMP + OmpF-MOMP at the surface were also tested for immunogenicity and protective efficacy, using heterologous prime-boost vaccination regimes in which systemic priming immunizations with subunit vaccine MOMP/CAF01 were followed by mucosal booster vaccinations with OMVs. The use of OMVs as a stand-alone intranasal vaccine in a genital *C. trachomatis* challenge mouse model raised strong antigen-specific cellular responses locally in the lung and to a lesser extent also in the ileac lymph node that drains the urogenital tract. This resulted in a low, but significant level of protection. OMVs were also tested as intranasal booster of subcutaneous priming with MOMP/CAF01. High-level systemic and iliac cellular responses were observed. Although specific immune responses were observed after immunizations with OMVs, the prime-boost groups seemed to be dominated by the strong subcutaneous MOMP/CAF01 immunizations. Homologous and heterologous prime-boost vaccination experiments were conducted with H56-expressing BCG. The heterologous vaccination with DNA/BCG::H56 combinations induced the highest levels of H56-specific cytokines, although bacterial loads and lung pathology were not significantly different to those of BCG vaccinated mice. Despite inducing appreciable immune responses to Ag85B and ESAT-6, dermal H56 cDNA tattoo immunization did not enhance the protective effect of BCG under the conditions tested. The broader response against mycobacterial antigens elicited by BCG and BCG::H56 appeared to be more effective at conferring protection than a specific response against H56 peptides.

An H56-based DNA vaccine was designed, constructed, tested and optimized an against *Mycobacterium tuberculosis* and this vaccine was tested for protective capacity in a murine tuberculosis model. The most robust H56-specific immune responses were induced by heterologous vaccination with DNA/BCG combinations. However, bacterial loads and lung pathology were not significantly different to those of BCG vaccinated mice.
Recombinant strains of the commensal bacterium *Streptococcus gordonii* expressing H56 were constructed and its immunogenicity was tested in different prime-boost schedules in mice (Fiorino et al. submitted ADITEC Research Topic Frontiers Immunology).
The efficacy of IDLV-NP immunization was assessed in aged mice. Young mice showed a higher NP-specific CD8+ T cell response compared to aged mice, suggesting an impairment of the immune response in the elderly using IDLV as a delivery system for influenza NP. It was demonstrated that the induction of T cell responses by IDLV is independent of TLR, of IRF 3/7 and of RIG-I/MDA5. In contrast to immunization with antigen mixed with a strong adjuvant such as CpG which induces strong CD8+ CTL responses which declined rapidly, the effector CTL responses induced by IDLV are still detectable several months after immunization, without requirement for re-stimulation.

Sub-project 2: Advanced Animal Models

Subproject 2 includes WP4 (Routes of Immunization), WP 5 (Prime-boost Strategies) WP6 (Advanced animal models), and WP7 (Early Life and Aging).
Different routes of immunization, such as mucosal (IN=intranasal; SL= sublingual; PO= peroral; and IVAG= intravaginal) and parenteral (TC= transcutaneous; and SC=subcutaneous) were studied for their ability to stimulate local and systemic immune responses (serum IgG and splenic T cell proliferation) using the ADITEC prototype antigens. Different adjuvants were compared for their ability of eliciting vaccine antigen-specific T helper responses upon a single SC or IN immunization. Early biomarkers of adjuvanticity after primary immunization were investigated using four different adjuvants combined with the chimeric tuberculosis vaccine antigen H56 administered by different routes. Clinical studies on safety and immunogenicity of sublingual vaccination healthy adults with were also conducted.
Combination of different vaccine formulations and routes of delivery for priming and boosting is a strategic approach for improving and directing vaccine-induced immune responses. To this aim several **prime-boost approaches** combined with specific immunization routes have been comparatively evaluated to allow an informed decision on their use. Prime-boost approaches using different adjuvants and vectors have been tested using the 3 ADITEC model antigens. Different prime-boost combinations using the H56 TB antigen and adjuvants CAF01, CpG, CTB-CpG, Squalene and alum have been studied (Ciabattini A. *et al.* Frontiers Immunology 2016, Ciabattini A. *et al.* Frontiers Immunology 2015, Prota G. *et al.* Vaccine 2015). Both B and T cell response have been characterized in terms of amount and frequency of activated cells, effector function, differentiation stages of B cell differentiation (plasmablasts, plasmacells, germinal centre-B cells) and humoral response. The CAF01 formulation has shown to elicit strong primary response that can ben efficiently boosted with both homologous vaccine formulations or o/w Squalene heterologous vaccine formulation. Prime-boost approaches for Chlamydia and influenza were also studied. Based on the promising challenge results of Chlamydia prime-boost combinations, these have been selected for progression into NHP studies and a collaborative clinical trial selected trough open call under WP 6.5.

Non human primate (NHP) studies A repeated low-dose *Chlamydia trachomatis* vaginal challenge model in macaques and a model of influenza virus infection in Cynomolgus monkeys have been established to identify correlates of protection and to assess the efficacy of the vaccine candidates developed by the consortium. Selection of ADITEC candidates to be advanced to *C. trachomatis* vaginal challenge in macaques has been successfully conducted and the protocol design developed. Studies were carried out to further compare the pathogenesis and the pathology of influenza in man and experimentally infected ferrets.

Thirty adult, female cynomolgus macaques (Macaca fascicularis) were immunized with different candidate vaccines against *Chlamydia tracomatis* (Table1).

			Immunization route/vaccine (dose)/adjuvant (dose)						Chlamydia trachomatis first challenge Strain/Dose	Chlamydia trachomatis second challenge Strain/Dose
			2016-01-19	2016-02-16	2016-03-15	2016-05-09	2016-05-24	2016-06-06	2016-09-05	2016-11-14
	Group	No animals	Week 0	Week 4	Week8	Week 16	Week 18	Week 20	Week33	Week43
1	**CTH552 +/- Alum** With IN	5	IM CTH522 (85µg) Alum (0.425mg)	IM CTH522 (85µg) Alum (0.425mg)	-	IM CTH522 (85µg) Alum (0.425mg)	IN CTH522 (30µg)	IN CTH522 (30µg)	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU
2	**CTH522 +/- CAF01** With IN	5	IM CTH522 (85µg) CAF01 (625µg/125µg)	IM CTH522 (85µg) CAF01 (625µg/125µg)	-	IM CTH522 (85µg) CAF01 (625µg/125µg)	IN CTH522 (30µg)	IN CTH522 (30µg)	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU
3	**CTH522 +/- CAF01** W/O IN	5	IM CTH522 (85µg) CAF01 (625µg/125µg)	IM CTH522 (85µg) CAF01 (625µg/125µg)	-	IM CTH522 (85µg) CAF01 (625µg/125µg)	-	-	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU
4	**DNA** (EP) / **CTH522+CAF01** (IM)	5	ID-EP DNA-MOMP (1mg)	ID-EP DNA-MOMP (1mg)	ID-EP DNA-MOMP (1mg)	-	-	IM CTH522 (85µg) CAF01 (625µg/125µg)	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU
5	**vectors** (ID-EP or IM) / **CTH522+CAF01** (IM)	5	ID-EP DNA-MOMP (1mg)	IM hAd5-MOMP 10^{11} vp	IM MVA-MOMP 4×10^8 pfu	-	-	IM CTH522 (85µg) CAF01 (625µg/125µg)	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU
6	**Control**	5	-	-	-	-	-	-	D/UW-3/Cx 5*10^7 IFU	D/UW-3/Cx 3,5*10^7 IFU

Table 1: Chlamydia immunization and challenge schedule

Following immunizations, all animals were challenged intravaginally with *Chlamydia trachomatis* serovar D/UW-3/Cx to evaluate the efficacy of candidate vaccines before specific treatment against chlamydia. Week 33 post first immunization with 5 x 10^7 IFU per animals and at week 43 post first immunization (10 weeks post first challenge) with 3.5 x 10^7 IFU per animals.

The T cell responses were assessed by intracellular staining (ICS) at base line and at week 22 post first immunization. At week 2 and week 3 post first challenge, 20 to 40% of animals from vaccinated groups were negative for chlamydia detection by PCR whereas all animals from the control groups are always positives. At week 5 post first challenge, all animals belonging to groups 4 and 5 are exempted of Chlamydia. After the second challenge, performed at week 10 post first challenge, all animals became more rapidly negative for chlamydia, including the control group. But the kinetic of clearance remained the same compared the first challenge. Animals from vaccinated groups were negative quickly compared to the control group. After the first and the second challenge, the difference between the group 4 (primed with DNA vaccine and boosted with MOMP protein) and the control group (group 6) was statistically significant.

Immune response in ageing and early life. The effects of new vaccine formulations have been analysed in **animal models at extreme of age**. Responses of young and old immune cells stimulated in vitro by novel compounds were assessed. Vaccine/adjuvant effects in neonatal, adult and aged mice have been defined. CAF01 was identified as a unique neonatal adjuvant. Adjuvanting influenza HA in the cationic liposome based adjuvant CAF01 induced robust primary humoral responses following a

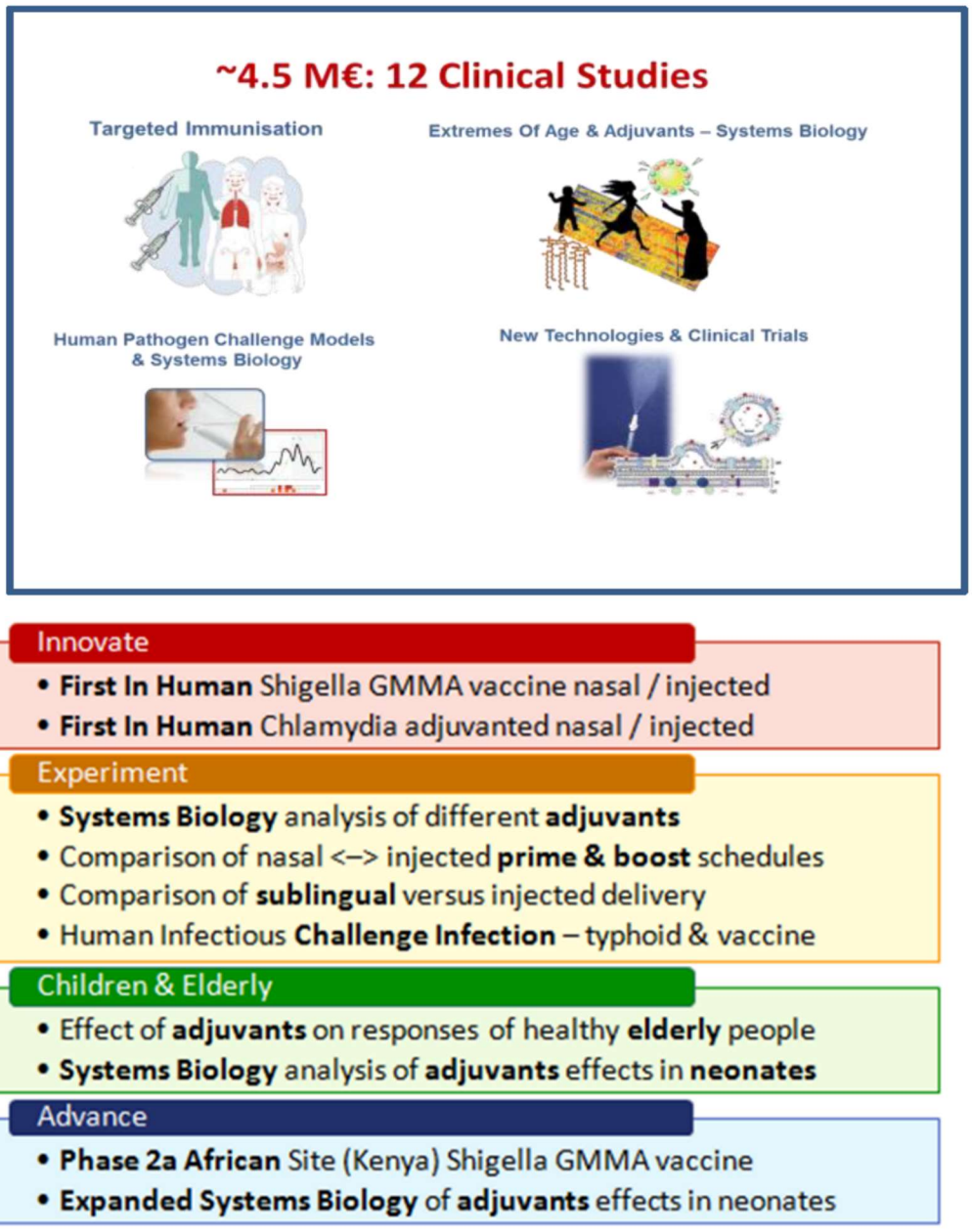
single neonatal immunization. Studies in neonatal mice have demonstrated that the monitoring of TFH cells may not be used as a reliable biomarker of early life GC activity.
Effects of registered or novel (MF59-adjuvanted) vaccines on **immune responses in toddlers, young and elderly adults** have been investigated. A pediatric influenza study enrolling 90 children randomised to receive 2 doses of either MF59-adjuvanted TIV or standard TIV was completed at the. Paper highlighting the striking differences of these results from previous studies in adults has been published (Nakaya H. *et al*. PNAS 2016). Phase IV clinical trial investigating primary and booster response to Hepatitis B vaccine in young and elderly adults has been completed.

Sub-project 3: Human Immunology

ADITEC has successfully conducted and completed **12 clinical studies and clinical trials** with over 700 subjects. Populations studied included very young infants, the elderly and healthy young adults. The programme ranged from experimental medicine studies using licensed vaccines or infectious pathogens as models of infection and immunization, through to Phase 1 clinical trials of novel vaccines including antigens translated within the ADITEC programme. Trials were conducted at the ADITEC participant institutions, but have also been translated from EU to endemic areas in Phase 1b. The programme envisaged an ambitious portfolio of around 12 studies / trials, including three Phase 1 trials that would be selected on the basis of a peer-reviewed competitive open call. The clinical trials were designed to "innovate" testing new vaccine candidates developed within the project, "experiment" testing different routes of immunization, prime boost-schedules, systems biology analysis of different adjuvants, infection challenge models; study different age groups "elderly and children" and "advance" vaccine candidates in Phase II clinical trials or in different age groups based on the results obtained in the first ADITEC clinical trials (Fig. 7).

- In total, ADITEC has sponsored 12 clinical trials, all completed.
- These trials have been carried out by 7 partners of which 5 are either academics or research institutions and they have enrolled approximately 700 patients across 6 unique sites.
- Over one third of the trials supported by ADITEC are looking at the responses to established vaccines in special populations such as the elderly, infants and women. They are identifying differences in the effects of the vaccines compared to the rest of the population.
- Across the ADITEC sponsored trials, there has been a special focus on systems biology including a trial (NCT01682369) focussing on systems biology of influenza adjuvanted and non adjuvanted vaccine in infants and its follow on trial (NCT02529904).
- 8 of the trials sponsored by ADITEC use technologies that are already available on the market; these trials vary in phase and design however all have a similar aim, to profile with the latest technologies the immune response elicited.
- While the majority of the clinical trials focused on established vaccines, 4 used novel vaccines. Four phase I trials focused on testing the safety of new vaccines in healthy volunteers. Lastly, trial (NCT02324751) run by Partners 6 and 39 (Oxford and NVGH) was a phase IIb study focusing on the effectiveness of a newly launched vaccine.
- The trials sponsored by ADITEC have been carried out by 5 work packages with these work packages having a total budget of € 4.5 million.

Fig 7. ADITEC Clinical trials strategy



In table 2 is reported a more detailed description of the clinical trials conducted and the main achievements

Table 2 ADITEC clinical trials conducted and the main achievements

N. of Clinical trial	Title	Primary Outcome	Phase	Subjets n.	Age (years)	Sponsor	Location	Main results
EudraCT: 2013-002589-38	Primary and booster vaccination in old age: Hepatitis A and Hepatitis B	Quantification of vaccine-induced immune response 6 months after the last dose of vaccine	IV	40	Adults 20-40 Elderly >60	University of Innsbruck	AT	Characterization of antibody responses and early expression profiles of lymphocytes in young adults and elderly after primary and booster vaccination.
NCT01682369	Multi-centre, Phase II, Open Labelled Randomised Control Trial to describe immune & transcriptomic responses to Trivalent Inactivated Vaccine (TIV) & MF59 Adjuvanted Influenza Vaccine (ATIV) in 14-26 Month Healthy Children	Descriptive analyses of gene expression profiles of participants following immunisation with TIV or ATIV in relation to baseline profiles.	II	90	14–26 months	University of Oxford	UK	Demonstrated safety and immunogenicity. First systems biology study of adjiuvanted influenza vaccine ± MF59 in infants. Important new insights about the dynamics of the innate and adaptive responses and identification of potential correlates of immunity to vaccination in children (Nakaya HI et al 2016).
NCT02529904	A Phase II, Open Label Study to describe immune & transcriptional tesponses to MF59 Adjuvanted Trivalent Influenza Vaccine (ATIV) in healthy 13-24 month children and adults 18-65 years	Early gene transcriptional responses to immunization with MF59-ATIV.Relationship between early gene transcriptional responses and haemagglutination inhibition responses	II	120	13-24 and 18-65 months	University of Oxford	UK	Demonstrated safety and immunogenicity. Data analysis for antibody, innate immune responses are complete, and transcriptomics analysis is ongoing.

NCT02032160	Study to Generate Exploratory Training Data Characterising Innate/ Adaptive Immune Responses Following 1st & 3rd Intra-muscular Immunisations With Fendrix/Engerix B Vaccines in Healthy Adult Males With no Pre-existing Immunity to Hep B	Change from pre-immunisation baseline values in global gene expression measured on whole blood samples.	IV	30	18 - 55	University of Surrey	UK	Immunization with two differently adjuvanted vaccines induced protective serum IgG titres over a several log-range. Both vaccines were demonstrated to be safe. Integration of PBMC transcriptomes with data from NHP study determined that including a TLR4 ligand as an adjuvant leads to more robust vaccine-induced peripheral innate inflammatory responses that correlate with antibody response magnitudes. Although distinct signatures for each adjuvant are not observed, the Alum+MPL adjuvant generally increases the magnitude and frequency of responses induced by Alum alone.
NCT02557802	Breadth of T-cell responses a after heterologous route immunological prime-boost using influenza antigens as a model system	Breadth of T-cell responses to influenza antigens measured by increases in frequency and phenotype of T cells synthesising or secreting cytokines, or proliferating in response to in vitro stimulation with influenza antigens.	IV	30	18-55	University of Surrey	UK	Dramatic differences seen in the serum IgG and IgA responses after IN LAIV or IM TIV immunisations. Blinded analysis of the immune responses to the two vaccines so far indicates a good spread of responses, including one complete non-responder. Evaluation of heterologous prime-boost schedule impact on breadth of CMI measured by T cell responses to defined epitopes now ongoing.
V114_01	Comparison of vaccine containing three men B protein antigens formulated with distinct adjuvants working through or independently of TLRs	Increased frequency of antigen-specific T cells and memory B cells after immunization with meningococcal B recombinant vaccine.	IV	20	Adults	Novartis V&D/ GSK V&D	IT	Analysis of vaccine-induced B and T cell immune responses in subjects receiving a recombinant meningococcus B vaccine (Bexsero).
NCT01710189	Phase 4 Clinical Trial of cervico-vaginal immune responses following three right deltoid or right thigh intramuscular immunisations with TicoVac (Tick Borne Encephalitis Virus [TBEV])	Proportion of subjects with a 15-fold or greater increase from pre-immunisation levels of anti-TBEV IgG in cervico-vaginal secretions at 28 days after final immunisation.	IV	40	18 - 49	University of Surrey	UK	Intramuscular immunisation of the leg of healthy female adults targeting rich lymphatic drainage of pelvis resulted in significantly higher IgA circulating B cell responses and increase of antigen-specific serum IgG compared with injection into the arm. Leg immunisation also resulted in higher serum IgA, and vaginal IgG and IgA antibodies. Confirmation that induces superior immune

	Vaccine in adult female participants							responses, especially genital tract mucosal responses, will require larger studies.
NCT00820144	Functional exploration of the Immune response Using the B-Subunit of Cholera Toxin (CTB) administered by mucosal way in healthy adult volunteer: potential role in development of vaccine processes	Comparison of the mucosal immune response after oral, nasal and sublingual administration of CTB . Production of immunoglobulin A1, A2 and G specific to the CTB in salivary secretions or peripheral blood.	I	40	18 - 50	Centre Hospitalier Universitaire de Nice	FR	Sublingual administration of cholera toxoid (CTB) in healthy adult volunteers was well tolerated. The mucosal immunogenicity of sublingual CTB was comparable to that of nasal CTB. However, administration of the licensed oral formulation (antacid buffered cholera vaccine) containing CTB led to more consistent mucosal and systemic responses.
NCT02324751	A Phase IIb, Observer-blind, randomised Controlled Trial to assess the immunogenicity and protective efficacy of Vi Conjugated (Vi-TCV) and Unconjugated (Vi-PS) Polysaccharide Vaccines in preventing typhoid infection compared to a control vaccine (Meningococcal ACWY), using a human challenge model of typhoid infection	Clinical (fever >38 degrees for more than 12 hours) or microbiologically (blood culture positive) proven typhoid infection following oral challenge with Salmonella Typhi.	II	99	18 - 60	University of Oxford	UK	Completed recruitment and follow-up, are finalizing analyses and reports

NCT02034500	A Phase 1, Randomized, Placebo Controlled, Single Center, Dose Escalation Study to Evaluate the safety and immunogenicity of 3 vaccinations with Shigella Sonnei vaccine (1790GAHB) administered either by intradermal, intranasal or intramuscular route in healthy adults.	Number of subjects with solicited and unsolicited local and systemic reactions after any vaccination.Geometric mean concentrations of anti-LPS antibody titers before and after immunization.	I	52	18 - 45	GSK Vaccines Institute For Global Health S.r.l	UK	These data, evaluated together with data from a parallel Phase 1 STOPENTERIC trial with the same vaccine, document safety and immunogenicity of Shigella sonnei Vaccine (1790GAHB) over a wide range of doses, and via different routes and delivery systems. Results have guided route and dose level selection for further clinical development. A dose of at least 25µg administered intramuscularly has been selected for testing in a population from a Shigellosis endemic country (the real target for the vaccine) in the phase 2a ADITEC trial NCT02676895 (Muturi-Kioi V. et al 2016)
NCT02676895	A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study to evaluate the safety, reactogenicity and immunogenicity of 2 doses of the GVGH 1790GAHB vaccine against Shigella Sonnei, administered intramuscularly in adult subjects from a country endemic for Shigellosis	Number of subjects with solicited local and systemic adverse reactions after any vaccination	II	72	18 - 45	GSK Vaccines Institute For Global Health S.r.l.	KE	Follow-on trial to Phase I trial NCT02034500 reported above. The study is still ongoing and results will become available in June 2017.
NCT02787109	A Phase I First in Human, Double-blind, Parallel, Randomised and Placebo Controlled Clinical Trial of the safety of adjuvanted Chlamydia Vaccine CTH522 in healthy women aged 18 to 45 Years	Evaluation of adverse events/reactions and laboratory safety of adjuvanted chlamydia vaccine.	I	35	18 - 45	Statens Serum InstitutCCollaborators: Imperial College London)	UK	Completed recruitment and follow-up, are finalizing analyses and reports

Systems Biology of Innate and Adaptive Immunity to Vaccination in Humans. The aim of these studies was to perform a system biological analysis of the innate and adaptive immune responses to vaccination. The concept of systems vaccinology applied in ADITEC is a shift from studies in a large number of subject analysing a limited number of parameters to trials with a limited number of subject analysing a large number of parameter and data (Fig. 8).

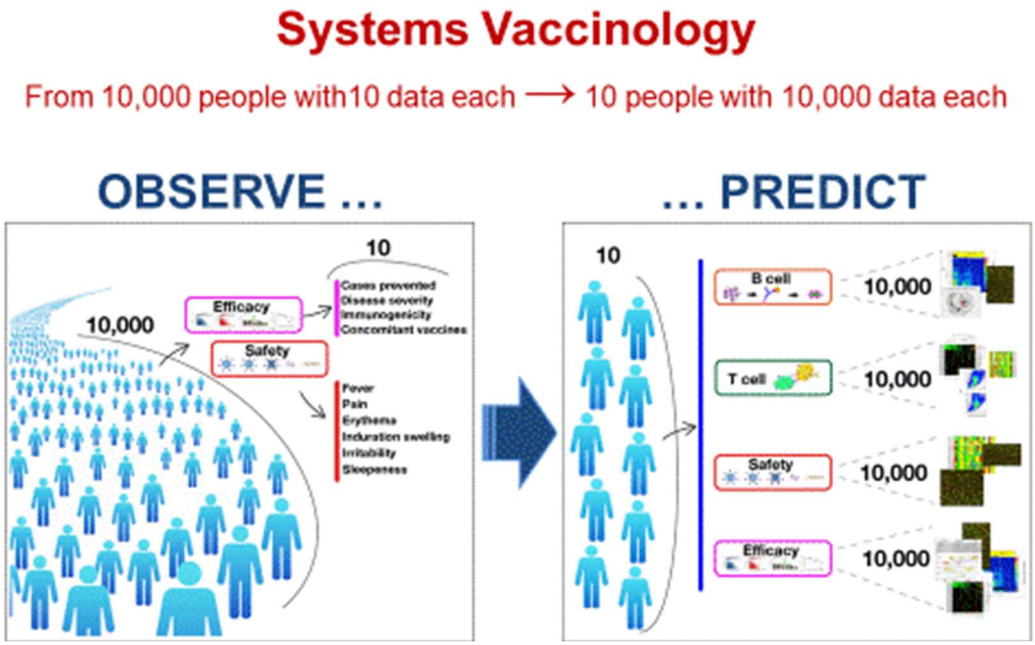


Fig. 8 The concept of systems vaccinology applied in ADITEC

The main focus was on studies with the influenza vaccines, either with or without adjuvants, conducted in WP10 and WP7. The overarching goal of this effort was to identify molecular signatures that correlate with, and predict, various key parameters of the innate and adaptive immune response. A secondary goal was to obtain insights into the molecular mechanisms driving innate and adaptive responses to vaccination. One of the main achievements is the conduction of the first study analysing through a systems biology approach, immunity to MF59-adjuvanted versus nonadjuvanted trivalent seasonal influenza vaccines in early childhood. Results of the study were published in PNAS (Nakaya H. *et al.* Proc Natl Acad Sci U S A. 2016)

Based on these results a further study examining responses to MF59 adjuvanted vaccine in children and adults was conducted and completed as follow up to the previous pilot study. Data analysis is complete for antibody and innate immune responses and the transcriptomics are being undertaken currently at Emory. Systems biology was also applied of the Hepatitis B Adjuvants Comparison Clinical Trial.

The Effects of Human Genetics, Gender and Disease on Vaccine Responses have been analysed. Recruitment of participants for the study on the effect of age, gender underlying disease and genetics on immune response to influenza vaccination has been completed with 1901 participants in total, whereof 584 in autumn 2015. 1853 or 97.5% of all participants provided both pre- and post-vaccination samples, and their HAI titers have been measured. Of these 1853 subjects who were included in the final analysis 1010 (54.5%) were women and 843 (45.5%) are men. Age distribution was wide (22-105 years of age, median age 54 years). All vaccinees have been genotyped and over 95% of the

vaccinees have returned the completed questionnaire. All 1853 vaccinees have given consent to access to relevant clinical information at health institutions, Lists from the 1853 vaccinees for genome wide association scans (GWAS) were generated. No sequence variants showing genome wide significant association with the vaccine response traits were identified, but several sequence variants show suggestive association with high or low response to influenza vaccination (Olafsdottir T et al Front. Immunol*., submitted).* Several sequence variants showing suggestive association with lack of response to smallpox vaccination have been identified.

Molecular Signatures of Immunity and Immunogenicity.

Vaccinology aims to understand what factors drive vaccine induced immunity and protection. For many vaccines, however, the mechanisms underlying immunity and protection remain incompletely characterized at best, and except for neutralizing antibodies induced by viral vaccines, few correlates of protection exist. Recent omics and systems biology big data platforms have yielded valuable insights in these areas, particularly for viral vaccines, but in the case of more complex vaccines against bacterial infectious diseases, understanding is fragmented and limited. To fill this gap, ADITEC featured WP8 on "Molecular signatures of immunity and immunogenicity", aimed to identify key molecular mechanisms of innate and adaptive immunity during effector and memory stages of immune responses following vaccination. Specifically, technologies were developed to assess the human immune response to vaccination and infection at the level of the transcriptomic and proteomic response, T-cell and B-cell memory formation, cellular trafficking, and key molecular pathways of innate immunity, with emphasis on underlying mechanisms of protective immunity. This work was integrated with efforts in other sections of the ADITEC project, such as a work package on immunity and vaccine-induced responses in early life and aging; and work packages on the human response to adjuvants in clinical and translational vaccinology. The main achievements of this WP were reported in the review "Molecular signatures of immunity and immunogenicity in infection and Vaccination" by Hanks MC et al published in the ADITEC Research Topic in Frontiers Immunology (doi: 10.3389/fimmu.2017.01563).

The main achievements can be summarised as follows:

1. An **extended multiplex transcriptomic profiling assay** (dual-colour Reverse-Transcription Multiplex Ligation-dependent Probe Amplification or dcRT-MLPA) was developed for bioprofiling the human immune response following vaccination against human infectious diseases, with particular emphasis on transcriptomic signatures of innate, adaptive, regulatory, inflammatory and memory responses (Joosten et al., 2012; Haks et al., 2015). Determination of optimal responses and dense kinetic transcriptomic response measurements will be helpful in optimizing novel immunization strategies
2. This platform was complemented by **global transcriptomic gene expression profiling (mRNA as well as micro-RNA) following vaccination with the live recombinant BCG (VPM1002),** which had been tested in infants and adults. Transcriptomic analyses were used to dissect novel mechanisms and correlates of immunity and safety, with particular emphasis on age and temporal changes following vaccination (Kaufmann et al., 2014). Superior protection by VPM1002 was later shown to relate to earlier recruitment of type 1 cytokine producing T-cells and a profound capacity to produce type 17 cytokine responses, which was not seen after BCG vaccination (Desel et al., 2011). Moreover, VPM1002 stimulates enhanced AIM2 inflammasome activation,

enhancing autophagy and secretion of (IL)-1β and IL-18 (Saiga et al., 2015). Experiments with immunodeficient mice revealed higher safety of VPM1002 as compared to parental BCG. Enhanced safety of VPM1002 is also illustrated by a lower incidence of abscess formation in immunized infants (Loxton et al., 2017).

VPM1002 has now completed phase I clinical trials in healthy adults (Grode et al., 2013; Loxton et al., 2017) and a phase IIa trial in South African new-borns. It is currently undergoing phase II trial assessment in HIV-exposed neonates (NCT01479972). Within the ADITEC project we have generated gene expression profiles from infants participating in the phase IIa trial. Two groups of 11 neonates each receiving either VPM1002 or parental BCG were included and gene expression profiles were analysed from samples taken at the time of immunization and at weeks 2, 6, 12, 18 and 26 post vaccination. Safety and immunogenicity data from this clinical trial revealed that safety parameters for VPM1002 and the parental BCG strain are comparable. Both vaccines induced IFNγ responses, while VPM1002 vaccination in addition resulted in an increased proportion of IL-17 producing CD8 T cells (Loxton 2017).

3. Complementing the work on transcriptomics, **quantitative proteomic profiles were determined using novel platform technologies, including Hydrogen Deuterium Exchange (HDX) coupled to Mass Spectrometry (MS)**. HDX-MS was used to define functionally active epitopes and antibodies in polyclonal sera following vaccination. We used Hydrogen Deuterium Exchange (HDX) coupled to Mass Spectrometry (MS) to allow the mapping of conformational epitopes (Malito et al., 2013; Faleri et al., 2014; Malito et al., 2014; Ciferri et al., 2015a; Ciferri et al., 2015b; Bertoldi et al., 2016; Cariccio et al., 2016). The approach was also compared to the most sophisticated and available approaches such as protein chip (Bertoldi et al., 2016; Cariccio et al., 2016), phage display (Malito et al., 2013; Bertoldi et al., 2016; Cariccio et al., 2016), X-Ray (Malito et al., 2013) and cryo-EM (Ciferri et al., 2015a; Ciferri et al., 2015b). As a result, the breadth of applications of the method, including epitope mapping, has expanded (Pirrone et al., 2015). The approach has was also successfully applied to map epitopes from NadA (Malito et al., 2014; Bertoldi et al., 2016) and NHBA (Cariccio et al., 2016), the two other protective epitopes of the vaccine against group B meningococcus, Bexsero. Hybrid approaches making use of HDX-MS and electron microscopy also evidenced the power of HDX-MS to map viral antigen epitopes of CMV gH-gL complex. In addition to better characterizing the immune response to vaccination and to support antigen design, this method that speeds up the elucidation of recognized epitope will contribute to increase the number of available structures of antigens and antigen-antibody complexes, opening new possibilities for the development of novel tools that might reliably predict protective epitopes.
4. Studies addressed the **role of T follicular helper (Tfh) cells** and explored their presence in the blood as possible biomarkers of protective vaccine efficacy. Tfh cells are a CD4+ T-cell subpopulation that is identifiable in lymph nodes and tonsils. Tfh cells are specialized in providing help to B-cells (Goodnow et al., 2010; Crotty, 2011; Shulman et al., 2013). The identification of a circulating counterpart of the Tfh subset in the blood (Bentebibel et al., 2013; Schmitt et al., 2014) would allow the measurement of these cells ollowing vaccination, which may help defining markers predicting vaccine efficacy. Therefore, the question was to investigate whether IL-21+ CD4+ T-cells induced by specific vaccination were detectable in human blood, if and how vaccination modulated their frequency, and whether their expansion correlated with increased titers of functional antibodies. Indeed, following vaccination with MF59-adjuvanted avian H5N1

vaccine, H5N1-specific IL-21+ CD4+ T-cells were detectable in the blood, expanded after vaccination and accumulated in the CXCR5−ICOS1+ subset. The rise of vaccine-specific ICOS1+IL-21+CD4+ T-cells appeared to predict the post vaccination increase of functional antibodies in these vaccines. Finally circulating CXCR5−ICOS1+ CD4+ T-cells contained increased numbers of T-cells able to help influenza-specific B-cell differentiation, such that they differentiated *in vitro* into antibody-secreting cells in a manner that was dependent on IL-21- and ICOS1 (Spensieri et al., 2013). More recent studies have confirmed these findings and have also shown that the early expansion of cells with a Tfh phenotype predicts the long term persistence of neutralizing antibodies against influenza virus (Spensieri et al., 2016). Thus, the expansion of antigen-specific ICOS1+IL-21+ CD4+ T-cells in the circulation may represent an early predictor of a vaccine's ability to stimulate vaccine-specific immunity and a useful surrogate marker of a vaccine's immunogenicity in human beings.

5. A key feature of vaccine induced immunity is the formation of **antigen specific memory Tcells and B-cells, and their ability to migrate to the correct tissue sites**. New technology platforms to analyse human B-cell and T-cell immune repertoires, suitable to interrogate large number of B-cells or T-cells for the first time, allowed a comprehensive analysis of the memory response in all its cellular components. A combination of experimental approaches (antigenic stimulation, TCR deep sequencing and cloning of Th1, Th2 and Th17 memory subsets) allowed the dissection of T-cell subset responses, showing that pathogen- or vaccine-induced T-cells are functionally heterogeneous and comprise both clones polarized towards a single fate, as well as clones whose progeny has acquired multiple fates. By immortalizing memory B-cells from donors upon influenza vaccination, a new type of rarely occurring influenza-neutralizing antibodies targeting a conserved site in the Hemagglutinin (HA) stem was found. We combined antigenic stimulation, TCR deep sequencing and cloning of human Th1, Th2 and Th17 memory subsets, to study the distribution and TCR repertoire of pathogen- and vaccine-specific Tcells in immune donors (Becattini et al., 2015).
6. Molecular **mechanisms regulating T-cell trafficking at mucosal sites in health and disease**: Chronic immune activation dampens leukocyte trafficking and calls for novel vaccination strategies.

   The homing of leucocytes in general, and antigen specific T-cells in particular, to peripheral tissues, mucosal sites, and secondary lymphoid organs is controlled, amongst others, by the local production of chemokines, the expression of chemokine receptors on the cell surface, and an efficient cytoskeleton machinery (Lira, 2005; Bachelerie et al., 2014). The characterization of the surface expression of the different chemokine receptors on T cells have guided the discovery of different T helper cell subsets, and is still a precious tool for the characterization of novel functional subsets. In health and disease, the microenvironment can further control cell migration, by releasing factors that cooperate with chemokines for enhancing cell responses (Schiraldi et al., 2012; Venereau et al., 2013; Cecchinato et al., 2016; Proudfoot and Uguccioni, 2016), or by producing natural chemokine antagonists that block chemokine-induced activities (Proudfoot and Uguccioni, 2016). In addition to the proteins produced by the microenvironment, systemic chronic immune activation can dampen T-cell responses to chemokines (Cecchinato et al., 2017).

   T-cell migration in HIV-1 infection was evaluated as a model of impaired migration to mucosal sites, as it is well known that anti-retroviral therapy (ART) therapy is not able to fully restore complete T-cell repopulation of the intestinal mucosa in HIV-1 infected patients. Both CCR6+

and CXCR3+ CD4+ T-cells from HIV-1 infected patients, albeit expressing the same number of chemokine receptor as in healthy individuals, were characterized by an inefficient polymerization of actin after receptor triggering, regardless of ART therapy. This impairment was also confirmed in a macaque SIV infection model. *In vivo* studies showed that persistent immune activation, but not the presence of the virus, was responsible for the alterations in the cytoskeleton machinery, resulting in altered cellular trafficking to the mucosal compartment. This could be redressed by pharmacological intervention, restoring effective lymphocyte migration. These results highlight the importance of evaluating the capability of Th cells to reach mucosal niches to support cell maturation and functional activity, particularly in individuals experiencing chronic immune activation, and thus suggest new molecular strategies for improved vaccination.

7. **Activation of innate immunity is key to adjuvant activity and expression of vaccine effector mechanisms.** Since plasticity and polarization are key components of innate immunity, key molecular markers of innate immunity were investigated in the context of adjuvant activity and expression of vaccine effector mechanisms. One focus was on the role of PTX3 (Bottazzi et al., 2016, a member of the pentraxin family involved in innate resistance, as an endogenous adjuvant in vaccination using outer membrane vesicles (OMV) from *Neisseria meningitidis* as a model vaccine. In addition, the role of PTX3 in innate resistance to pathogens, particularly in urinary tract infections was assessed. This work allowed the description of the association of *PTX3* human genetic polymorphisms with susceptibility to infections with *Aspergillus fumigatus* in immunocompromised patients and with uropathogenic *Escherichia Coli*. In addition the role of PTX3 in tissue remodeling was defined and novel markers associated with macrophage polarization discovered. Moreover PTX3 orchestrates complement activity (Doni et al., 2012) and, by regulating complement-dependent tumor promoting inflammation, can act as an extrinsic oncosuppressor gene in murine and human tumors (Bonavita et al., 2015). Finally, recent results obtained in different models of tissue damage highlighted a non-redundant role of PTX3 in remodelling and repair of tissue via its interaction with fibrin (Doni et al., 2015). This further supports the evidence that the recognition of matrix and microbial components are shared ancestral features of the humoral arm of the innate immune system.

   Activation of an innate inflammatory response is a key step in the mechanisms of action of adjuvants. In view of the up-regulation of PTX3 by selected adjuvants we investigated the role of this protein in the antibody response using a well-known model of vaccination with outer membrane vesicles (OMV) from *Neisseria meningitidis* (*Nm*). We found that PTX3 binds *Nm* as well as OMV derived from *Nm*, exerting a protective role in a model of infection with *Nm* in the infant rat. *Ptx3*-deficient mice vaccinated with OMV without any adjuvant developed a lower antibody response compared to WT mice. In addition, co-injection of PTX3 enhanced the antibody response, especially in *ptx3*-deficient mice. Recognition of the antigen is essential for the effect exerted by PTX3, since immunization with an antigen not recognized by PTX3, such as ovalbumin (OVA), induced similar response in WT and *ptx3-/-* mice (Bottazzi et al., 2015). This observation was confirmed by further investigations showing that *ptx3-/-* mice produced lower levels of IgM in response to administration of Pneumovax, a human vaccine containing capsular polysaccharide from multiple *Streptococcus pneumoniae* (*Sp*) serotypes (Chorny et al., 2016). The data outlined above provide a rationale to evaluate whether PTX3 could be a correlate of the shaping of the immune response induced by adjuvants in humans. In collaboration with D.J. Lewis and G. Del Giudice, PTX3 and CRP plasma levels were measured in a cohort of individuals injected with

placebo or with licensed influenza vaccines adjuvanted or not. This study received ethical approval from London - Surrey Borders Research Ethics Committee (REC Ref: 13/LO/0044), and was registered on ClinicalTrials.gov prior to enrolment (NCT01771367). Preliminary unpublished results evidenced an increase in PTX3 plasma levels at early time points in individuals injected with the adjuvanted vaccine, confirming in humans the effects on PTX3 gene up-regulation initially observed in mice. In addition, after immunization with an adjuvanted vaccine we observed an earlier induction of PTX3 (peak at 24 hrs post injection) compared to CRP (peak at 48 hrs post injection), likely due to the local expression of PTX3 versus the systemic production of CRP. Several single nucleotide polymorphisms (SNPs) have been described in the human *PTX3* gene, mainly located in its non-coding regions with the only exception of one exonic SNP causing an amino acid variation in position 48 (Asp48Ala). The protection associated haplotypes were also associated with higher PTX3 protein expression and circulating levels (Cunha et al., 2014; Wojtowicz et al., 2015), supporting an active role of PTX3 as non-redundant player involved in the innate defence against recognized pathogens. In summary PTX3, a molecule of the innate immune system, produced in response to proinflammatory mediators, not only acts as an antibody-like molecule, recognizing pathogens and promoting their removal, but also helps in antibody production by adaptive immunity, acting as endogenous adjuvant.

The main achievements of the ADITEC project are summarised in Figure 9

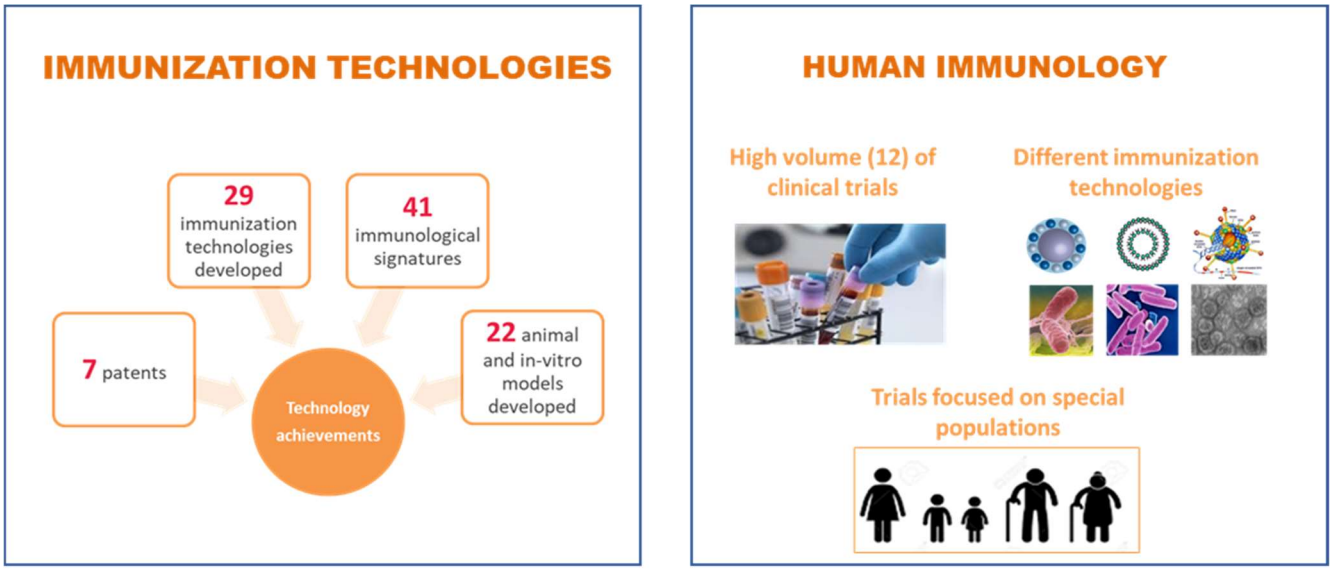
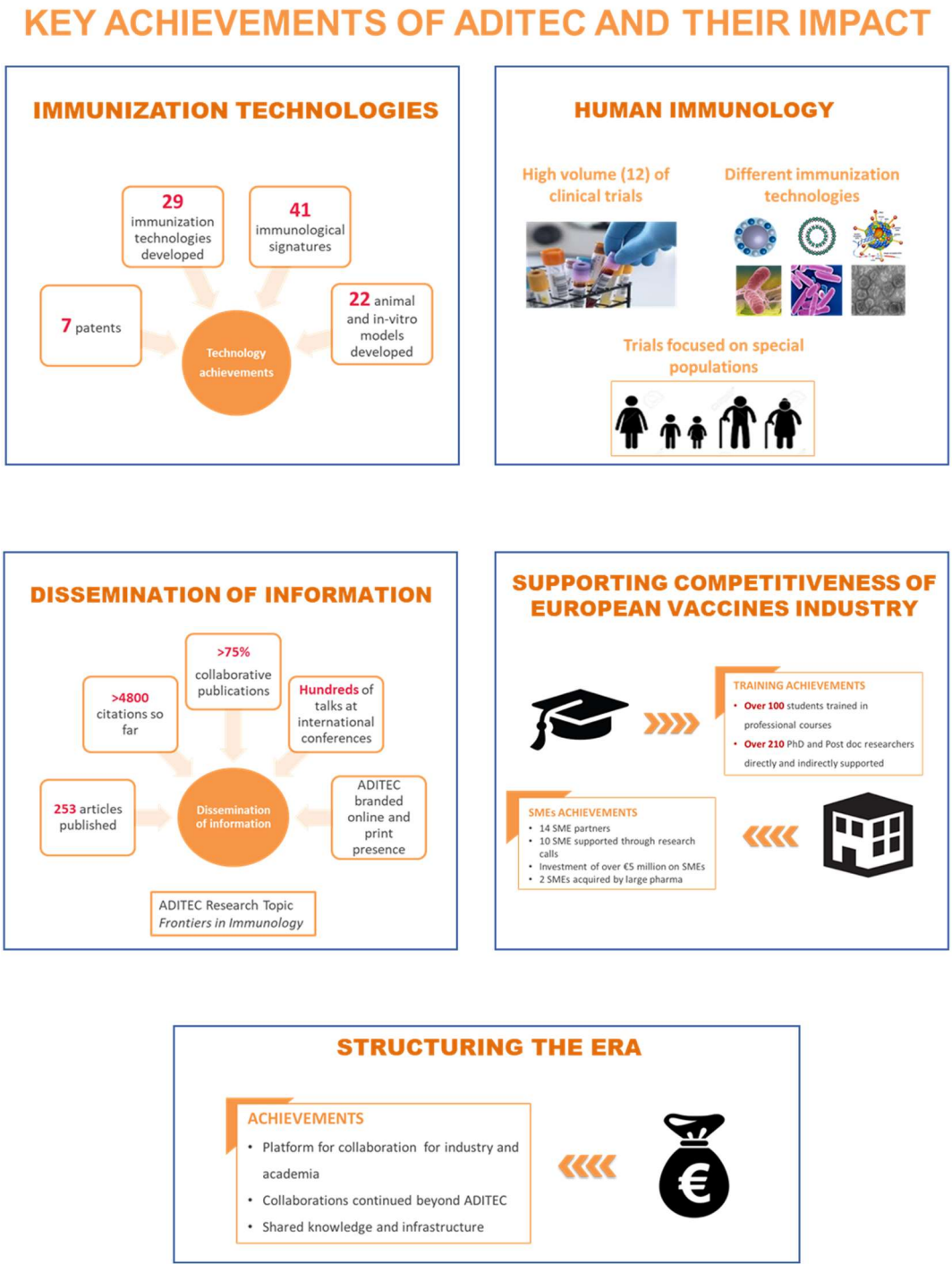


Fig. 9 Main achievements of the ADITEC project

4. The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

The ADITEC project has conducted an extensive Scientific and Socio-Economic Impact Assessment elaborated with Thomson Reuters (Attached). The report was first presented in November 2016 at the European Parliament with a special Event organized by STOA and dedicated to the ADITEC success and further updated and presented to the final Annual Meeting in September 2017.



The Scientific and Socio Economic Impact of ADITEC can be summarized as follows:

- 253 publications in peer reviewed journals
- 12 clinical studies conducted in 6 clinical centers enrolling 700 subjects
- 7 patent applications generated
- 29 immunization technologies
- 22 animal and *in-vitro* models developed
- 43 exploitable results
- 2 spin-off companies created
- 8 SMEs supported through research calls
- over 110 students trained in professional courses
- over 210 PhD and Post-Doc researchers directly and indirectly supported
- additional founds leveraged for 17,144,000.

Significant advances in the areas of advanced immunization technologies and human immunology. ADITEC has realised several innovations including new immunization technologies, new animal and in-vitro models, novel immunological signatures. To protect discoveries for future research, consortium members have generated 7 patents, with more in the pipeline. These are all positive indicators of the high throughput of the ADITEC project.

Specific results so far such as novel immunization technologies, adjuvants, vectors and delivery systems, optimized formulations and vaccination methods for different age groups, their rational combination in heterologous prime-boost schedules, all come together in a unique ***'toolbox of immunization technologies'***, enabling the best possible insight into fighting diseases. Several of these technologies have been used to further develop vaccines and advance to clinical trials. These new findings have also created commercial potential for some partners.

The ADITEC project is quite unique with **12 sponsored clinical trials completed**. The volume as well as the nature of the clinical trials is indicative of the high impact that this project has created. For example, about **700 subjects** were enrolled in the clinical studies conducted across **6 clinical sites**. Comparing the ADITEC project with other FP7 projects, ADITEC has the highest number of clinical trials.

Observing the nature of the trials provides an equally encouraging message in terms of diversity of immunization technologies used and **focus on unmet needs and different target populations** (i.e infants and elderly). A significant number of trials have applied novel systems biology approaches to fully decipher the vaccine immune response. Several completed trials studying novel vaccines technology and delivery have shown positive results in the early clinical phases leading to further clinical trials which have also been sponsored by ADITEC.

ADITEC project dedicated to the dissemination of information both scientific and non-scientific audiences ADITEC partners have published a total of **253** publications up until September 2017 (see Annex 4). The number of publications is more than double of FP7 funded projects in the area of Vaccines for Infectious Diseases (Table 2: FP7 Projects used as comparators). The ADITEC publications were **cited >4,800 times** and the citation impact is almost 4 times the average citation impact of all Web of Science papers published in "Immunology" area in the time range 2012-2017 (5.87). The **total impact factor is 1.740** with an average JIF for paper = 7,38 and **73% of papers** have

been published in journals **with impact factors in Quartile 1** of their respective subject categories (Section 6.2.1.2).

ADITEC training A huge gap is expected between the demand and supply of skilled researchers, scientists and engineers within the life sciences sector in Europe. ADITEC's **significant investment of over €1.4 million** in this area will have a clear benefit in increasing the skill set within vaccines research in Europe. ADITEC has already trained over **100 students** in professional level courses as well as supported over **210 PhD/ Post doctoral researchers**.

ADITEC project working towards increasing competitiveness of the European vaccine industry. ADITEC is promoting increased competitiveness in the European vaccines industry and has succeeded in creating a European Vaccine Enterprise. ADITEC is a true public-private partnership with 3 large pharma, 11 SMEs working closely together with 28 public research organizations (including research institutes, academics and not for profit organizations).

ADITEC has strongly supported EU SMEs encouraging their active participation in research activities. Since the start of the project **14 SMEs** have been involved as project partners with **2 SMEs being acquired by Large Pharma**. **Over € 4.6 million** (16% of total funding) has been spent on **promoting innovation within SME's** within and outside the ADITEC cohort. Several SME's have not only benefited from the funding but also from the sharing of technology, expertise and infrastructure. There have been multiple instances where technologies and infrastructures were made available to the SME partners within the consortium. Besides this, SMEs were not part of the project partners have also received similar support through **5 calls of proposals for SMEs commissioned research**. Through these calls **9 EU SMEs** have received services, supported by ADITEC funding, from 10 ADITEC partners providing access to the latest technologies and know how developed.

In addition to the SMEs, ADITEC brings together **3 Large Pharmas** strongly committed to the project. By combining different types of scientific cultures and ethos the ADITEC project contributes to increase European competitiveness and support the vaccine industry within Europe. In these perspectives this project represents a very successful experiment of public-private partnership that could become a model for future initiatives.

ADITEC project structuring the ERA as a centre of excellence for vaccines research. Large number of collaborations have been reported between partners. These collaborations have ranged from sharing of new scientific knowledge, infrastructure, joint research to publications. It is envisaged that these collaborations will provide an excelled platform which combines the knowledge and strengths of each organization to build a truly competitive environment for vaccines development in the long term. In addition to the above, **10 Memoranda of Understandings** (MOUs) with EU and US Organizations active in the Vaccine field were agreed during the duration of the project, these MOUs are expected to impact positively at various levels of innovation, improving competitiveness and structuring the ERA.

At least an additional **€11 million** has been raised **through in-kind support** and over **€17.1 million leveraged as additional funding**. However, given the scale of this project a significant amount of further funding will be required to keep up with the achievements. Now, further support is needed to capitalize on the great progress made on novel technologies for the vaccines of the future. Continued EU support would guarantee that the ADITEC initial success is fully exploited with an evident impact on health improvements and European economy.

From the above it is clear that the ADITEC project has delivered several successes. The following chapters in this report details out the achievements and the potential socio-economic impact as well as the road ahead for continuous acceleration of vaccines development.

4.1 Dissemination activities

ADITEC has successfully used a tailored approach to spread information to the scientific and non-scientific world using a variety of media. The main activities for dissemination are:

- Publications
- Conferences and Events
- Print media

Scientific Publications

ADITEC has published a total of **253 publications** (See list of publication in Table A1) up until September 2017. The number of publications per year shows an Average Annual Growth Rate of 50%, passing from 20 publications in 2012 (first year of the project) to 60 publications in 2016 (Fig. 10).

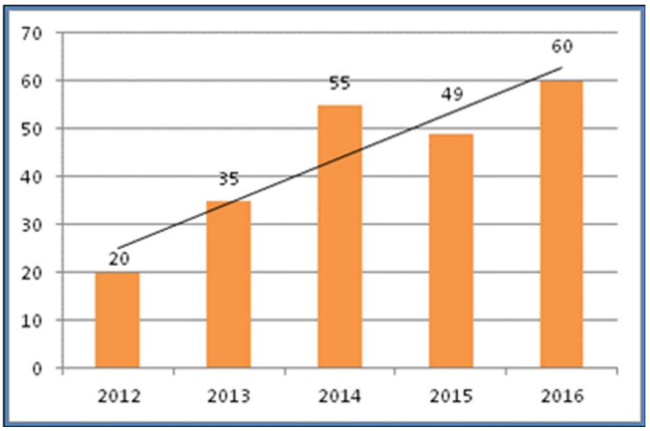


Fig. 10 Number of the ADITEC publications per year

At the moment, 30 papers have been published in 2017 whilst other 13 publications are currently in the "in press" status. Furthermore, a Research Topic dedicated to ADITEC with over 27 manuscripts is under publication in Frontiers Immunology. The bibliometric analysis reported below refers to the 240 published manuscripts.

187 of these publications (78%) are original research articles, and this shows a good rate of original and primary research (in all Web of Science – Core Collection database, articles account for around 60% of the total publishing production).

ADITEC publications have appeared in **97 different, peer reviewed journals**. Below the Top 10 journals, by number of publications, are reported (Fig 11).

Source Titles	records	% of 240
PLOS ONE	23	9.583
VACCINE	18	7.500
FRONTIERS IN IMMUNOLOGY	16	6.667
JOURNAL OF IMMUNOLOGY	9	3.750
EUROPEAN JOURNAL OF IMMUNOLOGY	9	3.750
PNAS	8	3.333
JOURNAL OF INFECTIOUS DISEASES	6	2.500
TRENDS IN IMMUNOLOGY	5	2.083
SCIENTIFIC REPORTS	5	2.083
SCIENCE TRANSLATIONAL MEDICINE	5	2.083

Fig. 11 Top 10 journals, by number of ADITEC publications

Over 50% of the ADITEC publications have been published in open access journals. More than 25% of all ADITEC publications have been published in Gold Open Access journals[2].
Looking at the country affiliation[3], the Top 10 countries most represented in the ADITEC set of papers are shown in Fig. 12:

Countries/Territories	records	% of 240
ITALY	73	30.417
GERMANY	55	22.917
NETHERLANDS	50	20.833
USA	42	17.500
FRANCE	40	16.667
DENMARK	38	15.833
ENGLAND	36	15.000
SWITZERLAND	18	7.500
SWEDEN	15	6.250
AUSTRIA	13	5.417

Fig. 12 Top 10 countries most represented in the ADITEC set of papers

Below a geographical view of all countries with at least one affiliation represented in the ADITEC set of papers. Counter clock wise are reported the TOP 5 ones.

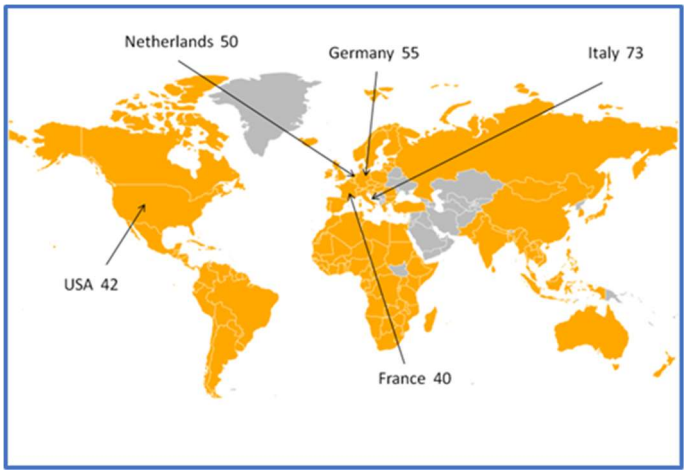


Fig. 13 Countries with at least one affiliation represented in the ADITEC set of papers

[2] Papers indexed on www.doaj.org
[3] Papers in which there is at least one author from one institution of that country.

54% of papers are the result of an international collaboration (the world average for international collaboration is around 19% overall and around 23% in the "immunology" area). Including the Research Partner Group, a total of 41 different countries were involved in authoring papers resulting from ADITEC sponsored research.
At least 116 papers (53% of the total) have resulted from collaborations with organizations outside the ADITEC Research Partner Group (42 organisations). 8 papers (3.5%) have been signed by a single author whilst 63 papers (26%) are the result of a collaboration of authors within a single institution (more than 75% of papers are "collaborative").
At least **45 papers (18.75%) are the result of cooperation with industry**. This percentage is well beyond the world average in the "Immunology" area (only considering big corporations, is around 2%).
For what concerns the categorization of papers, considering the "Research Areas[4]" classification available on Web of Science, it can be noticed that more than half of papers have been published in the "Immunology" area. The list of the TOP 10 Research Areas is reported in Fig. 14.

Research Areas	records	% of 240
IMMUNOLOGY	126	52.500
SCIENCE TECHNOLOGY OTHER TOPICS	40	16.667
RESEARCH EXPERIMENTAL MEDICINE	38	15.833
MICROBIOLOGY	27	11.250
INFECTIOUS DISEASES	19	7.917
BIOCHEMISTRY MOLECULAR BIOLOGY	15	6.250
CELL BIOLOGY	14	5.833
VIROLOGY	10	4.167
RESPIRATORY SYSTEM	10	4.167
BIOTECHNOLOGY APPLIED MICROBIOLOGY	10	4.167

Fig. 14 List of the TOP 10 Research Areas

Notwithstanding most of the papers have been published in very recent years, ADITEC set of publications is already quite highly cited, having accrued **4,835 citations**[5] (Fig. 15) for 240 articles, for an average citation impact of 20.15. This citation impact is almost 4 times the average citation impact of all Web of Science papers published in "Immunology" area in the time range 2012-2017 (5.87). Even more significant outcomes will probably be achieved in the next years. The percentage of self citations is absolutely in the norm (10.5%): the results can be considered fair and consistent.

[4] http://images.webofknowledge.com/WOKRS58B4/help/WOS/hp_research_areas_easca.html. Research areas are 151.
[5] Data from Web of Science Core Collection collected on September 2017

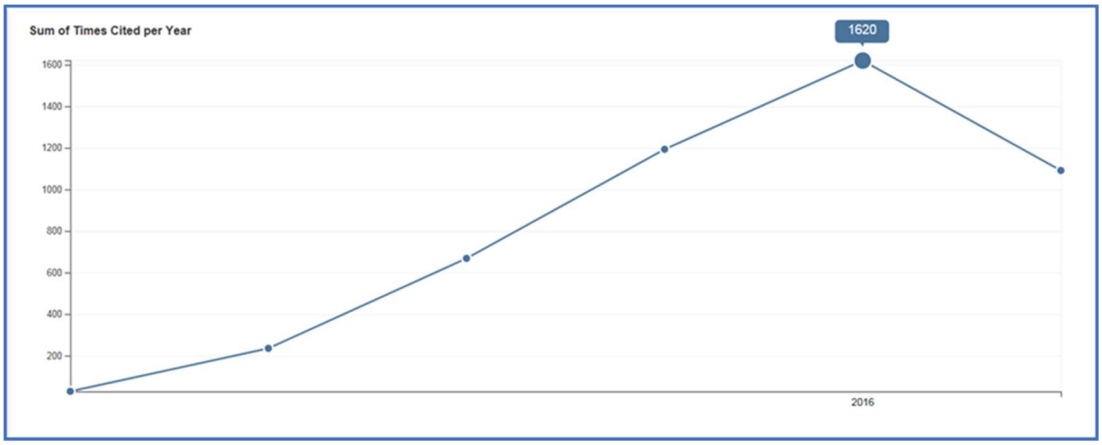


Fig. 15 ADITEC publications: sum of times cited per year

Among the 240 published ADITEC papers, 8 Highly Cited Papers[6] can be pointed out (3.33% of the total). This percentage is above the world average and the average in the "Immunology" area in the same years (0.58% and 0.62% respectively). Highly Cited Papers are papers that largely outperform their peer papers, i.e. papers that have been published in the same research area, in the same year and have the same document type. To be a Highly Cited one, a paper needs to be in Top 1% of its peer papers (or in other words to be 99 in percentile among peer papers). The list of current ADITEC Highly Cited Papers is reported in Fig. 16.

Authors	Title	Source	Year
Zak et alius	A blood RNA signature for tuberculosis disease risk: a prospective cohort study	LANCET	2016
Becattini et alius	Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines	SCIENCE	2015
Kaufmannet alius	Progress in tuberculosis vaccine development and host-directed therapies-a state of the art review	LANCET RESPIRATORY MEDICINE	2014
Cunha et alius	Genetic PTX3 Deficiency and Aspergillosis in Stem-Cell Transplantation	NEW ENGLAND JOURNAL OF MEDICINE	2014
Garlanda et alius	The Interleukin-1 Family: Back to the Future	IMMUNITY	2013
Galdiero et alius	Tumor associated macrophages and neutrophils in cancer	IMMUNOBIOLOGY	2013
Venereau et alius	Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release	JOURNAL OF EXPERIMENTAL MEDICINE	2012
Schiraldi et alius	HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4	JOURNAL OF EXPERIMENTAL MEDICINE	2012

Fig. 16 ADITEC Highly Cited Papers

The excellence of ADITEC set of publications impact can also be demonstrated looking at where those 235 papers have been published:

- **~73% papers have been published in Q1 journals**
- **~21% papers have been published in Q2 journals**

So, almost 93-94% % of papers have been published in impacted Journals with JIF beyond the median value

- Only ~5% papers have been published in Q3 journals
- Less than ~1% papers have been published in Q4 journals

[6] See for instance the Leiden Manifesto http://www.leidenmanifesto.org/

Based on the above, it is quite evident that ADITEC publications have been high in number, quality and widely cited within research. Lastly, as many published articles are still quite recent, the full impact of the research is not yet identifiable. Over the next months and years as dissemination grows, the impact of this project's research will continue to grow beyond the numbers we see today.

Research Topic "Advanced Immunization Technologies for Next Generation Vaccines"

A Research Topic "Advanced Immunization Technologies for Next Generation Vaccines", dedicated to the ADITEC project and its success is under publication in Frontiers in Immunology online journal (Impact Factor 6.429), Section Vaccines and Molecular Therapeutics (Fig. 17). The aim of this Research Topic is to assemble a series of reviews and original research articles, which highlight the advances made by the ADITEC project in the development of advanced immunization technologies and in the study of human vaccine immune responses through the latest generation methodologies. The special Issue will contain over 20 manuscripts that freely available online and collected in a single e-book.

The Research Topic and the e-book are sponsored by the ADITEC project that has covered the publication costs.

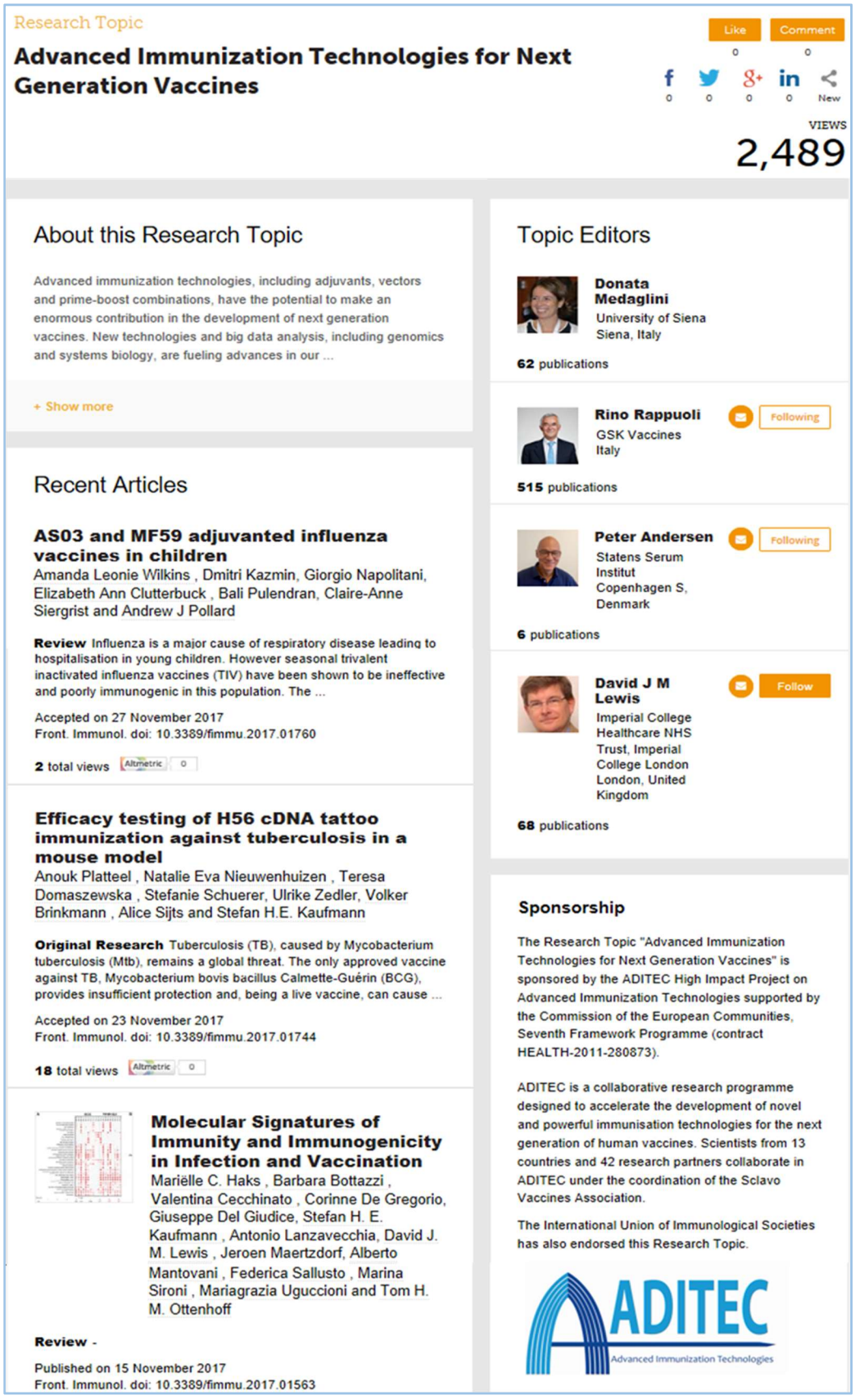


Fig. 17 Research Topic "Advanced Immunization Technologies for Next Generation Vaccines", dedicated to the ADITEC project and its success under publication in Frontiers in Immunology journal

Conferences and Events

Throughout the project ADITEC partners have contributed to a significant number of conferences and events. ADITEC partners have attended and presented ADITEC achievements in over 300 presentations at conference and workshops. ADITEC has organised several Project Meetings, Workshops, Conferences, Courses and Events. In addition to the above major events, there were several work package meetings, held in Porto, Paris, Goteborg, Brussels and Siena, as well as 34 steering committee meetings (either face to face or teleconferences) during the course of the project. The ADITEC consortium was invited to contribute to hundreds of international conferences including events such as the World Vaccine Congress and World Vaccines H2020.

The Annual Project meetings, held in Siena, Nizza and Brussels, were successfully organized by the coordinator with the involvement of over 100 participants each year. A Presidency Event dedicated to ADITEC was held at the Italian Embassy in Brussels in October 2014. In November 2016 a special event on "Vaccine Research and Development" was co-organized with the Science and Technology Option Assessment at the European Parliament in Brussels, highlighting the ADITEC success story. The event was attended by representatives of the European Commission, the European Parliament, European Union Member States and various organizations such as the EMA, EFPIA, IMI, large Pharmaceuticals, SMEs and Research and Academic Institutions (Fig. 18). The event at the European Parliament was followed by a reception dedicated to the ADITEC project organized at the Italian Embassy on the 8th November 2016.

Fig. 18 Event at the European Parliament dedicated to the ADITEC project (8th November 2016).

Print media and website presence

At least **133 print media articles** have been published to date. **16 ADITEC branded newsletters** between October 2012 and August 2017 have been published and are available on the ADITEC website (www.aditecproject.eu). Additionally, the ADITEC partners have collectively reported that **30 articles targeting** the non-scientific audience have also been published in a variety of formats such as newsletters, press releases and articles for newspapers.

Between October 2011 and September 2017, the **ADITEC website** hosted a total of 40,901 sessions with an average of 6.54 pages viewed per session. 75% of users visiting the website were new users indicating a growing interest in the project from a wider population (Fig. 19). Seven of the top 10 countries for hosting sessions are European but it important to highlight that the second country for hosting sessions are US and also India and Brasil are within the top 10, showing the strong attention to ADITEC also beyond Europe.

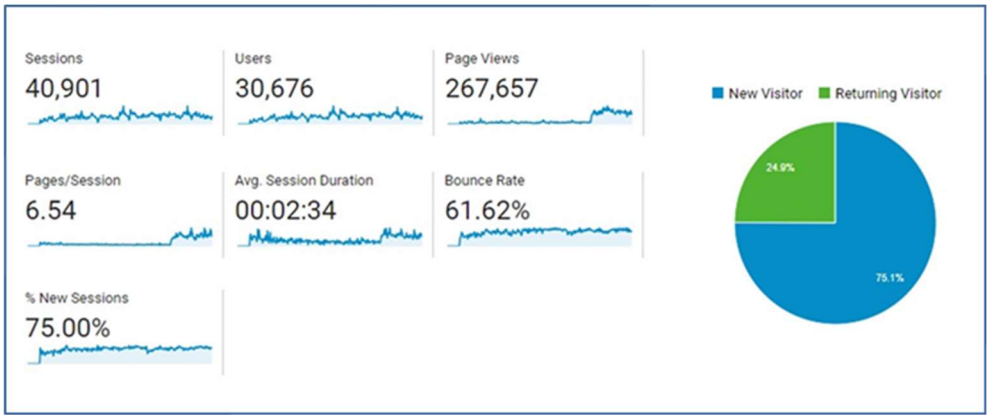


Fig. 19 Analysis of the ADITEC website activity

4.2 Training

ADITEC focused highly on training, with the objective of creating an internationally recognized educational programme on translational immunology and vaccinology thereby supporting knowledge exploitation by European industries.

The training program has been developed with three levels of training: master, post-graduate and specialized (Fig. 20).

Fig 20. The ADITEC training program

Component 1- **Training at Master level**: built upon postgraduate courses in Vaccinology and Pharmaceutical Clinical Development (NVGH and University of Siena). Overall 10 ADITEC students were recruited in 3 consecutive Masters (2013/14, 2015/16 and 2016/17). Students underwent a formal

examination after each module. At the end of the Master program the students discussed a thesis, assigned during the first year of the course. Students received a full scholarship from NVGH.
Component 2- **Training at post-doctoral and professional level organized within the Advanced Course of Vaccinology** – ADVAC (5). Five two-week courses were organized for scientists already involved in vaccine research, clinical trials or translational vaccinology (academia or private sector), with the participation of 31 ADITEC-supported students from academia and SMEs. They were provided with a broad training in essential aspects of vaccinology.
Component 3- **Training modules in "Adjuvants and vaccine formulations" and "Flow Cytometry Applied to Vaccinology"**. The Training module in "Adjuvants and vaccine formulations" comprised two types of one-week course (6). Two theoretical courses on vaccine development were organized, gathering 25 students. Two practical courses in vaccine formulation gathered 14 students. They aimed at training students on the methods of preparation of adjuvants including oil-in-water emulsions and aluminium gels, their formulation with antigens, and quality control of the resulting vaccines.
A training module "Advanced Course in Flow Cytometry Applied to Vaccinology" was organized in Siena from 11th to 14th September 2017. Fourteen participants from 6 different countries have attended the course, after an accurate selection of more than 30 applications received. The course has been structured on both practical and theoretical sessions, held by international experts in different field of flow cytometry and vaccinology. The course covers technical aspects (Standardization and quality assurance for multicolor flow cytometry, Validation of flow cytometric methods, Multi-dimensional data analysis), application of flow cytometry to profiling the B and T cell responses (in preclinical and clinical studies), detection of rare cells and analysis of their functionality, computational flow cytometry and combination of multicolor flow cytometry and multi-gene next generation sequencing (NGS).
Overall, ADITEC has supported the training of **118 students** to the courses listed above. In addition to the above, over **210 Post-Doctoral and PhD students** were supported by the ADITEC partners. In total, about € **1,462,402 million** was spent on training initiatives, about 5% of the total budget.

4.3 Promoting SMEs

The ADITEC project has brought together **14 SMEs** during the course of the project. Of these 14 SMEs, 11 are currently active and 2 are active but have been acquired by large Pharma.
To ensure that SMEs within the project were adequately supported 16% (over €4.6 million) of all European Commission funding has been granted to SMEs. This includes manpower support of 38.08 FTEs.
There has been a high involvement of SMEs across 7 different work packages focusing on innovation such as development of new immunization vectors, adjuvants and novel routes of administration.
In addition to the support given to the partner SMEs a separate initiative has been set up to provide technical support to SME organizations which are not currently part of the partner cohort. In total **10 SMEs have received support through ADITEC research open calls**, of which 7 are not part of ADITEC Consortium.

Commissioned vaccine/immunology research for SMEs
This initiative aims to provide interested SMEs, that may not be part of the consortium, free access to the ADITEC technologies, expertise and facilities which they may not have access to but are essential

to the progression of their research. All of these projects identify one or more ADITEC partners which have the relevant infrastructure in place to support the SME/PHO requesting the service and provides the service at no cost to the requesting organization.
Free access has been limited to a value of up to € 100,000 per proposal. Since the first call for proposals in 2013, ADITEC has received 24 proposals and **has funded 13 proposals amounting to total funds just around 1 million.**
10 SMEs received grants for the 13 proposals collectively. Of the organizations receiving grants 3 were ADITEC partners and the remaining 7 SMEs/PHOs were not part of ADITEC

4.4 Exploitation of results

43 exploitable project results have been reported from the above advances by all ADITEC partners7. From this it is clear that ADITEC research has served as a **'Toolbox of Technologies'** for further development both within the realms of the project as well as for external commercial development opportunities.
Looking at the exploitable results being developed within the project, a Chlamydia antigen vaccine adjuvanted by CAF01 was selected through a comparison of adjuvant formulations in pre-clinical studies. This formulation included the use of a novel technology in the experimental phases which has progressed first in ADITEC non-human primate studies and now to full clinical development in 2016 with a trial (NCT02787109) sponsored by ADITEC.
NVGH Partner 39 has seen a GMMA vaccine against Shigella sonnei undergo a phase I trial (NCT02034500) in 2014 and then successfully progress to a phase II trial (NCT02676895) starting in 2016. Both trials carried out have been sponsored by ADITEC, and now Bill and Melinda Gates foundation has further supported, with a 14 million grant, the further development of this vaccine candidate, addressing a neglected pathogen. Meanwhile SME partners such as Okairos (Reithera) and Duotol have reported a number of exploitable results which they have further developed from the initial research undertaken as part of the ADITEC initiative. Okairos (Reithera) has focused on the production and characterization of chimp Adenovector vaccines expressing prototypes antigens H56 and MOMP. The learning from this research has aided the development of two ChAd-based vaccines, one for RSV and another for Ebola, that have been reported as being in phase I in 2013 and phase I, II and III in 2014 trials respectively. While the research has been carried out as part of ADITEC, these trials are being sponsored independently by Okairos (Reithera). Duotol has reported the development and optimization of a preclinical preparation technology of a novel 2nd generation adjuvant. In addition to this, Duotol is conducting a clinical proof-of-principle for the sublingual administration of vaccines. Currently all of these technologies are being tested in further clinical studies for which Duotol has collaborated externally.
The ADITEC project has also led to the creation of **2 spin-off companies**. The Spin-off Adjuvatis has PLA particles designed by CNRS at the heart of the new company. The creation of new companies based on the ADITEC research is not only beneficial to the project but also to the industry as a whole as the research and products will be available to a wider audience.

[7] ADITEC Annual Report . November 2016

In addition to the reported developments, **7 patents** have been applied for by ADITEC partners (Table 3). As of 2016, one has been granted and this is still owned by the patent originator. Assuming all 7 patents are granted, they have a potential to be used by the originator or licensed-out thus creating future economic value.

Table 3 List of patents applied for by ADITEC partners

Number	Patents	Applicant
CN105377879 A	Vaccines against Chalmydia sp	Statens Serum Institut
US2014112979 A1	Methods for producing liposomes	Statens Serum Institut
US2016244488 A1	Cholera Toxin A-Like Polypeptide useful as adjuvant component	University of Gothenburg
WO 2014139587 A1	Improved poxviral vaccines	Okairos Ag
US 9321829 B2	Antibodies directed against Influenza	Emory University
US9469685 B2	Antibodies directed against influenza	Emory University
WO2017108902 A1	Oil-in-water emulsions including retinoic acid	Novartis AG

In summary, the ADITEC project has made several technological advances with commercial potential in a significantly short span of time and budget. However, most of the research is still in the early phases of development and significant amount of resources will be required to further advance these new technologies. It has been reported by work package leaders that the work carried out by the ADITEC project has the potential to fast-track the development of vaccines against emerging diseases so long as support, both financially and through expertise, is sustained going forward.

The technologies and scientific developments carried out by the ADITEC partners have led to them being in a position to carry out clinical testing of both novel and existing technologies (Fig. 21).

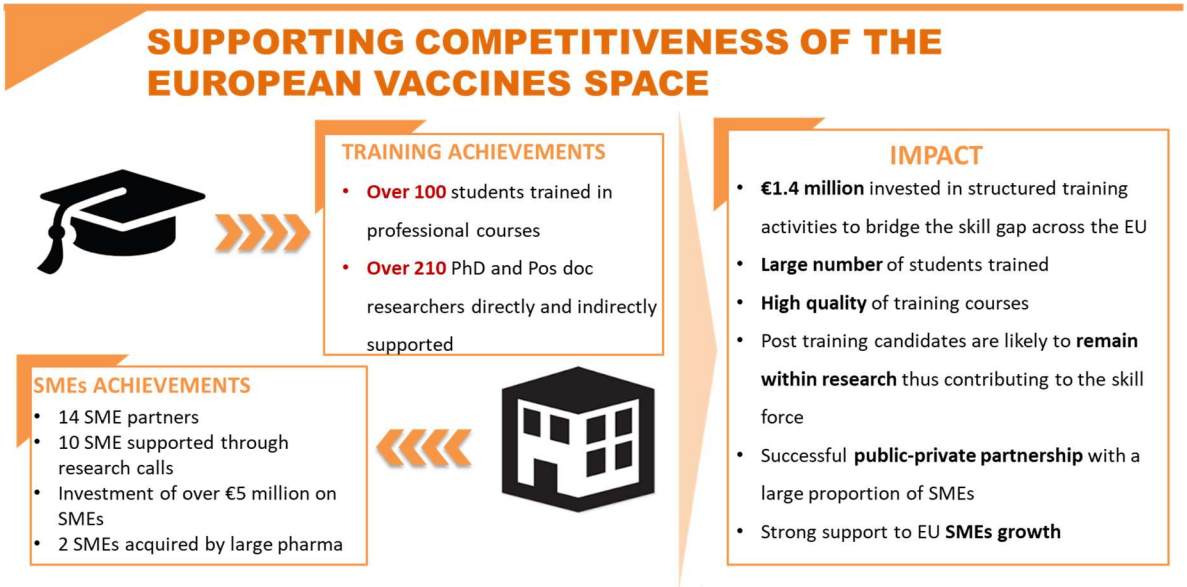


Fig. 21 Impact, SMEs and training activities of the ADITEC project

The **comparative analysis of the ADITEC projec**t against 10 selected FP7 projects in the area of vaccines has shown that ADITEC has produced over the double of publication and conducted 2.6 times more clinical trials per million of EU contribution. Furthermore the number of SMEs supported by ADITEC is equal to the number of the 10 projects put together and double the average of these 10 projects (see table below and page 15 of Socio-Economic Impact Report).

Acronym	Project Title	EC Contribution (Euro)	Clinical trials N.	Partners N. (of which SMEs)	Publications
NEWTBVAC	Discovery and preclinical development of new TB vaccines	11,900,000	0	35 (1)	144
CUT'HIVAC	Cutaneous and Mucosal HIV Vaccination	11,929,000	4	13 (0)	8
MULTIMALVAX	A Multi-Stage Malaria Vaccine	8,055,788	4	6 (1)	1
NGIN	Next Generation HIV-1 Immunogens inducing broadly reactive Neutralising antibodies	7,534,742	0	18 (3)	76
PEACHI	Prevention of Hepatitis C Virus (HCV) and HIV-1 Co-Infections	5,844,998	3	5 (1)	7
IHIVARNA	Therapeutic TriMIx/mRNA based Vaccine in Chronic HIV-1 Infected Patients on Antiretroviral Therapy	6,000,000	2	8 (2)	0
EuroNeut-41	EUROpean consortium on NEUTralising antibodies using gp41	12,000,000	1	18 (5)	17
REDMAL	Clinical development of a Pfs48/45-based malaria transmission blocking vaccine	2,999,998	0	6 (1)	11
TRANSMALARIABLOC	Malaria Transmission Blocking by Vaccines, Drugs and Immune Mosquitoes: Efficacy Assessment and Targets	2,993,964	0	7 (0)	54
STOPENTERICS	Vaccination against Shigella and ETEC: novel antigens, novel approaches	11,460,630	2	15 (0)	20
TOTAL		**80,719,120**	**16**	**126 (14)**	**338**
ADITEC	**Advanced Immunization Technologies**	**29,980,670**	**12**	**42 (14)**	**248**

The address of the project public website:
WWW.ADITECPROJECT.EU

Relevant contact details:
Sclavo Vaccines Association
Via Fiorentina 1
53100 Siena, Italy

ADITEC logo