BELLEROPHON FINAL REPORT

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Final publishable summary report

Executive summary

The bacterium *Staphylococcus (S.) aureus*, is one of the most important bacterial pathogens, causing skin lesions, and deep infections in both the community and in hospitals. Treatment is difficult and expensive and may require prolonged intravenous antibiotic therapy. Since there is no vaccine licensed by the US Food and Drug Administration (FDA) or European Medicines Agency (EMA), interception also relies heavily on antimicrobials to which antibiotic resistance is developing.

BELLEROPHON is a pan-European project that is addressing these shortcomings in the fight against *S. aureus* infections by designing and evaluating vaccine candidates against both methicillin sensitive and methicillin resistant (MRSA) strains of *S. aureus*. BELLEROPHON partners comprise four European institutions involved in vaccine development, each contributing with specialised expertise and technology. IMAXIO is a French biotech company focused on immunology. The Jenner Institute at the University of Oxford (UOXF), UK, is an academic institution with key expertise in *S. aureus* antigens and viral vector delivery systems, and is coordinating the overall project. The European Vaccine Initiative is assisting with project management tasks and advising on production and the clinical aspects of the project. The fourth member is Preclin Biosystems, a Swiss contract research organisation with strong expertise in preclinical efficacy models for various infectious diseases.

The overall aim of the BELLEROPHON project is to define the most protective composition for an efficacious vaccine candidate, targeting both cellular and humoral immune response against *S. aureus* infection. This aim was pursued via two main activities: first, to design, manufacture and assess in a phase I clinical trial a novel *S. aureus* vaccine candidate, and second, to identify additional antigens that could be combined with the main vaccine candidate in order to improve its protective efficacy.

Combinations of different antigens in two delivery systems (as recombinant proteins and as viral vectors) were assessed in several preclinical models for immunogenicity and protective efficacy. Several approaches in optimising the vaccine candidates were undertaken, such as: delivery of the antigens as single proteins or fusions, the order of the antigens within the constructs, different adjuvants were tested for the ability to enhance the induced immune response, addition of a signal sequence to enhance antigen expression from viral vectors. The protein vaccine candidates were successfully produced achieving high purity and yields and the manufacturing process was developed to be scaled up and prepared for technology transfer to a manufacturing organisation. The different vaccine candidates were evaluated in the following models: pneumonia model / post-influenza pneumonia model, renal abscess formation model and intraperitoneal sepsis model. As the original vaccine candidate showed a limited protection in pre-clinical models, it was not deemed sufficient to progress for clinical development. However, the BELLEROPHON consortium discovered and validated in pre-clinical studies new promising vaccine candidate antigens and, by developing and testing new antigen delivery methods, improved the targeting of the immune response and immunogenicity, including cellular response.

BELLEROPHON scientific achievements have been published in peer reviewed journals and presented at several conferences to the scientific and general public. Two patent applications have been filed.

Context and objectives

***S aureus* disease burden:** *S. aureus* causes a range of serious infections in humans. It is responsible for approximately 16,000 deaths annually in Europe and 19,000 in the USA. Additional studies suggest at least €380 million annual European costs attributable to *S. aureus*, as well as several billion US$ per annum in the USA. The emergence of highly antibiotic resistant *S. aureus* strains, such as Methicillin-resistant *S. aureus* (MRSA), are creating a serious global public health threat, which is an increasing economic burden. *S. aureus* is one of the most important bacterial pathogens, causing skin lesions, and deep infections in both the community and in hospitals. Treatment is difficult and expensive and may require prolonged intravenous antibiotic therapy. To date, there are no *S. aureus* vaccines approved by a major regulatory agency, despite a number of clinical trials. Therefore, prevention relies heavily on antimicrobials to which antibiotic resistance is developing.

Existing vaccine strategies: Several major pharmaceutical companies continue to pursue a vaccine containing a combination of antigens mediating protection against *S. aureus*. This strategy is compatible with preclinical data showing that in murine models, multi-antigen vaccines perform better than single antigen vaccines. Small animal preclinical studies remain a gateway to clinical studies of *S. aureus* vaccines, but one notable difference between human populations and murine models concern exposure to *S. aureus* prior to vaccination: all humans have been exposed to *S. aureus*, and in whom nasal colonisation is common, while in murine models, exposure to *S. aureus* (if any) in which exposure is less understood. Pfizer is conducting Phase II studies of capsular antigens in conjunction with Clumping Factor A and Manganese Transporter C, while both GlaxoSmithKline (GSK) and Novartis Vaccines (now GSK) have products at earlier stages of development. Development of all these products is supported by encouraging preclinical data.

Failure of previous Phase III clinical studies: Unfortunately, the likely clinical impact of these vaccinological approaches is difficult to evaluate, because two recent unsuccessful high profile Phase III clinical trials leave the field with questions about the nature of the correlates of protection in *S. aureus* disease. One randomised trial (from NABI, now GSK) was targeting common bacterial capsules (types 5 and 8). This yielded equivocal results, with both the variable expression of the related capsule antigens and manufacturing issues proposed as an explanation for its failure. The second trial, from Merck, was targeting IsdB protein, reported during the BELLEROPHON project and also did not generate efficacy despite boosting antibody response. Indeed, as below, a safety signal may have been generated. Reasons for failure remain unclear in this case. One possibility is that delivery of the non-adjuvanted protein may have not generated cellular immune response, whereas studies indicate that T-cell response may be required to induce protection in humans.

Lack of clinical efficacy has also been observed using strategies involving transfer of monoclonal or polyclonal antibodies against *S. aureus* into vulnerable populations and a requirement for a cellular immune response has been suggested as an explanation for their failure.

Safety signals observed in Phase III clinical studies: Unexpectedly, the phase III clinical trial (V710) with Merck's vaccine candidate IsdB was stopped due to severe side effects of vaccination. An unexpected increase in mortality in vaccinated individuals who subsequently developed *S. aureus* sepsis has shown, in *post hoc* studies, to be associated with a particular profile of serum cytokine responses at vaccination (low IL-17A, low IL-2). This phenomenon had not been observed previously and emphasised the uncertainty surrounding naturally occurring immune responses to *S. aureus* in pre-clinical models.

Challenges: Thus, safety and protection against *S. aureus* remain one of the major challenges in the field of vaccine design and development. It is likely that an immune response including both

humoral and cellular immunity is necessary. Multiple previous trials highlight an insufficient understanding of the nature of protective immunity, the absence of a well-defined predictive animal model.

Major objectives of the project: The BELLEROPHON consortium aimed to address these shortcomings in the fight against *S. aureus* infection by targeting the following main objectives:

- Design, manufacture, and phase I clinical trial assessment of a novel *S. aureus* vaccine candidate designed to protect against both methicillin resistant and more sensitive *S. aureus* strains, targeting both the cellular and humoral immune responses.
- Identification of additional antigens which could be combined with the product developed in 1) to improve protective efficacy.

The context in which the BELLEROPHON project operated is summarised below:

a) Novel antigens: Consortium partners has identified a previously unstudied antigen BitC, present in the core genome of *S. aureus*, which elicits potent protection and is highly conserved between *S. aureus* lineages.

b) Novel and potent immunogenic tags: The consortium's partners had previously identified safe and efficacious pro-immunogenic tag (IMX313), and modifications thereof (IMX313P), which provides increased immunogenicity, inducing both humoral and cellular immune response for some antigens.

c) Clinically deployable viral vectors to induce cellular immunity: The consortium's partners had used viral vectors (chimpanzee adenovirus (ChAdOx1) and Modified Vaccinia Virus (MVA)) as antigen delivery vehicles, primarily to stimulate the T cell immune response, but also to generate high titre antibodies. Adenoviruses have been used in Phase I and II trials in humans, but it is now recognised that the widespread seroprevalence of antibodies to common human adenovirus serotypes (e.g. AdHu5) limits the utility of these viruses in humans. Clinical data with multiple simian adenoviruses show that they are not subject to the same limitations, although the use of many is encumbered by intellectual property rights (IPR). Consequently, a new vector (ChAdOx1) hady been developed within UOXF at the Jenner Institute. Seroprevalence of antibodies to ChAdOx1 has been examined in European and other populations; no individuals with high titre neutralising antibodies were found in the European group. Immunogenicity of ChAdOx1 has been good in both pre-clinical and clinical tests. MVA has been deployed in over 100,000 individuals at the end of the smallpox eradication campaign, and recombinant MVA has been used in multiple clinical trials, as well as to boost immune responses in man. Viral vector based approaches have not previously been used in *S. aureus* vaccine development.

The main objectives of BELLEROPHON were summarised as below:

1. To produce preclinical batches of protein and viral vector reagents.
2. To optimise selected immunisation regimens using preclinical batches.
3. To compare optimised regimens in preclinical studies and characterise them in additional models (mouse strains, *S. aureus* infection models)
4. To select the best regimen for manufacturing and clinical trial.
5. To manufacture the vaccine candidate according to Good Manufacturing Practice and perform toxicology and stability studies
6. To conduct Phase I clinical trial in man

Results

The activities of the BELLEROPHON project were divided into seven work-packages. The main scientific and technical results are described by work-packages and task below.

WP1: Optimisation and validation of vaccine candidate

WP2: Good manufacturing practice (GMP) manufacturing

WP3: Preparation of clinical trial

WP4: Clinical trial

WP5: Additional antigen discovery

WP6: Dissemination and exploitation

1. **WP1 Task 1.1** *Produce preclinical batches of protein and viral vector reagents (UOXF, IMAXIO; M1-M3)*

Production of protein preclinical batches

Detailed preclinical studies were performed on a variety of *S. aureus* antigens, fused into a single recombinant protein. Ultimately, a construct named BCAP, which is a multivalent antigen, was identified which was readily produced and provided strong immunogenicity in challenge preclinical animal models.

Multiple constructs were produced with and without IMX313 pro-immunogenic tags, using an *E. coli* production system. In the initial phase of the project two problems were encountered. Firstly, antibodies against recombinant proteins including toxin(s) did not elicit neutralisation and secondly, some combinations of fusions antigens showed insolubility problems which would have precluded manufacturing.

Deployable solutions found containing both the multiple vaccine candidates while bypassing technical problems. Inclusion of toxin(s) in the vaccine was deferred. Following extensive literature review and intellectual property assessment, it was decided to add additional antigens to the initial combination. The modified protein, BCAP, was successfully produced, in which three *S. aureus* antigens are fused to an IMX-immunogenic tag. The newly generated recombinant protein candidate vaccine BCAP was produced in the standard expression system, *E. coli*, and found in the soluble fraction. A satisfactory yield of approximately 80 mg/ml purified protein was obtained from 1L of bacterial culture. BCAP is a 779.8 kilo Dalton (kDa) heptameric recombinant protein. The obtained preclinical batch was sufficient to perform all required characterisation, immunogenicity and protection studies.

The total work generated approximately fifty plasmid constructs, twenty *E. coli* expressions, more than ten purified proteins, and several immunological studies in mice.

Viral vectored vaccine candidate

A virus vectored vaccine candidate was constructed to contain the three *S. aureus* antigens with or without the IMX313 immunogenic tag. In addition, we inserted the tissue plasminogen activator signal sequence (TPA) in front of the expression cassette and found that this improved the vaccine immunogenicity significantly. We pursued a construct containing TPA.BCA, deleting all sequences that didn't significantly enhance the immune response in the viral vector system. In order to assess

the importance of antigen order and the role of component A, new constructs containing either BC or CB insert were created. Testing of these constructs in mice is described under Task 1.3 below.

Additionally, the antigenic composition was designed in viral vectors expression system using chimpanzee adenovirus (ChAdOx1) and Modified Vaccinia Virus (MVA). For the BCA antigen, constructs with and without IMX313 tags have been produced.

In the later stages of the BELLEROPHON project the work on *S. aureus* toxins restarted. The R&D laboratory in IMAXIO produced several truncated and/or mutated toxins recombinant proteins with and without the IMX313P pro-immunogenic tag. The objective was to obtain a new non-cytolytic heptameric Hla protein capable of inducing neutralizing antibodies. All generated proteins were characterized. The analytical methods routinely used were: purity by SDS-PAGE, concentration by UV absorbance at 280 nm, mass spectrometry, and identification by western blot and endotoxin detection by Limulus amebocyte lysate (LAL) and/or Liquid chromatography–mass spectrometry (LC-MS) (at LPS-Biosciences). The induction of protective antibody was investigated.

Development of the IMX313 technology

The IMX313 technology is a pro-immunogenic tag, described in the BELLEROPHON grant application. It is an oligomerization domain which can be genetically fused to the antigen candidate in order to increase both antibody and T-cell immune responses. During the BELLEROPHON project, IMAXIO continuously worked to improve the IMX313 technology. At the start of the project, IMAXIO was investigating the additional benefit of a second generation of the IMX313 technology, named IMX313T. A further improvement, IMX313P, showing increased immunogenicity over both IMX313 and IMX313T was later developed, replacing both previous versions. The IMX313P was implemented in the BELLEROPHON vaccine candidates.

During the last 18 months of the project, further development of IMXP3P took place, focusing on improving the Th1 immunogenicity. For this purpose, a sequence (encoding 11 amino acids containing universal CD4+ epitopes developed in the 1990s was integrated in different manners into IMX313P. This has generated 10 different pro-immunogenic tags which were produced and purified at IMAXIO. A first round selection of the best candidate based on specified physicochemical characteristics have permitted to retain only one construct called IMX854. The capacity of the IMX854 candidate to induce *in vitro* maturation of the dendritic cells was compared to the original IMX313P sequence (IMX853). Unfortunately no benefit was seen with the 854 sequence, these investigations were stopped and this sequence was not included in the BELLEROPHON vaccine candidates.

2. **WP1 Task 1.2** *Optimise selected immunisation regimens (UOXF, PRECLIN; M4-M7)*

Immunogenicity studies with the BCAP protein vaccine candidate

The initial work conducted on the BCAP construct showed that the BCAP fusion protein is highly immunogenic in mice. The added value of the antigen fusion in the BCAP construct as well as the fusion to IMX313P were demonstrated by:

- T-cell and humoral responses similar or higher compared to responses to the separate antigens
- Antigens better presented to the immune system as a fusion protein BCAP
- Higher immunogenicity with the addition of IMX313P

A study comparing a mix of single components of BCAP and BCAP recombinant protein administered in mice at optimal doses (previously determined) was performed with and without

Montanide ISA51 adjuvant (Seppic). The mix of the three antigens resulted in similar humoral and cellular immune responses to the fusion protein. The immune response was boosted by the adjuvant in all groups.

Novel routes of administration: intranasal vaccination

There was a strong rationale for testing this route with the protein vaccines in the context of the BELLEROPHON project:

- Mucosal surfaces provide an ideal site for effective local immune response: i.e. block pathogen entry into tissues/bloodstream thus reducing infectious burden.
- There is an expected added value of IMX313P by this route (cationic and oligomerization increasing the molecular weight of the antigen, both factors being favourable for nasal delivery).

The intranasal route was tested in two separate studies: i) with BCAP and single antigen vaccines; and ii) with detoxified monomeric and heptameric toxin antigens.

Intranasal vaccination with BCAP vaccines

BALB/c mice were vaccinated by the intranasal (IN) route 2 weeks apart with 3 doses of 30 μg of BCAP or single antigen without adjuvant. A group vaccinated intramuscularly with BCAP formulated with Incomplete Freund's adjuvant (IFA) (2 doses of 30 μg) as well as a negative PBS control group were added. The BCAP IN immunisation induced good spleen cellular responses (systemic) and lung (local) cellular responses, and also humoral IgG (systemic) responses. The level of response was similar to that obtained following intramuscular adjuvanted BCAP vaccination (with IFA). The single antigen IN vaccination led to a similar immune response compared to BCAP IN. Following these results, this approach was tested in the pneumonia challenge model. However, with a lethal *S. aureus* challenge dose ($1x10^9$ colony forming units (CFU)/BALB/c) in specific and opportunistic pathogen free (SOPF) Balb/c mice, no efficacy was observed with BCAP IN (see Task 1.4).

Intranasal vaccination with detoxified toxin

C57/BL6 mice were vaccinated by the IN route with 3 doses of 25 μg of heptameric detoxified toxin antigen or the monomeric version without adjuvant. A group vaccinated subcutaneously with the multimer formulated with IFA adjuvant (2 doses of 25 μg) as well as a PBS control group were added. The multimeric truncated protein elicits better T cellular immune response than the monomeric versions following intranasal vaccination in both spleen and lungs. The highest humoral response was obtained by the heptameric vaccine, especially when the protein was adjuvanted with IFA and administered subcutaneously. The sera were tested in vitro for the ability to neutralize the wild type toxin. Antibodies against the heptamer administered subcutaneously (SC) with IFA elicited strong neutralizing activity. No neutralization effect was observed following IN immunization with the different tested vaccines.

Additional immunological / mechanistic investigations

Despite the high immunogenicity observed with BCAP, it was not protective in multiple different mice challenge models (see task 1.3). To better understand this finding, we performed some additional assays to further investigate the presentation of BCAP to the immune system and to compare its behaviour to the behaviour of individual antigen(s) included in the BCAP vaccine.

<u>Peptide scanning study by epitope mapping</u>

The objective of this experiment was to map the epitopes recognized by the antibodies raised in mice after BCAP or single protein vaccinations. This study was subcontracted to Pepscan who synthesized an array with overlapping linear peptide epitope-mimics of the BCAP sequence, and

then measured the binding of serum to the peptides by Enzyme-linked Immunosorbent Assay (ELISA). Although BCAP and the individual antigens from BCAP fused to IMX313P generated global similar immune responses, the epitope mapping of the corresponding sera did not elicit similar linear epitopes. One could speculate that the fused antigens might be presented differently to the immune system compared to the individual antigens. This might have an impact on efficacy in challenge studies.

Internalisation and maturation of dendritic cells (in vitro study)

The objective of this *in vitro* study was to evaluate the impact of BCAP on dendritic cell (DC) maturation. Immature DCs were generated by *in vitro* cultures of mouse bone marrow cells. BCAP and an example of individual antigen were labelled with Alexa647 fluorochrome for this study. DCs were incubated with the labelled proteins in order to evaluate i) the internalisation of the proteins into DCs through Alexa fluorescence; and ii) the activation of DCs by following CD40 up-regulation. Either BCAP or single proteins were well internalized by different DC subsets, and triggered the maturation of both pDC and cDC.

Possibilities for combining different *S. aureus* antigens

The different constructs of truncated detoxified toxins in combination with other BCA antigens or novel antigens identified as part of the work package 5 activities are currently under development and evaluation.

3. **WP1 Task 1.3 and WP1 Task 1.4** *Compare optimised regimens, selecting the regimen for phase I clinical studies (UOXF, PRECLIN; M6-M9); Confirm protection of optimised model (UOXF, PRECLIN; M9-M12):*

The original plan envisaged screening of antigens in an initial animal model (Task 1.3) with confirmation in additional models (Task 1.4). Both activities were fully completed.

As an additional, confirmatory model a sepsis peritoneal model in mice has been tested. The experiment was conducted outside the consortium by sub-contracting at the Biomedical Institute of Seville, Spain, with technology transfer into the consortium. The challenge strain used was Newman. First, the bacterial minimal lethal dose causing 100% mortality was assessed using a serially diluted inoculum. The dose retained was $1x10^9$ CFU/mice. The animals were infected by the intraperitoneal route (minimal lethal dose). Readouts including reaching a humane endpoint (mortality), and in survivors, bacteremia and CFU in spleen.

Viral vector regimen:

The viral vector vaccine candidate was tested in 3 different models:

- Renal abscess model (Intravenous (IV) challenge with *S. aureus* (SA); read out: colon forming units (CFU) and abscess formation in kidneys).
- Sepsis model (intraperitoneal (IP) challenge with SA; read-outs : mortality, body weight loss, bacteraemia and CFU in spleen)
- Pneumonia model (intranasal challenge (IN) with SA; read-outs: mortality (if lethal dose used), clinical score, body weight loss, CFU in lungs, histology).

In renal abscess model studies, ChAdOx1 vector was administered as the prime and MVA vector was administered 8 weeks later as the boost. The *S. aureus* intravenous challenge was performed 2 weeks after the boost. High immunogenicity of the different constructs was observed against each antigen: both humoral (for B, C and A using Luciferase Immunoprecipitation System, LIPS) and cellular (IFNγ Enzyme Linked Immuno Spot Assay (ELISPOT) specific for B and C) responses were strongly induced by the vaccination.

Evidence of efficacy in preclinical models: Humoral response against one of the antigens was significantly enhanced by the addition of TPA leader sequence. The CFU in kidney was significantly and consistently after vaccination with TPA-BCA vectors in viral vectors. In the intraperitoneal sepsis model, a significant improvement of the survival rate was observed after vaccination. This is mainly due to an innate effect of the viral vectors (i.e. effect was observed also with the empty viral vector). Decreased bacteraemia, lower CFUs in spleen and better clearance of *S. aureus* in surviving animals was specifically observed for the TPA.BCA viral vectored vaccine. In the pneumonia model, using a non-lethal challenge dose of *S. aureus* ($1.85x10^6$ CFU per mouse, BALB/c SOPF strain), the group receiving the vaccine had a lower histopathology score in the lungs.

In the first study using the renal abscess model, a relationship between the T cell response and CFUs in kidney was demonstrated: a higher the cellular response correlated with a lower CFU in kidney. The impact of viral vector vaccination was studied in normal mice and in CD4 and CD8 depleted mice. T cell subset depletion experiments were carried out. Following the depletion of CD4 T cells, the bacterial load was still significantly lower for the vaccinated animals. This was not the case after depletion of CD8 T cells, where the same level of bacterial load was detected in both groups. Overall, these results suggest an involvement of the T cells in the *S. aureus* pathogenicity/protective effect of the vaccination. The cellular depletion experiments indicate that the protective efficacy observed with the BCA+/-TPA viral vector constructs might be due to CD8 T-cell dependent immune response. Little is known of CD8 responses to *S. aureus*, despite their critical role in defence against other intracellular bacteria (e.g. *Listeria*). This result is being further investigated using planned adoptive transfer experiments as, if confirmed, this will provide the first evidence of such protective efficacy in a *S. aureus* vaccine.

BC vs. BCA and relevance of protein order: TPA.BC, TPA.CB

In view of the protection observed in three models, the effect of the various components of the BCA fusion construct TPA.BCA was determined. We compared TPA.BCA with TPA.BC in an Ad/MVA prime boost vector regime. Furthermore to study the effect of order in the fusion protein on immunogenicity the construct TPA.BC was compared with TPA.CB.

Our results indicate that removing component A (BC vs BCA) seems to alter the cellular and humoral response to some antigens while increasing it for others. Protection similar to BCA was observed with the BC construct. The TPA.CB construct had different immunodominance of antigens and superior protection in the single experiment in which it was examined.

Protein vaccine candidate

The lead protein vaccine candidate BCAP was tested in the same 3 models as the viral vectors. In these studies, animals were primed with 25 μg adjuvanted BCAP protein, boosted once or twice with adjuvanted BCAP at 3 weeks interval and then challenged 2 or 3 weeks after the last boost.

- *In the renal abscess model*, a wide range of adjuvants were tested: CFA/IFA, alum, GLA-SE, CAF01, IC31. High immunogenicity of the adjuvanted BCAP was observed in terms of humoral response (against B, C and A) and a significant but variable cellular response (specific for B and C) was observed. The ELISPOT response was lower than the response obtained with BCA MVA.
- *In the intravenous challenge model*, no effect of vaccination on the CFU in kidney was observed, regardless of the adjuvant used.
- *In the intraperitoneal sepsis model*, no positive effect of the vaccination with BCAP adjuvant with CFA/IFA was observed on mortality, bacteraemia and CFU in spleen. Compared to the negative PBS control, a large protective innate effect of the adjuvant alone was evidenced. There may have been a tendency to better bacterial clearance in surviving BCAP

vaccinated animals was observed (decrease of bacteremia and CFU in spleen, but not significant).

- *In the pneumonia model, intramuscular delivery:* BCAP emulsified in CFA/ IFA or PBS emulsified in CFA/ IFA was compared when delivered twice by intramuscular vaccination. Animals utilized for this study were SOPF female BALB/c mice from Taconic, which were 7 weeks of age at the first immunisation. The infectious dose was 1x10e6 CFU of *S. aureus* Newman strain. No significant differences in clinical score, CFU in lung tissue or histopathological scores were observed.
- *In the pneumonia model, intranasal delivery:* We investigated the efficacy of per nasal immunized single antigen and BCAP in alleviating morbidity and mortality in a *S aureus* pneumoniae challenge model and compare it with the efficacy of IN administered PBS vehicle control and intramuscularly administered BCAP emulsified in IFA (1.14x10e9 CFU, Newman strain, SOPF BALB/c, Charles River). On day 1 post infection 30% survival were detected in groups of per nasal immunized BCAP. Only 10% survival was measured in groups administered with PBS vehicle control and intramuscularly administered BCAP emulsified in IFA. No significant differences in clinical score or protection were observed.

In summary, these data do not support substantial efficacy of BCAP protein vaccination in any regime studied. Intranasal delivery is a route which could be investigate more in future studies.

Issues noted with BCAP in pneumonia challenge models: relevance to vaccine safety

In one study, a statistically significant enhancement of lethality of BCAP in combination with alum vaccinated animals, or animals in which BCAP was co-injected with an MVA preparation, used as an adjuvant was detectable (90%, 100% lethality on day 1 post infection respectively) compared to PBS control treated animals (40% lethality on day 1 post infection) in a *S aureus* pneumonia model (BALB/C SOPF animals, Taconic, *S aureus* infectious dose 1.8* 10e8 CFU). This data might indicate a detrimental effect of BCAP in combination with alum or MVA vaccination compared to control PBS treated animals. We investigated this observation carefully.

Firstly, it was observed in multiple experiments that the relationship between dose administered and lethality was very steep. The implication is that small variations in dose administered might have large impacts on outcome, an undesirable feature in such models.

Secondly, in a repeat study, vaccination of PBS control with BCAP in combination with alum or viral vector MVA were compared in the *S aureus* pneumonia model in SOPF BALB/c and CD1 animals (animals were sourced from Charles River, *S aureus* infectious dose was 1*10e9 CFU). On day 1 post infection in BALB/c mice, none of the animals receiving the BCAP in combination with MVA survived, compared to 80% lethality found in BCAP in combination with alum or PBS treated animals. Additionally, in CD1 animals, on day 1 post infection a higher lethality was detected for BCAP in combination with alum or viral vector MVA (100% and 90% lethality at day 1 post infection, respectively) compared to PBS treated animals (80% lethality at day 1 post infection). None of these results reached statistical significance.

The purpose of this experiment was to investigate the safety of BCA combined with viral vector (Ad-BCA + MVA-BCA) vaccinated animals compared with Ad-GFP + MVA-GFP or PBS treated control animals. The infectious dose was 1.85x10e6 CFU *S aureus* Newman and the animals utilized for the study were SOPF BALB/c mice purchased form Charles River.

Measurement of clinical score provide a comparable minor increase of clinical score during study day 0.5-1.5 of viral vector (Ad-BCA + MVA-BCA) vaccinated animals and Ad-GFP + MVA-GFP or PBS treated control animals. This was not statistically significant. Viral vector (Ad-BCA + MVA-BCA) vaccinated animals had a slightly higher temperature reduction and a slightly increased

weight of total and right lung lobe measured on day 6post infection compared to Ad-GFP + MVA-GFP or PBS treated control animals. None of these observations reached statistical significance.

In summary, the detrimental effect of BCAP in combination with alum or viral vectors MVA vaccinated animals compared to PBS treated animals, which was seen in experiment 1, could not be repeated in a subsequent repeat studies with either ptoein or viral vector (Ad-BCA + MVA-BCA) vaccinated animals compared to relevant controls.

Other safety studies performed

To further investigate possible safety effects of the vaccines studies, we investigated:

- Loss of weight post intravenous challenge compared with controls
- Effect of IMX313P

In order to address issue 1) data collected until end 2015 was analysed as a function of experiment, strain, and vaccine regime. This analysis showed variation in weight loss post challenge variation in the viral vector control group, related to the experiment and the mouse strain. Variations were however not related to viral vector regime or antigenic insert used nor secular time. In summary there is currently no evidence that vectored BCA leads to weight loss nor that it is generating a severity signal.

We investigated the safety of IMX313P. IMX313P is a 55 amino acids sequence, distantly related to the oligomerization domain of the chicken C4bp but modified to contain an arginine-rich «protamine-like» sequence (P part). In this context, the following points may be underlined:

- No safety signal associated to IMX313P fused to different antigens ± different adjuvants vaccination in mice: e.g. no weight loss, no local inflammation
- An immune response toward the IMX313P moiety in mice, but no cross-reactivity with protamine
- No anaphylactic reaction after an intravenous protamine challenge (outsourced to Biotrial), after guinea-pig vaccination with an antigen fused to IMX313P
- An immune response towards the IMX313P moiety, but no cross-reactivity with hC4bp (in mice and rabbits), or in human using MVA-85A-IMX313 in the TB028 clinical trial
- In another project (Influenza), no safety signal after challenge in mice vaccinated with an antigen fused to IMX313P.

In conclusion, we did not note severity signals associated with IMX313P.

Experimental design – lessons learned on mouse strains, housing, microbiota, adjuvant, challenge dose

In the pneumonia model, *S. aureus* challenge outcome varies with mouse strain and supplier. BALB/c SOPF mice from Janvier and Taconic appear to be less susceptible to *S. aureus* infection than mice from Charles River. This might be due to the SOPF status of the animals as those from Charles River are colonised with more bacterial species other than *S. aureus.*

At Oxford, *S. aureus* carriage monitoring by stool sampling has shown cage-specific differences. This could apply to the total microbiome in the experimental animals and could be a confounder in evaluating the vaccine efficacy. To avoid a bias in the results, we modified our animal co-housing strategy: previously, we kept animals receiving the same treatment together but now we house at least one animal receiving each different vaccination (i.e. controls and *S. aureus* antigens) within each cage.

4. **WP1 Task 1.5** *Select the regimen for manufacturing and clinical trial (UOXF, IMAXIO; M12)*

Based on the results obtained from efficacy studies in different animal models and by taking into account the advice of the ISAC it has been decided not to continue with the manufacturing and clinical assessment of the vaccine candidates as part of the BELLEROPHON project.

5. **WP2 Task 2.1 GMP manufacturing** *Manufacturing and release of the Hla* recombinant protein (IMAXIO, PRECLIN, EVI; M13-M25)*

The objective of the WP2 of BELLEROPHON project is the manufacturing and analytical process for the GMP production and release of the vaccine candidate (BCAP recombinant protein and /or a recombinant viral vector).

The process development of BCAP recombinant protein using a two-step purification GMP compatible protocol was successfully completed at a small scale (1L) at IMAXIO's research laboratory and the protein was well characterized by SDS page, western-blot, mass determination by size exclusion chromatography, differential scanning fluorimetry and endotoxin and residual host cell DNA test. It could be transferred to a Contract Manufacturing Organisation (CMO) for scale up and manufacture. The product is characterised with > 90% purity and a concentration of 2 mg/ml. BCAP is heptameric in aqueous buffer. Preliminary study showed good short term stability. The protein is stable without any protein loss or degradation after three months at +5°C or -20°C.

Several potential CMOs (Q Biologicals, Eurogentec, Wacker, Novasep) were identified and contacted by IMAXIO with request for proposal. A call as part of the tender selecting procedure was also launched on the BELLEROPHON website and the IMAXIO and European Vaccine Initiative (EVI) websites. The requested steps were the following:

- To manufacture a pre-Master Cell Bank (Research Cell Bank (RCB)) of 50 vials with full raw materials traceability
- To manufacture 100 vials (minimum) of a GMP Master Cell Bank with full quality control and release. To carry out the cell bank storage
- To develop an upstream process in fermenters and adapt an already developed purification process at small scale, to be transferred from IMAXIO to the CMO
- To scale up the process at the GMP manufacturing scale
- To transfer / adapt analytical methods provided by IMAXIO
- GMP manufacturing of the bulk drug substance (DS) (1 to 3g or at least 10 litres of fermenter), with full quality control and release
- GMP manufacturing of the drug product (DP), fill & finish, with quality control (QC), quality assurance (QA) release
- DS and DP International Conference on Harmonisation (ICH) stability studies

Three full proposals from cGMP CMOs (Eurogentec, Q Biologicals and Wacker) were studied, and costs compared.

6. **WP2 Task 2.2** *Manufacturing of the viral vector components of the vaccine candidate (UOXF, PRECLIN, EVI; M13-M23)*

Viral vectors with ChAdOx1 or MVA backbone were constructed, containing the BCA antigens with or without the TPA (tissue plasminogen activator) signal peptide sequence, and with or without the IMX313P tag.

Manufacturing of viral vector components is proposed in the grant outline as an alternative to manufacturing of a protein. Construct TPA-BCA (no IMX313 tag) was selected as it was the most immunogenic in different pre-clinical models. Cell banks were identified for the production of the viral vectors: ChAdOx1 was grown in HEK293 cells and MVA in Chicken embryo fibroblasts (CEF) cells. GMP validation of Ox-HEK293 cell bank for production was undertaken.

Routes to GMP production for both of these viral vectors were investigated. If a decision to manufacture viruses had been made, we would have proceeded with the GMP production of a ChAdOX1 construct at the viral vector Biomanufacturing Facility at Oxford. The MVA construct GMP production would have been carried out at a contract research organisation with an established track record in MVA manufacture (IDT, Germany).

Routes to viral vector bio-manufacturing have been identified. However, given the insufficient proof of concept of BCAP protein in challenge tests, the ISAC recommended to the consortium not to continue with the development of the BCA candidate protein vaccine. Further enhancements have been made to the viral vector platform. As a result, the WP2 of the BELLEROPHON project has been halted and we will not proceed with the cGMP manufacturing of TPA-BCA viral vectored constructs for clinical trial.

Deviation from original plan

Our protein vaccine candidate BCAP exhibited potential severity effect in a mouse model, resulting in increased early death in the vaccinated animals. However, the BELLEROPHON consortium, supported by our Scientific Advisory Committee (SAC), decided to halt the preparations for the clinical testing of this vaccine candidate until more data underlying these adverse observations can be collected. Thereby, the committee responsible for the final selection of the CMO did not meet for the final choice of the subcontractor, and we did not proceed with the cGMP manufacturing of a product as part of the BELLEROPHON project. Notably, detailed investigations into the potential side effect of vaccination did not suggest a causal, consistent relationship, and such effects were not seen in two other disease models.

At the gateway time for clinical trials during the project, a clear benefit in pre-clinical models was not evident. Therefore, the consortium, supported by the SAC agreed not to seek approval for a clinical trial on the basis of this vaccine candidate.

Given the insufficient proof of concepts of BCAP in challenge tests, the independent scientific advisory committee recommended not to continue the development of the BCAP protein candidate vaccine. As a result, the WP2 of the BELLEROPHON project was stopped.

We continued with the development of a new potential vaccine candidate (WP1) comprising three *S. aureus* antigens in two immunisation platforms. Our results show a protective effect of the virus vectored BCA vaccine in two pre-clinical mouse models and we currently have ongoing experiments to confirm our initial findings and are applying for further funding in order to continue with the development of a new *S. aureus* vaccine candidate.

7. **WP3 Preparation of clinical trial / WP4 Clinical trial**

As above, we did not proceed to clinical trial.

Due to a decision not to continue with work packages relating to the preparation and implementation of a clinical trial (WP3 and WP4) further resources were reallocated from these WPs towards uncovering the basis of the observed safety severity signal (WP1) and new antigen discovery (WP5).

8. **WP5 Task 5.1** *Develop rapid methods for viral vector production (UOXF; M1-M4)*

We have optimised the production protocols for the manufacture of our viral vectors which will be employed as the antigen delivery vehicles for our candidate vaccines against *S. aureus*. The modifications introduced into each of the methods are detailed above.

The new optimised method for producing MVA results in a higher immunogenicity of the viral vector due to the change in the antigen insertion site, leaving the TK gene intact. The production process for adenoviruses has been optimised into a method that results in a faster and more efficient virus production, as demonstrated by the high number of constructs already produced at our Institute using the optimised protocol.

Employing both of these methods would improve the manufacture of our candidate viral vectored vaccines for the BELLEROPHON project.

9. **WP5 Task 5.2** *Screen additional antigens for protection in S. aureus abscess models (UOXF; M6-M22)*

Overview of antigens tested in WP5 to date

Within the antigen discovery work-stream of BELLEROPHON (WP5) several *S. aureus* antigens have been evaluated as potential vaccine candidates. Several criteria were considered for selecting these antigens:

- Known virulence factor or homologous to known factors
- Secreted or surface associated
- Conserved across *S. aureus* strains
- Non-toxic
- Not super antigenic

Antigens were identified according to the above criteria using a bioinformatics strategy. The potential of selected antigens was assessed by vaccinating mice in a ChAdOx1/MVA prime boost regime and testing their immunogenicity as well as bacterial recovery and abscess formation in an intravenous challenge model. With the most promising candidate three experiments in mice were performed and a significant effect on *S. aureus* growth, colonisation and abscess formation was observed. Combining all data from the three experiments shows a significant reduction in renal bacterial recovery. A patent related to this has been filed.

Intracellular *S. aureus*:

Phenotype of blood stream isolates differs systematically from skin/nasal isolates. In 2012 Young et al published a study using high-throughput genome sequencing on nasal isolates of *S. aureus* from long term carriers in a prolonged carriage study. Some participants of the study developed blood stream infection and genetic comparison of nasal and blood isolates identified a mutation in the AraC-family rsp gene of *S. aureus* to separate the blood from the nasal population. Rsp mutants show reduced toxicity and can survive longer in the intracellular space due to delayed cytotoxicity. Intracellularly, these mutants may be relatively protected from the humoral immune system and thereby exhibit an enhanced ability to survive.

At UOXF three bacterial pairs of strains (one of each pair being an rsp mutant) were tested for survival in healthy human blood *ex vivo* and furthermore lethality was tested in a mouse murine infection model. The results of those experiments can be summarised as follows:

- Rsp affects gene expression across the bacterial genome, repressing mainly surface and secreted protein encoding genes
- Mutating *rsp* reduces cytotoxicity and haemolysis of *S. aureus* across different strains
- Rsp is irrelevant for the survival of *Staphylococci* in human blood *ex vivo*
- In mice infected with *rsp* mutant, *S. aureus* lethality and weight loss is reduced
- Capability to infect, survive within the host and form abscesses are unaltered in mutants

These results indicate that the intracellular reservoir of *S. aure*us is crucial for maintenance of infection. To answer the questions of 'How can infected cells be identified? Which epitopes are present on infected cells?' currently an MHC - Immunopeptidomic approach (mass spectrometry to identify epitopes presented on MHC class I/II) is being followed. This method will allow identification of new antigens and dominant antigens presented on the surface of infected cells. To date, it has been shown that MHC II expression increases in infected cells and a number of new antigens were identified.

Other antigens being tested:

Following our discovery of a 'blood stage phenotype' adopted by *S. aureus* in severe human infection (BELLEROPHON output: Das S 2016 PNAS), several antigens upregulated during the 'blood stage phenotype' are under study and viral vectors against these have been produced. Targeting this pathway is a novel and highly promising strategy, particularly since one of these differentially regulated proteins (EsxA) displays some protective efficacy.

S. aureus structures with adjuvant activity have been identified as potentially involved in stimulation of innate immunity. These "scaffolds" possess the ability to promote oligomerisation and thus additional enhancement of the immune response. Several of these structures have been tested as fusions to *S. aureus* antigens. Most promising results in terms of oligomerisation and enhanced immune response were obtained with the SAR1376 protein, and mutants thereof. New constructs with this tag are currently under development. A patent related to this has been filed.

***S. aureus* carriage in mice:**

Disease free carriage of *S. aureus* can act as a reservoir from which *S. aureus* disease develops. Vaccines reducing carriage (e.g. Prevenar) have been shown to reduce disease. Consequently, control of *S. aureus* carriage is a relevant objective. Animal models of carriage are limited. In order to study carriage we established a small animal model of experimentally induced *S. aureus* carriage, in which *S. aureus* can be repeatedly isolated over months in the absence of antibiotic selection, something which has not previously been described in literature. In this model mice can be colonised by environmental contamination with *S. aureus*.

The colonisation experiments showed in summary that maintenance of bowel carriage is dependent on 'environment' as assessed by 3 different methods:

- Observations of small groups (3, 4 or 6) co-housed animals
- Exchange of animals within those communities
- Introduction of organism by environmental contamination

Furthermore the immune response to *S. aureus* in carriage was assessed. *S. aureus* carriage induces anti–*S. aureus* IgG1 antibodies in serum and nasal tissue and an altered cytokine profile in nasal tissue. However, despite development of immune responses to experimental *S. aureus* colonisation, colonisation did not result in protection from intravenous challenge (renal abscess model). In terms of vaccinology it would be very useful to explore the nature of host resistance to carriage.

Recombinant proteins

Following the promising preliminary results obtained with our newly discovered vaccine candidate in viral vectors at Oxford, we tried at IMAXIO to also obtain these antigens as recombinant proteins. For this purpose, the genes encoding the antigens were synthesised (at DNA2.0), then cloned at IMAXIO's facilities in the *E.coli* bacteria for protein production. 4 purified proteins were obtained and characterized (SDS-PAGE, concentration by UV, mass spectrometry, LAL).

10. **WP5 Task 5.3** *Determine whether novel antigens identified also protect from pulmonary disease and through recombinant protein regimen (Preclin, IMAXIO; M22-M27)*

As the most promising results were obtained just before the end of project, there was not sufficient time to set up efficacy studies in the pulmonary model.

11. **WP5 Task 5.4** *Determine whether novel antigens identified increase protection over current combinations (OXFORD; M22-M27)*

We have investigated a large number of candidate antigens for immunogenicity and protective efficacy against *S. aureus* infection. Our results indicate that the intracellular reservoir of *S. aureus* is crucial for maintenance of infection and a vaccine needs to address this (i.e. cytotoxic T cell response).

A fusion our newly discovered vaccine candidate protein used in an AdHu5-MVA prime boost vaccine regimen is efficacious against *S. aureus* infection. It reduced the number of abscesses formed during the infection. These antigens could form a part of protective combination vaccine together with other antigens tested in BELLEROPHON.

In Rsp we have identified a pleiotropic transcriptional regulator/non-coding RNA virulence regulatory system that controls hemolysis and cytotoxicity, and a low-cytotoxic phenotype which plays a central role in invasive *S. aureus* infection. Genes regulated by *rsp* potentially represent good vaccine candidates (currently under investigation).

Impact, exploitation and dissemination activities

This project has established a highly efficacious route for delivery of *S. aureus* antigens involving the use of viral vectors. It has shown this approach to be superior to the use of similar proteins with a range of adjuvants. It has also discovered a range of promising additional antigens which can form part of multi-antigen vaccines. Overall, this project has brought the trial of a novel vaccination modality for *S. aureus,* and novel multi-antigen vaccines, to a state where they are now ready for Phase I trial in man.

As described in 'Section A' of this document the results of BELLEROPHON have been disseminated during conferences and in form of scientific publications. Several further results from the BELLEROPHON project are already submitted or are still in preparation. Two patent application are undergoing currently submission. While the BELLEROPHON project did not progress a vaccine candidate into clinical development, the scientific achievements paved the way to better understand *S. aureus* immunity and the prerequisites to be taken into account while developing *S. aureus* vaccine candidate. Based on the discoveries from the BELLEROPHON project a clinical development plan was developed. Should further funding opportunities arise, the best vaccine candidates designed and evaluated within BELLEROPHON will be further optimised by the addition of the novel antigens discovered within BELLEROPHON and further developed according to the clinical development plan.

The progress and success of the BELLEROPHON project was complimented by its independent scientific advisory committee. The committee was impressed by the achievements of the project in *S. aureus* vaccine development and with BELLEROPHON becoming an internationally competitive consortium within a remarkably short time period. It further remarked that the BELLEROPHON approach is needed in the scientific community especially with regard to the research done targeting *S. aureus* immunology.

There are several findings of the consortium that will be of special impact to the scientific community in the research for a vaccine against *S. aureus*:

(1) When non-protective antigens (protein + adjuvant) were administered via viral vector constructs, such antigens showed limited protection in relevant pre-clinical models. This is due to a combination of adjuvant effects of the viral vectors and the induction of antigen-specific immunity and it will be of interest to continue to investigating these mechanisms.

(2) During the BELLEROPHON project several *S. aureus* antigens have been selected using bioinformatics strategy and have been evaluated as potential vaccine candidates. These antigens were assessed in viral vectors regime for immunogenic and protective properties. All antigens tested are highly immunogenic but only one viral vector construct resulted in a reduction in bacterial recovery from kidneys after intravenous challenge. This construct was further investigated and the reduction in bacterial recovery (compared to controls) from kidneys was found to be consistent and reproducible, with higher number of IFN-gamma secreting cells after stimulation of PBMCs with vaccine candidate peptides from vaccinated animals. In addition, a significant decrease in the number of abscesses formed in the kidneys of vaccinated animals was observed. Notably, there was a significant decrease in the proportion of mice positive for *S. aureus* carriage in stools in the vaccinated group. This is an unprecedented achievement, suggesting that a strategy involving 'colonisation control' may be possible using these proteins and viral vector regimes. The developed vaccine candidates in both recombinant proteins and viral vectors induced strong humoral and cellular immune responses. This effect was enhanced for the viral vector constructs by the addition of TPA. Following *S. aureus* intravenous challenge infection the bacterial load in the kidney, expressed as CFU per gram tissue, was significantly lower after immunisation with TPA.BCA viral vector. The ability of the TPA.BCA viral vector regime to induce strong humoral and cellular immune response was confirmed in sepsis peritoneal model in Balb/c mice.

(3) Boxing effects and the mechanism of antigen induced adverse advents were studied by the consortium and will be of help for further studies for a safe vaccine.
(4) In an attempt to improve our vaccine candidates we focused on the detoxified Hla, one of the most promising vaccine candidates: some anti-Hla antibodies have the ability to neutralise Hla toxicity, and such antibody has been described as protective in cutaneous and pulmonary *S. aureus* disease models (e.g. Fiashi L. et al. 2016). It has been reported that when the stem domain of the Hla protein is removed, the Hla monomer self-assembles into heptamers and loses its ability to bind specific receptors and to exhibit haemolytic activity. In the BELLEROPHON project several Hla truncated and mutated proteins have been generated and more are currently under development both as recombinant proteins and in viral vectors.

The BELLEROPHON consortium established a clear communication plan that was followed through the life cycle of the project. Materials for general public dissemination were generated and distributed on different occasions. Scientific achievements were communicated to scientific and general public communities during international conferences and events. A logo for the project was designed and used on all communication material. The BELLEROPHON website was successfully implemented: http://www.bellerophon-project.eu. Project background, objectives and achievements on the website are readily accessible to both specialists and the general public. The website has been continuously updated with information on relevant project events, scientific progress and an up-to-date reference list of publications related to the project. EVI issued a leaflet giving an overview of the project background, objectives, milestones and up to date achievements to be distributed to the general public during international scientific or public events. In addition a postcard including a short summary of the project and contact details of the consortium was issued at the beginning of the project.

Project progress and major achievements were published annually in the EVI annual report for donors and stakeholders thus giving visibility of BELLEROPHON to other potential donors and funding agencies.

BELLEROPHON

Please find further information about the BELLEROPHON project at www.bellerophon-project.eu

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Use and dissemination of foreground

Section A

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS; STARTING WITH THE MOST IMPORTANT ONES										
NO.	Title	Main author	Title of periodical series	Number, date or frequency	Publisher	Place of Publication	Year of Publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication?
1.	Natural mutations in a Staphylococcus aureus virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation.	Das S, Lindemann C	Proc Natl Acad Sci U S A	31 May 2016	US National Academy of Sciences	Washington, USA	2016	113-122	http://www.pnas.org/content/113/22/E3101.long	Yes
2.	MRI Based Localisation and Quantification of Abscesses following Experimental S. aureus Intravenous Challenge: Application to Vaccine Evaluation.	Allen ER	PLoS One	26 May 2016	Public Library of Science	San Francisco, USA	2016	11-15	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0154705	Yes

[1] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[2] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

3	Population variation in anti-S. aureus IgG isotypes influences surface protein A mediated immune subversion	Julia Whitehouse; Yuko Yamaguchi	Vaccine	01 April 2016	Elsevier BV	Netherlands	2016	1792-9	http://www.sciencedirect.com/science/article/pii/S0264410X16001973	Yes
4	Risk factors for dermatitis in submariners during a submerged patrol: an observational cohort study	Amy Flaxman	BMJ	01 June 2016	BMJ Publishing Group	United Kingdom	2016	1-7	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4893864/	Yes

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
1.	Conference poster at ISSSI 2014	Claudia Lindemann	In vivo extraction of *Staphylococcus aureus* RNA from multiple tissue background	23-29 Aug 2014	Chicago, USA	Scientists and clinicians	600	Global
2.	Conference poster at ISSSI 2014	Elisabeth Allen	MRI based quantification of abscess volumes in mice following *S. aureus* i.v. challenge	23-29 Aug 2014	Chicago, USA	Scientists and clinicians	600	Global

3.	Conference poster at ISSSI 2014	Yuko Yamaguchi	Evidence for control of S. aureus nasal carriage by innate lymphocyte populations in humans: a cross sectional study	23-29 Aug 2014	Chicago, USA	Scientists and clinicians	600	Global
4	British Society for Immunology	UOXF	Evidence for control of S. aureus nasal carriage by innate-like T lymphocyte populations in humans: a cross sectional study Move back Print [log in to your personal itinerary]	02 December 2014	Brighton, UK	Scientists and clinicians	200	International
5.	Conference poster at ISSSI 2014	Pauline van Diemen	Partial protection against *Staphylococcus aureus* i.v. challenge with a vector vaccine	23-29 Aug 2014	Chicago, USA	Scientists and clinicians	600	Global
6.	Conference poster at Gordon Conference 2015	Amy Flaxman	Microenvironment-specific changes in microflora and immune parameters identified during a study of murine *S. aureus* carriage	12-17 July 2015	Lucca, Italy	Scientists and clinicians	600	Global
7.	Conference poster at Gordon Conference 2015	Claudia Lindemann	A naturally occuring inactivation of a major transcription factor elicits bacterial immune subversion	12-17 July 2015	Lucca, Italy	Scientists and clinicians	600	Global
8.	Conference poster at ECI 2015	Claudia Lindemann	A naturally occuring inactivation of a major transcription factor controls early lethality but not bacterial reproduction	6-9 Sep 2015	Vienna, Austria	Scientists and clinicians	200	Global

9.	Conference poster Florey Institute Symposium 2015	Amy Flaxman	Microenvironment-specific changes in microflora and immune parameters identified during a study of murine *S. aureus* carriage	11 Sep 2015	Sheffield, UK	Scientists and clinicians	100	UK
10.	Conference poster at ISSSI 2016	Amy Flaxman	A novel experimental murine *Staphylococcus aureus* colonisation model	31 Aug 2016	Seoul, South Korea	Scientists and clinicians	600	Global
11.	Conference presentation at ISSSI 2016	Claudia Lindemann	Natural mutations in a S. aureus virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation	02 Sept 016	Seoul, South Korea	Scientists and clinicians	600	Global
12.	Web site call	IMAXIO / EVI	Call for process development and GMP manufacture of a vaccine candidate	June 2015	IMAXIO/ web site	GMP CMOs		Global
13	Oral presentation to a wider public	EVI/IMAXIO	Combining cellular and humoral immune responses as a vaccine strategy against Staphylococcus aureus pathogen	December 2013	European Vaccine Initiative Rendez-Vous, Heidelberg, Germany	Scientific community (higher education, Research) - Industry - Policy makers	100	International
14	Oral presentation to a wider public	EVI/IMAXIO	Combining cellular and humoral immune responses as a vaccine strategy against Staphylococcus aureus pathogen	December 2014	European Vaccine Initiative Rendez-Vous, Institut Pasteur, Paris, France	Scientific community (higher education, Research) - Industry - Policy makers	100	International
15	Oral presentation to a wider public	EVI/UOXF	Staphylococcus aureus – antigen discovery	December 2015	European Vaccine Initiative Rendez-Vous Institut Pasteur, Paris, France	Scientific community (higher education, Research) - Industry - Policy makers	100	International

16	Press release	IMAXIO	Bellerophon Project' awarded EUR 5.5 million by EU to develop Staphylococcus aureus vaccine	01 July 2013	IMAXIO/website	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias		Global

Section B

Part B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP rights	Confidential Yes/No	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant(s) as on the application
None	None	None	None	None	None

Part B2

Type of Exploitable Foreground	**Description of Exploitable Foreground**	**Confidential Yes/No**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licenses)**	**Owner & Other Beneficiary(s) involved**
Patentable IP	Immunogenic tag derived from staphylococcal protein that enhanced	Yes	01/06/2018	Vaccines for infectious disease	Vaccine development	2-5 years	Patent application in progress	University of Oxford

	vaccine efficacy							
Patentable IP	New S. aureus candidate vaccine antigens	Yes	01/06/2018	Vaccine against S. aureus	Vaccine development	2-5 years	Patent application in progress	University of Oxford

Immunogenic tag

This is an antigen multimerising tag that we have demonstrated can improve the immunogenicity and/or efficacy of different types of vaccines (DNA, protein, viral vector). Such finding has a potentially broad application in vaccine development as it is transferable to a number of vaccination platforms. We have filed a United Kingdom provisional patent application. Further research includes the development of this tag further towards clinical use. This tag could find application in a commercial vaccine if shown to significantly add to the vaccine efficacy.

New candidate vaccine antigens

These are *S. aureus* antigens which we have found represent promising candidates for *S. aureus* vaccine development. This finding could be exploited in the design of novel vaccines against S. aureus, combining these novel antigens with other immunogenic antigens. We have filed a United Kingdom provisional patent application. Further research includes the development of novel vaccines comprising these two (and other) antigens towards clinical use and first-in-man testing.

Report on social implications

A General Information	
Grant agreement number:	**601783**
Title of Project:	**Bellerophon**
Name and Title of Coordinator:	**Dr. David Wyllie**

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	**YES**
2. Please indicate if your project has involved any of the following issues (tick box):	**No**
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue/Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction?)	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	☑
• Were those animals transgenic small laboratory animals?	☑
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	

RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to the local community (capacity building, access to healthcare, education ect)?	
DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of position	Number of Women	Number of Men
Scientific Coordinator	**0**	**1**
Work package leaders	**2**	**2**
Experienced researchers (i.e. PhD holders)	**11**	**5**
PhD Students	**2**	
Other9	**9**	**4**

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**7**
Of which, indicate the number of men:	**2**

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

☑ Yes
☐ No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective				**Very effective**
☑ Design and implement an equal opportunity policy	☐	☐	☐	☐	☑
☐ Set targets to achieve a gender balance in the workforce	☐	☐	☐	☐	☐
☐ Organise conferences and workshops on gender	☐	☐	☐	☐	☐
☑ Actions to improve work-life balance	☐	☐	☐	☐	☑

☐ Other:

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed? N/A

☐ Yes- please specify

☑ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

☐ Yes- please specify

☑ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

☑ Yes- please specify – BELLEROPHON website; summary sheet, Postcard

☐ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

☐ Main discipline[3]: 3.1

☐ Associated discipline: 1.5 ☐

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	☑ ☐	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

☐ No

☐ Yes- in determining what research should be performed

☐ Yes - in implementing the research

☑ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator;	☐ ☑	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

☑ No

☐ Yes- in framing the research agenda

☐ Yes - in implementing the research agenda

Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

☐ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)

☐ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)

☑ No

[3] Insert number from list below (Frascati Manual).

13b If Yes, in which fields?

Agriculture Audiovisual and Media Budget Competition Consumers Culture Customs Development Economic and Monetary Affairs Education, Training, Youth Employment and Social Affairs	Energy Enlargement Enterprise Environment External Relations External Trade Fisheries and Maritime Affairs Food Safety Foreign and Security Policy Fraud Humanitarian aid	Human rights Information Society Institutional affairs Internal Market Justice, freedom and security Public Health Regional Policy Research and Innovation Space Taxation Transport

13c If Yes, at which level?

- ☐ Local / regional levels
- ☐ National level
- ☐ European level
- ☐ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?		**4**
To how many of these is open access[4] provided?		**4**
How many of these are published in open access journals?		**4**
How many of these are published in open repositories?		**0**
To how many of these is open access not provided?		
Please check all applicable reasons for not providing open access:		
☐ publisher's licensing agreement would not permit publishing in a repository ☐ no suitable repository available ☐ no suitable open access journal available ☐ no funds available to publish in an open access journal ☐ lack of time and resources ☐ lack of information on open access ☐ other[5]: ……		
15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**2**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	
	Registered design	**2**
	Other	

[4] Open Access is defined as free of charge for anyone via Internet

[5] For instance: classification for security projects

17. How many spin-off companies were created / are planned as a direct result of the project?	**0**
Indicate the approximate number of additional jobs in these companies:	

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

☑ Increase in employment, or	☑	In small & medium-sized enterprises
☐ Safeguard employment, or	☐	In large companies
☐ Decrease in employment,	☐	None of the above / not relevant to the project
☐ Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:

Indicate figure: **9** ☐

The table below is an indicator of the positive effect of the FP7 program on employment / work at IMAXIO:

	Number of hours	FTE
In 2013	3904	2,4
1st BELLEROPHON period 09/2013-02/2015	6735,3	2,8
2nd BELLEROPHON period 03/2015-08/2016	10318,25	4,3

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

☐ Yes ☑ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

☑ Yes ☐ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

☑	Press Release	☑	Coverage in specialist press
☐	Media briefing	☐	Coverage in general (non-specialist) press
☐	TV coverage / report	☐	
☐	Radio coverage / report	☐	Coverage in national press
☑	Brochures /posters / flyers	☐	Coverage in nternational press
☐	DVD /Film /Multimedia	☐	
		☑	Website for the general public / internet
		☑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

☐	Language of the coordinator	☑	English
☐	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2. ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group