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1 Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.
4.1 Final publishable summary report

This section must be of suitable quality to enable direct publication by the Commission and should preferably not exceed 40 pages. This report should address a wide audience, including the general public.

The publishable summary has to include 5 distinct parts described below:

- An executive summary (not exceeding 1 page).
- A summary description of project context and objectives (not exceeding 4 pages).
- A description of the main S&T results/foregrounds (not exceeding 25 pages),
- 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 (not exceeding 10 pages).
- The address of the project public website, if applicable as well as relevant contact details.

Furthermore, project logo, diagrams or photographs illustrating and promoting the work of the project (including videos, etc…), as well as the list of all beneficiaries with the corresponding contact names can be submitted without any restriction.
The TRAUMAKINE project was aimed at obtaining EU Marketing Authorisation for the orphan indication of the biopharmaceutical interferon-beta (IFN-beta), to treat moderate and severe acute respiratory distress syndrome (ARDS). This goal was highly ambitious and had a significant impact as there are no approved pharmacological treatments for ARDS in Europe and the burden of ARDS is very heavy for the patients, their families and society. Despite the improved mechanical ventilation techniques and supportive therapies, ARDS has a high mortality (30-45%) and kills approximately 125,000 Europeans annually. The predominant feature of ARDS is vascular injury in lungs that increases capillary permeability leading to influx of protein-rich fluid and loss of lung function. Prevention of this vascular leakage has not been attempted previously in treatment of ARDS, even though it is known to be one of the very first pathological steps.

It is known that endothelial barrier function is physiologically maintained by adenosine, which is also capable of reducing extra-vascular lung water and down regulates inflammation, all of which could significantly help ARDS patients. During inflammation, multiple cell types release adenine nucleotides with CD73 acting as a rate-limiting enzyme controlling local adenosine availability. The key finding, leading to the initiation of the TRAUMAKINE project, was the identification of IFN-beta as an activator of the CD73 gene, leading to induced numbers of CD73 molecules on endothelial cell surfaces. Thus, IFN-beta would increase the local adenosine levels leading to enhanced endothelial barrier function and amelioration of the disease symptoms. This hypothesis was tested in a phase I/II study conducted by Faron Pharmaceuticals in 2008-11. The results were highly encouraging with 82% reduction in the odds of all cause mortality at day 28 (Bellingan et al., Lancet Res. Med. 2014: 2: 98).

Based on these results, the decision to proceed with the clinical development of IFN-beta was made and the TRAUMAKINE project team of four partners was established. In order to obtain the EU Marketing Authorisation for the treatment of ARDS using IFN-beta, the TRAUMAKINE project was divided into four main objectives: the 1st main objective was the successful execution of the pan-European phase III clinical study called the INTEREST Study. This study was designed to be a pivotal study capable of supporting the Marketing Authorisation Application. The 2nd main objective involved all bioanalytical assessment of the INTEREST Study samples. The 3rd main objective was the development of a new CD73 assay and a fit-for-purpose ARDS biomarker panel to follow disease progression and response to treatment. The 4th main objective was directed towards genetic analyses of the CD73 gene and its regulatory elements. In addition to these main objectives, daily management of the TRAUMKINE project and the dissemination of the project progress and results to the public were important factors.

The conduct of the INTEREST Study took slightly longer than originally planned, but all the preparatory work proved very well done as the study closure and analysis of the top line data was done in a very short time. Unexpectedly and extremely disappointingly, the INTEREST Study did not meet the primary composite end point for efficacy. As soon as this result became known, all partners initiated a complete root cause analyses to identify the underlying reason for this. As the biomarker data was analysed in more detail, it became apparent that there was a sub-group of IFN-beta treated patients who did show reduced mortality and a trend towards an increase in ventilator free days with correlation to biomarker response. This was highly important finding as it showed that the original treatment hypothesis was correct. Further data analyses have revealed concomitant corticosteroid use to interfere with Traumakine action and to increase mortality. This unexpected finding will have significant impact to ICU practices and any future Traumakine trial will exclude this concomitant corticosteroid use.
The TRAUMAKINE project was aimed at obtaining EU Marketing Authorisation for the orphan indication of the biopharmaceutical interferon-beta (IFN-beta), to treat moderate and severe acute respiratory distress syndrome (ARDS). ARDS is a sudden clinical syndrome of life-threatening acute respiratory failure with bilateral pulmonary infiltrates of non-cardiac origin with severe hypoxemia resulting in high mortality of these patients (30-45%). The primary aetiology of ARDS varies: approximately one third of the diagnosed cases have a background of sepsis; one third have pneumonia and the rest are mostly caused by traumas or aspiration. The predominant feature of this condition, in all cases, is vascular injury in lungs that increases capillary permeability leading, initially, to formation of interstitial protein-rich lung fluid followed by leukocyte migration from the blood circulation. The final stage of this deterioration is fibril accumulation, scarring and permanent loss of lung function. Despite the improved mechanical ventilation techniques and supportive therapies, ARDS kills approximately 125,000 Europeans annually. Many different approaches, and numerous clinical trials, have failed in treating ARDS and this condition still has no approved pharmacological treatment in Europe, even if the suspected underlying cause is being treated.

Prevention of vascular leakage has not previously been targeted in treatment of ARDS, even though it is known to be one of the very first pathological steps leading to infiltration of inflammatory cells and accumulation of fibrils. Adenosine is one of the physiological regulators of endothelial cell permeability, but its therapeutic use has been very limited due to its short half-life in circulation. Adenosine reduces extra-vascular lung water, increases endothelial cell barrier and down regulates inflammation, all of which significantly help ARDS patients. During episodes of inflammation, multiple cell types release adenine nucleotides with CD73 acting as a rate-limiting enzyme controlling local adenosine availability.

A key finding, leading to the initiation of the TRAUMAKINE project, was the identification of IFN-beta as an activator of the CD73 gene, leading to increased de novo synthesis of CD73 and its expression on the surface of endothelial cells. The increased presence of CD73 on the cell surface has been shown to result in increased adenosine levels and enhanced endothelial barrier function. Thus, it was proposed that IFN-beta could be used to significantly prolong the beneficial effects of adenosine in the circulation.

These findings led Faron Pharmaceuticals (Faron) to conduct a phase I/II study to test safety, tolerability and initial efficacy of IFN-beta in the treatment of patients with ARDS. Phase I tested the safety and pharmacokinetics of IFN-beta using four different doses and phase II continued with the discovered optimal tolerated dose (OTD). Results from this study were highly encouraging with key findings showing a strong reduction in the odds of all cause mortality at day 28 (by 82%), never seen previously in ARDS trials. Only three of the 37 patients treated with the IFN-beta died (8.1%) compared to adequate control population with best care and with 32.1% mortality. Also, many secondary end points were met which supported the efficacy of IFN-beta treatment. In addition, no drug related adverse events were observed with the OTD (Bellingan et al. The Lancet Res.Med., 2014, 2(2):98-107).

Based on these results, Faron decided to proceed with the clinical development of biopharmaceutical IFN-beta and a high-class scientific and clinical partner network was established. The core team for the next steps of the TRAUMAKINE project was quickly formed with four partners (Faron, University College London Hospitals (UCLH), University of Rome (U. Rome) and University of Turku (U.Turku)) and we were privileged to receive the European Union 7th Framework Programme grant for the
In order to obtain the EU Marketing Authorisation for the orphan indication of the biopharmaceutical IFN-beta, to treat moderate and severe ARDS, the TRAUMAKINE project was divided into four main objectives. The 1st main objective was the successful execution of the pan-European phase III clinical trial (FPCLI002) for the treatment of ARDS using IFN-beta. This FPCLI002 trial called the INTEREST Study was designed to be the pivotal study to support the filing of the Marketing Authorisation Application. The 2nd main objective was the analysis of the clinical and biomarker data collected from the INTEREST Study to support the finalisation of the clinical study report and preparation of publications reporting the INTEREST Study results. Thus, the objective was highly important as here the assays supporting the INTEREST Study sample assessments reached the required level and all processes for seamless sample management and data reporting were established. The 3rd main objective was the development of a new CD73 ELISA assay and the required reagents. In addition, development of an ARDS biomarker panel was an important deliverable. The 4th main objective involved genetic analysis of the CD73 gene, NT5E, in ARDS patients. This analysis was designed to investigate how the genetic makeup may influence the ARDS disease progression and response to treatment. In addition to these four main objectives, the project management and continuous dissemination activities were of high priority. All these main objectives were divided into deliverables and milestones to proactively manage the TRAUMAKINE project progression (see Figure 1 for TRAUMAKINE project structure).

The 1st main objective contained work starting from the set-up of the INTEREST Study to the clinical study report and Marketing Authorisation Application (MAA). The work began with the establishing of the INTEREST Study steering committee (SC). This SC consisted of key opinion leaders from each of the participating country (Belgium, The Czech Republic, Finland, France, Germany, Italy, Spain and UK) and had an important advisory role throughout the study. As ARDS is a rare and very complex syndrome it is extremely important to establish a high degree of standardisation in the clinical study design, the study protocol and in the actual clinical conduct of the study. European Medicines Agency (EMA) have a guideline for the clinical investigation of medicinal products in the treatment of ARDS and this set the rules for the main efficacy studies. Due to the heterogeneous patient population and their life-threatening condition the guidance document can only provide general aspects, which need to be considered in the planning of the clinical study. In order to ensure this and meet all the regulatory requirements, Faron have had three scientific advice procedures with the EMA. The INTEREST Study protocol was finalised based on this advice by the TRAUMAKINE project members with the assistance from the SC. Study sites were also selected from each country based on the recommendations by the SC. After the INTEREST Study protocol was finalised and the sites were selected, the site contracting process was initiated. The site contracting process varied between the participating countries and even at the site level. Also, the duration of the contracting process varied significantly. In addition, in some of the countries, the contracting process was made parallel with ethical and competent authorities approval processes, but in other countries the approvals were needed before the contracting process could begin. During this time, clinical trial study groups were established in each country and they also had their first meetings. These country specific study groups were led by the respective SC members and formed the national bodies of the INTEREST Study. As soon as the contracting process was finalised for the 70 sites in the eight participating countries, and all approvals were in place for each site - they began screening for patients. This was a key milestone marking the beginning of the INTEREST Study. The first patient was enrolled in December 28th, 2015.

The INTEREST Study was designed to recruit 300 patients in total. These patients were randomised equally to receive either the active drug or placebo in addition to the best standard of care. The patient enrolment phase lasted longer and required significantly more resources from all stakeholders than...
originally planned. The last patient was enrolled on December 8th, 2017, and therefore the study soon entered the exciting reporting phase with deliverables involving steps leading to the clinical study report and MAA. The next key step was to finalise and lock the database. For this purpose, all data needed to be entered to the database and verified as soon as possible in a quality-controlled manner. Sites entered all clinical data to the electronic case report forms which were source data verified before entered into the database. Laboratories also quality controlled their data before data entry. After the data has been inputted, the database was closed and a blinded review of the data including the tables and listings was conducted. Observed final discrepancies in the data were queried and corrected and the database integrity and programming were confirmed followed by the data base lock on April 23rd, 2018. Roughly 5% of the data was re-verified and was found to be correct. Total data points were around one million, indicating the challenging nature of the trial. The database lock allowed authorization of data unblinding and the outcome analysis of the INTEREST study began.

First, the data analysis concentrated on top line data on primary and secondary efficacy end points and of course safety aspects i.e. the key parameters of the INTEREST Study. Then, the data analysis progressed to biomarker and other efficacy scores. The INTEREST Study top line data was announced to the public as soon as it was available on May 8th, 2018. Presently, more detailed statistical data analysis is ongoing, and the clinical and laboratory data are being correlated. Once, all data analysis is ready also the clinical study report can be finalized. All study sites have now been closed and future plans for the clinical development of the biopharmaceutical IFN-beta are being made.

The 2nd main objective was designed for clinical sample analysis and data reporting. This objective supported the INTEREST study and was conducted simultaneously. Several different analytes were assessed from the INTEREST Study patient samples including CD73 (5’-nucleotidase), MxA (Myxovirus resistance protein A), binding and neutralizing antibodies against IFN-beta, 27 different inflammatory markers and genetic markers. Before the clinical study start, all sample collection related materials were selected, packed and provided for the sites. The sample preparation and storage instructions were prepared, and sampling schedules were designed for the study protocol and sample logistics were established. The assessments were designed, established, transferred to the central laboratory service providers and validated before the sample analysis began. Assessment of CD73 levels during and after the dosing period was highly important due to the treatment hypothesis as described earlier. The CD73 ELISA assay was developed by U.Turku as an outcome of the 3rd main objective. The MxA is the best-known biomarker for IFN-beta bioactivity and therefore also highly important to assess during and after the dosing period. Detection of binding and neutralizing antibodies against IFN-beta after the dosing regime was a regulatory requirement and provided important information about the immunogenity of IFN-beta in these patients. The 27 different inflammatory markers assay was established and conducted by U.Turku. The resulting data was important to understand the patient’s disease severity, progression and outcome and guided the development of the ARDS biomarker panel in the 3rd main objective. All this preparatory work was well done as the sample analysis and data reporting progressed as planned in a time and cost-effective manner. The database finalisation and lock were reached recently, and the data analysis and reporting are ongoing.

The 3rd main objective had two important deliverables: the first was the development of a new CD73 ELISA assay for monitoring the CD73 levels in patients during and after the dosing period and the second was development of an ARDS biomarker panel. CD73 levels have previously been measured with a chromatographic method which could not be used for measurement of thousands of samples or be properly validated. Therefore, a new immunoassay was developed by partner 4. For this purpose, novel human CD73 specific antibodies were generated and best combination for use in the
assay were selected. Simultaneously, a recombinant human CD73 molecule manufacturing process was established. This purified human recombinant CD73 was used as a reference standard molecule for the immunoassay development. During the assay development, several different sample series were analysed to characterise the assay behaviour and to study CD73 levels in different conditions. Once the CD73 ELISA assay was properly developed, it was subsequently transferred to a central laboratory service provider and validated for INTEREST Study sample analysis. The markers for the fit-for-purpose ARDS biomarker panel, which could be used to predict the disease severity, ARDS progression and length of hospitalization as well as response to IFN-beta and other supportive therapies have been selected based on the data analysis. The optimisation and validation of this panel will be done in the future.

The 4th main objective involved in-depth analysis of genetic polymorphisms/mutations in the CD73 gene, NT5E, and its regulatory elements, and their effect on ARDS disease and IFN-beta treatment efficacy. For this purpose, genetic samples were collected in the INTEREST Study with separate patient consent documentation. U.Turku purified the genomic DNA from all samples that were consented and conducted sequencing activities. After sequencing, the previously known and unknown single nucleotide polymorphism (SNPs) within NT5E gene itself and its regulatory elements were assessed against specific databases to study the number and location of the variants. This genetic variant data was correlated with the CD73 and MxA levels in the INTEREST Study patients to investigate if there are unique variants in the patients linked with different outcomes to IFN-beta treatment. These unique variants will be studied further to identify if a specific mutation can be identified to be responsible for a patient to be either responsive or non-responsive for the treatment or if there are genetic makeups that predispose patients for ARDS.

The daily management of the TRAUMAKINE project and the INTEREST Study were largely done by Faron. Management of a pivotal pan-European phase III clinical study is a significant undertaking and involves numerous stakeholders. The preparation and conduct phases of the INTEREST study took longer and required more resources than originally planned. For example, Faron hired seven new employees to manage the study in addition to the original four team members. Management of the TRAUMAKINE project was done proactively and communication between partners was very active and constructive.

The dissemination of TRAUMAKINE project and INTEREST Study progress and results were actively communicated to the public. This communication accelerated towards the end of the project, as more important results and milestones were reached. During the early phases of the project much of the communication was mainly towards scientific community, but later on, the ARDS disease awareness, health and economic burden of ARDS and treatment approach using biopharmaceutical IFN-beta were communicated also towards the public.
A description of the main S&T results/foregrounds (not exceeding 25 pages).

The overall goal of the TRAUMAKINE project was to develop biopharmaceutical IFN-beta for the treatment of moderate and severe ARDS. This was an ambitious and highly important goal as there is no approved pharmacological treatment in Europe for ARDS, even if the suspected underlying cause is being treated. Despite the improved mechanical ventilation techniques and other supportive therapies, ARDS kills approximately 125,000 Europeans annually. The burden of ARDS to the patients and their families and the society is very heavy. Almost all of the patients that survive ARDS suffer from cognitive impairment at hospital discharge and only about half of them are able to return to work within one year. They also suffer from higher rates of acute kidney injuries (approximately 40%) and roughly one third of them suffer from anxiety and/or post-traumatic disorders. The hospital costs are also significant as the patients are treated on average for 25 days in intensive care units and in total for 47 days in hospitals. Regardless of the obvious demand for pharmacological treatment for ARDS, there have not been many clinical development programs on ARDS. This could be due to the complexity of the condition, multiple aetiologies and low incidence (ARDS is classified as an orphan disease in Europe i.e less than 5 patients in 10000). Therefore, ARDS may not be considered an attractive drug development target, without a novel treatment approach.

Faron had exactly that – a novel approach to treat moderate or severe ARDS using a biopharmaceutical IFN-beta to restore the lung endothelial barrier function. This treatment approach was based on the following rational: Although the primary aetiology of ARDS varies with approximately one third of the diagnosed cases have a background of sepsis, one third have pneumonia and the rest being mostly caused by traumas or aspiration, the predominant feature of this condition, in all cases, is vascular injury in lungs that increases capillary permeability. This initially leads to the formation of interstitial protein-rich lung fluid followed by leukocyte migration from the blood circulation. The final stage of this deterioration is fibril accumulation, scarring and permanent loss of lung function. This prevention of vascular leakage had not been targeted previously even though it is known to be one of the very first pathological steps leading to infiltration of inflammatory cells and accumulation of fibrils. By treating the patients intravenously with IFN-beta, the CD73 expression on the surface of endothelial cells will increase, resulting in enhanced adenosine levels. Adenosine has been shown to reduce extra-vascular lung water, enhance endothelial cell barrier function and down regulate inflammation, all of which significantly help ARDS patients. This treatment approach using intravenously dosed IFN-beta was initially evaluated in a phase I/II trial to study safety, tolerability and initial efficacy of IFN-beta in the treatment of ARDS patients. As the results were highly encouraging, the TRAUMAKINE project was initiated to develop the first pharmacological treatment for ARDS.

The main scientific and technological results and foregrounds of the TRAUMAKINE project therefore involved the development of the biopharmaceutical IFN-beta, the set-up, conduct and actual outcome of the INTEREST Study and the new scientific know-how based on the analytical work involving the supportive pharmacodynamic and genetic assessments.

During the INTEREST Study set-up, a significant amount of work and scientific expertise was required to develop the study protocol. This work started at the beginning of the project when the TRAUMAKINE project partners and the newly established steering committee began to design the trial. The trial design was based on the EMA guideline for the clinical investigation of medicinal products in the treatment of ARDS (2007). This guidance sets the rules for the main efficacy studies by including the relevant primary and short term and long-term secondary endpoints in addition to the request of enrolment of heterogenous patient population to avoid restricted final indication. The
guidance also summarizes the important aspects that should be attended in the clinical trials: high rate of mortality, relative high rate of disability in survivors, heterogeneity of trial populations, need for controlled data, trend for increasing survival with improved ventilator technique, failure of existing medicinal therapy to influence outcome, confounding influences of co-morbid conditions and multi-organ failure. All these aspects mean that there needed to be a high degree of standardisation in the study design. The guidance also required that the proposed treatment needed to show the test items impact on the quality of life, on neurological and respiratory functions. All these parameters were incorporated in the protocol design. Due to the heterogenous patient population and their life-threatening condition, the guidance document can only provide general aspects, which need to be considered in the development of the clinical protocol. Therefore, it was of paramount importance to get the regulatory authorities involved in the design and contents of the protocol as well as in the overall clinical development. This authority involvement was achieved by having the scientific advice process with EMA. The outcome of the scientific advice process was implemented to refine the protocol. The INTEREST Study was also the first study to utilise the Berlin Definition for ARDS developed by the ARDS Definition Task Force (JAMA, June 20, 2012, Vol 307, No.23). This updated definition was designed to address the limitations of the previous definitions using consensus discussions and empirical evaluation to allow and facilitate enrolment of a consistent patient phenotype into clinical trials. Many of the patient inclusion and exclusion criteria were based on the Berlin definition and the study was designed to enrol 300 moderate (100mmHg<PaO$_2$/FiO$_2$<200mmHg), or severe (PaO$_2$/FiO$_2$<100mmHg) ARDS patients. All the above-mentioned aspects were considered by the partners and the SC members before the protocol was finalised. The finalisation of the INTEREST study protocol was an important milestone prior the actual study could begin. The final protocol was published during the study conduct (Bellingan et al. Trials, 2017, 18(1): 536). This protocol constitutes a significant foreground and can be exploited when future ARDS studies are designed. The INTEREST Study protocol publication also contained statistical analysis plan, which was designed along the protocol. The statistical analysis plan describes the statistical methods and processes, according to which all data collected during the study are analysed. Thus, this plan also contains significant amount of intellectual effort and is an important foreground, which can also be utilised in the future when similar trials are designed.

In addition to the study protocol and statistical plan, novel patient eligibility confirmation process and an electronic case report form (eCRF) were developed before the study start. Both of these are also important foregrounds that can be used in future ARDS studies. The patient eligibility process was designed to maximise possibility of enrolling only patients truly fulfilling inclusion/exclusion criteria and allowed direct communication between the site investigators and study medical monitors to discuss the eligibility of each patient if any concerns were raised during the screening process. This process utilised the educational x-ray image material accompanying the Berlin definition for ARDS. The eCRF was an online tool, programmed to enable the eligibility process and to capture every data point entered at the sites from each of the patient. This tool contained other functions required for efficient study monitoring in a Good Clinical Practise compliant manner: it allowed the clinical research associates to monitor the data points, place queries on data points, allowed data corrections by the sites and finally data sign-offs while maintaining the audit trail.

During the study set-up phase Faron developed the active pharmaceutical ingredient of Traumakine (biopharmaceutical IFN-beta) into a format that could be rapidly used at the intensive care units together with the contract manufacturing organisation. For this purpose, a lyophilised pharmaceutical formulation and its use was established and patented (WO2017149199A1). This lyophilized formulation (FP-1201-lyo) comprises a pharmacologically effective amount of Interferon beta-1a as an active pharmaceutical ingredient, disaccharides as a bulking agent and a non-ionic surfactant to
ensure the good recovery of IFN-beta after reconstitution. Reconstitution of the lyophilized IFN-beta is made using a pre-filled syringe of water for injection and a needleless MixJect device. After reconstitution, the composition can be administered intravenously to the severely ill ARDS patients in a quick and accurate manner.

Significant knowledge was also acquired about intensive care units in the participating countries: Belgium, The Czech Republic, Finland, France, Germany, Italy, Spain and UK. Site selection was based on the recommendations of the SC members from each country. SC members recommended sites from their countries based on previous ARDS study experience, forecasted monthly numbers of eligible patient, available equipment and training levels and expected duration of ethical approval and contracting processes. From a pool of over a hundred sites, 70 sites in total were eventually selected for the INTEREST Study. Even though optimal sites were selected, significant variance between site performance was observed. This was partly because of national differences in the ethics review processes but also the contracting processes varied within countries. Even more striking variance was observed between the sites in their ability to enrol patients. The best sites managed to enrol almost twenty patients, whereas some sites struggled to enrol their first patient (see Figure 2 for patient enrolment at sites which did recruit patients). Due to low patient enrolment at some of the sites, the actual study conduct lasted slightly longer than originally planned. This created the need for additional resources. Therefore, the INTEREST Study site network is a valuable starting point when future pan-European ARDS studies are planned. Much was also learned about methods to enhance patient recruitment in ARDS studies, which contained some unique characteristics. As the condition is very sudden, patients cannot be addressed beforehand, and advertising the study is of little use. Thus, to the extent possible, all effort should be concentrated at the sites to facilitate patient screening and enrolment.

During the INTEREST Study set-up, the study has undergone independent ethics reviews in compliance with EU Clinical Study Directive 2001/20/EC as it is specified in the national/local regulations of each participating country. During the study, substantial amendments done to the study protocol had been submitted to the ethics committees and favourable opinions were awaited before implementation of the amendment. In addition, other substantial changes have been submitted to the relevant ethics committees as required, e.g. addition of a study site, change of site Principal Investigator etc. Trial summary results will be posted in EudraCT within the set timeframe of 12 months after the study end. Safety reporting to the ethics committee has been done in blinded/unblinded manner throughout the study according to current requirements in the participating countries. This reporting included SUSARs, updates to Investigator Brochure, yearly reports and Development Safety Updates Reports.

Even though the patient enrolment lasted longer than originally planned, the last patient was enrolled in December 2017, two years from the first recruit (see Figure 3 for patient enrolment rate). The study thereafter entered the exciting final stage of data analysis and result reporting. All the preparatory work conducted in the four main objectives proved to be well-done as the final biomarker analysis and data entry to the database was done in a few weeks. The database finalization process was also well planned and all the important quality verification steps including source data verification and query checks, reconciliation of all Serious Adverse Events, blinded review of data tables and listings, final data checks and principal investigator sign-offs were done very efficiently resulting in the database lock on 23rd of April, 2018, followed by the authorization of statistical analysis. The first statistical analysis concentrated on analysing the all cause mortality rates between the treated and placebo groups. This result was to define the success of the INTEREST study i.e. was the IFN-beta treatment effective in the moderate or severe ARDS patients.
On May 8th, 2018, the top line data from the pan-European INTEREST Study were announced to the public:

Treatment with Traumakine did not result in an increased number of ventilator free survival days or a reduced mortality rate when compared to placebo.

- The median number of ventilator free days at Day 28 was 10 days in patients treated with Traumakine and 8.5 days in the placebo group.
- All cause mortality at Day 28, another important efficacy endpoint, was 26.4% for Traumakine and 23.0% for the placebo group.
- At Day 90 all cause mortality in the Traumakine group was 32.6% compared to 31.6% in the placebo group.
- None of these differences were statistically significant.

Safety was continually monitored throughout the study and there were no clinical concerns following the repeated administration of Traumakine. This negative result of study was completely unexpected and incredibly disappointing for everyone involved with the INTEREST Study and the TRAUMAKINE project. After the topline data became available, all partners initiated a thorough root cause analysis to understand why the outcome was negative. This root cause analysis was supported by the many vendors involved in the INTEREST Study and Traumakine development. Shortly after, on May 11th, it was announced that:

- Early analysis of certain biomarker indicators suggest that the treatment did not produce the expected interferon-beta bioactivity in the treatment group that was previously seen in Faron's Phase I/II trial for Traumakine.

All partners continued to investigate the biomarker data further and simultaneously conducted thorough checks on the Interferon beta-1a manufacturing process, blinding and randomization processes and analysing data to understand the outcome better. On June 14th, 2018, a third company press release was announced on the biomarker analysis:

- The biomarker data confirms that Traumakine treatment did not produce consistent interferon-beta bioactivity across the treatment group. A retrospective stratification of Traumakine treated patients has been conducted, based on subjects in the INTEREST trial that demonstrated a defined biomarker response. These were defined as patients with a 2-fold increase in CD73 serum levels during the first seven days of treatment and 3-fold MxA activation (during the first four days of treatment) in peripheral blood cells.

- This sub-group of patients (n=48) demonstrated a reduced D28 all-cause mortality, with a mortality rate of 14.6% compared to 32.3% in the remaining patients (n=96) in the Traumakine treatment arm (p=0.02). In addition, this sub-group of patients demonstrated a trend toward an increase in ventilator free days at D28, with 16 ventilator free days (VFDs) compared to 6.5 days (p=0.06).

- While these remain initial findings, this data suggests a correspondence to previous results observed in the Phase II study. In the Phase II trial patients with an elevated MxA and CD73 biomarker response also demonstrated an improved D28 mortality and reduced need for ventilation compared to patients with low or no increase in biomarkers.

At the moment, it can be summarized that the overall outcome of the INTEREST Study was negative, but there clearly was a subgroup of patients who showed expected CD73 and MxA responses and improved clinical outcome based on the D28 all cause mortality and increase in ventilator free days.
constructs were transfected to Chinese Hamster Ovary (CHO) cells and the best construct was

glycosylphosphatidylinositol anchor

form

assay

recombinant human CD73
deplete

success of depletion was confirmed using immunoaffinity chromatography

Human serum matrix without any CD73 was produced by depleting large quantities of type AB serum

using immunoaffinity chromatography with the human CD73 specific antibody clone 4G4. The success of depletion was confirmed using concentration and activity measurements. This CD73 depleted serum matrix was to be used as negative control for the ELISA assay. The purified recombinant human CD73 was required as a positive control and reference standard material for the assay. The idea was to produce CD73 resembling as closely as possible the natural soluble CD73 form found in serum. Thus, different CD73 gene constructs were made lacking the
glycosylphosphatidylinositol anchor sequences using standard recombinant DNA techniques. These constructs were transfected to Chinese Hamster Ovary (CHO) cells and the best construct was

was important to prepare human serum matrix material which did not contain any human CD73 and other important components of the assay were also developed be made a fine pair with the 4G4 clone for

of the new clones, 40 and 118, were found to be highly specific for human CD73 and the clone 118

CD73 together with an established 4G4 clone for an optimal antibody pair for a sandwich assay.

The purified recombinant human CD73 that could be used as a reference standard material for the assay was required.

Recent additional analyses of biomarker responses in connection to concomitant medication suggest possible prevention of IFN-beta bioeffect by concomitant corticosteroids. This will be carefully explored as this finding could have direct impact in current ICU practices. This information is also needed to design any future ARDS trials.

These events show that the preparatory work invested in the set-up and validation of the biomarker assessments was highly important as the data provided vital information on the outcome and insight for the subgroup analysis. It can be concluded from the subgroup analysis that robust induction of CD73 expression was present in those patients who did benefit from IFN-beta treatment and thus it seems to be the main pre-requisite for successful ARDS treatment. This very strongly supports the original science and approach based on using IFN-beta to treat hypoxic conditions by restoring the endothelial barrier function.

The above mentioned scientific and technological results/foregrounds mainly involved the 1st and 2nd main objectives. However, significant progress was also made involving the 3rd and 4th main objectives i.e. the development of CD73 ELISA assay and ARDS biomarker panel and genetic analysis of the CD73 gene, NT5E, and its regulatory elements, respectively. A fit-for-purpose method to follow CD73 levels in patients treated with IFN-beta was required to monitor treatment efficiency. An increase in soluble CD73 levels in patient serum was the expected outcome for IFN-beta dosing based on the treatment hypothesis. Previously CD73 levels have been measured using a thin-layer chromatographic activity measurement which could not have provided high enough throughput to analyse thousands of samples and would have been extremely difficult to validate to the required level. For these reasons, development of a new enzyme-linked immunosorbent assay (ELISA) was planned for the TRAUMAKINE project. This assay development and optimization work began from the screening of previously cloned novel mouse monoclonal antibodies (mAb) specific for human CD73 together with an established 4G4 clone for an optimal antibody pair for a sandwich assay. Two of the new clones, 40 and 118, were found to be highly specific for human CD73 and the clone 118 made a fine pair with the 4G4 clone for the ELISA assay. During the antibody screening process, two other important components of the assay were also developed before further assay optimization: It was important to prepare human serum matrix material which did not contain any human CD73 and also purified recombinant human CD73 that could be used as a reference standard material for the assay was required.

Why this was only seen in the subgroup and not in all treated patients is still currently unknown. Therefore, additional data analysis has been completed with very unexpected findings. The post hoc statistical analysis showed a strong correlation between corticosteroid use on these patient and increased mortality, even in the placebo group. These results will be communicated by Drs. Bellingan and Ranieri to the ICU community at the 31st ESICM (European Society of the Intensive Care Medicine) meeting in Paris on October 22nd followed by a main article of the study outcome. Also, a press release will be released on the same October day for the markets.

The screening of previously cloned novel mouse

planned for the TRAUMAKINE project.

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Human serum matrix without any CD73 was produced by depleting large quantities of type AB serum using immunoaffinity chromatography with the human CD73 specific antibody clone 4G4. The success of depletion was confirmed using concentration and activity measurements. This CD73 depleted serum matrix was to be used as negative control for the ELISA assay. The purified recombinant human CD73 was required as a positive control and reference standard material for the assay. The idea was to produce CD73 resembling as closely as possible the natural soluble CD73 form found in serum. Thus, different CD73 gene constructs were made lacking the glycosylphosphatidylinositol anchor sequences using standard recombinant DNA techniques. These constructs were transfected to Chinese Hamster Ovary (CHO) cells and the best construct was
selected for stable production based on the reactivity with different CD73 antibodies and on the production level of the soluble form into the culture supernatant. The best construct was again transfected to CHO cells and this time a stable expression cell line was established. The purification of the soluble CD73 from the culture supernatant was developed based on Concanavalin A and AMP affinity chromatography steps. As a result, the successfully manufactured soluble human CD73 could be used as a positive control and as reference standard material for the CD73 ELISA assay.

When the CD73 depleted serum matrix and CD73 reference standard materials were available, further optimization of the human CD73 specific ELISA was continued. Different reagents including coating, diluent and blocking buffers were tested at several concentrations. Also, detection reagent concentrations and different incubation times were optimized leading to the final optimized assay. This optimised assay provided linear response to CD73 reference standard material and was sensitive enough to allow the measurement of normal soluble CD73 levels even in healthy volunteer samples i.e. in the absence of CD73 induction by the IFN-beta treatment. When the results between the CD73 activities measured using the old chromatographic method were compared to the CD73 concentrations measured using the new ELISA, they were found to correlate very well. Thus, the optimized CD73 ELISA was considered suitable and fit-for-purpose for analysing large sample cohorts.

As the new human CD73 specific ELISA assay was successfully developed and optimized, different types of patient samples were measured to gain more experience on the assay and to learn more about the CD73 levels in patients. The assay was first used to study the admission samples from 161 patients suffering from acute pancreatitis. This patient cohort resembled ARDS patients quite well as acute pancreatitis can also lead in to moderate or severe ARDS. The samples were collected on admission from patients with acute pancreatitis and both the CD73 concentrations and activities were measured using the optimized ELISA and the old thin-layer chromatographic methods, respectively. In these samples, the concentrations and activities showed again positive correlations and the circulating levels of soluble CD73 were significantly higher in patients with acute pancreatitis than in healthy reference subjects. The soluble CD73 levels on admission to hospital correlated inversely with the severity of acute pancreatitis. The soluble CD73 activity predicted the development of severe acute pancreatitis among all patients, patients with moderately severe and severe acute pancreatitis and most interestingly among the patients without signs of organ failure on admission, with better accuracy compared to CRP and creatinine. Thus, it could be concluded that the CD73 activity on admission to hospital had prognostic value in predicting the development of the severe form of acute pancreatitis. These results were published in Critical Care Medicine Journal (Maksimow et al. Crit Care Med, 2014, Vol 42, Issue 12).

The optimized CD73 ELISA assay was subsequently transferred to a central laboratory service provider for validation according to current guidelines. Simultaneously, the human MxA specific ELISA assay was also transferred for validation. This same central laboratory service provider also validated the IFN beta binding and neutralizing antibody assays according to the most recent guidelines. All these validations were finalized before the INTEREST Study sample measurements began.

The CD73 ELISA assay was also used to study the soluble CD73 levels and their evolution in critically ill patients with severe sepsis and to assess the potential association of soluble CD73 levels with acute kidney injury and 90-day mortality. In this study, plasma samples of 588 patients admitted with severe sepsis/shock or with developing severe sepsis were analyzed at 0h (Intensive Care Unit admission) and 24h, and additionally, on day 3 or day 5 from a subset of the patients. These samples were analysed post-hoc of the prospective, observational FINNAKI study conducted in 17 Finnish
From the first initial discussions with ARDS key opinion leaders concerning the treatment hypothesis of using biopharmaceutical IFN-beta to restore the endothelial barrier function and prevent vascular leakage, it became apparent that there were not many established biomarkers used to monitor the patients. Therefore, one of the aims already in phase I/II clinical study was to measure possible inflammatory markers in ARDS patients. This aim was also included and extended in the TRAUMAKINE project to develop a fit-for-purpose ARDS biomarker panel, which could be used to predict the disease severity, ARDS progression and length of hospitalization as well as response to IFN-beta and other supportive therapies. Based on the original treatment hypothesis, CD73 could be considered as the treatment target molecule and therefore an important candidate for the biomarker panel. If CD73 is induced as a response to the IFN-beta dosing, the amount of adenosine would be also increased and all its effects in prevention of vascular leakage should benefit the patients. Similarly, MxA protein was a likely candidate for the ARDS biomarker panel as it is the best-known biomarker for IFN-beta bioactivity. Thus, the MxA levels should be increased as a response to IFN-beta treatment. In addition to CD73 and MxA, several possible inflammatory markers were measured already from the patient samples collected in the phase I/II study. These results from the phase I/II study were already highly encouraging: CD73 and MxA were strongly induced during the IFN-beta dosing and they both gradually declined towards the baseline values after the six consecutive dosing days. Important proinflammatory cytokines, like interleukin-6 and interleukin-8, were present at high levels at the ARDS diagnosis, but declined in the survivors within the first few days after intensive care begun (Bellingan et al. The Lancet Res.Med, 2014, 2(2):98-107). These results were considered during the development of the INTEREST Study protocol and it was decided that MxA, CD73 and possible inflammatory markers samples should be collected at the baseline (before the first IFN-beta dose) and daily until day 14 from all patients. These and other important observations resulted in important foreground patented by Faron (EP2956772 patent).

For the INTEREST Study, the CD73 and MxA assays were validated and the sample analyses were conducted by the central laboratory service provider. U.Turku established and conducted the possible inflammatory marker analyses using a multiplex biomarker panel consisting of 27 different cytokines, chemokines and growth factors. In total, more than 3200 MxA, CD73 and possible inflammatory marker samples each were collected and analysed followed by the entry of verified data into the database. In total, these sample analyses resulted in almost 100.000 data points. The importance of CD73 and MxA results in the INTEREST Study result analysis was already discussed previously and highlighted by the identification of the patient sub-group, with clear induction of both biomarkers, that did benefit from the IFN-beta treatment. Thus, the selection of CD73 and MxA into the ARDS biomarker panel became self-evident. As the data analysis progressed, interleukine-6 and interleukine-8 were also selected for the biomarker panel (interleukine-6 is an important mediator of fever and acute phase response and interleukine-8 attracts phagocytic cells to the sites of inflammation and induces their phagocytic activity once they have arrived). Both of these analytes
have been previously associated with ARDS disease severity and outcome and their levels declined quickly within the first few days in both active and placebo treated patients. Thus, together these four analytes could form a robust fit-for-purpose ARDS marker panel as induction of MxA, and even more importantly CD73 provided insight of IFN-beta bioactivity after dosing and treatment efficacy, respectively. On the other hand, high baseline values of interleukine-6 and interleukine-8 are associated with disease severity and poor prognosis and fast decline their levels are indication of treatment efficiency and improved condition i.e. together these four markers provide insight for the disease severity, prognosis, treatment efficiency and outcome of the severely ill ARDS patients. This selection of the final set of analytes for the ARDS biomarker panel is also well within the scope of the previously reported EP2956772 patent by Faron.

This ARDS biomarker panel will be further optimized to predict the disease severity, ARDS progression and length of hospitalization as well as response to IFN-beta and other supportive therapies. In future ARDS studies, this panel could be used to monitor patient condition and treatment efficacy in real time.

The 4th main objective was directed towards the genetic analysis of the CD73 gene, NT5E, and its regulatory elements in the INTEREST Study patients. For this purpose, a genetic sample was collected in the INTEREST Study with a separate patient consent documentation. In total, 234 patients consented for the genetic sample. U.Turku had done significant amount of preparatory work for the patient sample analysis and thus the actual clinical study sample preparation and analysis was quickly done. U.Turku purified the genomic DNA from all samples that were consented and performed the sequencing. The quality of the sequencing data in general was high and the alignment provided uniform coverage enabling reliable variant calling. Altogether, over 8000 variants with 3% in the coding region of the NT5E gene across the patients were found. The initial detailed analysis was concentrated to compare the patient sub-groups who either responded or did not respond to the IFN-beta treatment. The distribution of the known (approximately 90 %) and novel (10 %) variants was the same between responders and non-responders. Next, the distribution of the identified variants in respect to regulatory regions was studied. It was found that among the novel variants almost all are located in regulatory non-coding region in non-responders and responders compared to approximately 60 % of the known variants locating to the regulatory non-coding region in non-responders and responders. This indicates that in these patients the novel variants are enriched to the regulatory region of NT5E gene. The analysis of these variants will be studied in detail in the future. Next, the unique variants, i.e. those that were present at least once in the whole data set were studied. It was found that almost 50 variants were only present in non-responders and almost 100 variants were only present in responders indicating that there are specific variants in these patient groups. These will be selected for more detailed investigation and it is possible that a genetic makeup is found that could explain why some patients are more likely to benefit from the IFN-beta treatment. This type of knowledge would be beneficial for future clinical trials. The complete analysis of this genetic information will require a significant amount of effort and will likely provide a significant amount of new publishable information.

Even though the outcome of the INTEREST Study was negative, the data collected during the study is of high value. The overall database is very large as it contains over one million data points. It can be said that the detailed analysis of the dataset has just begun, and the main publications and the clinical study report will become available only later. The overall results will most likely provide help for these severely ill ARDS patients in the future.
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 (not exceeding 10 pages).

The potential impact of the TRAUMAKINE Project was set very high from the beginning – to develop the first pharmacological treatment for ARDS and thus save lives of critically ill patients suffering from a condition with 30-45% mortality. For this purpose, a pan-European phase III clinical trial for the treatment of ARDS using IFN-beta, called the INTEREST Study, was designed to be the pivotal study to support the filing of the MAA. To reach this significant overall goal, the project was divided into four main objectives involving many important aspects and steps required to reach this overall goal. These activities included the development of biopharmaceutical IFN-beta, the set-up, conduct and actual outcome of the INTEREST Study and the development and validation of the analytical methods and the actual conduct of the pharmacodynamic and genetic assessments of the INTEREST Study samples. All scientific and technological results and foregrounds were utilized to the extent possible and much of the data analysis will continue after the end of the project. All scientific and technological results and foregrounds have been published in appropriate scientific journals or patented and the results have been actively communicated to wider audiences in scientific meetings and to the public using press releases and presentations.

All of the preparatory work required to initiate, conduct and report the INTEREST Study results and to file for the MAA for the treatment of ARDS using IFN-beta proved to be well done as all study components worked seamlessly and efficiently. One of the most important foregrounds gained during the INTEREST study set-up was the protocol. This protocol was designed and finalised by all project partners together with the study SC. The protocol design was based on four main components: EMA guidelines for the clinical investigation of the medicinal products in the treatment of ARDS, scientific advice process with EMA, the new Berlin Definition of ARDS redefining the ARDS criteria and the vast experience of the steering committee members. The EMA guideline sets the overall framework for the main efficacy studies including primary, secondary and pharmacoeconomic endpoints. More detailed contribution from EMA was acquired through the scientific advice process where specific items were agreed. The Berlin Definition for ARDS updated the ARDS criteria and many of the study inclusion and exclusion criteria were based on this to ensure and facilitate enrolment of a consistent patient population with moderate or severe ARDS. Therefore, the final study protocol contained a significant amount of scientific expertise and constitutes a framework that can be considered and utilized by all future ARDS studies. The article describing this INTEREST Study protocol was published and thus made available quite recently (Bellingan et al. Trials, 2017, 18(1): 536). This protocol publication also contained statistical analysis plan, which was designed along the study protocol. The INTEREST Study statistical analysis plan described the statistical methods and processes, according to which all data collected during the study were analysed and thus is an important foreground as well, which can also be utilised when future studies are planned.

In addition to the important and published INTEREST Study protocol and statistical analyses plans, other significant insights were also gained which the TRAUMAKINE project team can exploit in the future. For example, the INTEREST Study eCRF was an online tool developed based on the study protocol to collect every data point from each study patient. This tool also contained several data management functions for efficient study monitoring while maintain the audit trail. Also, a novel patient eligibility conformation process was developed for the INTEREST Study utilizing the Berlin definition for ARDS x-ray image material. This process was designed to maximise the possibility of enrolling patients truly fulfilling the inclusion/exclusion criteria to the study. This process also allowed direct and
timely communication between the investigators and the medical monitors to discuss all possible questions regarding the eligibility of each patient.

All of these are important when future ARDS studies are designed. For the TRAUMAKINE Project team, these tools are readily available and can be taken to use as such or with only minor modifications for future ARDS studies. Therefore, significant savings in time and resource can be gained when similar clinical studies are designed and managed.

Development of the lyophilized formulation of interferon beta-1a (FP-1201-lyo) was done by Faron and has significant value for the company. This type of IFN-beta product containing the lyophilized IFN-beta vials, prefilled water for injection for the reconstitution and the needleless MixJect Devise can be used to deliver the medication rapidly and accurately to the severely ill ARDS patients in intensive care units. This IFN-beta formulation and its use were patented by Faron (WO2017149199A1). This type of formulation is also suitable and practical for treatments of other conditions which benefit from the IFN-beta treatment approach i.e. restoration of endothelial barrier functions. The manufacturing of the IFN-beta drug substance and the lyophilized drug product (FP-1201-lyo) are established and conducted within EU and thus have the potential to make a significant economic impact for all the companies involved in the supply chain.

During the INTEREST Study conduct, important information was also gathered about the characteristics of conducting pivotal clinical trials on moderate or severe ARDS patients in each of the participating countries. The database of countries and sites are valuable when similar studies are planned in the future. Using this information, optimal strategies for timing the submissions for ethical reviews and contracting processes can be designed. In addition, the individual site performance information is of high value: for future studies, only the sites with the best capability of enrolling suitable patients and reporting data of a very high quality will be selected. This should also lead to major savings in resources and time in the future. Important experience was also gained on how to enhance patient enrolment in ARDS studies. As the condition is very sudden, all effort should be focused to facilitate patient screening and enrolment at each site. They should have sufficiently large, well trained and motivated study teams available every day and the study protocol, eCRF and eligibility confirmation process should be designed to facilitate the enrolment to the extent possible.

After the last patient has visited for the last time, the database verification, finalization and lock processes were initiated and finally the statistical analysis of the INTEREST Study began. A few weeks later, on May 8th, 2018, the top line data of the study became available and was announced to the public: Treatment with Traumakine did not result in an increased number of ventilator free days or a reduced mortality rate when compared to placebo. This negative result was completely unexpected and incredibly disappointing for everyone involved with the INTEREST Study and TRAUMAKINE project. This result meant that the INTEREST study could not support the filing of the MAA. Instead, Faron and all other TRAUMAKINE project team members initiated a thorough root cause analyses, with the help form many of the collaborators on the study, to understand why the pivotal study result was negative. The study result analysis continued, and it was soon announced on May 11th, 2018, to the public: Early analysis of certain biomarker indicators suggest that the treatment did not produce the expected interferon-beta bioactivity in the treatment group that was previously seen in Faron’s Phase I/II trial for Traumakine. Among all other checks, the biomarker and study outcome data analysis continued and a retrospective stratification of Traumakine treated patients was conducted, based on subjects in the INTEREST trial that demonstrated a defined biomarker response. These were defined as patients with a 2-fold increase in CD73 serum levels during the first seven days of treatment and 3-fold MxA activation (during the first four days of treatment) in peripheral blood cells. These data were announced on June 14th, 2018, to the public:
This sub-group of patients (n=48) demonstrated a reduced D28 all-cause mortality, with a mortality rate of 14.6% compared to 32.3% in the remaining patients (n=96) in the Traumakine treatment arm (p=0.02). In addition, this sub-group of patients demonstrated a trend toward an increase in ventilator free days at D28, with 16 ventilator free days (VFDs) compared to 6.5 days (p=0.06). This was highly important and encouraging observation as it demonstrated that the original treatment hypothesis is correct: Patients who demonstrated the expected induction in MxA and CD73 levels showed improved mortality by day 28 and reduced need for ventilation compared to patients without biomarker response – exactly in line with the treatment hypothesis. The INTEREST study data analysis is still ongoing and the clinical study report and the main publications are being prepared by the TRAUMAKINE project team. The root cause analysis showed corticosteroid interference of Traumakine action, explaining at least in part the INTEREST trial results. Thus, a new clinical trial can be initiated exploiting all the existing results generated during the TRAUMAKINE project. These exploitable results also include all the work conducted involving the bioanalytical work.

The analysis of the INTEREST data will continue and any possible correlations of patient mortality, concomitant medication and biomarker response hopefully will reveal further advise for the next trial design.

The identification of the subgroup of patients benefitting from the IFN-beta treatment with the expected increase in the MxA and CD73 biomarkers strongly emphasized the importance of the preparatory work conducted for the development and validation of the biomarker assays. One of the goals of the TRAUMAKINE project was to establish and validate a fit-for-purpose novel human CD73 specific ELISA assay. This aim was driven by the need to develop an assay that could replace older method based on thin-layer chromatographic method which would not have had required the throughput required to analyse all the samples collected form the INTEREST Study. In addition, this type of assay could not have been validated properly for use in a central laboratory service provider. The set-up of the human CD73 specific ELISA started really from the beginning by selecting a well-functioning pair of human CD73 specific antibodies. Luckily, a pair of antibodies were found originally developed by U.Turku. This was very important as continuous access and supply of the antibodies was also secured this way. Important other components of the assay were also developed by U.Turku including the CD73 depleted human serum matrix and a recombinant soluble human CD73 molecule reference standard material. These components were required for the assay to serve as the negative and positive controls, respectively. When these components became available, the final assay optimization was done. Once the human CD73 specific ELISA was properly established it was transferred to a central laboratory service provider validated it to required level. This same laboratory also validated human MxA specific ELISA and assay for detection of interferon beta-1a specific binding and neutralizing antibodies according to the latest guidelines. All this work has high scientific and commercial value as the assays are ready to be used for analyzing samples from clinical trials (see Figure 4 for the overall logistics related to sample management in the INTEREST Study). Faron is already exploiting these results in its other ongoing trials. As the central laboratory service provider is located in EU, the economic impact remains in EEA.

The human specific CD73 ELISA assay was used to study CD73 levels in acute pancreatitis and in sepsis and acute kidney injury patients. Both these studies provided important scientific information and insights and have been published in peer reviewed journals. The on admission acute pancreatitis samples were measured using both the old thin-layer chromatographic and the new ELISA methods, and the results correlated well supporting the use of the ELISA method for analyzing larger cohort samples. Results also showed that CD73 levels were significantly higher in patients with acute
pancreatitis than in healthy reference subjects. It was concluded that the CD73 activity has prognostic value predicting the development of the severe form of acute pancreatitis at the admission to hospital. These results are important to broaden the understanding of the roles different molecules have on acute pancreatitis development and progression and could be exploited in future by developing acute pancreatitis diagnostics further to aid the early treatment decisions.

The CD73 levels were also studied in critically ill patients with severe sepsis/shock and to assess the potential association of CD73 levels with acute kidney injury and 90-day mortality. In these patients, the CD73 levels were low at the early time-points compared to normal population and increased by day five. In kidney injury patients, the early CD73 levels were higher than in patients without kidney injury. Also, non-survivors with severe sepsis, but without septic shock, had higher CD73 levels in all time-points compared to survivors. However, after multivariable adjustments CD73 levels did not associate independently with acute kidney injury nor 90-day mortality. Thus, CD73 levels in this patient population did not seem a beneficial marker for predicting the disease progression or mortality. These types of results provided important insight to better understand CD73 levels in different conditions, which is important for the potential use of CD73 biomarker diagnostics.

The development of a fit-for-purpose biomarker panel was another important goal for the TRAUMAKINE project. Research to meet this goal started already soon after the TRAUMAKINE project start. Important work was done already when the phase I/II study samples were analysed and very exciting findings were made. These findings were patented by Faron (EP2956772) and were exploited when the INTEREST Study was designed and the patient sampling schedules were designed. U.Turku established the possible inflammatory marker panel to analyse 27 different cytokines, chemokines and growth factors and conducted the INTEREST Study sample analysis. These assessments provided significant amount of data and the data analysis is still ongoing. However, the selection of biomarkers for the potential ADRS biomarker panel has already been made. As previously emphasized, the roles of MxA and CD73 in the assessment of IFN-beta bioactivity and treatment efficiency were fundamental and their selection to the panel was self-evident. As the analyses of other biomarkers progressed, interleukine-6 and interleukine-8 became also selected for the biomarker panel. Both these analytes have been found to be important for monitoring the disease severity and progression. Together these four analytes could form a robust fit-for-purpose ARDS panel: Induction of MxA and CD73 would report the IFN-beta bioactivity and the treatment effectivity according to the treatment hypothesis, respectively, and on the other hand, the decline of interleukine-6 and interleukine-8 from their base line levels would suggest of improved condition. The next steps in exploiting this foreground is to combine these four markers in to a single biomarker panel that would be easy and robust to use at the intensive care units. This biomarker panel would then need to be optimized and then rigorously tested using patient samples. This type biomarker panel could be a significant asset in future clinical trials involving IFN-beta treatment and in intensive care units to monitor ARDS progression and treatment efficacy.

The 4th main objective of the TRAUMAKINE project was the genetic analysis of the CD73 gene, NT5E, and its regulatory elements in the INTEREST Study patients. This assessment was done by U.Turku using the genetic samples consented by the patients in the INTEREST Study. This analysis yielded large amount of data and the analysis is still ongoing. It has been found already that many of the novel variants are enriched to the regulatory region of NT5E gene. The analysis of these variants will be studied in detail in the future. Importantly, it was also found that almost 50 variants were only present in non-responders and almost 100 variants were only present in responders indicating that there are specific variants in these patient groups. These variants will be analysed further to investigate if a genetic makeup could be found that would identify patients more likely to benefit from the IFN-beta
treatment. This type of foresight could be exploited in future by developing a genetic test to select patients for this type of treatment.

Important work has also been made to disseminate the scientific and technological results to the public and to report the study progression and most importantly to make the potentially lethal ARDS better known within the industry, scientific community, investors and public. Since ARDS is acute and very severe condition with a mortality rate of 35-40%, the ARDS patient associations or advocacy groups are not established in a similar manner as for patients with much more common chronic diseases. Therefore, there are not established networks through which the public awareness could be improved in the EU. For these reasons, all partners first started with presentations about TRAUMAKINE program for the scientific community. Then gradually more and more options to inform wider audiences became available, which were used to the extent possible. As Faron was continuously applying for more funding, the investor networks began to understand the condition better. When Faron listed in in the fourth quarter of 2015 on the Alternative Investment Market of the London Stock Exchange, the possibilities to communicate to wider audiences was significantly improved. Similarly, the public interest increased. The following presentations have been given by the participants to describe the scientific rational behind the TRAUMAKINE approach and ARDS around the world:

- Jalkanen S. “Biological Drugs modifying the immune system- new ways to fight against harmful inflammations and cancer”, Biological Drugs Symposium, Helsinki, 3.3.2013
- Bellingan G. Which control group?, ESICM September 2014, Barcelona, Spain 25.9.-1.10.2014
- Bellingan G. Novel therapies for ARDS, 35th ISICEM Meeting, Brussels, Belgium 17-20.3.2015
- Bellingan G. New immunomodulatory therapies for ARDS, Dublin summer ESICM 2015, Dublin, Ireland 11-12.6.2015
- Bellingan G. Putting all that together, ESICM October 2015, Berlin, Germany 2-7.10.2015
- Jalkanen S. New molecules controlling the endothelial barrier. 35th ISICEM Meeting, Brussels, Belgium 17-20.3.2015
- Jalkanen S. New tools to fight against harmful inflammations. Doctor Promotion, University of Eastern Finland, Kuopio, Finland 4.6.2015
- Jalkanen S. New possibilities to treat ARDS. Department of Lung Diseases, Turku University Hospital, Turku, Finland 13.10.2015
- Bellingan G. "Interferon beta in ARDS - new therapies in the pipeline", 36th ESICM meeting, Brussels, 15 -18.3.2016
- Jalkanen J. "New pharmacologic interventions for organ protection after major operations" ISICEM meeting, Brussels, 23.3.2017
• Jalkanen S. “New tools to fight against harmful inflammations and cancer” Science Days, Helsinki, 14.1.2017
• Jalkanen S. “New tools to fight against harmful inflammations and cancer” Symposium of the Pharma Industry Finland, Helsinki 27.4.2017
• Jalkanen S. “Endothelial cells as targets to modulate tumor immunity” ESM-EVBO Meeting, Geneva, 28.5-1.6. 2017
• Faron Pharmaceuticals Ltd also organized a R&D Day directed towards British Investors in London 14.6.2016
• Jalkanen S. “How to prevent vascular leakage and save lifes”, sTARTUp Day, Tarto, 8.12.2017
• Jalkanen S. “Control of vascular leakage: a target to fight against harmful inflammations”, Stanford University, USA, 11.1.2018,
• Bellingan G. Interferon-beta?, ISICEM, Brussels, 20-23.3.2018
• Jalkanen S. Japan-Finland seminar on Personalized Medicine and Health. “Control of vascular integrity: a target to fight against harmful inflammations”, Tokio, 9-11-4.2018,

More local communication has also taken place for the public:

• Jalkanen S. "Uusin keinoin tulehduksia vastaan", Rotary Club 1.10.2017 Turku, Finland
• Jalkanen S. "Uusin keinoin tulehduksia vastaan", Local Martta Club 3.11.2017 Turku, Finland
• Jalkanen S. has also been interviewed for four Finnish news papers: Mediutiset, Ylioppilaslehti, Eeva, Kauppalehti
• Jalkanen M. was interviewed for the Finnish TV program Akuutti regarding the developing of treatment for ARDS.
• Jalkanen S. receive the European Innovator award in Brussels and was interviewed for local TV channels two times.

In late 2017 and during 2018, Faron, assisted by the other partners, were preparing for market access with a disease awareness and public knowledge campaign. The main three educational components were 1) The causes leading to ARDS: pneumonia, influenza, sepsis and trauma etc. 2) The health and economic burden of ARDS to the society. 3) The role of the compromised endothelial barrier resulting in vascular leakage and building of fluid into the lung airspace. The main event in the spring 2018 was the International Symposium on Intensive Care and Emergency Medicine (ISICEM) in Brussels (20-23.3.2018) were Faron had a significant presence with company’s first booth, ARDS leaflets (ARDS Infographic.pdf and Traumakine Clinical Backgrounder.pdf) and two disease awareness videos (ARDS Disease Awareness Video.mp4 and Traumakine ARDS Animation.mp4). UCLH provided an editorial for the event (Traumakine INTEREST Study Advertorial.pdf). The booth proved to be a huge success and an excellent venue for communicating the disease awareness.

As ARDS patient associations or advocacy groups are not well established in the EU, Faron participated in a global web and social media-based campaign, by the US based ARDS foundation, where real life stories of ARDS survivors were presented (ARDS Survivor Stories.pdf).

After becoming a publicly listed company, Faron has had active communication towards the investors and therefore for the public as well. These activities are listed below:

• Jalkanen M. Presentation for One2One Investor Forum, Proactive investors, London, 26.1.2017
• Jalkanen M. Recorded interview, Proactive investors, London, 9.2.2017
Jalkanen M. Presentation on Full Year Results, Faron/Consolium, London, 29.3.2017
Jalkanen M. Recorded interview, Proactive investors, London, 29.3.2017
Jalkanen M. Presentation on Interim Results, Faron/Consolium, London, 6.9.2017
Jalkanen M. Recorded interview, Proactive investors, London, 6.9.2017
Jalkanen M. Presentation for One2One Investor Forum, Proactive investors, London, 5.10.2017
Jalkanen M. Presentation in Bio-Europe Conference, Berlin, 6-8.11.2017
Jalkanen M. Presentation in Biotech & money Conference, London, 14.11.2017
Jalkanen M. Recorded interview, Biotech Showcase, 25.1.2018
Jalkanen M. Presentation, Faron R&D Day, London, 21.2.2018
Jalkanen M. Faron stand launch, 38th ISICEM meeting, Brussels, 20-23.3.2018
Jalkanen M. Presentation, Investor Events Evening, Proactive investors, London, 5.4.2018
Jalkanen M. Presentation on Full Year Results, Faron/Consolium, London, 8.5.2018

In summary, it can be concluded that the TRAUMAKINE project did reach almost all milestones that were originally planned. One of the most important goals was the execution of the INTEREST Study, designed to be a pivotal study capable of supporting the Marketing Authorisation Application for treatment of moderate and severe ARDS using IFN-beta. Unexpectedly and very disappointingly the overall result of the INTEREST Study was negative as the study did not meet the primary end-point. The root cause analysis of the negative result and data analysis is still ongoing, and the clinical study report is being prepared. However, huge amounts of important scientific and technological data and results and insights have been made already. There is also a significant amount of data analysis still ongoing and the main scientific papers are currently being written and so therefore the impact of the TRAUMAKINE project is expected to be very strong and widely felt. The most important findings thus far from the INTEREST Study have been that the IFN-beta treatment was not associated with major safety concerns in the study safety population and that a sub-group of patients responded as expected to the IFN-beta treatment. If further data analyses can identify the characteristics of the responding patient sub-group, then a new pivotal clinical study can be designed. In this case, all the insights gained thus far, would tremendously facilitate the set-up and conduct of this new clinical study. We hope that this clinical study would be successful and able support the MAA and thus finally deliver the first pharmacological treatment for the future ARDS patients.
The address of the project public website, if applicable as well as relevant contact details.

www.traumakine.eu
Figure 1

Figure 1. TRAUMAKINE project structure. The four main objectives of the TRAUMAKINE project and their deliverables are rough timelines are presented above. Deliverables in the blue boxes have been reached already, deliverables in yellow boxes are currently being conducted and deliverables in red boxes will be reached in the future. The red arrows indicate dependencies between main objectives and deliverables.
Figure 2. Patient enrolment at each site that did manage to enrol patients. The final recruitment status in chronological order for both countries and sites, starting at the top with the first country having recruited a patient. Within country, the order of sites that have recruited patients is shown with the first recruiting site on top. The duration of site being open for patient enrolment varied in length as sites and countries became activated at different times. Thus, some sites were fast in patient enrolment but managed to get only few patients.
Figure 3. Patient enrolment. The blue diamonds present the enrolment of each patient for the INTEREST Study. Patient enrolment lasted slightly longer than planned due to site initiation timelines and the actual patient enrolment rate. During the first months of the study, sites were still being opened and only few sites were screening patients. As more and more sites became active, also the enrolment rate improved. Some seasonal variation in ARDS can also be seen in the picture, as ARDS is more common during the flu-season i.e. the winter months.
Figure 4

The overall logistics related to sample management in the INTEREST Study. Logistics and Central Laboratory service providers distributed all required materials for sample preparation for all the study centres (blue boxes). The sites prepared all samples and stored the samples until they were shipped to Central Laboratory service provider (yellow boxes). Central Laboratory service provider conducted sample reconciliation and analysed MxA, CD73 and anti-drug antibody samples. They also shipped PIM and genetic samples to U.TURKU for analyses (green boxes). Both U.TURKU and Central Laboratory service provider uploaded their results to the database (red box). Red arrows indicate physical material shipments and blue arrows the electronic data entries.