RESEARCH PROTOCOL

Anti-COVID19 AKS-452 Phase I/II VaccinaTion Study

(ACT-study)

Anti-COVID19 VaccinaTion (ACT) Study

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Every decade in the twenty-first century has experienced a new major coronavirus epidemic; SARS in the 2000s, MERS in the 2010s, and now (in 2020) Coronavirus Disease 2019 (COVID-19) caused by the SARS-COV-2 virus. This novel COVID-19 is a severe and acute respiratory illness caused by infection with the SARS-CoV-2 virus. The first COVID-19 case was reported in Wuhan, China in December 2019 and as of 18 November 2020, there has been approximately 76 million (M) cases world-wide to date (quantified as SARS-Cov-2 virus confirmed and unconfirmed "probable"), in which there are 18.5M active cases, 36M recovered cases, and 1.7M fatal cases attributed to COVID-19 (*COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University*; https://www.covidtracker.com/). Consequently, to address this pandemic crisis, there is an immediate need for solutions that can accurately quantify the level of neutralizing anti-SARS-CoV-2 antibodies (Abs) in individuals and therapeutically induce and/or amplify the level of neutralizing anti-SARS-CoV-2 Abs across the population. The expectation of the foreseeable future is that natural and vaccine-induced immunity most likely will not be long-lived [4, 7-10], and therefore a cost-effective and safe vaccine administered as frequently as every 6 months, if necessary, is required to maintain robust immunity among the population. Due to the apparent increased transmissibility of SARS-CoV-2, a global security priority is to advance and stockpile coronavirus vaccines as quickly as possible, inevitably requiring significant international funding and relaxing of regulatory paths in a responsible manner. Given the challenges of a recombinant SARS-Cov-2 Spike Protein (SP) subunit vaccine to induce a strong protective immune response in an immunologically naïve human population, the SP Ag must be modified and/or formulated with additional immune-enhancing features to overcome the activation thresholds of naïve T and B cells. Akston, the subsidizing party of the study, has implemented the following features into its COVID-19 vaccine that are major advantages over most other such vaccines in development, in which the Therapeutic Product Profile (TPP) describes details of its clinical candidate, AKS-452:

1. The use of the smaller focused antigenic portion of SP, the RBD
2. Recombinant fusion of RBD with human IgG1 Fc (SP/RBD-Fc)
3. Emulsification of SP/RBD-Fc in the water-in-oil adjuvant, Montanide ISA 720

In summary, the Fc moiety on AKS-452 is designed to act as a mild adjuvant via inducing activation signaling to the antigen-presenting cell (APC) via FcγRs and is designed to work in concert with a strong classical adjuvant, such as Montanide ISA 720, to enhance the duration of Ag exposure to APCs and perhaps direct Ag entry into lymph nodes locally and systemically where additional APCs reside. As a consequence, the Fc moiety in combination with an adjuvant is expected to create a dramatic dose-sparing potential for both the Ag and adjuvant such that the risk of reactogenicity (a safety concern) is dramatically reduced; i.e., too much adjuvant that over-activates many APCs and other innate immune cells can lead a systemic inflammatory reaction termed *reactogenicity*. Such reactogenicity is induced acutely after injection and is not mediated by T and B cells.

Objective: Evaluation of safety and initial efficacy of a novel fusion-protein based anti-COVID19 vaccine in healthy volunteers.

Study design: Single center, open-label, phase I dose-finding and safety study combined with a phase II, safety / efficacy study, on the biological activity against COVID-19 under study to warrant more extensive development towards a phase III clinical study.

Study population: Healthy human volunteers, 18 - 65 years old

Intervention: Phase I safety/dose-finding study (6 cohorts), three dose-levels (22.5, 45 or 90 µg) administered via subcutaneous (SC) route with a 1 or 2 dose regimen to 10 subjects per cohort in which safety parameters and neutralizing IgG titers will be reviewed after the 1st and 2nd doses. If safety and immunogenicity are acceptable per subject, the respective subject will continue with a 2nd dose on day 28, or will not receive a 2nd dose as is the case with the single-dose cohorts (Cohort 1,3,5). Phase II safety/initial efficacy study will be initiated after safety review and immunogenicity assessment at which time the decision will be made in which a 1-dose regimen and a 2-dose regimen will be chosen for phase II. For phase II the participating subjects will be randomized between the 1- and 2 dose regimen. A statistical re-assessment will be done after the interim analysis of phase I to determine the definitive number of subjects for phase II based on anti-SARS-CoV-2 SP receptor-binding domain (RBD) IgG Ab titers. Based on the current assumptions, we estimate that both cohorts in phase II will consist of 58 subjects each, with a total of 116 subjects.

Main study parameters/endpoints: Phase I: safety/immunogenicity, phase II immunogenicity and initial efficacy; immunogenicity will be expressed by SP/RBD-specific IgG titers.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden of participating in the study will be the number of site visits and possible travelling for subjects in phase I/II, study investigations such as blood samples for measurement of immunogenicity, physical examination prior to inclusion / exclusion, and physical discomfort related to the subcutaneous injection of AKS-452. The risks associated with exposure to a novel vaccine for a 1st in-human phase I/II clinical study are pain, swelling, redness, bleeding, infection, and granuloma formation at the SC injection site, mild fever, chills, feeling tired, headache, muscle and joint aches, syncope, and an allergic reaction which are all well within the tolerable range for a novel vaccine like AKS-452. The benefit, in the case of a safe and sufficient immunogenicity provoking vaccine, is for protecting health care workers, future vulnerable and frail elderly, and patients undergoing large surgical procedures for instance oncology, transplantation etc. Moreover, providing protection in co-morbid citizens (i.e., diabetes, overweight, cardiovascular disease, etc.) and ultimately, creating another leverage to returning societies back to their previous health care system capacities and economic growth world-wide.

1. INTRODUCTION AND RATIONALE

1.1 COVID-19 overview and the SARS-Cov-2 vaccine landscape

Every decade in the twenty-first century has experienced a new major coronavirus epidemic; SARS in the 2000s, MERS in the 2010s, and now (in 2020) Coronavirus Disease 2019 (COVID-19) caused by the SARS-COV-2 virus. This novel COVID-19 is a severe and acute respiratory illness caused by infection with the SARS-CoV-2 virus. The first COVID-19 case was reported in Wuhan, China in December 2019 and as of 18 December 2020, there has been approximately 75 million (M) cases world-wide to date (quantified as SARS-Cov-2 virus confirmed and unconfirmed "probable"), in which there are 18.5M active cases, 42M recovered cases, and 1.7M fatal cases attributed to COVID-19 (*COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University*; https://www.covidtracker.com/). At this time, there are two specific vaccines approved for COVID-19 (Pfizer/BioNTech and Moderna). Given the insufficient natural immunity to COVID-19 among the world population, the high basic reproduction number (R0 - R naught) of at least 2.2, the high probability of asymptomatic transmission, and the high death rates estimated at 2-10 times higher than that of influenza, extreme social distancing and widespread shutdown of human activity and interactivity are the only solutions currently available to prevent the spread of infections and minimize the number of serious illnesses and fatalities (reviewed in [1]). The consensus among experts is that society cannot return to normal unless and until there is a sufficient level of immunity conferred on the population. Achieving natural herd immunity is estimated to require at least 70% of the population to have been infected [1] which would result in millions of deaths worldwide, an ethically unacceptable outcome. Furthermore, early surveillance studies using Ab testing has shown that over 20% of the population in some areas, such as New York City, have antibodies (Abs) to SARS-CoV-2 [2]. However, within that group, many in the population appeared to have borderline to low levels of IgG titers and at present public health officials are unsure of what levels or class of Abs confer neutralizing activity or long-lasting protection against the SARS-CoV-2 virus [3]. Likewise, a more recent screening of 365,000 adults in the general population of England (the *REACT2* study) identified 17,500 that were positive for SARS-Cov-2 Ab titers (i.e., 6% of the general adult population), in which the prevalence of these titer-positive subjects rapidly declined by 27% at 3 months after recovery from infection [4]. These results, combined with early reports from South Korea that some recovered COVID-19 patients have re-presented with measurable SARS-CoV-2 viral loads [5], raises the following key questions: i) what percentage of individuals infected with SARS-CoV-2 develop appreciable levels of Abs, ii) how long do these Abs to SARS-CoV-2 remain in the body after infection, and iii) for how long do they prevent reinfection, if at all? [6]. Consequently, to address this pandemic crisis, there is an immediate need for solutions that can accurately quantify the level of neutralizing anti-SARS-CoV-2 Abs in individuals and therapeutically induce and/or amplify the level of neutralizing anti-SARS-CoV-2 Abs across the population.

The expectation of the foreseeable future is that natural and vaccine-induced immunity most likely will not be long-lived [4, 7-10], and therefore a cost-effective and safe vaccine administered as frequently as every 6 months, if necessary, is required to maintain robust immunity among the population. Due to the apparent increased transmissibility of SARS-CoV-2, a global security priority is to advance and stockpile coronavirus vaccines as quickly as possible, inevitably requiring significant international funding and relaxing of regulatory paths

in a responsible manner. Indeed, regulatory agencies appear to embrace this COVID-19 crisis with a unique urgency to allow for an abbreviated preclinical and clinical timeline. A SARS-CoV-2 vaccine could serve as a pan-coronavirus vaccine and appears scientifically feasible if modelled after those previously developed for SARS (caused by the SARS-CoV virus) because both coronaviruses bind the same host target protein, angiotensin converting enzyme 2 (ACE2), expressed in human lung epithelium, endothelial cells, and neuronal cells [11, 12], both exhibit genomes of approximately 30 kb, and SARS-CoV-2 exhibits approximately 89% nucleotide similarly to SARS-like.

Some, but not all, of these features are being implemented in over 170 SARS-CoV-2 vaccine candidates currently in development, including live viruses, nucleic acids, and recombinant protein subunits that may ultimately offer promise as preventive vaccines against COVID-19. However, each vaccine strategy has unique advantages and challenges with respect to manufacturing, safety, and efficacy that must be simultaneously managed in an optimal manner during investigational new drug (IND)-enabling studies such that registration trials can be achieved in a timely manner [14-18]. The following is a summary of the advantages and disadvantages of the current COVID-19 vaccine programs that are most advanced in clinical development.

1. Live-attenuated, inactive whole virus or non-SARS-CoV-2 viral vector-based vaccines represent a classic group of strategies. One viral vector-based approach, which is being deployed by Johnson & Johnson, uses Janssen's AdVac® adenoviral vector that is manufactured in a PER.C6® cell line technology to generate their lead vaccine, JNJ-78436735. Similarly, AstraZeneca with Oxford University have developed their AZD-1222 vaccine produced using a chimpanzee adenoviral vector, ChAdOx [19, 20]. Both are now in Phase 3 trials, but have encountered significant serious adverse events (SAEs) that caused clinical trial pauses [21]. A major advantage of whole virus vaccines is their inherent immunogenicity and ability to stimulate toll-like receptors (TLRs) including TLR 3, TLR 7/8, and TLR 9. However, live virus vaccines often require extensive additional testing to confirm their safety. This is especially an issue for coronavirus vaccines, given the findings of increased infectivity (see below: Antibody Dependent Enhancement, ADE) following immunization with live or killed whole virus SARS coronavirus vaccines [16]. In addition, it is well documented that viral vector vaccines, especially those composed of adenoviruses, induce humor immunogenicity to the vector leading to neutralization of the vaccine itself although use of chimpanzee tropic adenoviruses has temporarily overcome such concerns [22]. Another challenge with whole virus vaccines is the relatively low manufacturability throughput (and therefore COGs) due to either chicken egg-based production or cell expression systems [22].
2. Nucleic acid expression vector vaccine platforms for COVID-19 encode the major coronavirus target antigen (Ag), the Spike Protein (SP), that mediates the virus' infective mechanism via its binding the host receptor, ACE2. ACE2 is expressed on lung epithelium, blood vessel endothelium, and specific neuronal cells that appears to account for the dominant clinical manifestations of COVID-19, including pulmonary, cardiovascular, and neurological complications, respectively [26]. The major biotech/pharmaceutical companies that have advanced such vaccines to phase 3 trials, include mRNA vaccines encoding the full-length SP developed by BioNTech/Pfizer, BNT162b2 [23, 24], and Moderna Therapeutics, mRNA-1273 [25, 26]. Indeed, both vaccines have reported very positive interim phase 3 results with efficacy in protecting

from symptomatic SARS-Vov-2 viral infection of >90% [27, 28]. Both companies have recently gained regulatory approval in the Unites States and the European Union. The concept of immunizing with RNA or DNA began with promising results in mice in 1993 showing protective immunity against influenza, but for decades, these findings have not translated to similar findings in humans. Moreover, while non-replicative, many of these RNA and DNA expression vector vaccines continue to endogenously produce the target viral Ag well after induction of the intended immune response, an aspect that could ultimately create immune tolerance to the virus which is a growing concern and may become a practical risk with such current COVID-19 vaccine. Other challenges of these nucleic acid vaccines are the low durability of the response that may require too frequent dosing, and an unfavorable COGs due to cumbersome manufacturability via chemical synthesis.

3. Recombinant subunit vaccines for both SARS coronaviruses rely on eliciting an immune response against the SP to prevent its docking with the host target protein, ACE2 [16, 29, 30]. Novavax has developed and produced immunogenic virus-like nanoparticles based on recombinant expression of SP, NVX-Cov2373, that is formulated with a saponin-based adjuvant system, Matrix-M™, while Clover Biopharmaceuticals is developing a subunit vaccine consisting of a trimerized SARS-CoV-2 SP using their patented Trimer-Tag® technology [31]. Note that the full-length SP target Ag is known to have low expression yields in cell-expression systems and when used in SARS vaccines is known to induce anti-SP IgG titers against non-neutralizing epitopes of SP that mediate increased viral infectivity (i.e., ADE) and inflammation caused by lung eosinophilia (i.e., Th2-mediated immunopotentiation, discussed below). Therefore, a consortium led by Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine has developed and tested a subunit vaccine comprised of only the receptor-binding domain (RBD) of the SARS SP [16], and when formulated with alum, this RBD-based vaccine can elicit high levels of protective immunity upon homologous virus challenge, in addition to avoiding ADE and immunopotentiation [16]. Initial findings that the SARS and SARS-CoV-2 RBDs exhibit more than 80% amino acid similarity and bind the same ACE2 target offer an opportunity to develop either protein Ag as a subunit vaccine. Indeed, such a subunit vaccine proof-of-concept has been successfully demonstrated with coronavirus SP/RBD Ag's of MERS and SARS infections [18, 29, 32, 33].

1.2 Akston's SP/RBD subunit vaccine rationale

As discussed above, the recombinant protein-based subunit vaccine approach has an advantage of safety and multiple-booster dosing relative to inactivated or live-attenuated virus and nucleic acid vector-based vaccine formats, in addition to allowing for the selective use of the most dominant epitopes to generate potent neutralizing Ab titers [15, 17]. However, the relatively smaller size of the recombinant proteins may pose a problem of lower immunogenicity compared to a whole virus Ag, and therefore require additional features to enhance immunogenicity. The following is a brief discussion of the immunological mechanisms that form the basis of Akston's approach for developing its *immune-enhanced* recombinant subunit vaccine, AKS-452, and emphasizes its distinguishing factors from other closely related vaccine programs.

With respect to a basic immune response, injection of any protein an Ag can, and most likely will, induce an immune response, the magnitude and type of which is highly dependent on the "status" of the respective immune system. For example, injection of a foreign Ag relative to a self Ag will induce a greater immune response in an immune system that maintains central and peripheral tolerance mechanisms, while self Ag can elicit significant immune responses in an immune system with broken tolerance mechanisms, such as an autoimmune condition. Moreover, foreign or self Ag administration to an immune system that has been primed to previous exposure to the respective Ag (e.g., a viral infection or an autoimmune disease) will lodge a more rapid and elevated immune response relative to that of an Ag-naïve system. The immunological basis of this priming is two-fold; 1) an Ag-naïve immune system has naïve B and T lymphocytes that have a much higher threshold of activation than do the Ag-primed "memory" cells of a Ag-primed immune system, such that the antigen-presenting cells (APCs) that present Ag require much less Ag to activate primed memory T cells, and 2) due to expansion of memory T cells during the Ag priming exposure, there are inherently greater numbers of such cells upon re-exposure to an injected Ag. Note that dominant APCs are dendritic cells (DCs) and macrophages that present Ag in complex with Major Histocompatibility Complex (MHC) molecules on their surface to T cell Ag receptors (**Figure 1**). It is these APCs that can influence both the "magnitude" and "type" of response to Ag; e.g., the Th1 cell response is required to clear most viral and bacterial infections, in which virus-like or bacterial-like substances (non-Ag in nature) condition APCs to express key cytokines and surface co-stimulatory molecules that, during Ag presentation, drive T cells to become the Th1 type. In fact, this APC activation is the conceptual basis of many immune enhancing substances called *adjuvants*. Some adjuvants are designed to trick the immune system into reacting to the injected vaccine Ag as if it were part of an on-going infection (i.e., infectious agents provide such natural viral or bacterial adjuvant substances). Therefore, adjuvants activate APCs for greater Ag-presentation capabilities necessary to overcome the high activation threshold of naïve T cells, in addition to shaping their development into the Th1 response to effectively clear the respective infection. Note that such T cells provide critical help to B cells that specifically bind the respective Ag to produce Ag-specific antibody (Ab) titers (**Figure 2**).

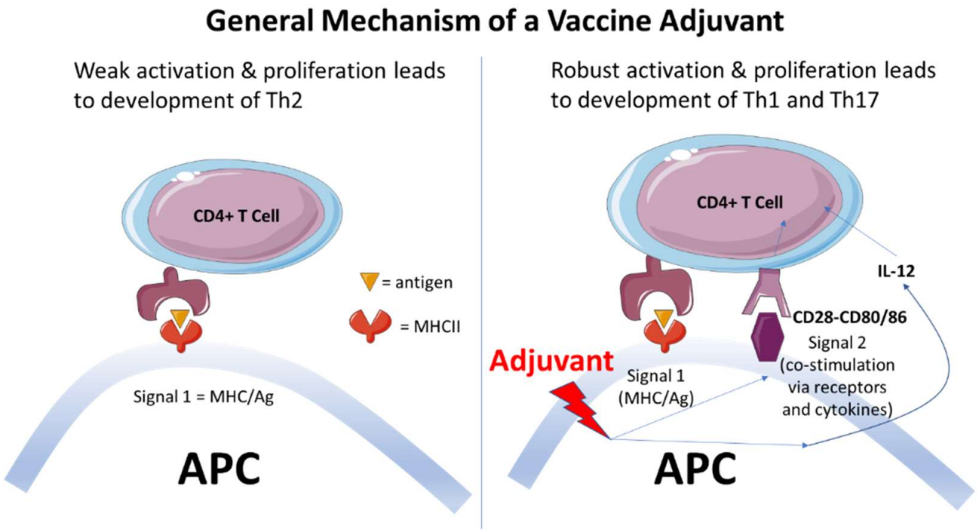

Figure 1: A general schematic of how an **adjuvant enhances T cells responses** that help B cell Ab responses. APC, Antigen-presenting cell; Th cell, T helper cell, MHC, major histocompatibility complex; Ag, antigen, IL-12, interleukin-12

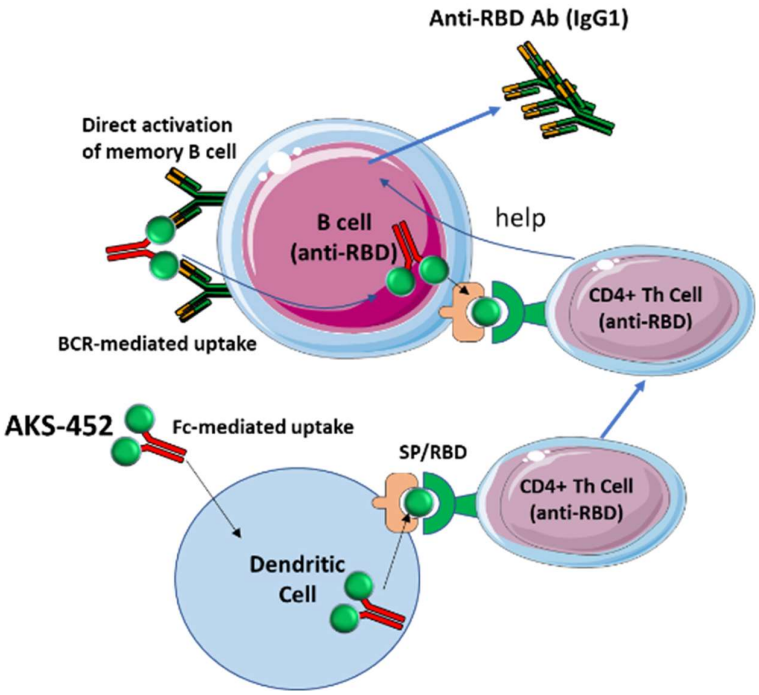

Figure 2: Mechanism of action by which AKS-452 is taken up and processed by an Ag-presenting dendritic cell that presents peptide fragments of SP/RBD to Th cells that help B cells produce anti-SP/RBS-specific Abs. AKS-452 can also directly bind existing SARS-CoV-2-specific memory B cells via their B cell receptors (BCRs) in a bivalent fashion which can crosslink BCRs enhancing proliferation, activation, and Ab production in the absence of Th cells.

Given the challenges of a recombinant SARS-Cov-2 SP subunit vaccine to induce a strong protective immune response in an immunologically naïve human population, the SP Ag must be modified and/or formulated with additional immune-enhancing features to overcome the activation thresholds of naïve T and B cells. Akston has implemented the following such

features into its COVID-19 vaccine that are major advantages over most other such vaccines in development, in which the Therapeutic Product Profile (TPP) describes details of its clinical candidate, AKS-452 (Table 1):

1. The use of the smaller focused antigenic portion of SP, the RBD
2. Recombinant fusion of RBD with human IgG1 Fc (SP/RBD-Fc)
3. Emulsification of SP/RBD-Fc in the water-in-oil adjuvant, Montanide ISA 720

The following are explanations of the above features:

1. The focused immunogenicity of the RBD Ag leads to only those Abs that bind this region on SARS-CoV-2 SP to prevent virus binding to the ACE2 target protein on host cells, thus inhibiting infection. This is in contrast to the use of a whole SP Ag vaccine that risks the generation of non-RBD-binding Abs that actually facilitate viral infection by tagging the virus for Fcγ receptor (FcγR)-mediated uptake by macrophages that act as cellular factories for viral replication (i.e., ADE). Perhaps of even greater value is that the small size of RBD provides for at least a 10-fold greater production yield relative to SP (Akston's unpublished observation).
2. However, simply injecting such a small foreign protein fragment alone as a vaccine Ag would not be expected to induce a strong enough B cell (Ab) or Th1 cell response from a naïve immune status. Therefore, Akston created the subunit vaccine, AKS-452, comprised of a bivalent analog of RBD recombinantly fused to a human IgG1 Fc moiety (**Figure 3**) that (i) facilitates the focused delivery of the RBD Ag to local APCs that internalize SP/RBD-Fc via FcγRs, and then process and present RBD fragments (**Figure 4**) [34] to $CD4^+$ Th cells that in turn promote ("help") B cell activation and anti-SARS-CoV-2 RBD IgG (i.e., Ab) production (**Figure 2**). In addition, a more direct and unique mechanism of AKS-452 is its direct binding to existing SARS-CoV-2-specific memory B cells through their Ag-specific B cell receptors (BCRs). Such binding triggers activation signals upon BCR cross-linking via the RBD bivalency feature of AKS-452 that leads to enhanced proliferation and anti-SARS-CoV-2 IgG production in the absence of $CD4^+$ Th cells (**Figure 2**). Indeed, fusion of IgG Fc with a different RBD fragment derived from the SP of the SARS virus (i.e., SARS-CoV) has been demonstrated to impart significant adjuvant activity relative to the very low immunogenicity of the SARS-RBD fragment alone [15, 17]. In fact, this human IgG Fc-fusion enhancing approach has been demonstrated with the development of a MERS vaccine containing recombinant protein of a truncated MERS SP/RBD fragment (residues 377-588) fused to human IgG Fc that increased immunogenicity via FcγR-binding on APCs, in addition to increasing the in vivo half-life and stability [18, 32]. That is, Fc enhances the systemic half-life and bioexposure of RBD to more APCs residing throughout the body due to binding the neonatal FcR (FcRn) expressed on endothelial cells that enables long serum half-lives of most monoclonal Ab (mAb) therapeutics. Another advantage of fusing RBD with Fc is the bivalency of the Ag per Fc molecule (i.e., two RBD fragments to one Fc fragment) that improves the stoichiometric quantity of Ag delivered to APCs.
3. However, the Fc feature of Akston's vaccine Ag may have limited use to only RBD-primed individuals who had a prior infection of SARS-Cov-2, both asymptomatic and symptomatic for COVID-19. That is, FcγR binding and activation signals in APCs, while known to provide significant signals for Ag presentation, are typically not strong enough to achieve the activation threshold of naïve lymphocytes, although these signals would be expected to re-activate the low-threshold of memory T and B cells of primed

individuals (**Figure 1**). In addition, the weak signaling of FcγR in naïve lymphocytes does not ensure commitment to Th1 development. To overcome these limitations, Akston enhanced vaccine potency by formulating the SP/RBD-Fc Ag in the adjuvant, Montanide ISA 720, which is a water-in-oil substance containing the Th1-promoting and human-safe squalene oil. Squalene is a natural organic compound originally obtained for commercial purposes (primarily from shark liver oil), is a biochemical intermediate in plants and animals (including humans) and has been approved as an adjuvant component in several human vaccines [35]. Therefore, Montanide ISA-720 was developed for its low reactogenicity in humans and its closely related form, ISA 51, is an EU-approved adjuvant for a cancer vaccine [36-38]. Note that Montanide ISA 720 has been used in more than 200 clinical trials involving cancer, AIDS, malaria or autoimmune disease vaccines involving an accrual of more than 20,000 patients and has demonstrated an excellent clinical safety profile in addition to its strong promotion of immunogenicity and Th1 responses [37-48]. In summary, the Fc moiety on AKS-452 is designed to act as a mild adjuvant via inducing activation signaling to the APC via FcγRs and is designed to work in concert with a strong classical adjuvant, such as Montanide ISA 720, to enhance the duration of Ag exposure to APCs and perhaps direct Ag entry into lymph nodes locally and systemically where additional APCs reside. As a consequence, the Fc moiety in combination with an adjuvant is expected to create a dramatic dose-sparing potential for both the Ag and adjuvant such that the risk of reactogenicity (a safety concern) is dramatically reduced; i.e., too much adjuvant that over-activates many APCs and other innate immune cells can lead a systemic inflammatory reaction termed *reactogenicity*. Such reactogenicity is induced acutely after injection and is not mediated by T and B cells.

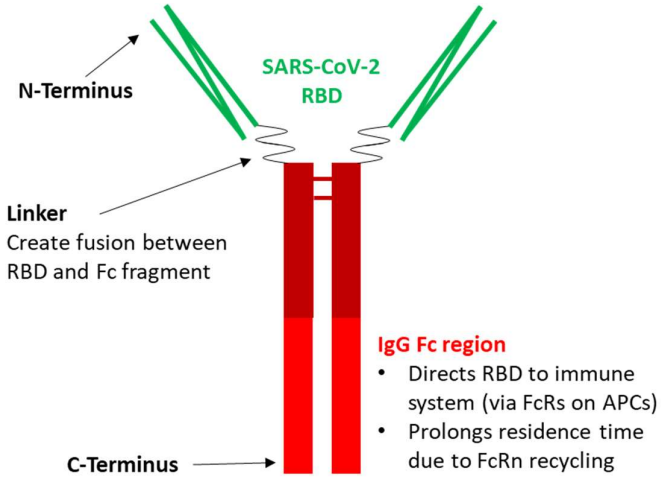

Figure 3: Schematic representation of AKS-452: 1. SARS-CoV-2 SP/RBD – enables COVID-19-specific Ag presentation to the immune system, 2. Linker – 21 amino acid sequence creating the fusion between the SP/RBD and the Fc fragment, 3. Human IgG1 Fc fragment – directs antigen presenting cells (APCs) to take up and process the SP/RBD Ag via FcγRs and enhances residence time via FcRn recycling.

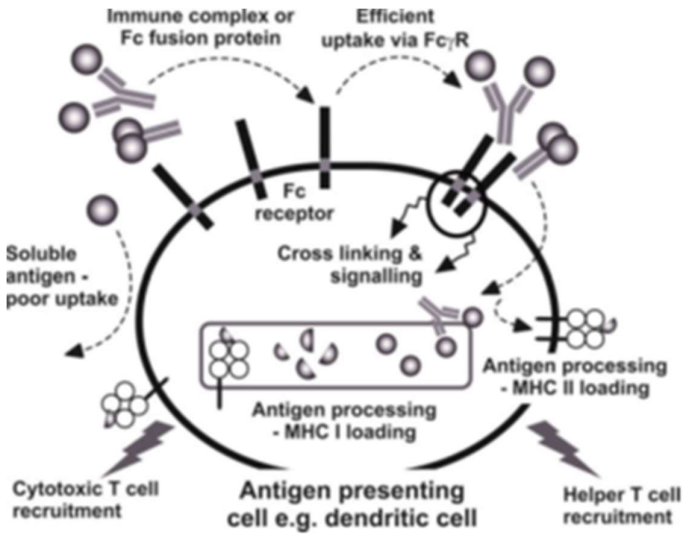

Figure 4: Fc-Ag fusion protein binds to antigen presenting cells (APCs) via the Fc_gRs and is processed efficiently in the cytoplasm where Ag processing loads Major Histocompatibility Complex (MHC) class II molecules while endosomal processing loads MHC class I molecules, triggering the respective predominant cellular responses (i.e., $CD4^+$ T cell with MHCII and CD8+ T cell with MHCI) [34].

1.3 Pre-clinical experience

1.3.1 Summary of pre-clinical immunogenicity studies

Akston Biosciences' clinical development vaccine candidate, AKS-452, is a biologically engineered SARS-CoV-2-SP/RBD-Fc fusion protein that is intended to be an injectable therapy for inducing and/or augmenting neutralizing IgG antibody (Ab) titers against the novel SARS-CoV-2 virus in patients to address the COVID-19 pandemic. Akston's SP/RBD-Fc protein vaccine has been evaluated in mouse, NHP, and rabbit immunization studies for capacity to safely induce high-titer neutralizing Ab responses using different dosing strategies. The SP/RBD-Fc vaccine antigen (AKS-452) has been evaluated in formulations containing a panel of adjuvants designed to amplify immunogenicity in which the water-in-oil adjuvant, Montanide ISA 720, performed optimally and was selected for clinical evaluation with AKS-452. AKS-452 emulsified in ISA 720 adjuvant was initially evaluated in BALB/c mice for immunogenicity and the capacity to induce production of antibodies (Abs) that bind and neutralize the SARS-CoV-2 virus Spike Protein (SP). Upon binding to the Receptor Binding Domain of the SP (SP/RBD), these vaccine-induced Abs prevent the virus from attaching to the host target protein, ACE2, expressed on a variety of cell types including endothelial cells of the lung, blood vessels and neurons. After a single injection of 1 to 100 µg in ISA 720, substantial neutralizing Abs were induced in mice, NHPs, and rabbits that 1) bound to recombinant SP/RBD, 2) inhibited recombinant ACE2 from binding recombinant SP/RBD, and 3) prevented the SARS-CoV-2 virus from infecting live VERO-E6 cells that naturally express ACE2. Notably, the potency of the AKS-452 vaccine to induce neutralizing Abs in each of the above animal models was on par or above the neutralization capacity of human serum obtained from convalescent COVID-19 subjects, setting the expectations that AKS-452 combined with ISA 720 adjuvant should induce sufficient protection in humans. The animal model experiments and additional research assays were used to demonstrate that the optimal dose level in adjuvant was in the 10 to 100 µg range, that two doses given either s.c. or i.m. induced maximum immunogenic responses, and that 3 doses of 100 µg in ISA 720 given 14 days apart showed no toxicities or serious adverse effects in a GLP toxicology study in rabbits. It was noted that s.c. administration in preclinical models gave a trend of higher titers as compared to the i.m. groups, but this difference was not found to be statistically significant. Therefore, the focus of the clinical study will solely focus on subcutaneous administration. In terms of preclinical safety, no significant findings were demonstrated, though mild and transient injection site reactions, likely due to the ISA 720 adjuvant, were observed.

2. OBJECTIVES

2.1 Primary Objective:

To evaluate the safety, tolerability and humoral immunogenicity profile (i.e., SP/RBD-specific IgG titers) of AKS-452 following a one-injection regimen and a two-injection regimen administered 28 days apart in a combined Phase I/II single-center, open-label clinical study.

2.2 Secondary Objectives

1. To evaluate the inhibitory/neutralization potency of the SP/RBD-specific IgG titers induced by AKS-452 and to estimate peak titers and duration of the response.
2. To evaluate the Th1/Th2 immune response profile.
3. To achieve these objectives, the following will be measured:
    a. Anti-SARS-CoV-2 SP/RBD IgG titers at days 0, 28, 56, 90, and 180.
    b. Serum titer inhibition of recombinant ACE2-SP/RBD binding and/or neutralization of live SARS-CoV-2 virus infection of live cells (Plaque Reduction Neutralization Test, PRNT) at days 0, 28, 56, 90, and 180.
    c. T-cell responses measured ex vivo using PBMCs to measure SP/RBD-specific T cell production of IFN-g and Th1/Th2/Th17 related cytokines via ELISpot or other Ag-specific flowcytometric-based assays on days 0, 28, 56, 90, and 180.

3. STUDY DESIGN

The study is designed as a combinatorial single-center open-label phase I and II clinical study design:

I. a phase I dose-finding and safety study combined with,
II. a phase II safety / efficacy study on the biological activity against COVID-19

If successful, the proposed Phase I/II study will warrant more extensive development towards a phase III clinical study. The study will have a duration of approximately 6 months and will be executed at the University Medical Center Groningen (UMCG), The Netherlands, and the study is financially supported by the subsidizing party Akston Biosciences.

Akston's clinical SP/RBD-Fc vaccine candidate is AKS-452, although Akston has experience with several protein antigen variations that have been initially evaluated in mouse immunization studies for capacity to induce high-titer neutralizing Ab responses using different dosing strategies. Such SP/RBD-Fc proteins have been evaluated as stand-alone vaccines and in formulations containing different adjuvants for screening and selection of a lead adjuvant. Key findings in mice and other species described below in the preclinical experience, Section 1.3. demonstrate the immunogenic effectiveness of the Fc moiety in addition to the strong immunogenic nature of Montanide ISA 720 that supports its use as an adjuvant.

The UMCG / Akston clinical trial strategy follows those of other organizations currently entering or having finalized Phase III trials with COVID-19 vaccines [20, 31, 49, 50] (A more detailed description of the clinical study design is illustrated in **Figure 5**).

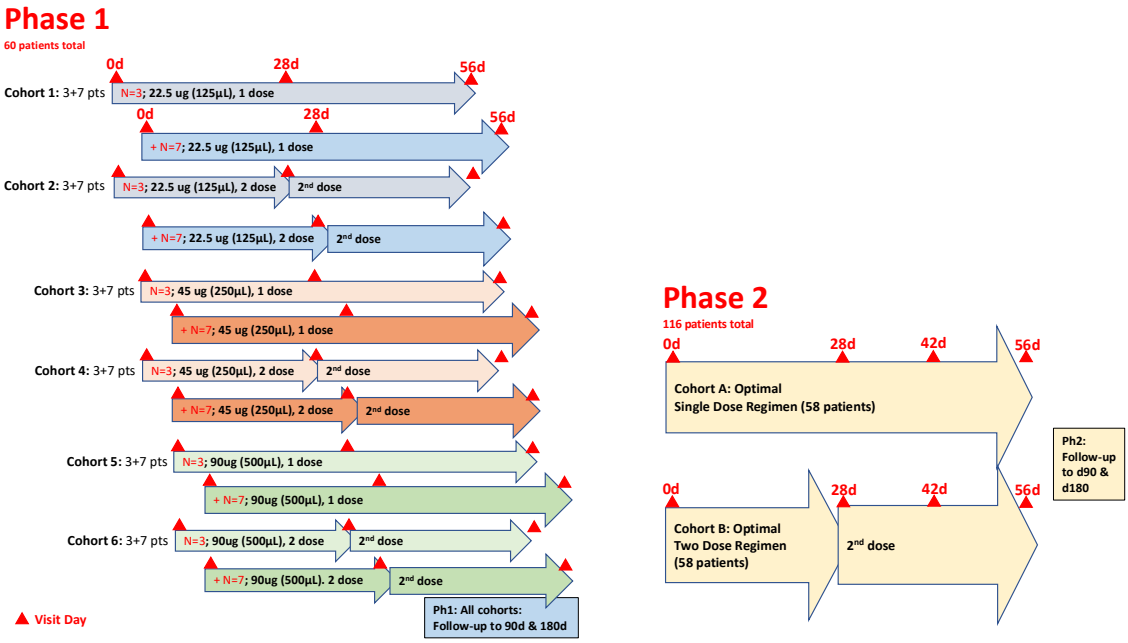

Figure 5: Strategic design of the Phase I/II safety and immunogenicity trial cohort arms for adjuvanted AKS-452. In the Phase I study, cohorts 1 and 2 (22,5 µg) will be initiated simultaneously. After 3 successive subjects with acceptable cohort safety, the remaining 7 subjects are included and cohorts 3 and 4 (45 µg) will be initiated simultaneously. In an identical way, cohorts 3 and 4 will be expanded to 10 subjects per group and cohorts 5 and 6 (90 µg) will be initiated. In an identical way, cohorts 5 and 6 will be expanded to 10 subjects per group. After an interim analysis of Phase I will occur after the last patient of Cohort 6 completes their day 56 visit, and the optimal

dose regimen will be selected according to a predefined decision-making flowchart (**Figure 6**). The decision is based on safety (number of AE's and SAE's attributable to the candidate vaccine AKS-452) and the number of subjects with seroconversion per cohort. For both the single and two-dose regimens the optimal dose will be studied simultaneously in Phase 2 with 58 subjects each for Phase 2 Cohorts A and B. For phase II the participating subjects will be randomized between the A and B cohort.

In Phase I, three dose-levels (22,5, 45 or 90 µg) will be administered subcutaneously (to 10 subjects per Cohort, for six Cohorts numbered 1-6). For each dose level, two dose regimens will be examined, one with a single injection of adjuvanted AKS-452 and a second cohort with a second injection of adjuvanted AKS-452 administered 28 days after the initial dose. In all cohorts, safety parameters, anti-SARS-CoV-2 spike protein RBD IgG titers and neutralizing IgG titers will be evaluated at pre-dose and also at 28, 56, 90 and 180 days after the first dose for Cohorts 1, 3 and 5. Similarly Cohorts 2, 4 and 6 will be evaluated on the same visit days, but on day 28, Cohorts 2, 4 and 6 will receive a second dose of adjuvanted AKS-452.

The Phase II portion of the study will be initiated only after a safety review and immunogenicity data assessment that is conducted after the last patient in Cohort 6 completes their day 56 visit, at which time the optimal single-dose and optimal two-dose regimens will be selected for Phase 2 (Cohorts A and B in **Figure 5**). The selection and continuation decision-making shall be based on the flowchart in **Figure 6**. Upon successful review, the Phase II arm of the study will include 58 subjects per cohort with all subjects receiving adjuvanted AKS-452 vaccine, with the rationale for the number of patients provided below in Section 4.4 'sample size calculation'. A statistical re-assessment will be conducted after the interim analysis of phase I in order to determine the definitive number of subjects for phase II based on anti-SARS-CoV-2 SP/RBD IgG Ab titer data from Phase I. For phase II the participating subjects will be randomized between the A and B cohort to get the most homogeneous distribution.

To evaluate safety, the Phase I arm of the proposed study has proposed the use of a sentinel dosing strategy with dose escalation. Three dose levels (DLs) selected from preclinical studies will be evaluated in a 6x3 adaptive, safety/dose-finding design that comprises an initial sentinel dosing and dose escalation phase using just 3 subjects per cohort, and a second part in which an additional 7 subjects per cohort are enrolled to a maximum cohort size of 10 subjects per cohort.

The Phase II study will be initiated with 58 subjects per cohort (all subjects will receive the adjuvanted AKS-452 vaccine). It is considered unethical, in the light of the presence of an approved vaccine in the community (i.e. the recent PfizerNBiotech and Moderna vaccine approvals), to include a placebo arm in the phase II part of the clinical study, which is supported by Dal-Ré et al. [54], The outcomes of this 4-month Phase I/II adaptive trial design will allow for the initiation of a pivotal Phase III clinical study, possibly a non-inferiority study design due to the presence of an already approved vaccine in the community, for registration [53]

Successful completion of the Phase I/II trial in addition to a sufficient scale-up of the GMP manufacturing process will enable production of a sufficiently large quantity of doses for Phase III and the future wide scale vaccine treatment of the world population with AKS-452 in ISA 720. Akston's current estimates indicate that a single 2,000 L bioreactor production train run could yield enough material for such an expected 45 µg dose of drug substance to treat approximately 100 million people receiving a single dose. A 2,000L bioreactor production train running ten times per year would therefore supply over 1 billion doses of AKS-452 at 45 µg per dose. This manufacturing capacity is extremely significant and far surpasses the production throughput and costs of the other viral-based, nucleic acid-based, and full-length recombinant SP subunit-based vaccines. The potency, manufacturability, and mechanism-of-action of the

AKS-452 Fc-fusion protein formulated with adjuvant, therefore, offer an opportunity to immunize billions of people globally as frequently as necessary to maintain high levels of neutralizing anti-SP/RBD Ab titers throughout the population, regardless of COVID-19 status; i.e., boosting of those with prior SARS-Cov-2 infection or of those who received a prior vaccination.

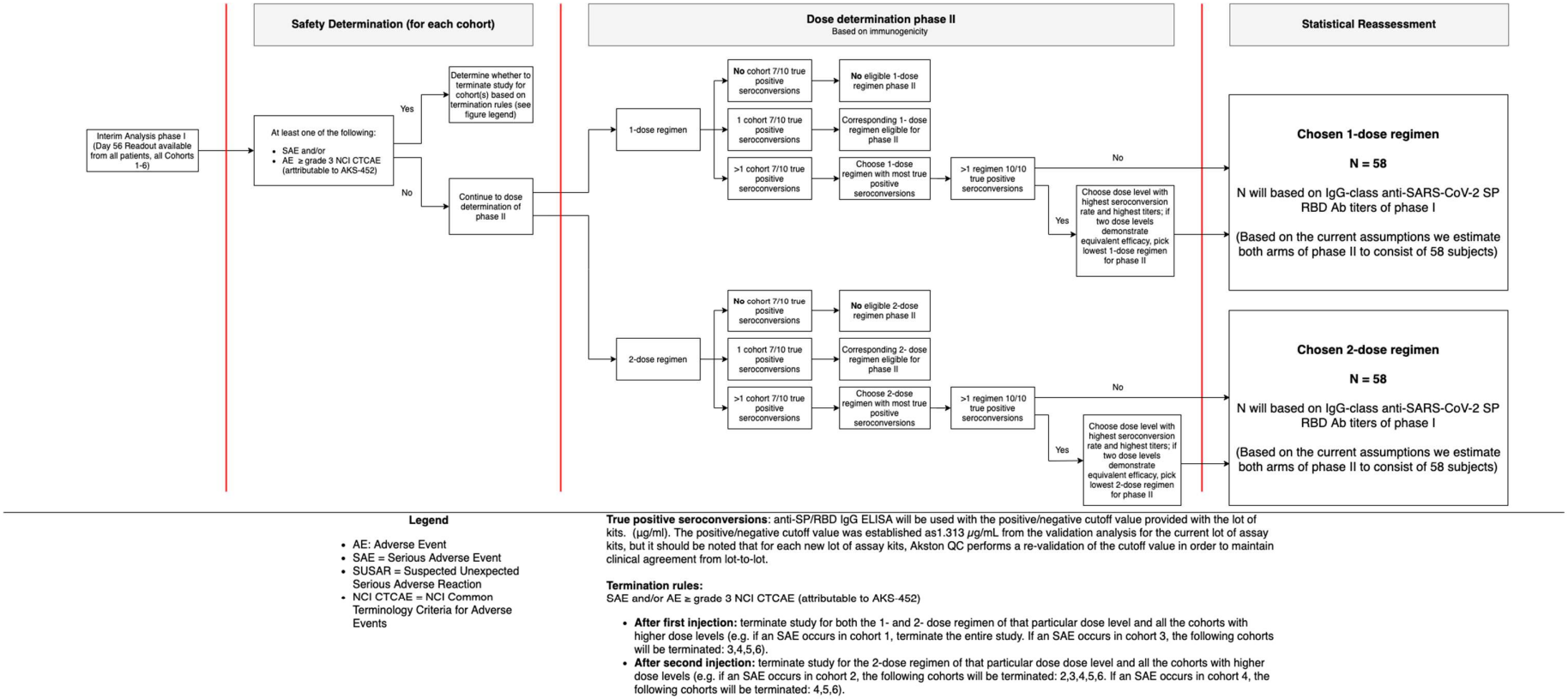

Figure 6: Decision-making flowchart for the dose selection for both the single and two dose regimens for Phase II, based on predetermined safety and efficacy criteria. True positive seroconversion is based on the anti-SP/RBD IgG ELISA positive/negative cutoff value criteria with the quantitative cut-off value defined by the specific batch of the assay kit expressed as µg/mL. The positive/negative cutoff value was established as 1.313 µg/mL from the validation analysis for the current lot of assay kits, but it should be noted that for each new lot of assay kits, Akston QC performs a re-validation of the cutoff value in order to maintain clinical agreement from lot-to-lot (historically the cutoff value has ranged between a narrow range).

Table 1: Target product profile (TPP) of the SARS-CoV-2 vaccine, AKS-452: a recombinant human IgG Fc fusion protein with the SARS-CoV-2 spike protein receptor-binding domain (SP/RBD-Fc) emulsified in the water-in-oil adjuvant, Montanide ISA 720.

Item	Desired target
Drug structure and formulation	A subunit vaccine comprised of: • a human IgG Fc moiety • a linker • a recombinant SP/RBD • the water-in-oil adjuvant, Montanide ISA 720
Indications	• A primary vaccine to protect against lethal infection caused by the SARS-CoV-2 virus • A boosting immunization for anti-SARS-COV-2 SP/RBD Ab positive individuals from prior infection (or immunization) to maintain immunity
Mechanism	• Induction of SP/RBD-neutralizing antibodies without causing undesired immunopotentiation such as: o Th2-type immunopathology, including lung eosinophilia, and/or o Antibody Dependent Enhancement (ADE) of infectivity • Fc moiety focuses SP/RBD Ag to immune cells that express Fc receptors in addition to increasing Ag half-life allowing for “extended immune stimulation” to achieve single dosing • The water-in-oil adjuvant, Montanide ISA 720, enhances the “potency” of the Fc-based Ag that allows for dose-sparing
Target population	• Adults and children >18 years of age • Suitable for adult healthcare workers • At-risk, adults > 65 years old • Individuals with underlying diabetes or hypertension
Route of administration	Subcutaneous
Product presentation	Multi-dose vials; 1.0 mL fill with ≥0.8 mL extractable volume
Dosage	• 10 µg to 100 µg of the SP/RBD-Fc antigen, presented as the emulsion with ISA 720.
Dosage schedule	• Maximum of two immunizations regardless of age, with the second injection given 4 weeks after the first immunization • Potential booster immunizations no more frequently than every 6 months
Warnings and precautions/ pregnancy and lactation	• Mild-to-moderate local injection site reactions, such as erythema, edema and pain, the character, frequency and severity of which is similar to licensed recombinant protein vaccines. • Less than 0.01% risk of urticaria and other systemic allergic reactions. • Incidence of SAEs no more than licensed comparator vaccines

Expected efficacy	Minimum of 70% efficacy in inducing protective Ab titers, potentially leading to herd immunity and a significant reduction in SARS-CoV-2 associated infections
Co-administration	All doses may be co-administered with antiviral drugs and/or other vaccines used in public health emergencies.
Storage	Refrigeration between 2-8°C. Can be out of refrigeration (at temperatures up to 25°C) for up to 2 weeks or longer, based on current stability studies.
Shelf-life	12 months at -80°C, currently one month at room temperature upon thaw or one month at 2-8°C upon thaw.
Product registration	Licensure by European regulatory agencies and FDA in the US

4. STUDY POPULATION

4.1 Population (base)

Healthy volunteers, aged 18-65 years old, are recruited for this study.

The study population will be a representation of the general population in terms of characteristics as sex, ethnical background etc. The seroprevalence for COVID19, the percentage of people from the general population who have antibodies against COVID19 and thus have had COVID19, ranges between 3-5% according to the RIVM. (https://www.rivm.nl/pienter-corona-studie/resultaten) Therefore, we expect to be able to recruit a sufficient number of subjects.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age 18-65 years (extremes included), males and females
- SARS-CoV-2 serology (an anti-SARS-Cov-2 SP-specific IgG ELISA):
    - Tests negative for IgG titer and no known prior SARS-Cov-2 infection
- Body mass index (BMI) between 19.0 and 30.0 kg/m^2, inclusive
- General good health, without significant medical illness, as determined via physical exam findings, ECG or vital signs
    - Note: one retest of vital functions and ECG is allowed within the screening window
- No clinically significant laboratory abnormalities as determined by the investigator
    - Note: one retest of lab tests is allowed within the screening window
- Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the subject understands the purpose and procedures required for the study and is willing to participate in the study
- Willing to adhere to the prohibitions and restrictions specified in this protocol
- No invitation is received to get a registered vaccine within the first 2 months after the moment of participation in this study.
- Negative hepatitis panel (including hepatitis B surface Ag and anti-hepatitis C virus Abs) and negative human immunodeficiency virus Ab and Ag screens at screening
- Female subjects should fulfil one of the following criteria:
    - At least 1 year post-menopausal (amenorrhea >12 months and/or follicle-stimulating hormone >30 mIU/mL) at screening;
    - Surgically sterile (bilateral oophorectomy, hysterectomy, or tubal ligation);
    - Will use adequate forms of contraceptives from screening to discharge.
- Female subjects of childbearing potential and male subjects who are sexually active with a female partner of childbearing potential must agree to the use of an effective method of birth control from screening to discharge
    - Note: medically acceptable methods of contraception that may be used by the subject and/or partner include combined oral contraceptive, contraceptive vaginal ring, contraceptive injection, intrauterine device, etonogestrel implant, double barrier, sterilization and vasectomy
- Female subject has a negative pregnancy test at screening and upon check-in at the clinical site.
    - Note: pregnancy testing will consist of a serum pregnancy test at screening and urine pregnancy tests at other (dosing) visits, in all women.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Pregnant or breast-feeding females
- Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, hematologic, rheumatologic, endocrine, autoimmune, or renal disease
- Any laboratory test which is abnormal, and which is deemed by the Investigator(s) to be clinically significant
- Behavioral or cognitive impairment or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and cooperate with the study protocol
- Current alcohol/illicit drug/nicotine abuse or addiction: history or evidence of current drug use or addiction (positive drug screen for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, or opiates) or excessive use of alcohol at screening and Day -2.
- Presence of any febrile illness (T > = 38.0°C or lab confirmed viral disease (PCR)) or symptoms suggestive of a viral respiratory infection within 1 weeks prior to vaccination. Patients will be screened for SARS-Cov-2 with an EUA-approved PCR test at screening, and at day 0, 28, and 56 at a minimum
- Use of corticosteroids (excluding topical preparations for cutaneous or nasal use) or use of immunosuppressive drugs within 30 days before inoculation
- A history of anaphylaxis, history of allergic reaction to vaccine, known allergy to one of the components in AKS-452. Mild allergies without angio-edema or treatment need can be included if deemed not to be of clinical significance (including but not limited to allergy to animals or mild seasonal hay fever)
- A history of asthma within the past 10 years, or a current diagnosis of asthma or reactive airway disease associated with exercise
- Receipt of a licensed vaccine within 4 weeks prior to viral inoculation
- Received any (experimental) SARS-CoV-2 vaccine or drug
- Receipt of blood or blood-derived products (including immunoglobulin) within 6 months prior to vaccination.
- Receipt of another investigational agent within 30 days or 5 times the product half-life (whichever is longest) prior to vaccination
- Shares household with/works with immunocompromised individual(s), adults with significant cardiopulmonary disease, persons with significant asthma, institutionalized elderly or elderly with functional disability
- Deprived of freedom by an administrative or court order or in an emergency setting
- Any condition that in the opinion of the principal investigator (PI) would jeopardize the safety or rights of a person participating in the trial or would render the person unable to comply with the protocol.

4.4 Sample size calculation

The study consists of a combined Phase I/II clinical study. Phase I will be a 6x3 dose-finding design, after which we will assess safety after each dosing cohort (n=3 subjects). The following stopping-rule will be applied on a cohort-by-cohort basis (see also flow-chart, figure 6): for a particular cohort, any SAE or AE $\geq$ 3 (according to NCI Common Terminology Criteria for Adverse Events [CTCAE]) attributable to AKS-452.

In the case where none of the included subjects in a given cohort has an AE $\geq$ 3 or SAE attributable to AKS-452, we will expand the remainder of that dosing cohort with an additional 7 patients. After completing the 6 cohorts with a total of 10 patients each (overall 60 patients), a safety assessment during the interim analysis between Phase I and II will be executed. On the basis of this safety assessment conducted on each cohort and a minimum project seroconversion rate of 7/10 for each cohort (where seroconversion is defined as a true positive based on the SP/RBD IgG ELISA assay positive/negative cutoff criteria using the quantitative cut-off value defined by the assay kit batch expressed in µg/mL. The positive/negative cutoff value was established as 1.313 µg/mL from the validation analysis for the current lot of assay kits, but it should be noted that for each new lot of assay kits, Akston QC performs a re-validation of the cutoff value in order to maintain clinical agreement from lot-to-lot), we will determine the optimal single-dose and the optimal two-dose cohorts for the single-dose s.c. regimen and two-dose s.c. regimen, respectively, for the Phase II study. If two dosing cohorts are eligible for the subsequent phase II study, then the lowest dose-regimen will be selected. During phase II the subjects will be randomized between the single-dose and two-dose regimen by using an balanced number generator.

The sample size of the two cohorts in phase II is determined based on the following justification. We assume an experiment in which the proportion of seroconversion will be measured. We want to power at 0.8 on a true seroconversion with a 95% certainty that a true seroconversion rate is above 0.7. This is similar if the lower limit of the 95% Confidence Interval (CI) of seroconversion is above 0.7. If the measured seroconversion is around 0.84, we need 58 subjects, with a sufficient small CI to confirm this statistically, as described below:

The sample size calculation is based on a non-inferiority test:

Suppose the AKS-452 vaccine has a target for a minimum proportion of seroconversion of 70%. Clearly, higher proportions are better, but we want to design a test to prove that the drug is not appreciably inferior to this target with an α=0.05 and with a power equal to 0.80.

The hypotheses of the non-inferiority test are

H0: p <= p0
H1: p > p0
where p0=0.70

As it is of interest to see the sample sizes required can vary significantly for a range of possible seroconversion proportions from 0.80 to 0.92, and therefore we suggest the following (See **Table 2** and **Figure 7**):

Regarding the fact that no human data yet exist on the proportion of seroconversion based on the AKS-452 vaccine, we propose the following: i) assume a seroconversion rate of 0.84 for a total of 58 subjects per each final dosing cohort (single-dose and two-dose cohort), totaling 116 patients for phase II, ii) introduce an adaptive Final Statistical Re-assessment between I and II, based on the actual antibody titers derived from the selected cohorts in Phase I, as depicted in the Flow-Chart in **Figure 6**.

Subjects will be recruited from March 2021 onwards, until a maximum number of 176 subjects (total Phase I plus Phase II) is reached.

Table 2: The POWER procedure Z test for binomial proportion

Fixed Scenario Elements	
Method	Normal approximation
Number of Sides	U
Null Proportion	0.7
Nominal Power	0.8
Variance Estimate	Null Variance
Alpha	0.05

Computed N Total			
Index	**Proportion**	**Actual Power**	**N Total**
1	0.80	0.800	119
2	0.82	0.802	81
3	0.84	0.803	58
4	0.86	0.803	43
5	0.88	0.806	33
6	0.90	0.812	26
7	0.92	0.802	20
8	0.94	0.807	16

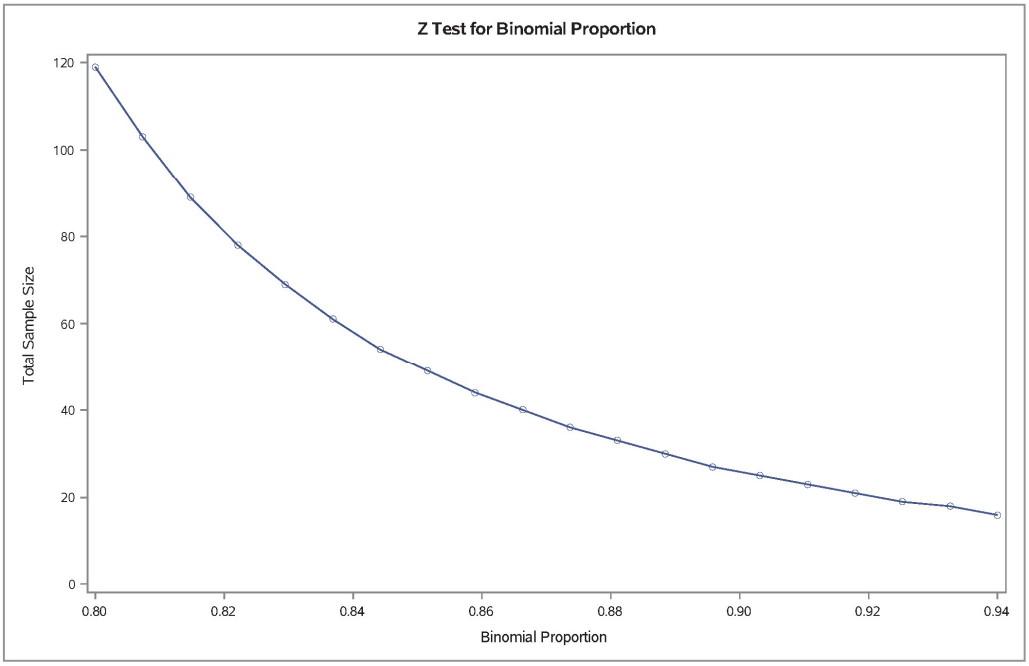

Figure 7: The POWER procedure Z test for binomial proportion

5. TREATMENT OF SUBJECTS

The subjects will receive an immunological treatment in the form of a vaccination.

5.1 Investigational product/treatment

The investigational product is a fusion-protein based vaccine.

5.2 Use of co-intervention (if applicable)

There are no-specific life-rules, diet or co-medication for the duration of the study when subjects participate.

5.3 Escape medication (if applicable)

In case of an allergic reaction, the Standard Operating Procedure ‘Anaphylactic Reaction’ is activated and in summary consists of the following escape medication: anti-histamine medication (e.g. cetirizine 10 mg p.o., dexamethasone (4 mg p.o.) and in case of a severe reaction epinephrine, oxygen and alarm UMCG Emergency Crash Team at the ward.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

AKS-452 is a recombinant fusion protein comprising a portion of the SARS-CoV-2 SP/RBD and an Fc fragment containing a portion of the hinge, and the full CH2, and full CH3 domains of the human IgG1 Fc fragment that are connected via a covalent peptide linker sequence (**Figure 3**). The resulting SARS-CoV-2 SP/RBD-Fc fusion protein, when made in CHO cells, is expressed as the homodimer of two identical Fc fusion protein chains that are connected via multiple disulfide bonds. The homodimer is encoded by a single nucleic acid molecule, much in the same way that a monoclonal antibody heavy chain is encoded and expressed in CHO cells. Detailed information on the production of AKS-452 can be found in the IMPD, section 2.1.S Drug Substance and 2.1.P Drug Product

6.2 Summary of findings from non-clinical studies

A summary of findings from non-clinical studies can be found in the Investigators Brochure (IB) and Investigational Medicinal Product Dossier (IMPD), in:

- IB AKS-452 version 2.0, section 4
- IMPD AKS-452 version 2.0, section 2.2

6.3 Summary of findings from clinical studies

This protocol represents a first-in-human study and therefore, no data or findings from clinical studies are available.

6.4 Summary of known and potential risks and benefits

No clinical data on AKS-452 is available. The repeated dose toxicity study in New Zealand White Rabbits revealed that no significant mortality or organ weight changes were observed at the main study termination. Mild effects, such as increases in fibrinogen, were most likely due to the Montanide ISA 720 adjuvant during the Dosing Phase which subsided during the recovery phase. The expected macroscopic and microscopic transient redness of the injection site were also likely due to adjuvant. These findings from the animal toxicity studies on the adjuvant are in line with clinical findings of adverse events in clinical trials. A potential risk is an allergic reaction after the injection of AKS-452. In this trial, to guard the subjects' safety during and after AKS-452 injection, they will be closely observed until 2 hours post injection by a medical doctor and qualified nurses, who will be ready to promptly intervene. Vital signs and will be monitored and recorded during this period.

6.5 Description and justification of route of administration and dosage

In the phase I first-in-human trial, three different dosages will be investigated. Based on the preclinical trials in rabbits and non-human primates we anticipate that two doses (separated by 28 days) of either 22.5, 45, or 90 µg or one dose of either 22.5, 45, or 90 µg will generate seroconversion to protect against SARS-CoV-2 infection, although the optimal dosage will be derived from the study outcome. The route of administration for the human clinical setting is subcutaneous injection based on preclinical studies. The trial consists of six study arms (**Figure 5**), where the subjects in the first arm will be administered one subcutaneous injection of 22.5 µg AKS-452 mixed with Montanide ISA 720 in a final volume of 125 µl. The second cohort will receive the same dose, but with two injections per subject. The two injections will

be administered 28 days apart. In an identical way a 45 µg dose (with a final volume of 250 µl) will be examined in a 1 and 2 dose regimen in cohort 3 and 4. In cohort 5 and 6 the 90 µg dose (500 µl total volume) will be tested with both dose regimens.

6.6 Dosages, dosage modifications and method of administration

AKS-452 will be mixed with Montanide ISA 720 and doses of 22.5 µg (with a final volume of 125 µl), 45 µg (with a final volume of 250 µl) or 90 µg (with a total volume of 500 µl) will be injected subcutaneously or intramuscularly. For more details see 6.5.

6.7 Preparation and labelling of Investigational Medicinal Product

AKS-452 has been produced to GMP standards. The final product has been verified by an identity method based on SEC-HPLC. The product has been filled in vials under GMP conditions to a final concentration of 600 µg/mL in phosphate buffered saline (pH 7.2). The vials are stored frozen at -80°C, but are thawed on day of use. A copy of the primary and secondary label according to GMP standards is provided in the IMPD.

6.8 Drug accountability

The investigational agents are stored at the (co-)investigators pharmacy (Department of Clinical Pharmacy, University Medical Center Groningen, The Netherlands). If investigational agents would need to be shipped (for example after creating aliquots of the study compound by an external subcontractor), they will be shipped to the Principal Investigator's authorized designee, who will check and record the amount and condition of the agents received. All shipments of investigational agent will include an enclosed packing slip; the slip must be signed by the designee and returned to the shipper to verify receipt of investigational agent. At the end of the study or as directed, all used and unused supplies, including partially used or empty containers, will be disposed of or transferred in accordance with local written procedures, if applicable. Any disposal or transfer of investigational agent shall be noted on the investigational drug disposition log and signed-off by a second person.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable

7.2 Summary of findings from non-clinical studies

Not applicable

7.3 Summary of findings from clinical studies

Not applicable

7.4 Summary of known and potential risks and benefits

Not applicable

7.5 Description and justification of route of administration and dosage

Not applicable

7.6 Dosages, dosage modifications and method of administration

Not applicable

7.7 Preparation and labelling of Non-Investigational Medicinal Product

Not applicable

7.8 Drug accountability

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

To evaluate the safety and tolerability and humoral immunogenicity profile of AKS-452 following one injection or two injections administered 28 days apart in a combinatorial phase I/II clinical study.

- Main study parameter for phase I is safety and dose-finding in a 6x3 dose-finding design, with an expansion to 10 subjects per dosing-cohort (6x10 in total).
- Main study parameter for phase II is safety and initial efficacy measurement as a preparatory study towards a sufficiently powered phase II study design comprising two study arms, each containing 58 subjects, with all subjects receiving the active vaccine.

8.1.2 Secondary study parameters/endpoints

- To evaluate the inhibitory/neutralization potency of the SP/RBD-specific IgG titers induced by adjuvanted AKS-452 and to estimate peak titers and duration of the response.
- To evaluate the Th1/Th2 immune response profile.

To achieve these objectives, the following will be measured:

- Anti-SARS-CoV-2 SP RBD IgG titers at days 0, 28, 56, 90, and 180
- Serum titer inhibition of recombinant ACE2-SP/RBD binding and/or neutralization of live SARS-CoV-2 virus infection of live cells (Plaque Reduction Neutralization Test, PRNT) at days 0, 28, 56, 90, and 180
- T-cell responses measured ex vivo using PBMCs to measure SP/RBD-specific T cell production of IFN-γ and Th1/Th2/Th17 related cytokines via ELISpot or other Ag-specific flowcytometric-based assays on days 0, 28, 56, 90, and 180.

8.1.3 Other study parameters (if applicable)

Not applicable.

8.2 Randomisation, blinding and treatment allocation

The study is a single-centre, open-label Phase I/II clinical vaccine-evaluation study. For Phase I No randomisation is applicable, nor blinding. All subjects included will receive the active vaccine. The Phase II study will be initiated with 58 subjects per cohort (all subjects will receive the active vaccine). For Phase II randomization of the subject will take place to get the most homogeneous distribution. It is considered unethical, in the light of the presence of an approved vaccine in the community (i.e. the recent PfizerNBiotech and Moderna vaccine approvals), to include a placebo arm in the phase II part of the clinical study, which is supported by Dal-Ré et al. [54], The outcome of the proposed 4-month Phase I/II adaptive trail design will allow for the initiation of a pivotal Phase III clinical study, possibly a non-inferiority study design due to the previous mentioned presence of an already approved vaccine in the community, for registration [53].

8.3 Study procedures

- AKS-452 will be mixed with Montanide ISA 720 at the pharmacy and doses of 22.5 (with a final volume of 125 µl), 45 µg (with a final volume of 250 µl) or 90 µg (with a total volume of 500 µl) will be injected subcutaneously.
- In the Table below, the Proposed clinical Schedule is provided.

	Screening Visit	**Visit 1 (4-hr) (first dose)**		**Visit 2 (boost)**	**Visit 3 (Boost + 28 days)**	**Visit 4**	**Visit 5**	**Discharge or Early Termination Visit[d]**
	Days -28 to -2[a]	**Visit 1a (day 0)**	**Visit 1b (Day 1)**	**Day 28±1**	**Day 56±1**	**Day 90±5**	**Day 180±5**	**Unscheduled**
Screening/Administrative/Other Assessments								
Informed consent	X							
Demography	X							
Eligibility criteria	X	X		X				
Medical and medication history	X							
Drug/alcohol screen[a]	X	X		X				
Serology[a]	X							
COVID-19 PCR	X	X		X	X	X	X	X
Discharge		X		X[c]				
Safety Assessments								
Physical exam	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Supine 12-lead ECG	X			X				X
Blood pregnancy test[b]	X							X
Urine pregnancy test		X		X				
Blood for safety	X[i]	X		X	X	X	X	X
Symptom Questionnaire		X	X	X	X			X
Concomitant therapy, AEs/SAEs	X	X	X	X	X	X	X	X
Study Agent Administration/Pharmacokinetics and Immunology Assessments								
Fc-conjugation Vaccine administration[c]		X		X				
Peripheral blood for humoral responses		X[e]		X[e]	X	X	X	X

Peripheral blood for PBMCs (cellular responses)		X[e]		X[e]	X	X	X	X
Peripheral blood for cytokine estimations	X	X[f]	X	X[f]	X	X	X	X

a) See Laboratory assessments for list of tests.
b) Pregnancy tests will be performed for all female subjects
c) After first and second (booster) vaccination, subjects are to be kept under medical supervision for 2h.
d) Subjects who terminate the study early will be asked to return to the clinical site within 14 days after discontinuation for safety assessments
e) Sample to be taken pre-vaccine administration
f) Samples to be taken pre and 2 hours post vaccine administration

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Subjects can also leave the study if the option to receive an already registered vaccination, prior to or after the administration of the candidate vaccine AKS-452, becomes available to him/her. Such participants will then be considered as drop-outs of this study and are kindly asked to complete the follow up period of 180 days for reasons of safety and efficacy. It will also provide the investigators with crucial information about protection against viral infection after multiple vaccine administrations. The request to complete the follow up will only apply when the subjects have received the AKS-452 vaccine prior to the approved vaccine. The time between the last injection with the candidate vaccine AKS-452 and the first injection of another already registered vaccine has to be at least 28 days.

8.4.1 Specific criteria for withdrawal (if applicable):

Not applicable.

8.5 Replacement of individual subjects after withdrawal

Subjects who are withdrawn may be replaced until the intended number of subjects is reached by this study. Study subjects that will receive an approved vaccine will be considered as drop-outs, although withdrawn subjects will be followed up as planned only when they have received the AKS-452 vaccine. The cohorts will be filled up to 10 subjects which only received the AKS-452 vaccine until day 180.

8.6 Follow-up of subjects withdrawn from treatment

After administration of the active vaccine, (serious) adverse events that occur in subjects that are withdrawn from the study procedure and preferably antibody titers, will still be recorded if possible.

8.7 . Premature termination of the study

Termination based on safety aspects: A multidisciplinary team with study investigators ([co]PI's and dedicated PhD-candidates) together with the sponsor will discuss safety aspects during the study procedures. Results will be reported immediately in case of any SUSAR to the external Data Safety Monitoring Board (DSMB).

The CTCAE stopping rules for first in human studies will be leading to grade a possible SAE and how to proceed if an SAE occurs.

The multidisciplinary team together with the sponsor will decide if cohorts need to be expanded after inclusion of 3 subjects for Phase I per cohort (Cohorts 1-6). The termination rules are as follows: When an SAE and/or AE ≥ grade 3 NCI CTCAE (attributable to AKS-452) occurs after the first injection: terminate study for both the 1- and 2- dose regimens of that particular dose level and all cohorts with higher dose levels (e.g. if an SAE occurs in cohort 1, terminate the entire study. If an SAE occurs in cohort 3, the following cohorts will be terminated: 3,4,5,6).
After second injection: terminate study for the 2-dose regimen of that particular dose level and all the cohorts with higher dose levels (e.g. if an SAE occurs in cohort 2, the following cohorts will be terminated: 2,3,4,5,6. If an SAE occurs in cohort 4, the following cohorts will be terminated: 4,5,6). Similar methodology shall be applied to the other cohorts in a like manner.

Termination based on other aspects: The study will be suspended based on urgent medical or ethical considerations as decided by the principal investigators (additional to section 8.7). In case of termination of the study, the sponsor, the institution, regulatory authorities (CCMO), and DSMB will be informed. Moreover, sponsor's medical monitor (or designee) may stop or suspend the trial due to safety concerns from the sponsor. Furthermore, sponsor may stop the trial due to other concerns.

8.8. Decision-making rules to proceed from Phase I to Phase II for each regimen type (single-dose and two-dose regimens)

Criteria Group 1 (Safety): Safety evaluation, including blood chemistry, and (S-)AEs. The scope shall include all blood work and (S-)AEs collected up to and including the Day 56 visit for all patients (including any patient reported (S-)AEs that occur after Day 56 and before the decision making period).

- Mandatory: A cohort must demonstrate that it has passed the criteria for (S-)AEs specified in section 8.7.

Criteria Group 2 (Efficacy): Use Day 56 seroconversion rates to determine if a cohort dose level and regimen is eligible for Phase 2 as follows.

- Mandatory: A cohort must demonstrate a minimum of 70% of subjects (7/10 subjects for all completed cohorts (in case a subject dropouts, he/she will be replaced with another subject and thus always totaling 10 patients per cohort for final analysis), that have seroconverted to IgG and have anti-SARS-CoV-2 SP/RBD IgG titers above the assay cut-off value for positive/negative determination as determined by Akston's validated ELISA assay.
- Mandatory: Give preference to cohorts with maximum seroconversion rates within a given regimen (either single-dose or two-dose regimens; up to 10/10 for a given cohort).

Preference Group Items:

- Safety: Evaluation of the frequency or type of (non-)serious AEs within a given cohort.
- Efficacy:
    - Evaluation of maximal antibody titers: potential preference for the cohort(s) with the highest Day 56 median numerical anti-SARS-CoV-2 SP/RBD IgG titers as determined on Akston's validated ELISA assay.

    - Evaluation of minimal antibody titer spread within cohorts: potential preference for cohorts that exhibit the smallest group variance with respect to the Day 56 titer cohort dataset (e.g. the smallest difference between the median and the first interquartile below the median).
- Dose level: if two or more cohorts have similar safety and efficacy, preference may be given to the lowest dose level cohort.

Thus, the best cohort from the single-dose regimens in Phase I that advances to Phase II is the one that meets the mandatory criteria for Criteria Group 1 and the mandatory Criteria from Group 2. However, if more than one cohort meets these criteria, then a careful evaluation of the above Preference Group Items will be performed to determine which one of the cohort dose levels shall be moved forward into the Phase II portion of the study.

Similarly, the best cohort from the two-dose regimens in Phase I that advances to Phase II is the one that meets the mandatory criteria for Criteria Group 1 and the mandatory Criteria from Group 2. However, if more than one cohort meets these criteria, then a careful evaluation of the above Preference Group Items will be performed to determine which one of the cohort dose levels shall be moved forward into the Phase II portion of the study.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance with section 10, subsection 4, of the WMO, the sponsor will suspend the study if there are sufficient grounds that continuation of the study will jeopardise the subject's health or safety. The sponsor will notify the CCMO without undue delay of a temporary halt, including the reason for such an action. The study will be suspended pending a further positive decisions by the CCMO. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product / trial procedure / the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. Subjects will be monitored throughout the study for AEs, from the time of first dose on Day 0 up to the assessments of Day 180. However, for clarity, only the AEs up to and including the Day 56 visit shall be included for the purposes of the decision making rules as to which dose levels and regimens proceed to the Phase II portion of the study. Untoward events that occur prior to first dose of study vaccine will not be recorded as an AE but should be recorded as medical history. Adverse events that are identified at the last assessment visit (or the early termination visit) as specified in the protocol must be recorded on the AE CRF with the status of the AE noted, and the AE must be followed until resolution. AEs that are resolved at the final study visit should be recorded with a stop date. All AEs should be followed to resolution whenever possible. Action taken will be categorized as none, study drug discontinued, dose modified, required concomitant medication, required procedure, or other. Event outcome at resolution or time of last evaluation will be recorded as event resolved, resolved with sequelae, ongoing, or death.

Severity. For evaluating severity and relatedness to AKS-452, CTCAE will be used (CTCAE V5.0). Adverse events will be graded by a numerical score according to the defined NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) and version number specified in the protocol. Adverse events not specifically defined in the NCI CTCAE will be scored on the Adverse Event log according to the general guidelines provided by the NCI CTCAE and as outlined below:

- Grade 1: Mild
- Grade 2: Moderate
- Grade 3: Severe or medically significant but not immediately life threatening
- Grade 4: Life threatening consequences
- Grade 5: Death related to the adverse event

Relationship to treatment

The relationship of the event to the study drug should be determined by the Investigator according to the following criteria:

- Not related: The event is most likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs, and does not follow a known response pattern to the study drug, or the temporal relationship of the event to study drug administration makes a causal relationship unlikely.

- Possibly related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug, but is more likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs.
- Probably related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug but could be explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs.
- Definitely related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug and cannot be reasonably explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing in subjects' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.
- An elective hospital admission will not be considered as a serious adverse event.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited CCMO that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the CCMO:

- SUSARs that have arisen in the clinical trial that was assessed by the CCMO;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the CCMO.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the CCMO. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life-threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the CCMO, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

An independent external Data Safety Monitoring Board of experts is established to perform ongoing safety surveillance and to evaluate interim analyses on the safety data. This board is considered necessary considering the 1st in-human application of AKS-452, see chapter 13.

The independent members of the DSMB are:

- Prof. dr. J.E. Tulleken, MD, PhD, Internist-Intensivist (chairman)
- Prof dr. R.T. Gansevoort, MD, PhD, Internist-Nephrologist
- Dr R. Bokkers, MD, PhD, Intervention Radiologist
- M.L. Toren-Wielema, PharmD, Hospital Pharmacist
- F. Sollie, Senior Biostatistician PRA Health Sciences

The members of the DSMB have expertise from different scientific areas and are experienced in serving on a DSMB. They have no conflicts of interest with the conducted trial or principal investigators of the study. They have no financial interest in the outcome nor will be authors of future publications of this study.

The investigators involved in this study will perform an interim analysis on safety data, after completion of the 6x3 dosing-cohorts, and in-between phase I (6x10 subjects per cohort) and II. Phase II will be initiated after safety review and immunogenicity assessment after the last patient of Cohort 6 completes the Day 56 visit, at which time two decisions will be made;

1) selection of the optimal dose for a 1- dose regimen based on (S)AEs and seroconversion

2) selection of the optimal dose for a 2- dose regimen based on (S)AEs and seroconversion.

Phase II will include 58 subjects per cohort with all subjects receiving active vaccine.

The DSMB will consider essential parts of study conducts like protocol adherence, patient withdrawal and safety. The DSMB will work according to Standard Operating Procedures (SOPs). A DSMB charter is available.

The responsibilities of the DSMB include:

- Monitor protocol compliance by subjects and investigators and monitor patient withdrawal as early indicators for problems with respect to safety or feasibility (receive summary after phase I);
- Monitor safety of AKS-452 (e.g. toxicity data, AEs, SAEs, deaths);
- Advice on the need for dose adjustments because of safety issues;
- Advise on protocol modifications suggested by investigators or sponsors (e.g. to inclusion criteria, trial endpoints, or sample size);
- Monitor compliance with previous DSMB recommendations;
- Considering the ethical implications of any recommendations made by the DSMB;
- Provide recommendations regarding study modification, continuation or termination, based on the results of the interim analysis;
- Decide whether to recommend that the trial continues to recruit subjects or whether recruitment should be terminated either for everyone or for some participant subgroups.

The DSMB will discuss the results of the interim-analyses and advice the PI's. The DSMB provides recommendations regarding study modification, continuation or termination. Discontinuation of the trial is advised by the DSMB according to the pre-defined stopping guidelines stated in paragraph 8.7. The advice(s) of the DSMB will only be sent to the PI's of the study. Should the PI (after a joint decision with the sponsor) decide not to fully implement the advice of the DSMB, the PI will send the advice to the reviewing CCMO, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. STATISTICAL ANALYSIS

10.1 Analysis Populations

Safety Population: The Safety Population will consist of all subjects who received any dose of AKS-452.

10.2 Descriptive statistics

Patient demographic characteristics (such as age, sex, race, ethnicity, BMI, medical history and morbidity) will be displayed as: (geometric) means with standard deviations, medians with range and frequencies.

Continuous variables will be inspected for normal distribution by histograms and if non-normally distributed, attempts will be made to transform the data to obtain a normal distribution.

10.3 Primary study parameter(s)

The primary aim of this vaccination study is to evaluate the safety, tolerability and humoral immunogenicity profile of AKS-452 for Phase I and II.

Safety: Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

Evaluation of Safety and Tolerability:
Safety and tolerability will be assessed through AEs, clinical laboratory, vital signs, ECGs and physical examination findings, and any other parameter that is relevant for safety assessment. Adverse Events: A listing of all individual AEs will be provided. Summary tables of TEAEs will be presented by system organ class based on the MedDRA terminology list (preferred terms): containing the number of TEAEs (frequency of occurrence, number of subjects experiencing the event) by treatment and containing the number of drug-related TEAEs (frequency of occurrence, number of subjects experiencing the event) per treatment. Additional tables of total counts by treatment and relationship and by treatment and severity will be given.

Phase I will be a classical 6x3 dose-finding design, after which we will assess safety after each dosing cohort (n=3 subjects). The following stopping-rule will be applied on a per-cohort basis (see also flow-chart): any SAE or AE $\geq$ 3 (according to NCI Common Terminology Criteria for Adverse Events [CTCAE]) attributable to AKS-452.

In the case where none of the included subjects has an AE $\geq$ 3 or SAE attributable to AKS-452, we will expand each dosing cohort with an additional 7 patients. After completing the 6 cohorts with a total of 10 patients each (overall 60 patients), again a safety assessment will be conducted during the interim analysis between Phase I and II will be executed. On the basis of this safety assessment and a minimum project seroconversion rate of 7/10 (where seroconversion is defined as a true positive based on the SP/RBD IgG ELISA assay pass/fail criteria using the quantitative cut-off value defined by the specific batch of the assay kit expressed as µg/ml), we will determine the two final optimal dosing cohorts for respectively the

one injection s.c. regimen and two-dose s.c. regimen. If two dosing cohorts are eligible for the subsequent phase II study, the lowest dose-regimen will be selected.

Laboratory data: Clinical laboratory data will be listed accompanied by an indication if the parameter is outside the reference range. A summary of all data outside the reference range of the clinical laboratory will be provided. Clinical laboratory data will be presented descriptively, where applicable.

Once a participant decides that he/she will drop out in favor of an already registered COVID-19 vaccine, he/she will be considered as a drop out, as discusses above. We will kindly ask them to complete the follow up period of this study, which encompasses 180 days (only in the case when they received the AKS-452 vaccine). The consequences for the statistical analysis plan will be that only follow-up data and blood sample results (e.g. antibody titers) until the last moment of follow-up can be used for that time-point in the overall study data. However, the safety data and blood sample analysis data (e.g., antibody titers) obtained up to 4 weeks after the first dose and 4 weeks after the second dose, prior to the subject having received an approved vaccine, do remain valid for final analyses. If and when a subject has received the first dose of an approved vaccine other than AKS-452, none of the subsequently obtained data can be used for the proposed endpoints for safety and efficacy. As there are no data on combining AKS-452 and an approved vaccine, we will nevertheless be allowed to continue to collect data to support an important side-study for determining safety profile and antibody response for the combination of AKS-452 and any other approved vaccine. In such cases where a subject chooses to drop out and receives an approved vaccine, no data will be collected past the pre-determined termination of the AKS-452 study protocol.

10.4 Secondary study parameter(s)

- To evaluate the inhibitory/neutralization potency of the SP/RBD-specific IgG titers induced AKS-452 and to estimate peak titers and duration of the response.
- To evaluate the Th1/Th2 immune response profile.

  To achieve these objectives, the following will be measured:
  - Anti-SARS-CoV-2 SP RBD IgG titers at days 0, 28, 56, 90, and 180
  - Serum titer inhibition of recombinant ACE2-SP/RBD binding and/or neutralization of live SARS-CoV-2 virus infection of live cells (Plaque Reduction Neutralization Test, PRNT) at days 0, 28, 56, 90, and 180
  - T-cell responses measured ex vivo using PBMCs to measure SP/RBD-specific T cell production of IFN-γ and Th1/Th2/Th17 related cytokines via ELISpot or other Ag-specific flowcytometric-based assays on days 0, 28, 56, 90, and 180.

10.5 Other study parameters

Not applicable

10.6 Interim analysis (if applicable)

An interim analysis towards the key safety parameters including AE's, SAE's and SUSARS that occurred during the trial will be performed after completion of the 6x3 dosing-cohorts in

phase I and after completion of inclusion of 10 subjects per cohort, prior to initiation of phase II.
The investigators involved in this study will perform an interim analysis on safety data and tolerability and immunogenicity. Phase II will be initiated after safety/tolerability review and immunogenicity assessment at after the last patient in Cohort 6 completes their Day 56 visit, at which time two decisions will be made;

1) selection of the optimal dose for a 1- dose regimen based on (S)AEs and seroconversion

2) selection of the optimal dose for a 2- dose regimen based on (S)AEs and seroconversion

After completion of phase I, the DSMB will be asked to review (or in an earlier time if safety data are in doubt by PI and/or sponsor) and report on (dis)continuation of the next phase. (there is a DSMB charter in place and sections 8.7 and 9.5 of this protocol).

A simultaneous interim analysis towards the secondary endpoint will be conducted by the investigators involved in this study after the Phase I study patients for all six cohorts have completed their Day 56 visits.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, 2013 amendment) and in accordance with the medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. The protocol has been written and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ICH E6). The protocol will be approved by the National Ethics Committee (CCMO) as part of a fast trajectory process for COVID-19 studies.

11.2 Recruitment and consent

Potential eligible subjects are recruited by posting announcements on social media and through local or regional newspapers. Eligibility is assessed by the research physician (in Dutch: arts-onderzoeker) in consultation with the treating physician / PI by checking in- and exclusion criteria based on the available data (according to paragraph 4.2 and 4.3). If the patient is eligible to participate in the study, the treating physician discusses the option to participate in the study. After this, subjects will receive a letter containing information about the study and study procedures during the consultation or they will receive it by mail when the letter is sent to the subject together with the standard information each patient receives prior before the scheduled operation (see patient information leaflets for UMC Groningen).

The subjects will be informed about the aims of the study, the possible adverse events, the procedures and possible hazards to which they will be exposed before enrolment into the study. They will be informed as to the maintenance of confidentiality of their patient data.
Subjects will be contacted by phone, mail or e-mail to ask if they are interested in participating in this study by one of the investigators (research physician) involved in this study.

Each patient will be given the opportunity to ask questions and will be informed about the right to withdraw from the study at any time without prejudice. See the patient information sheet and patient informed consent statement for the UMC Groningen. When subjects affirm their intent to participate, a pre-treatment consultation is planned and subjects will be asked to bring the signed informed consent form, which will be received by the research physician.

Informed consent

Documented informed consent must be obtained for all subjects included in the study before they are registered in the study. Subjects must be given adequate opportunity to read the information and enquire about details of the study before consent is given. The informed consent procedure conforms to the ICH guidelines on Good Clinical Practice. This implies that the written informed consent form will be signed and personally dated by the patient or by the patient's legally acceptable representative. The informed consent statement will be signed and dated by the research physician afterwards and the patient will receive a copy. The general physician of each patient will be informed about the enrolment of the patient to the study.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

11.4 Benefits and risks assessment, group relatedness

For the participating subjects, there is formally no objective diagnostic, preventive or treatment benefit related to the study, as this is a 1st in-human phase I/II clinical study. Since all participants receive the active vaccine, there is the anticipated effect of protection against a COVID-19 infection, although the safety and efficacy need to be confirmed. Participation may possibly produce useful scientific data for the future and the impact for fighting the COVID-19 pandemic. The risks related to AKS-452 administration are described in section 6.1, the IB and IMPD.

The risk associated with exposure to a novel vaccine for a 1st in-human phase I/II clinical study are pain/swelling/redness/bleeding/infection/granuloma formation at SC injection site, hematoma due to the venepuncture for blood sampling, mild fever, chills, feeling tired, headache, muscle and joint aches, syncope, and an allergic reaction related to AKS-452, which are all well within the tolerable range for a novel vaccine like AKS-452 as based on the animal toxicology data. The benefit, in case of a safe and sufficient immunogenicity provoking vaccine, is for protecting health care workers, future vulnerable patients and frail elderly, and patients undergoing large surgical procedures for instance oncology, transplantation etc. Moreover, providing protection in co-morbid citizens (i.e., diabetes, overweight, cardiovascular disease etc) and ultimately, creating another leverage to returning societies back to their previous health care system capacities and economic growth world-wide.

11.5 Compensation for injury

As this study is an investigator-initiated study, the investigators employer has liability insurance which is in accordance with article 7 of the WMO. This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

For each day of patient related study procedures, the subjects will receive compensation for travelling expenses (€ 0.19/km) and a ticket for free parking. Furthermore, a participation fee of € 500 euros will be given to every participant taking part in this study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Case Record Forms (eCRF) - REDCap will be used for clinical data management. eCRFs are designed within REDCap to collect and store study data. A paper CRF will be used to collect data during procedures. Afterwards, this data is entered in REDCap. The investigators are responsible for the legibility, completeness and correctness of the CRF. The Principal Investigator (PI) can track subjects and lock patient data when all study duties are fulfilled. Error, changes and/or additions to the CRF are tracked by the program.
The PI and main investigators will have all access to data, all other investigators who help during measurements will only be allowed to add data to the CRF.

Data storage - Data of subjects will be handled confidentially and a coded identification number (study protocol name ‘’) followed by the patient number of inclusion (for example ‘01’) will be used to link the data to the specific patient. A decoding file of the data will be stored by the PI and is only accessible by the PI. The handling of the personal data complies with the EU law: General Data Protection Regulation. These data will be stored at the specific site for at least 25 years. Coded study data will be made available to relevant partners within the project.

Data sharing - Imaging data and medically relevant information will be coded and shared with Akston Biosciences under the terms of the clinical trial agreement (CTA) that was recently signed between UMCG (the Sponsor) and Akston Biosciences (the subsidizing party) as UMCG is the owner of the data under the agreement. Akston Biosciences will not receive the key that safeguards the data, as this will remain in the possession of the principal investigator. In this way no privacy sensitive information will be shared with Akston Biosciences. This is also stated in the patient information provided for this study. The UMCG beholds the publication right.

12.2 Monitoring and Quality Assurance

On-site monitoring will take place conform the NFU (Nederlandse Federatie van Universitair Medische Centra)-guideline “Kwaliteitsborging van mensgebonden onderzoek 2020” by the independent and qualified monitor. For this study, the risk classification is considered “negligible”, which implies intensive independent monitoring of at least 3 visits per year, dependent on the patient inclusion speed. This study will be monitored by independent certified monitors, employed at the Service Desk Clinical Research Office (UMCG). The monitors will perform source data verification on the research data by comparing the data entered into the CRF with the available source documentation and other available documents. Source documents are defined as the patient’s hospital medical records, clinician notes, laboratory print outs, digital and hard copies of imaging, memos, electronic data etc.

12.3 Amendments

Amendments are changes made to the research after a favorable opinion by the CCMO has been given. All amendments will be notified to the CCMO that gave a favorable opinion. A ‘substantial amendment’ is defined as an amendment to the terms of the CCMO application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;

- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the CCMO and to the competent authority. Non-substantial amendments will not be notified to the CCMO as competent authority but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the CCMO as the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. The sponsor will notify the CCMO immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the CCMO and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the CCMO as the Competent Authority

12.6 Public disclosure and publication policy

The study will be registered in a public trial registry (www.clinicaltrials.gov). The sponsor of the study is University Medical Center Groningen, subsidized by Akston Biosciences Corporation. All research contracts as required by the University Medical Center Groningen are signed and stored. The study results will be published in academic journals.

13. STRUCTURED RISK ANALYSIS

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
The burden of participating in the study will be the number of site visits and thus travelling for subjects in phase I/II, blood samples for measurement of immunogenicity, physical examination prior to inclusion / exclusion, physical discomfort related to the subcutaneous or intramuscular injection of AKS-452. The risk associated with exposure to a novel vaccine for a 1st in-human phase I/II clinical study are pain/swelling/redness/bleeding/infection/granuloma formation at SC injection site, mild fever, chills, feeling tired, headache, muscle and joint aches, syncope, allergic reaction.

13.1 Potential issues of concern

Level of knowledge about mechanism of action

The recombinant protein-based subunit vaccine approach has an advantage of safety and multiple-booster dosing relative to inactivated or live-attenuated virus and nucleic acid vector-based vaccine formats, in addition to allowing for the selective use of the most dominant epitopes to generate potent neutralizing Ab titers [15, 17]. However, the relatively smaller size of the recombinant proteins may pose a problem of lower immunogenicity compared to a whole virus Ag, and therefore require additional features to enhance immunogenicity. The following is a brief discussion of the immunological mechanisms that form the basis of Akston's approach for developing its *immune-enhanced* recombinant subunit vaccine, AKS-452, and emphasizes its distinguishing factors from other closely related vaccine programs.

With respect to a basic immune response, injection of any protein Ag can, and most likely will, induce an immune response, the magnitude and type of which is highly dependent on the "status" of the respective immune system. For example, injection of a foreign Ag relative to a self Ag will induce a greater immune response in an immune system that maintains central and peripheral tolerance mechanisms, while self Ag can elicit significant immune responses in an immune system with broken tolerance mechanisms, such as an autoimmune condition. Moreover, foreign or self Ag administration to an immune system that has been primed to previous exposure to the respective Ag (e.g., a viral infection or an autoimmune disease) will lodge a more rapid and elevated immune response relative to that of an Ag-naïve system. The immunological basis of this priming is two-fold; 1) an Ag-naïve immune system has naïve B and T lymphocytes that have a much higher threshold of activation than do the Ag-primed "memory" cells of a Ag-primed immune system, such that the antigen-presenting cells (APCs) that present Ag require much less Ag to activate primed memory T cells, and 2) due to expansion of memory T cells during the Ag priming exposure, there are inherently greater numbers of such cells upon re-exposure to an injected Ag. Note that dominant APCs are dendritic cells (DCs) and macrophages that present Ag in complex with Major Histocompatibility Complex (MHC) molecules on their surface to T cell Ag receptors (**Figure 1**). It is these APCs that can influence both the "magnitude" and "type" of response to Ag; e.g., the Th1 cell response is required to clear most viral and bacterial infections, in which virus-like or bacterial-like substances (non-Ag in nature) condition APCs to express key cytokines and surface co-stimulatory molecules that, during Ag presentation, drive T cells to become the Th1 type. In fact, this APC activation is the conceptual basis of many immune enhancing substances called *adjuvants*. Some adjuvants are designed to trick the immune system into

reacting to the injected vaccine Ag as if it were part of an on-going infection (i.e., infectious agents provide such natural viral or bacterial adjuvant substances). Therefore, adjuvants activate APCs for greater Ag-presentation capabilities necessary to overcome the high activation threshold of naïve T cells, in addition to shaping their development into the Th1 response to effectively clear the respective infection. Note that such T cells provide critical help to B cells that specifically bind the respective Ag to produce Ag-specific antibody (Ab) titers (**Figure 2**).

Given the challenges of a recombinant SARS-Cov-2 SP subunit vaccine to induce a strong protective immune response in an immunologically naïve human population, the SP Ag must be modified and/or formulated with additional immune-enhancing features to overcome the activation thresholds of naïve T and B cells. Akston has implemented the following such features into its COVID-19 vaccine that are major advantages over most other such vaccines in development, in which the Therapeutic Product Profile (TPP) describes details of its clinical candidate, AKS-452 (Table 1):

- The use of the smaller focused antigenic portion of SP, the RBD
- Recombinant fusion of RBD with human IgG1 Fc (SP/RBD-Fc)
- Emulsification of SP/RBD-Fc in the water-in-oil adjuvant, Montanide ISA 720

The following are explanations of the above features:

The focused immunogenicity of the RBD Ag leads to only those Abs that bind this region on SASRS-Cov-2 SP to prevent virus binding to the ACE2 target protein on host cells, thus inhibiting infection. This is in contrast to the use of a whole SP Ag vaccine that risks the generation of non-RBD-binding Abs that actually facilitate viral infection by tagging the virus for Fcg receptor (FcgR)-mediated uptake by macrophages that act as cellular factories for viral replication (i.e., ADE). Perhaps of even greater value is that the small size of RBD provides for at least a 10-fold greater production yield relative to SP (Akston's unpublished observation).

However, simply injecting such a small foreign protein fragment alone as a vaccine Ag would not be expected to induce a strong enough B cell (Ab) or Th1 cell response from a naïve immune status. Therefore, Akston created the subunit vaccine, AKS-452, comprised of a bivalent analog of RBD recombinantly fused to a human IgG1 Fc moiety (**Figure 3**) that (i) facilitates the focused delivery of the RBD Ag to local APCs that internalize SP/RBD-Fc via FcγRs, and then process and present RBD fragments (**Figure 4**) [34] to CD4+ Th cells that in turn promote ("help") B cell activation and anti-SARS-CoV-2 RBD IgG (i.e., Ab) production (**Figure 2**). In addition, a more direct and unique mechanism of AKS-452 is its direct binding to existing SARS-CoV-2-specific memory B cells through their Ag-specific B cell receptors (BCRs). Such binding triggers activation signals upon BCR cross-linking via the RBD bivalency feature of AKS-452 that leads to enhanced proliferation and anti-SARS-CoV-2 IgG production in the absence of CD4+ Th cells (**Figure 2**). Indeed, fusion of IgG Fc with a different RBD fragment derived from the SP of the SARS virus (i.e., SARS-CoV) has been demonstrated to impart significant adjuvant activity relative to the very low immunogenicity of the SARS-RBD fragment alone [15, 17]. In fact, this human IgG Fc-fusion enhancing approach has been demonstrated with the development of a MERS vaccine containing recombinant protein of a truncated MERS SP/RBD fragment (residues 377-588) fused to human IgG Fc that increased immunogenicity via FcgR-binding on APCs, in addition to increasing the in vivo half-life and stability [18, 32]. That is, Fc enhances the systemic half-life and bioexposure of RBD to more APCs residing throughout the body due to binding the neonatal FcR (FcRn) expressed on

endothelial cells that enables long serum half-lives of most monoclonal Ab (mAb) therapeutics. Another advantage of fusing RBD with Fc is the bivalency of the Ag per Fc molecule (i.e., two RBD fragments to one Fc fragment) that improves the stoichiometric quantity of Ag delivered to APCs.

However, the Fc feature of Akston's vaccine Ag may have limited use to only RBD-primed individuals who had a prior infection of SARS-Cov-2, both asymptomatic and symptomatic for COVID-19. That is, FcgR binding and activation signals in APCs, while known to provide significant signals for Ag presentation, are typically not strong enough to achieve the activation threshold of naïve lymphocytes, although these signals would be expected to re-activate the low-threshold of memory T and B cells of primed individuals (**Figure 1**). In addition, the weak signaling of FcgR in naïve lymphocytes does not ensure commitment to Th1 development. To overcome these limitations, Akston enhanced vaccine potency by formulating the SP/RBD-Fc Ag in the adjuvant, Montanide ISA 720, which is a water-in-oil substance containing the Th1-promoting and human-safe squalene oil. Squalene is a natural organic compound originally obtained for commercial purposes (primarily from shark liver oil), is a biochemical intermediate in plants and animals (including humans), and has been approved as an adjuvant component in several human vaccines [35]. Therefore, Montanide ISA-720 was developed for its low reactogenicity in humans and its closely related form, ISA 51, is an EU-approved adjuvant for a cancer vaccine [36-38]. Note that Montanide ISA 720 has been used in more than 200 clinical trials involving cancer, AIDS, malaria or autoimmune disease vaccines involving an accrual of more than 20,000 patients, and has demonstrated an excellent clinical safety profile in addition to its strong promotion of immunogenicity and Th1 responses [37-48]. In summary, the Fc moiety on AKS-452 is designed to act as a mild adjuvant via inducing activation signalling to the APC via FcgRs and is designed to work in concert with a strong classical adjuvant, such as Montanide ISA 720, to enhance the duration of Ag exposure to APCs and perhaps direct Ag entry into lymph nodes locally and systemically where additional APCs reside. As a consequence, the Fc moiety in combination with an adjuvant is expected to create a dramatic dose-sparing potential for both the Ag and adjuvant such that the risk of reactogenicity (a safety concern) is dramatically reduced; i.e., too much adjuvant that over-activates many APCs and other innate immune cells can lead a systemic inflammatory reaction termed reactogenicity. Such reactogenicity is induced acutely after injection and is not mediated by T and B cells.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Not applicable

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Yes, the vaccine is potent at inducing high titers of neutralizing Abs after single and double low-dose administrations via s.c. routes in mice, rabbits and non-human primates.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The selectivity has been extensively tested and evaluated in human cells and animals (see IB / IMPD), but not yet in human beings.

e. Analysis of potential effect

The vaccine is composed of two main components: the SP/RBD-Fc fusion protein antigen and the Montanide ISA 720 adjuvant. The SP/RBD-Fc antigen is given at extremely low dose levels because of its design to function locally in the proximity of the injection site. Therefore, this component does have any associated serious practical risks. The Montanide ISA 720 adjuvant is designed to be less reactogenic in humans relative to other approved adjuvants, and therefore, the volumes given, 125 µl, 250 µl and 500 µl, have been demonstrated to not have drug associated SAEs in at least 20,000 subjects in various clinical trials over the past 20 yrs. The only AEs are a tolerability concern of some transient redness and mild erythema at the injection site.

f. Pharmacokinetic considerations

Because such low doses of the vaccine are administered, the vaccine does not achieve any detectable systemic levels.

g. Study population

Healthy volunteers

h. Interaction with other products

Not applicable

i. Predictability of effect

The levels of neutralizing Abs measured in the ACE2-SP/RBD binding ELISA that are as high as those in human convalescent serum are a good predictability marker of efficacy, which is the case with other vaccines already evaluated in Phase III COVID-19 efficacy trials. In addition, the induction of SP/RBD-specific Th1 T cells, measured via IFN- γ ELISAPOT assay, are confirmation that the vaccine induces the SARS-Cov-2 protective T cell response.

j. Can effects be managed?

For the following burden / side-effects the effects can be managed, although the effects are estimated to be low <1%.

- Pain / swelling / redness, at SC injection site: painkiller and cold packages
- Bleeding / inflammation / infection /granuloma formation, at SC injection site: bandage, observation / anti-inflammatory drug – antibiotics. Granuloma formation in general can be observed for regression – ultimately in case of discomfort after 3 –6 months a surgical removal of the granuloma
- Mild fever / chills / feeling tired / headache / muscle and joint aches during/after SC or IM injection: anti-inflammatory drugs (acetaminophen 500 or 1000 gram p.o., max 4000 gram per day)
- Syncope: observation / supine bed positioning
- Allergic reaction: In case of an allergic reaction, the Standard Operating Procedure ‘Anaphylactic Reaction’ is activated and in summary consists of the following escape medication: anti-histamine medication (e.g. cetirizine 10 mg p.o., dexamethasone (4 mg p.o.) and in case of a severe reaction epinephrine, oxygen and alarm UMCG Emergency Crash Team at the ward.

13.2 Synthesis

The vaccine AKS-452 has been extensively tested in a broad range of cells and animals (including non-human primates) without any significant toxicity. It can be concluded from the data presented in the IB / IMPD that the pharmacological profile of AKS-452 is fully within the range of clinical translation towards a phase I/II clinical study.

The risks associated with exposure to a novel vaccine for a 1st in-human phase I/II clinical study are pain/swelling/redness/bleeding/infection/granuloma formation at the SC injection site, mild fever, chills, feeling tired, headache, muscle and joint aches, syncope, and an allergic reaction which are all well within the tolerable range for a novel vaccine like AKS-452. The benefit, in case of a safe and sufficient immunogenicity provoking vaccine, is for protecting health care workers, future vulnerable and frail elderly, and patients undergoing large surgical procedures for instance oncology, transplantation etc. Moreover, providing protection in co-morbid citizens (i.e., diabetes, overweight, cardiovascular disease etc) and ultimately, creating another leverage to returning societies back to their previous health care system capacities and economic growth world-wide.

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Pharmacy Manual

Study Title:

Anti COVID 19 AKS-452 Phase I/II VaccinaTion Study (ACT-Study)

Trial Identifier (e.g. EudraCT No.:)	2020-05997-82
Trial Name:	ACT-Study
Reference:	Research Protocol ('Onderzoeksprotocol') in its currently valid version
Phase:	I/ II
Investigational Medicinal Product:	Fusion protein vaccine
Version	V2.0
Date:	30-APR-2021

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Approval by University Medical Center Groningen, UMCG, Representative:

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Herewith I confirm that I have forwarded the new information to the appropriate personnel. Representative of the Pharmacy *****

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Confirmation of invalidation of the Pharmacy Manual by a representative of the Pharmacy

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Document History

Second version / April 30th, 2021:

Small changes in the used disposables.

Reason: the initially recommended disposables were not available.

Table of Contents

List of Abbreviations

APC	Antigen-presenting Cells
API	Active Pharmaceutical Ingredient
AKS	Akston
°C	Degree Celsius
CRA	Clinical Research Associate
CMO	Contract Manufacturing Organization
CoA	Certificate of Analysis
CoC	Certificate of Conformity or Certificate of Compliance
CRA	Clinical Research Associate
CRO	Contract Research Organization
CSM	Clinical Study Manager
RP	Clinical Study Protocol
DP	Drug Product
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
h	Hour
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
mg	Milligrams
mL	Milliliter
mm	Millimeter
MSDS	Material Safety Data Sheet
µl	Microliter
µg	Micrograms
NaCl	Sodium Chloride

OoS	Out of Specification
PF	Pharmacy File
PPN	Pharmacy Product Number
QAA	Quality Assurance Agreement
QP	Qualified Person
RT	Room Temperature
TMF	Trial Master File
AKS-452	IMP of this Clinical Trial, the AKS-452 fusion protein vaccine

1. Contact Details

The sponsor is responsible for monitoring the activities at the pharmacy. Nevertheless, general questions and issues concerning the preparation of the fusion protein vaccine should be addressed to the CMO as well as to the Drug Substance Manufacturer.

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2. Introduction

The purpose of this manual is to serve as a guideline for Contract Manufacturing Organizations (CMOs), pharmacies and trial sites regarding the receipt, storage, handling, inspection, preparation and destruction of the investigational medicinal product (IMP).

All handling of IMP has to be conducted in compliance with the research protocol (RP), GCP and ICH Guidelines as well as required in local and regional regulations and guidelines. The RP can be updated independently of the Pharmacy Manual; in case changes in the RP also affect the Pharmacy Manual, a new version of the Pharmacy Manual will be provided. Appendices to this Pharmacy Manual can be updated independently of the Pharmacy Manual.

3. Trial Design

ACT is a first-in-human, open label phase I/ II Clinical Trial testing a COVID-19 directed vaccine in healthy volunteers. Volunteers will be vaccinated according to the flow chart in Figure 1. Vaccines will be administered by means of subcutaneous injection. The vaccinations will be administered with a 28 day time-lag between each vaccination, or as a single administration.

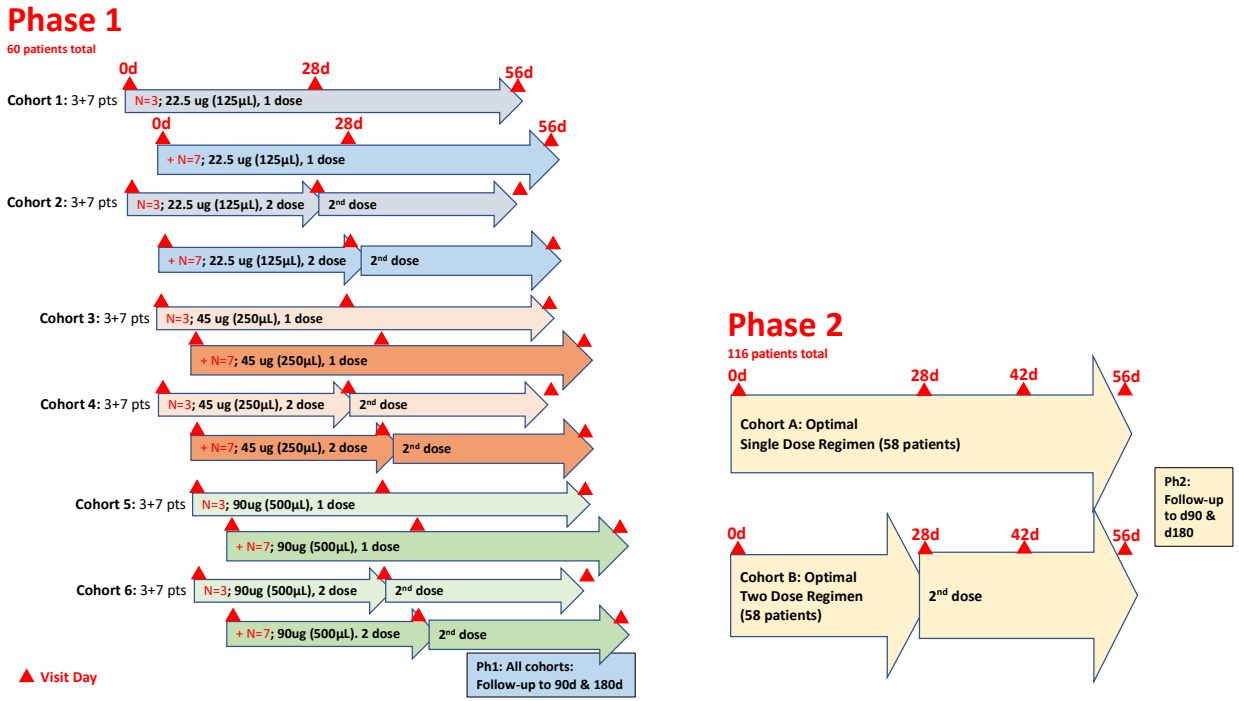

Figure 1: Workflow of the ACT Phase I/II study. After Phase I, a decision point is made as to whether to continue to Phase II and if continuing, a selection of the Phase II dose levels and regimens is made before proceeding.

4. Investigational Medicinal Product (IMP)

4.1. General Information

The concept of the ACT study is based on the induction of a COVID-19 directed immune response by treatment with a fusion protein vaccine, AKS-452, depicted in Figure 2.

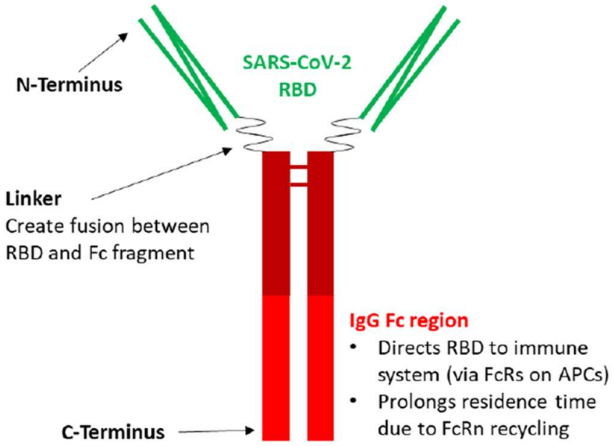

Figure 2: AKS-452, the fusion protein vaccine in this clinical trial.

Table 1: Components needed for reconstitution of the IMP for s.c.. injection.

Material/Equipment	Manufacturer
1 vial containing ~ 3 mL MONTANIDE™ ISA 720 VG STERILE	Seppic S.A. (Paris, France)
1 vial containing AKS-452 drug product ~1 mL (extractable volume ≥ 0.8 mL)	Akston Biosciences (Beverly, MA, USA); filled and QP released by PRA Health Sciences (Gronigen, Netherlands)
1 empty 2.0 ml vial (remark: vial may contain up to 4 ml)	Greer, SE2508020G
4 vial adapters (13 mm)	West Pharma
2 luer lock syringes (5 mL)	Norm-Ject ® 5 ml Luer Lock syringes (ref NJ-4606710-02)
1 Adapter luer-lock	(MEDEX: ref MX494)
Up to 20 luer lock syringes (1 mL)	BD Luer-Lok syringe (Netherlands); Ref: 309628
Up to 20 25G 5/8” subcutaneous injection needles	BD (Netherlands); Ref: 305122

4.2. Reconstitution

The IMP consisting of AKS-452 is reconstituted in a three-step process: (1) Products loading, (2) Emulsification, and (3) Loading into suitable syringes.

Each component has a label approved by the respective competent authority. If necessary, the labels are additionally approved by the institutional review board according to regulatory requirements. The Montanide™ ISA 720 is sourced as a QP released medicinal product which carries the label of the manufacturer.

Relabeling single components is exclusively possible due to an extension of the shelf life after receiving a written order from PRA in co-operation with Akston and the Sponsor. Relabeling has to take place under GMP-compliance and in compliance with local rules and the requirements of PRA, Akston and the Sponsor. If necessary, labels can be provided by PRA or the Pharmacy involved.

If required, the pharmacy adds specific information (e.g. Subject Number) as additional information on the labels.

If the IMP is not reconstituted directly at the site of injection, the pharmacy is responsible for labeling the reconstituted IMP for the study site. Reconstituted IMP should at least contain

information about the period it can be applied to the volunteer, dose and batch number. Corresponding labels can be requested and provided by the Pharmacy.

Expiry Date

The expiry date of components also limits the period for administration of the IMP and adjuvants that are reconstituted from these kits. The expiry date represents the date after which the corresponding IMP must not be administered anymore.

4.3. Request, Shipment and Receipt of IMPs

4.3.1. Transport from Manufacturer of Kits to Pharmacy

The shipment of the vaccine to the respective pharmacy for training purposes occurs by request and is organized by PRA in co-operation with the UMCG.

Shipment of AKS-452 for clinical use is flexible, and shipment dates and batch size depend on expected recruitment rates, shelf life, and pharmacy storage capacity. The pharmacy staff will be informed about shipping amounts and dates in advance.

Shipment will be performed by a qualified carrier. A QP release certificate will be provided by PRA with each shipment of IMPs and must be filed in the Pharmacy File (PF).

The carrier will stop the temperature logger in the transport box and check together with the involved pharmacy staff for potential deviations (temperature, duration) during shipment (see also Chapter 7).

The proper and complete receipt will be documented upon handover of AKS-452 as well as the MONTANIDE™ ISA 720 VG STERILE in the “Shipping Request Form” by dated signature and shared with PRA electronically.

4.3.2. Transport from Pharmacy to Investigator Site

The investigator site will inform the pharmacy in electronic format about the required reconstitution of the IMP for a patient two days in advance by using the “Request for IMP preparation Form”. This form contains information on dose cohort, volume to administer as well as date and time for planned injection. Whenever possible, the prepared IMP should be transported to the investigator directly after preparation and administered immediately.

4.4. Shipping and Storage Conditions

4.4.1. AKS-452 and MONTANIDE™ ISA 720 VG STERILE

AKS-452 and MONTANIDE™ ISA 720 VG STERILE have to be shipped and stored under temperature control as follows:

Table 2: Shipping and storage temperature of AKS-452 and MONTANIDE™ ISA 720 VG STERILE.

Component	Shipping temperature [°C]	Storage temperature [°C]
AKS-452	-80 ± 10	-80 ± 10
AKS-452 (after thawing)	+5 ± 3 (shelf life=1 month at this temperature)	+5 ± 3 (shelf life=1 month at this temperature)
MONTANIDE™ ISA 720 VG STERILE	+20 ± 5	+20 ± 5

Every day, temperature has to be documented. It is necessary to ensure that all refrigeration appliances are attached to a controlled safety system (emergency power supply, monitored). Consumables have to be stored according to the manufacturer specifications.

4.4.2. Reconstituted IMP

The reconstituted IMP needs to be stored and shipped at temperature-controlled conditions at +20 ± 5 °C. It was shown that the reconstituted IMP can be administered within 24 h. Nevertheless, the storage time of the reconstituted IMP should be kept as short as possible. Whenever possible, the reconstituted IMP should be transported to the investigator and injected directly within **8 hours of reconstitution and further dilution.**
Transport from pharmacy to investigator site should take place at +20 ± 5 °C via the standard transport system. In order to confirm that transport operations do not impair the product quality of the reconstituted IMP the transport checklist (Appendix I) has to be filled in once for the initial transport by the person handling the IMP.

If the IMP is not administered immediately after receipt it should be kept at room temperature (+20 ± 5 °C) at the investigator site as well. Storage temperatures for short-term storage at the pharmacy have to be documented in temperature logs by using the pharmacy´s own logs or temperature logs provided by the sponsor. Temperature logs should include min/max values for every day. In any event, stored IMP should be discarded 8 hours after reconstitution.

5. IMP Reconstitution

The reconstitution of the IMP AKS-452 for a clinical trial must only be conducted by trained pharmacy staff members and only if the investigator site has sent the form "Request for IMP preparation to the pharmacy.

5.1. Material needed for IMP Reconstitution

All consumables approved for reconstitution of the IMP are summarized in Table 1. The materials required per dose are listed in Table 3. The overview of the different doses and respective volumes are listed in Table 5.

Table 3: Material needed per injection.

AKS-452	MONTANIDE™ ISA 720 VG STERILE	Consumables
1 vial containing AKS-452 drug product ~1 mL	1 vial containing ~ 3 mL MONTANIDE™ ISA 720 VG STERILE	4vial adapters (13 mm) 2 luer lock syringes (5 mL) 1 Adapter luer lock 1 empty 2 ml vial Up to 20 luer lock syringes (1 mL) Up to 20 25G 5/8" subcutaneous injection needles

5.2. Reconstitution of AKS-452 using MONTANIDE™ ISA 720 VG STERILE

Overview:

The following protocol is used to manufacture 2.50 mL of the AKS-452 w/o emulsion in a 70/30 volumetric ratio (1.75 mL of Montanide™ ISA 720 for 0.75 mL of AKS-452 drug product).

Before starting the procedure, the AKS-452 (stored at -80°C) is thawed for 1-2 h at 20 ± 5 °C. Thawing should be done under gently swirling and thawing should be complete before loading can start.

STEP 1: Products loading

Start loading 0.75 mL of the aqueous phase (AKS-452) into one syringe and 1.75 mL of Montanide™ ISA 720 into the second syringe using vial adapters as follows:

1) Remove the cover from the vial adapter package while keeping the vial adapter in the blister package.
2) Place the adapter onto the vial; use the blister package to handle the adapter. Connect the adapter on the vial by pushing down until the spike penetrates the rubber stopper and the adapter snaps in place. Remove and discard the blister package.
3) Connect the luer lock syringe to the adapter.
4) Withdraw 0.75 mL of the aqueous AKS-452 from the vial into the first syringe and remove air. Keep the syringe plugged on the vial.
5) Repeat the same operation with a new vial adapter and the second syringe: withdraw 1.75 mL of Montanide™ ISA 720 into the second syringe.
6) Remove the second syringe from the adapter and twist it onto the Adapter luer lock.
7) Push the plunger very slowly in order to drain air as much air as possible from the system.
8) Remove the first syringe from the adapter and twist it into the Adapter luer lock.
9) The system is now ready for emulsification.

STEP 2: Emulsification

The emulsification process is performed in 2 steps:

- A pre-emulsification step at very slow speed,
- An emulsification step at high speed.

Make sure both syringes are firmly and tightly attached at both ends of the Adapter luer lock. Hold firmly the system (syringe/ Adapter luer lock /syringe) to guarantee a constant connection. Thumbs will be used to push the plungers apart. Do not push simultaneously with both of the thumbs to avoid leakage.

1) Push completely on the plunger of the syringe containing the aqueous phase (the drug) in order to get both phases in one syringe (the one filled with the Montanide™ ISA 720 adjuvant).
2) Start emulsifying by transferring alternatively very slowly the formulation from one syringe to the other.
3) One cycle represents the passage of the entire formulation from one syringe to the other through the connector, and back to the first syringe. The first 20 slow cycles are done with a slow rhythm: around 4 seconds to transfer the premix from one side to the other. Thus, a complete cycle will require an average of 8 seconds. This first part will give a "pre-emulsion," and this full process should take at least 2 minutes.

At the end of this first stage, the speed is dramatically increased. The following 60 fast cycles are made at high speed, as fast as possible.

When the emulsion starts to form, a resistance can be felt when applying pressure to the syringe plunger. The mixture takes on a creamy viscous appearance at this time. The overall emulsification process will need 80 cycles and should take around 3 minutes.

STEP 3: Finish and load into injection syringes

After the 20 low-speed and 60 high-speed cycles of the emulsifying process, transfer the entire emulsion into one syringe:

1) Disconnect the empty syringe from the Adapter luer lock and discard.
2) Disconnect the full syringe from the Adapter luer lock .Take the empty 2 ml vial and the vial adaptor. Remove the cover of the adaptor package, keep the head of the vial adaptor in the sterile package.
3) Place the vial adaptor on the empty vial: move the vial adaptor downwards and let the needle penetrate the stopper of the vial. The adaptor will connect with a click to the vial.

4) Connect the full syringe with vaccine emulsion to the vial. Tighten the syringe to the vial adaptor by screwing.
5) Empty the contents of the syringe into the vial. Subsequently, remove the vial adaptor from the vial.
6) Allow the vial with the emulsion to stand for 15 min in order to stabilize the vaccine emulsion.
7) Connect a new vial adaptor to the vial containing the emulsion.
8) Connect a 1 ml Luer lock to the vial and withdraw the prescribed dose for the subject.
9) In order to correct for the void volume in the needle, 0.06 ml should be additionally withdrawn in the syringe.
10) Check whether the emulsion in the syringe is homogeneous, opalescent and without any agglomerates.
11) Repeat actions under 8-10 as many times as needed for the amount of doses prescribed for one particular day.

In case of aggregation and/or precipitation or sedimentation of the reconstituted IMP is observed, the reconstitution was not successful.

In this case, the emulsion must not be used. The reconstitution has to be repeated with unused AKS-452 and Montanide™ ISA 720. The used vials need to be stored.

The event needs to be documented and the Study Team needs to be informed immediately.

6. Training and Qualification of Pharmacy Staff Members

Before the pharmacy is allowed to reconstitute the IMP, it is recommended that each staff member of the pharmacy, responsible for reconstituting the IMP within the clinical trial, will undergo an initial training.

The initial training and qualification involves:

- Demonstration of IMP reconstitution including further preparation of syringes with IMP by a qualified representative of the Study Team.
- Reconstitution of IMP by the pharmacy staff members.
- Training and delegation logs have to be filed in the PF.

Further trainings (e.g. training of new pharmacy staff members) can be performed by already successfully trained pharmacy staff, even if they were not present at the initial qualification executed by the Study Team. All trainings have to be documented in writing using training logs. After trainings are performed, delegations have to be documented in writing using the delegation log. Training and delegation logs have to be filed in the PF. Pharmacy staff members that were already trained in preparation of IMP for one clinical trial are considered as qualified to prepare IMPs for additional clinical trials with equal preparation procedures.

7. Deviations

Deviations from defined operating procedures and processes have to be documented and communicated to the Study Team and the sponsor. Deviations are evaluated together by the sponsor and The Study Team and where appropriate by the pharmacy.

In case any deviation occurred during reconstitution, storage or transport (e.g. temperature, duration), PRA and the Study Team has to be informed immediately. The kit or reconstituted IMP has to be stored under quarantine in accordance with the storage conditions listed in section 4.6 until further procedure has been named by PRA.

Parties that contain their own deviation management system are allowed to use their own forms for reporting. Cause analysis, classification and evaluation of the deviation as well as determination and authorization of CAPAs takes place in accordance with valid SOPs and in consultation with the party concerned.

8. Destruction and/or Return of IMPs

All compounds independent of their use will be stored by the pharmacy until accountability has been finalized and the accountability log has been signed by the pharmacist and the responsible

CRA (or comparable). All used vials and incompletely used, damaged or expired kits are destroyed according to sponsor's requirements after written authorization by the sponsor or a CRO delegated by the sponsor.

Redundant labels provided by PRA or the Pharmacy Department are destroyed on site.

If required, syringes containing the reconstituted IMP can be destroyed (e.g. short-term cancellation of administration). Destruction has to be well documented.

9. Recalls

Possible problems with the quality of consumable materials, raw materials or kits and the components contained require a recall for the batch concerned. Product recall is the responsibility of the sponsor. Kits that are returned as part of a recall have to be documented in the Drug/Non Drug Accountability Log (pharmacy level) prior to the shipping.

10. Potential Preparation-Associated Risks and Risk Management

Protective clothing as usually applied in pharmacies (lab coat and gloves) has to be worn. It has to be operated in accordance with good industrial safety and hygiene practice.

There are no indications of potential severe harmful risks which could be traced to the IMP drug products or Montanide™ ISA 720 in the applied concentrations (see MSDS).

Information on irritation or toxicity of any form are known. In case of contact with skin or mucosa, the affected area has to be thoroughly rinsed with water. In each case the involved sponsor physician has to be consulted and if needed the event needs to be documented.

In case of a possible stab wound with a needle, the wound has to be disinfected, rinsed with water and one of the involved physicians has to be consulted.

In case of a potential contamination of the working surface with IMP, the IMP has to be cleaned and be disposed of according to the usual terms of the pharmacy.

11. Monitoring and Audits

The pharmacy will be visited by the responsible CRA (or comparable) of the sponsor or a CRO delegated by the sponsor on a regular basis.

During regular monitoring visits, the "*Drug/Non Drug Accountability"* of used and not used IMP is performed. If necessary, destruction and/or return of IMP will be prepared (see section 8). Prior to destruction and/or return, Drug/Non Drug Accountability for the corresponding IMP is performed, discrepancies are clarified, documented and signed by the responsible pharmacy

member and the responsible CRA (or comparable).

Team quality assurance of sponsor or a delegate will perform audits at regular intervals and/ or if required in order to check the conformity of the processes performed by the pharmacy.

Inspections are performed by the competent authorities and can be carried out during the ongoing trial. In case of an announced inspection the sponsor, Akston and PRA have to be informed when the inspection will be applicable to the study.

12. Drug/Non Drug Accountability

The pharmacist must maintain adequate and accurate records of the amounts and dates for AKS-452 and Montanide™ ISA 720 received, dispensed during the study, and unused kits that were returned or destroyed. This inventory record must be available for inspection by the sponsor at any time. AKS-452 and Montanide™ ISA 720 are to be used only in accordance with the clinical study protocol in its valid and approved version and under the supervision of the investigator.

Incoming compounds will be documented by the pharmacy staff member with signature and date within the “Drug/Non Drug Accountability Log (pharmacy level)“ containing information like medication number, Kit number, batch number, expire date and date of receipt at the pharmacy.

Additionally, via the “Drug/Non Drug Accountability Log per Subject (pharmacy level)“ consumption per subject is documented. (e.g. AKS-452, Montanide™ ISA 720, etc.).

All fully completed logs have to be dated and signed contemporary by a pharmacy staff member. Each change which has been made has to be signed with date and signature. If a change was done afterwards by a different pharmacy staff member, the subscribing pharmacy staff member has to sign the log again.

13. Pharmacy File

Prior to the start of the clinical trial, the sponsor will provide the pharmacy with the PF which contains all documents which are necessary for the study and the pharmacy staff members. During the trial the pharmacy is responsible for completeness and accuracy of the PF. The CRA (or comparable) responsible for the trial verifies for completeness and accuracy of the PF during the regular monitor visits. Missing documents will be provided by the CRA (or comparable) if possible.

- Clinical Study Protocol and amended versions
- Approval of the Competent Authority and Ethics Committee on the study and valid amendments

- Training certificate
- Certificate of Analysis (CoAs) AKS-452
- Certificate of Compliance (CoCs) AKS-452
- Certificate of Analysis (CoAs) MONTANIDE™ ISA 720 VG STERILE
- Certificate of Compliance (CoCs) MONTANIDE™ ISA 720 VG STERILE
- Drug - Non Drug Accountability Log (pharmacy level)
- Drug - Non Drug Accountability Log per Patient (pharmacy level)
- Temperature Logs (Templates)
- Pharmacy Manual incl. Appendices in valid and invalid versions
- MSDS
- Request for preparation of IMP Form/comparable document
- Request and Shipment Form/comparable document

14.Forms/Template

The listed Forms and Templates will be provided by the Sponsor and are to be used as described within this pharmacy manual.

- Request for preparation of IMP Form/comparable document
- Request and Shipment Form/comparable document
- Training certificate
- Temperature Logs
- Drug - Non Drug Accountability Log (pharmacy level)
- Drug - Non Drug Accountability Log per Patient (pharmacy level)
- Annex 1: Transport Checklist
- Add/remove missing/non valid entries

15. Update/Invalidation

If there are any updates to the Pharmacy Manual, a document of the PF or a Form/Template is needed, the corresponding document will be provided to the Pharmacy by the sponsor or the CRO delegated by the sponsor (e.g. shared electronically). The initial document at the pharmacy will be invalidated by the CRA (or comparable) of the sponsor or a CRO delegated by the sponsor unless a different arrangement has been agreed in writing with signature and date.