TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A 2-part randomized, double-blind (sponsor-unblinded), placebo-controlled, ascending dose and parallel group study of TLR4 agonist (GSK1795091) administered to healthy subjects

Compound Number: GSK1795091

Development Phase: 1

Effective Date: 12-APR-2017

Protocol Amendment Number: 03

Author (s):

PPD

Clinical Pharmacology Science & Study Operations, Australia
Oncology Research and Development, USA
Clinical Biomarkers, IOC DPU, UP, USA
GSK R&D PCPS, CPMS, USA
Clinical Statistics, USA

Copyright 2017 the GlaxoSmithKline group of companies. All rights reserved.
Unauthorised copying or use of this information is prohibited.

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2015N236402_00	2016-MAR-11	Original
2015N236402_01	2016-NOV-29	Amendment No. 1
This protocol amendment is to clarify several sections of protocol text regarding the dose selection process and associated *in-vitro* and *in-vivo* data to support the dose escalation regime. Additional text was clarified by outlining replacement subjects, review of data from Part 1, obtaining ethics approval before starting Part 2 and additional assessments added to the Time and Events table. All changes are detailed in Appendix 7.		
2015N236402_02	2016-DEC-02	Amendment No. 2
This protocol amendment is to correct administrative formatting in Section 5.2 to ensure the numbers were consecutive for the exclusion criteria.		
2015N236402_03	2017-APR-12	Amendment No. 3
This protocol amendment is revising Part 1 dose escalations and subject numbers following a halt in dosing after one subject experienced a transient heart rate increase from 68 bpm to 125 bpm, meeting protocol-defined stopping criteria as a grade 3 change. For Part 1, this protocol amendment is intended to generate additional clinical data while applying the available clinical data to maximize the safety of subjects enrolled in subsequent cohorts. Repeating the 60 ng cohort will further characterize the frequency and magnitude of heart rate increases and temperature increases before taking any additional steps. The stopping rules have been modified to, now, include an effect threshold as well as an increase from baseline is supported by the good tolerability profile of GSK1795091 in cohorts 1 – 3. In turn, because clinical signs and symptoms are now being observed, future dose increments are smaller and the potential top dose has been lowered. Minor typographical errors and other sections have been amended for clarification. All changes are detailed in Appendix 7, Section 12.7.3.		

SPONSOR SIGNATORY

PPD

PPD

Axel Hoos

Senior Vice President, TA Head Oncology R&D

12-APR-17

Date

PPD

MEDICAL MONITOR/SPONSOR INFORMATION PAGE

Medical Monitor/SAE Contact Information:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number	Site Address
Primary Medical Monitor	Dr PPD	PPD			1250 S. Collegeville Road, Collegeville, PA 19426
Secondary Medical Monitor	Dr PPD				1250 S. Collegeville Road, Collegeville, PA 19426
SAE contact information	Medical Monitor				

Sponsor Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): EudraCT 2016-000759-28

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number 204685.

I confirm agreement to conduct the study in compliance with the protocol, as amended by this protocol amendment.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

1. PROTOCOL SYNOPSIS FOR STUDY 204685

Rationale

GSK1795091 is a synthetic Toll-like receptor (TLR4) agonist that is being developed as an immunological adjuvant to be administered in combination with immune system modulators for the treatment of cancers. GSK1795091 is being evaluated in this ascending dose first-time-in-human (FTIH) study to provide safety, pharmacokinetic (PK), and pharmacodynamic (PD) data in healthy subjects. The results will support the design of future clinical trials of GSK1795091 administered to subjects with advanced malignancies in combination with other immune system modulators.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of GSK1795091 when administered to healthy subjects.	• Safety data comprising adverse events, vital signs, laboratory tests, and 12-lead ECGs.
Secondary	
• To evaluate the systemic pharmacokinetics of GSK1795091 following administration of a single intravenous dose to healthy subjects.	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t), AUC(0-last), AUC(0-∞), CL, Vd, and t1/2.
• To evaluate the pharmacodynamic effects of GSK1795091 following administration as a single intravenous dose to healthy subjects.	• Vital signs, CRP measurements, WBC and differential.
• To evaluate the systemic pharmacokinetics of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose.	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t), AUC(0-last), and AUC(0-τ), CL, Vss, t1/2 and accumulation and time invariance (as possible).
• To evaluate the clinical and pharmacodynamic effects of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose.	• Vital signs, CRP measurements, WBC and differential, and laboratory tests.

Objectives	Endpoints
Exploratory	
• To evaluate immune system effects following intravenous administration of GSK1795091.	• Cytokine measurements in plasma and immune cell phenotyping of leukocytes.
• To evaluate the pharmacodynamic effects of intravenous administration of GSK1795091 on gene signature analysis of Peripheral Blood Mononuclear Cells (PBMCs) from part 2 healthy subjects.	• Gene signature analysis
• To explore the relationship between pharmacokinetics, pharmacodynamic markers, and adverse events.	• Correlation between pharmacokinetics and pharmacodynamic markers, such as vital signs and CRP, and adverse events
• To characterize the metabolic profile of GSK1795091	• Collection of samples to characterize the metabolites in plasma and urine.

Overall Design

The study will be conducted in two parts.

Part 1 will be a randomized, double-blind (sponsor-unblinded), placebo-controlled, single-center, single-dose escalation, sequential-group evaluation of intravenously administered GSK1795091 to evaluate the safety and tolerability in healthy subjects.

Part 2 will be an open-label, parallel-cohort evaluation of 2 doses of GSK1795091 administered, either 1 week apart (Part 2, Cohort 1) or 2 weeks apart (Part 2, Cohort 2). In Part 2, on Day 1 subjects will receive intravenous GSK1795091 at a dose determined by results from Part 1.

Treatment Arms and Duration

The duration of the study for each subject, including screening, is approximately 10 weeks.

Subjects will be evaluated in the clinical unit for a screening visit and, if eligible, will receive study product within 30 days. Subjects will be admitted to the clinical unit two days prior to dosing (Day -2).

Part 1

Part 1 is planned to enrol up to 7 sequential cohorts of 8 subjects per cohort (at protocol Amendment 03, the planned number of cohorts is reduced). Subjects enrolled in Part 1 will participate in the study for approximately 60 days from screening to the follow-up visit.

In Part 1, on Day 1 subjects will be randomised in a 3:1 ratio to receive intravenous GSK1795091 or matching placebo. Subjects will be observed as inpatients for 96 hours at which time they will be discharged after assessments have been performed by the investigator. Subjects will have a follow up visit as an outpatient on Day 7 and a final follow up contact by phone or by visit on Day 30.

Part 2

Part 2 is planned to enrol 2 parallel cohorts of 6 subjects per cohort. Subjects enrolled in Part 2 will participate in the study for approximately 67 to 75 days from screening until the follow-up visit.

In Part 2, on Day 1 subjects will receive intravenous GSK1795091 at a dose determined by results from Part 1. Subjects will be observed as inpatients for at least 96 hours, after assessments have been performed by the investigator. Subjects in Cohort 1 will remain in-house until Day 12. Subjects in Cohort 2 will be discharged after completion of the Day 5 (96 hour) assessments and will return to the clinical unit for a second inpatient visit on Day 13 (Part 2, Cohort 2). If AEs attributed to the first dose of GSK1795091 have resolved, subjects will receive a second dose of GSK1795091 on Day 8 (Part 2, Cohort 1) or Day 15 (Part 2, Cohort 2; Otherwise, subjects will not receive the second dose and will be followed according to the schedule for Part 1). Subjects will be followed according to the same schedule as after the first dose of GSK1795091.

Type and Number of Subjects

Approximately 68 subjects may be enrolled in the study. Between 8 and 56 subjects will be enrolled in Part 1. Twelve (12) subjects will be enrolled in Part 2.

Part 1 is planned to enrol up to 7 cohorts of 8 subjects per cohort.

Part 2 is planned to enrol 2 cohorts of 6 subjects per cohort.

Subjects may not participate in more than one cohort.

Analysis

Primary Analyses

Clinical safety observations will include Adverse Events (AEs), vital sign measurements, 12-lead ECG and clinical laboratory assessments. Safety data will be tabulated and where appropriate, analysed by the use of descriptive statistics. Safety data will be tabulated for safety population.

The probability of safety events (e.g. liver toxicity defined by an ALT greater than 3xULN, QTcF > 500 msec, change from baseline QTcF > 60 msec) will be estimated and with associated 95% corresponding confidence intervals for each cohort using the binomial distribution.

Secondary Analyses

Drug concentration and pharmacokinetic parameters will be listed and summarized by dose cohorts in Part 1 and Part 2. Concentration-time data will be presented in graphical form both individually and by cohort as mean and median values. If data permit, dose proportionality, accumulation ratio, and time invariance will be determined.

Exploratory Analysis

Exploratory graphical PK/PD analyses may be performed to examine the relationship between GSK1795091 PK and response as measured by vital signs (e.g., body temperature, heart rate, etc.) or PD biomarkers (e.g., cytokines/chemokines) or other endpoints if warranted by the data (e.g., grade, onset and duration of AEs). The relationship(s) between PD endpoints and PK parameters initially will be explored graphically. Plots of PD endpoint versus PK parameters of GSK1795091 will be generated.

Plasma for cytokine analysis, blood for immunophenotyping, and PBMCs for gene signature analysis will be collected for analysis. Immunophenotyping of blood will be done on an on-going basis, whereas cytokine analysis and gene signature analysis will be done after the study completion. Graphical representations will be created based on the exploratory biomarker data.

2. INTRODUCTION

Toll-like receptors (TLRs) are a family of cell surface proteins primarily expressed on immune and epithelial cells that function as activators of innate immunity in response to microbial-related molecules known as Pathogen-Associated Molecular Patterns (PAMPs). PAMPs include molecules such as nucleic acids, flagellar proteins, and lipopolysaccharide (LPS) (the natural ligand for TLR4). TLR engagement results in the production of various inflammatory cytokines/chemokines such as TNFα, IL6, G-CSF, and type I interferons (IFNα, IFNβ) and enhanced uptake, processing, and presentation of antigens. Accordingly, TLR agonists are being developed for a variety of therapies where immune modulation is desirable such as for vaccine adjuvants and for treatments for allergy and asthma, chronic viral infections, and cancer. GSK1795091 is a synthetic TLR4 agonist that is being developed as an immunological adjuvant to be administered in combination with immune system modulators for the treatment of cancers.

2.1. Study Rationale

GSK1795091 is being evaluated in this ascending dose first-time-in-human (FTIH) study to provide safety, pharmacokinetic (PK), and pharmacodynamic (PD) data in healthy subjects. The results will support the design of future clinical trials of GSK1795091 administered to subjects with advanced malignancies in combination with immune system modulators.

The study will be conducted in two parts.

In Part 1, sequential cohorts of healthy subjects will receive single ascending doses of GSK1795091. The safety and tolerability will be characterized. PK and PD data will also be collected. Results will inform the design of a first-in-cancer-patient clinical trial.

In Part 2 of the study, two parallel cohorts of subjects will receive 2 doses of GSK1795091 at a dose determined by results from Part 1 (Section 4.2), either 1 week apart (Part 2, Cohort 1) or 2 weeks apart (Part 2, Cohort 2). The second dose will provide clinical and laboratory results that can be compared to those following the first dose in order to understand if tolerance (i.e., a diminished pharmacologic response upon repeat administration of drug) to GSK1795091 is observed, as has been reported with repeat doses of other TLR agonists [Astiz, 1995; de Vos, 2009]. Thus, Part 2 of the study will provide data relevant to selection of an appropriate dosing interval to be evaluated in the first-time-in-cancer-patient study.

Conduct of the FTIH study in healthy subjects is preferable to a study in cancer patients. The preclinical safety profile of GSK1795091 and historical experience with the drug class readily accommodate development in healthy subjects. Preclinical safety data for GSK1795091 do not suggest risks for adverse events that are irreversible or that cannot be monitored. Clinical safety data for the drug class is characterized by a predictable tolerability profile of fever and flu-like symptoms (e.g. chills, nausea, malaise, etc.) [Bahador, 2007]. Meanwhile, GSK1795091 would not be expected to demonstrate anti-tumor activity in a FTIH study in patients with cancer. TLR agonists have been studied extensively in cancer patients, as systemically-administered single agents, and robust

anti-tumor activity has not been observed [Guha, 2012]. Additionally, because GSK1795091 stimulates the immune system, dose escalation must start from a very low level (i.e. minimum anticipated biologic effect level; MABEL). Given the need for a low starting dose and the anticipated lack of monotherapy, anti-tumor activity, a FTIH study in healthy subjects is the preferred development approach to avoid exposing subjects with advanced malignancies to an ineffective investigational product.

The safety, PK, and PD results from the FTIH study will facilitate the design of a clinical trial in cancer patients where the benefits of GSK1795091 are more likely to be realized as an adjuvant in combination with a checkpoint modulator. The results of this FTIH study will maximize the safety and potential for efficacy in a first-time-in-cancer-patient study.

2.2. Brief Background

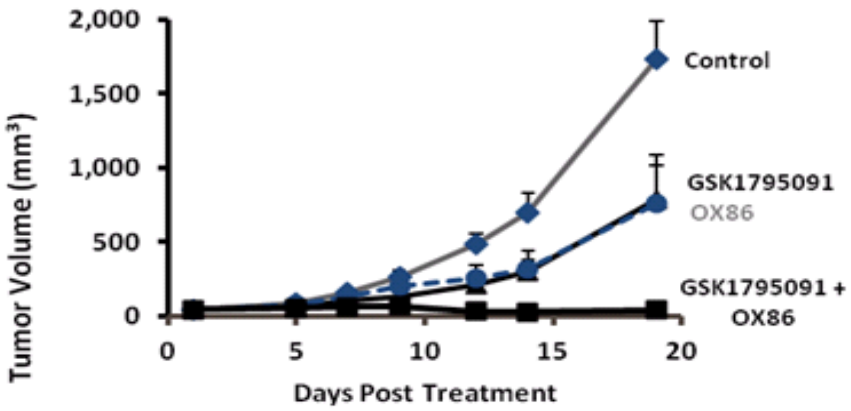
TLR biology

TLRs engage pathways responsible for innate immune system responses to pathogen-associated molecular patterns (PAMPs) such as microbial nucleic acids, flagellar proteins, or lipopolysaccharide. TLRs are primarily expressed in myeloid cells, monocytes, macrophages and dendritic cells (DC), where they are involved in immune surveillance. Many vaccine adjuvants and stand-alone immunomodulators appear to interact with members of the TLR family. TLR engagement activates intracellular signalling pathways via interaction with intracellular adapter molecules such as MyD88, TRIF, TIRAP, and TRAM. These adapter molecules can regulate the expression of inflammatory cytokines/chemokines such as TNFα, interferons and many others.

GSK1795091 was developed in the course of structure-activity studies on LPS, the naturally occurring ligand of TLR4. GSK1795091 is a monosaccharide immunomodulator from the aminoalkyl glucosaminide 4-phosphates (AGPs) class of compounds intended for use as a vaccine adjuvant or an immune modulator. Additional details are provided in the Investigator's Brochure.

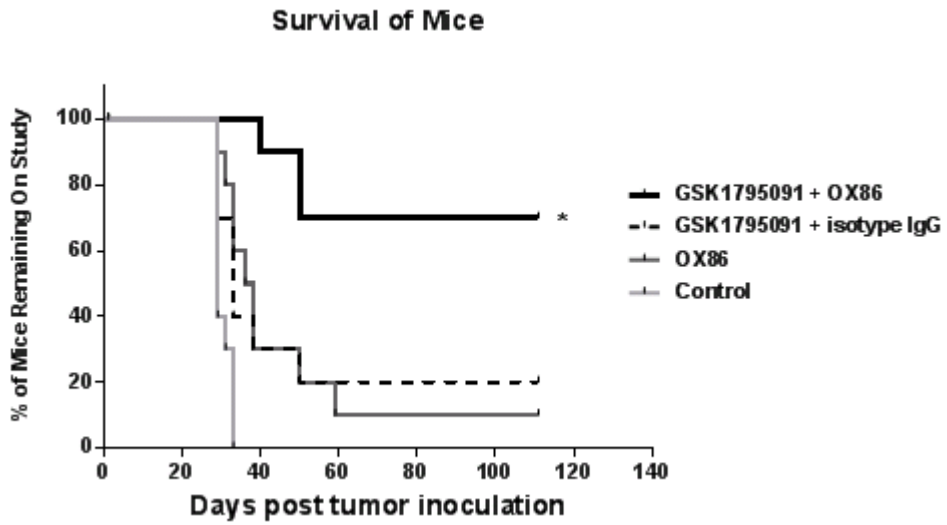
GSK1795091 is an agonist of TLR4 that induces immunologic responses *in vitro* and *in vivo*. GSK1795091, as a single-agent, stimulates cytokine production (*in vitro* and *in vivo*), changes in immune cell populations (*in vivo*) and generates fever response (*in vivo*). When administered by intravenous bolus and combined with the immune checkpoint modulator, OX86, mouse surrogate OX40 agonist antibody, induced a potent immunologic response and significant tumor shrinkage in a syngeneic mouse model of cancer. Four (4) groups of 10 Balb/c mice (with intact immune systems) were implanted with syngeneic CT-26 tumors. Mice received either placebo, GSK1795091, OX86, or the combination of GSK1795091 and OX86. While the monotherapies had modest effects on tumor growth (Figure 1), the combination produced durable responses.

Figure 1 CT26 Tumor Volume



The reduction in tumor volume translated to significant improvement in the survival of animals (Figure 2). Approximately, 70% of animals that received the combination of GSK1795091 and OX86 survived more than 100 days. By contrast, only 10-20% of the animals that received either monotherapy survived 100 days.

Figure 2 Survival of mice



This model provides support for the evaluation of this combination in patients with cancer. Additional details are available in the Investigator's Brochure.

Healthy subject experience with TLR4 agonists

TLR4 agonists have been evaluated extensively in healthy subjects. Following administration, subjects experience mild to moderate flu-like symptoms and increases in body temperature and heart rate. Clinical changes are attributable to increases in cytokines and typically peak within several hours and subside quickly. Overall, the drug

class is well-characterized and not associated with toxicities that are irreversible or that cannot be monitored.

The prototypical TLR4 agonist, LPS, has been administered for decades to healthy subjects and to patients for experimental and therapeutic objectives. The typical dose of LPS administered in clinical trials is 2 to 4 ng/kg at which adverse effects such as fever, tachycardia, headache, nausea, and vomiting may be observed. A review of studies comprising 1000's of healthy subjects that have been administered LPS notes that long-term toxicities have not been described, [Bahador, 2007].

LPS has been evaluated in an ascending dose study designed to investigate biomarker and clinical findings at doses below the commonly investigated 2 to 4 ng/kg range [Dillingh, 2014]. Healthy subjects were administered LPS at doses ranging from 0.5 to 2.0 ng/kg. All adverse events were reported to be mild, and the most commonly reported adverse event was headache. Dose dependent changes in vital signs were observed, with peak changes approximately 3 to 4 hours after LPS administration. At the highest dose, mean temperature increased by 1.5^{o}C and mean heart rate by 28 beats per minute. Dose dependent increases from baseline were observed for TNFα, IL6, and IL8. IL1β levels trended higher with increasing dose, however, many measurements were below the limit of quantitation and no statistical analysis was performed. C-reactive protein, synthesized by the liver in response to IL6 and IL1β, also increased with dose and remained elevated 24 hours after LPS administration, by virtue of a longer half-life relative to cytokine markers [Clyne, 1999]. Total white blood cell count and neutrophil count transiently increased and peaked 4 hours after LPS administration. Multiple other hematologic parameters, including haemoglobin, lymphocyte count, and platelet count, transiently decreased. Clinical and laboratory parameters, except as noted, returned to baseline in 12 to 24 hours. Thus, when administered to healthy subjects, at doses up to 2.0 ng/kg, LPS produced mild adverse events and transient, dose-dependent changes in vital signs and laboratory parameters.

Synthetic TLR4 agonists, such as monophosphoryl lipid A (MPL), have similarly been studied in ascending dose studies [Astiz, 1995]. MPL is used as a vaccine adjuvant in Fendrix, a hepatitis B vaccine, and Cervarix, a human papillomavirus vaccine [Kanzler, 2007]. Early in development of MPL, healthy subjects received escalating intravenous doses from 1 to 20 µg/kg. The 20 ug/kg dose was considered safe and biologically active. Systemic adverse events and cytokine increases were observed after doses of 10 µg/kg. At the highest doses tested, 6 of 6 subjects experienced adverse events including fevers, chills, headache, myalgia, and pain at the injection site. Adverse events were mild to moderate in severity. Changes in vital signs, as compared to control, included increases in mean temperature of 2^{o}C and increases in mean heart rate of 30 beats/min. Changes in respiratory rate and blood pressure were not observed. Vital sign changes peaked within 3 to 4 hours and returned to normal within 12 hours. Total white blood cell count transiently increased and total lymphocyte count transiently decreased. Laboratory biomarkers, TNFα, IL6 and IL8 peaked within 2 to 4 hours. Clinical and laboratory parameters returned to baseline in 6 to 24 hours. A second cohort of subjects received 20 µg/kg MPL followed by a dose of endotoxin 24 hours later. Subjects that received endotoxin 24 hours after MPL experienced diminished cytokine release and improved tolerability consistent with drug tolerance which has been described following

repeat dosing of TLR agonists [de Vos, 2009]. This study provides an example of a synthetic TLR4 agonist that was safely administered intravenous (IV) in ascending doses to healthy subjects and produced transient changes in clinical and laboratory measurements.

Rare serious adverse events have been reported in clinical trials of LPS administered to healthy subjects. One group, with experience administering LPS to approximately 2200 healthy subjects, observed 2 cases of severe hypotension and sinus arrest and identified 2 similar cases in the literature [van Eijk, 2004]. Although all subjects recovered quickly, the report highlights that these subjects had a prior history of syncope in 3 of 4 cases and lacked pre-hydration. The authors conclude that administration of LPS is "a safe means of studying inflammation in humans" but recommend hydration and exclusion of subjects with a history of vagal reactions from participation in clinical trials.

At extreme doses (1 mg; >3500-fold above the standard dose administered to healthy subjects) LPS has caused symptoms similar to septic shock [Taveira da Silva, 1993]. In a case report, an individual presented with fever (40^{o}C), hypotension (42mm/20mm), and tachycardia (114 bpm) 2.5 hours after self-administering 1 mg of endotoxin. The subject received intravenous fluids and pressors. All cytokines that were measured peaked within 24 hours (e.g. TNFa 3.6 hours, IL6 and IL8 6.8 hours, and GCSF 22.5 hours). Remarkably, the subject was reported to be "alert, oriented, and afebrile" within 44 hours and discontinued pressors after 50 hours. At that time, the subject required diuretics for pulmonary edema. Within approximately 4 days, clinical parameters including vital signs and haematological analytes had returned to normal. Thus, although massive doses of LPS produced excessive cytokine elevations and severe hypotension, the case report indicates that over-activation of TLR4 produces toxicities that are qualitatively similar to those observed at lower doses.

TLR4 agonist development plan

The development plan for GSK1795091 includes a healthy subject clinical trial to optimize collection of safety, PK and PD data and to inform the design of future combination therapy trials in cancer patients. The combination of TLR4 agonists with novel immunotherapies holds promise in treating patients with cancer. Systemically administered TLR agonists, including TLR4 agonists, have not demonstrated robust anti-tumor activity as monotherapies [de Bono, 2000; Isambert, 2013; Guha, 2012]. However, one model of a "cancer-immune cycle" describes a series of steps that positively feed-forward to enhance anti-tumor activity [Chen, 2013]. These steps include:

- Release of cancer cell antigens
- Cancer antigen presentation
- Priming and activation
- Trafficking of T cells to tumors
- Infiltration of T cells into tumors
- Recognition of cancer cells by T cells
- Killing of cancer cells

It is possible that immunotherapies acting at different steps in the cycle could have improved therapeutic indices over currently available monotherapies. Thus, a TLR4

agonist may enhance anti-tumor effects of checkpoint modulators by its actions on dendritic cells and antigen presenting cells. This hypothesis is supported by nonclinical data (Figure 1 and Figure 2).

Relevance of a healthy subject study to future development in cancer patients

Part 1 of this study in healthy subjects will provide relevant monotherapy safety, pharmacokinetic and pharmacodynamic information for GSK1795091 to support a subsequent trial in patients with cancer. To ensure the safety of subjects in a FTIH study of an immune system agonist, whether the trial is performed in healthy subjects or in cancer patients, the starting dose is conservative and anticipated to have minimal biological activity. As dose escalation progresses, pharmacologically relevant doses can be identified safely in healthy subjects based on monitorable and reversible, mild to moderate clinical effects (e.g. changes in body temperature, heart rate, and flu-like symptoms, etc.). Subsequently, a pharmacologically active dose could reasonably be explored as a starting dose in a study in cancer patients. Thus, by beginning development of GSK1795091 in healthy subjects, it will be possible to conduct the FTIH study safely while avoiding the exposure of cohorts of cancer patients to pharmacologically inactive doses of the study drug.

The pharmacodynamic markers to be measured in this FTIH study are directly relevant to a subsequent study to be conducted in cancer patients. For example, we will measure IL-1, TNFα, IL-6 cytokines as markers of TLR4 signaling and immune activation; these markers will also be measured in patient studies to confirm TLR4 signaling and to explore the relationship between the markers and clinical activity. In study 204685, we will also measure immune cell activation and immune cell expression of target proteins of interest (e.g., OX40, ICOS, and others) in peripheral blood. This cellular phenotype information will inform our understanding of the pharmacologic effects of GSK1795091 on circulating immune cells and may suggest additional immunotherapy combinations of interest. By conducting study 204685 in healthy subjects, it will enable detailed PK and PD assessments (e.g., multiple time points to characterize time-course) that would be more difficult to obtain from a study in cancer patients due to blood-draw volume considerations and the inconvenience.

A primary goal of Part 2 of study 204685 is to optimize the dosing interval for GSK1795091 in future studies. Administration of TLR agonists can be associated with tachyphylaxis when the dosing interval is short. Therefore, two parallel cohorts of subjects will receive 2 doses of GSK1795091, either 1 week apart (Part 2, Cohort 1) or 2 weeks apart (Part 2, Cohort 2). The pharmacodynamic effects of the second dose will be compared to those of the first dose using biomarker endpoints. This part of the study will provide a more robust assessment of the dosing interval for GSK1795091 than would be possible in a cancer patient study.

This study in healthy subjects will provide the relevant monotherapy safety, pharmacokinetic and pharmacodynamic information to support a combination immunotherapy trial in patients with cancer.

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of GSK1795091 when administered to healthy subjects.	• Safety data comprising adverse events, vital signs, laboratory tests, and 12-lead ECGs.
Secondary	
• To evaluate the systemic pharmacokinetics of GSK1795091 following administration of a single intravenous dose to healthy subjects.	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t), AUC(0-last), AUC(0-∞), CL, Vd, and t1/2.
• To evaluate the pharmacodynamic effects of GSK1795091 following administration as a single intravenous dose to healthy subjects.	• Vital signs, cytokine measurements, WBC and differential, immune cell phenotype.
• To evaluate the systemic pharmacokinetics of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose..	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t) , AUC(0-last), and AUC(0-τ), CL, Vss, t1/2 and accumulation and time invariance (as possible).
• To evaluate the clinical and pharmacodynamic effects of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose.	• Vital signs, cytokine measurements, WBC and differential, immune cell phenotype, and laboratory tests.
Exploratory	
• To evaluate immune system effects following intravenous administration of GSK1795091.	• Cytokine measurements in plasma and immune cell phenotyping of leukocytes.
• To evaluate the pharmacodynamic effects of intravenous administration of GSK1795091 on gene signature analysis of PBMCs from part 2 healthy subjects.	• Gene signature analysis
• To explore the relationship between pharmacokinetics, pharmacodynamic markers, and adverse events	• Correlation between pharmacokinetics and pharmacodynamic markers, such as vital signs and CRP, and adverse events
• To characterize the metabolic profile of GSK1795091	• Collection of samples to characterize the metabolites in plasma and urine

4. STUDY DESIGN

4.1. Overall Design

The study will be conducted in two parts.

Part 1 will be a randomized, double-blind (sponsor-unblinded), placebo-controlled, single center, single dose escalation, sequential group evaluation of intravenously administered GSK1795091 to evaluate the safety and tolerability in healthy subjects.

Part 2 will be an open-label, parallel group evaluation of 2 doses of GSK1795091 administered, either 1 week apart (Part 2, Cohort 1) or 2 weeks apart (Part 2, Cohort 2). GSK1795091 will be administered at a dose determined by results from Part 1 (Section 4.2).

Figure 3 Part 1 study schematic

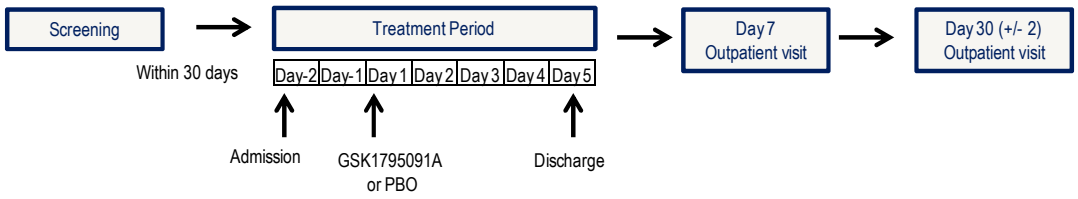
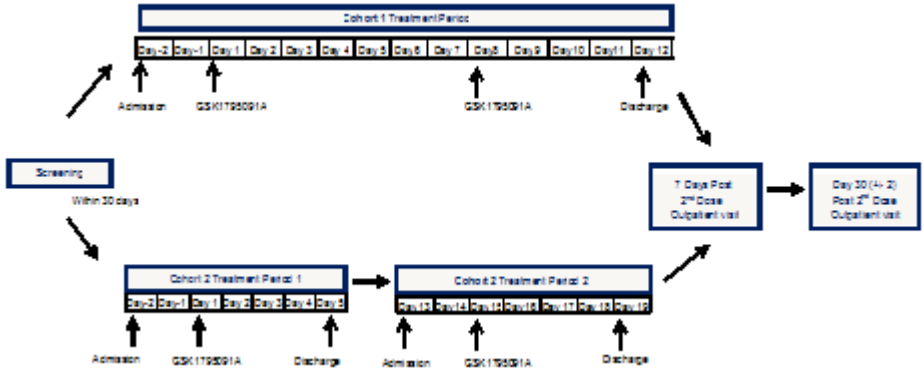


Figure 4 Part 2 study schematic



- Subjects will attend the clinical unit for a screening visit and, if eligible, will attend to participate in the study within 30 days.
- For both Part 1 and Part 2, subjects will be admitted to the clinical unit 2 days prior to dosing (Day -2). On Day -1, adverse events and vital signs will be recorded according to the schedule on Day 1.

- In Part 1, each subject will be randomised to receive an intravenous dose of GSK1795091 or matching placebo in a 3:1 ratio. Subjects will then be observed as inpatients until discharge on Day 5, after assessments have been performed.
- For Part 1, subjects will return on Day 7 for clinic visit and have a follow up contact on Day 30.
- In Part 2, on Day 1 subjects will receive intravenous GSK1795091 administered at a dose determined by results from Part 1 (see Section 4.2).
- Subjects in Part 2 Cohort 1 will be observed as inpatients and remain inhouse until discharge on Day 12.
- Subjects in Part 2 Cohort 2 will be observed as inpatients until discharge on Day 5, after assessments have been performed. They will return to the clinical unit for a second inpatient visit on Day 13.
- Each subject will receive a second dose of GSK1795091 on Day 8 (Part 2, Cohort 1) or Day 15 (Part 2, Cohort 2) (unless AEs attributable to the first dose of GSK1795091 have not resolved, in which case the subject will not receive the second dose).
- For Part 2, subjects will return to the clinical unit for a clinic visit 7 days after the second dose of GSK1795091 and have a follow up contact 30 days after their second dose of GSK1795091.

Details of the assessments and procedures subjects will undergo are listed in Section 7. The timings and time windows for the visits are provided in the Time and Events table, (Section 7.1).

4.2. Treatment Arms and Duration

Overview

The duration of the study, including screening, is approximately 10 weeks.

Part 1 is planned to enrol up to 7 sequential cohorts of 8 subjects per cohort (at protocol Amendment 03, the planned number of cohorts is reduced, and the dose of GSK1795091 for cohorts 5, 6 and 7 lowered. Cohort 4 is a repeat of the 60 ng dose; refer to Section 4.5.2). Subjects enrolled in Part 1 will participate in the study for approximately 60 days from screening to the follow-up visit.

Part 2 is planned to enrol 2 parallel cohorts of 6 subjects per cohort. Subjects enrolled in Part 2 will participate in the study for approximately 67 to 75 days from screening until the follow-up visit.

Subjects will be evaluated in the clinical unit for a screening visit and, if eligible, will receive study product within 30 days. Subjects will be admitted to the clinical unit 2 days prior to dosing (Day -2).

Part 1

In Part 1, on Day 1, 8 subjects will be randomised in a 3:1 ratio to receive intravenous GSK1795091 or matching placebo. Subjects will be observed as inpatients for 96 hours at which time they will be discharged after assessments have been performed by the investigator. Subjects will have a follow up visit as an outpatient on Day 7 and a final follow up contact by phone or by visit on Day 30.

In Part 1, dosing of study product for each cohort will be staggered, with approximately 1 day of observation between at least 4 subgroups. No more than 2 subjects will be dosed per day, and administration of study product will be separated by at least 1 hour when subjects are dosed on the same day. A sentinel group of 2 subjects will receive study product in a ratio of 1 GSK1795091: 1 placebo. The remaining 6 subjects will receive study product in a ratio of 5 GSK1795091: 1 placebo.

In Part 1, the Principal Investigator should pause dosing of new subjects, within a cohort, for review with the GSK Medical Monitor in the following cases:

- If 1 or more subjects experience a Grade 3 or greater adverse event that can reasonably be attributed to GSK1795091 or a serious adverse event or QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline, dosing of additional subjects within the cohort should stop.
- If 1 or more subjects experience a Grade 2 or greater adverse event that lasts more than 24 hours and can reasonably be attributed to GSK1795091, dosing of additional subjects within a cohort should be held until the adverse event has resolved to Grade 1 or less.

In Part 1, cohorts will be opened sequentially after evaluation of adverse events, vital signs, laboratory tests, and 12-lead ECGs by the Principal Investigator (blinded), GSK Medical Monitor (unblinded), GSK statistician (unblinded). All available safety and tolerability data (adverse events, vital signs, 12-lead ECG and safety laboratory assessments) will be reviewed from ongoing and preceding cohorts. Safety data from 96 hours after administration of study product must be available for a decision to dose-escalate to be taken. Additionally, safety data from at least 5 of the 6 subjects who were randomized to receive GSK1795091 must be available in order for a decision to dose-escalate to be taken. If subjects prematurely discontinue from the study, additional replacement subjects may be enrolled and assigned to the same treatment at the discretion of the Sponsor in consultation with the Investigator. Up to 8 subjects may be replaced during the study.

Progression to the next higher dose level will be stopped if any of the following occur:

- 1 or more subjects experience a serious adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a Grade 3 or greater adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a transaminase increase 3xULN (see Section 5.4.2).

- 1 or more subjects experience QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.
- 3 or more subjects in a cohort experience Grade 2 adverse events that can reasonably be attributed to GSK1795091.
- Any other event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

The grading of adverse events will be guided by the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Appendix 2) with the exception of fluctuations in white blood cell counts (which are anticipated and may be considered adverse events only after >24 hours) and fever and tachycardia which also require a change from time-matched baseline (per table below).

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Temperature	>0.5°C change AND >38.0°C	>1.5°C change AND >38.5°C	>3 °C change (<24 hours) AND >39.0°C	>3°C change (≥24 hours) AND >40°C
Heart rate	>15 bpm change AND >101 bpm	>30 bpm change AND >116 bpm	>45 bpm change AND >130 bpm	Emergency intervention required

Following blinded review of safety data and attribution assessments by the study investigator, the medical monitor may determine that the investigator can be unblinded to study treatment in cases where adverse events in a placebo-treated subject might stop dose escalation.

If dose escalation stops, all safety data will be reviewed to determine whether (1) additional subjects should be enrolled at the current dose level or at a lower level to confirm safety findings or (2) Part 1 of the study should be considered complete. PD data may be reviewed.

Following completion of Part 1, the safety data and proposed dose level for Part 2 will be submitted to the Ethics Committee for review. Once the Ethics Committee vote is received, Part 2 will begin.

Part 2

In Part 2, on Day 1 subjects will receive intravenous study product GSK1795091 at a dose determined by results from Part 1.

If dose escalation in Part 1 was stopped for one of the following reasons, GSK1795091 will be administered in Part 2 at a dose one level below the highest dose evaluated in Part 1:

- 1 or more subjects experienced a serious adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experienced a Grade 3 or greater adverse event that could reasonably be attributed to GSK1795091.
- 1 or more subjects experienced a transaminase increase 3xULN.
- 1 or more subjects experience QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.
- Another event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

If dose escalation in Part 1 was stopped for the following reason, GSK1795091 will be administered in Part 2 at the highest dose evaluated in Part 1:

- 3 or more subjects in a cohort experienced Grade 2 adverse events that could reasonably be attributed to GSK1795091.

In Part 2, dosing of study product will be staggered, with approximately 1 day of observation between at least 3 subgroups and no more than 2 subjects within each cohort dosed per day. Administration of study product will be separated by at least 1 hour when subjects are dosed on the same day.

Subjects will be observed as inpatients for at least 96 hours, after assessments have been performed by the investigator. Subjects in Cohort 1 will remain inpatients until Day 12. Subjects in Cohort 2 will be discharged after completion of the Day 5 (96 hour) assessments and will return to the clinical unit for a second inpatient visit on Day 13 (Part 2, Cohort 2). If AEs attributed to the first dose of GSK1795091 have resolved, subjects will receive a second dose of GSK1795091 on Day 8 (Part 1, Cohort 1) or Day 15 (Part 2, Cohort 2). Subjects will be followed according to the same schedule as after the first dose of GSK1795091. In addition, the Principal Investigator should review all available safety data following the second dose of GSK1795091 and interrupt subsequent dosing (within or between cohorts) if any of the following occur

- 1 or more subjects experiences a serious adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a Grade 3 or greater adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a transaminase increase 3xULN (see Section 5.4.2).
- 1 or more subjects experience QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.

- Any other event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

In the event that dosing is interrupted, administration of GSK1795091 in Part 2 will only resume after approval by the Ethics Committee and Higher Health Authority following a substantial amendment.

Table 1 Part 2 dosing regimen (parallel cohorts)

Cohort	Dose Interval	Subjects
1	1 week	6
2	2 weeks	6

Details of the assessments and procedures, including acceptable windows, are described in Section 7 and the Time and Events Table (Section 7.1).

4.3. Type and Number of Subjects

Approximately 68 subjects will be enrolled in the study. Between 8 and 56 subjects will be enrolled in Part 1. Twelve (12) subjects will be enrolled in Part 2.

Part 1 is planned to enrol up to 7 cohorts of 8 subjects per cohort.

Part 2 is planned to enrol 2 cohorts of 6 subjects per cohort.

If subjects prematurely discontinue from the study, additional replacement subjects may be enrolled and assigned to the same treatment at the discretion of the Sponsor in consultation with the Investigator. Up to 8 subjects may be replaced during the study.

4.4. Design Justification

GSK1795091 is being evaluated in this single ascending dose FTIH study to provide safety, PK, PD, and exploratory biomarker data in healthy subjects.

A healthy subject population is an appropriate population for the FTIH study. A healthy subject study will allow for robust PK and PD data to be collected in a population free of confounding co-morbidities, concomitant medications, and other intrinsic and extrinsic factors that increase variability of these data. Meanwhile, the preclinical safety profile of GSK1795091 and historical experience with the drug class support development in healthy subjects. Preclinical safety data do not suggest that subjects are at risk for adverse events that are irreversible or that cannot be monitored. Likewise, experience with the drug class has shown that TLR agonists have a predictable tolerability profile characterized by fever and flu-like symptoms. Finally, a FTIH study in healthy subjects avoids exposing subjects with advanced malignancies to an investigational product unlikely to have anti-tumor activity when administered systemically as a monotherapy.

Adverse event grading is based upon Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (see Appendix 2),

consistent with the use of TLR agonists as immune adjuvants and the anticipated adverse event profile. Fever and tachycardia are graded according to changes from baseline values, with Grade 2 event thresholds approximating the mean increases for tolerated doses reported for other TLR4 agonists. Thus, the anticipated adverse events in this trial should not exceed the severity of the toxicities reported in other trials of TLR agonists administered to healthy subjects.

In Part 1, a standard dose escalation design is planned. The starting dose selection and dose escalation increments are based upon preclinical safety, PK and PD data and *in vitro* comparisons to LPS as described in Section 5.2. The starting dose was selected conservatively, as described below (Section 4.5.1). The dose increments between cohorts progressively decline from 3-times to 1.5-times the previous dose and progressively increase overlap of GSK1795091 exposures.

A placebo control and double-blind (sponsor-unblinded) design are intended to reduce bias in the reporting and collection of subjective data.

The staggered dosing in Part 1 is planned as a conservative measure because GSK1795091 has not been previously administered to humans. The 1 day interval between subgroups is supported by the transient preclinical pharmacodynamic effects of GSK1795091 and rapid (<24 hour) resolution of clinical and laboratory effects of other TLR4 agonists (Astiz, 1995; Dillingh, 2014). The inpatient observation period of 96 hours provides an opportunity to ensure that adverse events have resolved or are resolving prior to discharge of subjects from the inpatient unit. Additionally, the extended inpatient period provides a controlled environment that reduces the likelihood of subjects engaging in routine daily activities associated with adverse events similar to those caused by TLR agonists (e.g. headache, myalgia, etc.) The review of safety data and the stopping rules and adverse event grading are consistent with standard FTIH designs.

In Part 2, a second dose will be administered based upon the definition of a tolerated dose level in Part 1. A second dose will only be administered if subjects have recovered from adverse events considered at least possibly related to study product. The cohorts in Part 2 will be conducted in parallel given that the safety of the second dose is expected to be similar to or better than the first dose (due to the known tolerance that develops with repeat doses of TLR agonists) (Astiz, 1995; de Vos, 2009). However, dosing will be staggered so that a second dose will only be administered to 2 subjects on a single day.

4.5. Dose Justification

4.5.1. Starting dose selection

The proposed starting dose for the study was determined after due consideration of all available *in vitro* and *in vivo* preclinical data, in accordance with EMEA and FDA regulatory guidance for first-time-in-human studies.

4.5.1.1. Calculation of Minimum Anticipated Biological Effect Level

The dose is calculated using the *in vivo* Minimum Anticipated Biological Effect Level (MABEL) in the 6-week cynomolgus monkey study, and secondarily, supported by rabbit pyrogenicity studies and *in vitro* potency comparisons to LPS. The cynomolgus monkey was chosen as the *in vivo* toxicology model based upon greatest sensitivity and highest similarity to humans as determined by cytokine production following exposure of whole blood from several species to GSK1795091 *ex vivo*. Five (5) cytokines were measured *in vivo;* these cytokines were selected for evaluation based upon high *ex vivo* sensitivity of whole blood to GSK1795091 (IL10 and IP10) or based upon increases following administration of TLR4 agonists to healthy subjects in published clinical trials (TNFa, IL6, and IL8) [Astiz, 1995]. Vehicle or GSK1795091, at doses of 0.05, 2, and 200 µg/kg, was administered to cynomolgus monkeys. IP10 and IL10 were the most sensitive pharmacodynamic markers and were detectable in all evaluable monkeys at 0.05 µg/kg. There were minor decreases in lymphocytes, monocytes, eosinophils, and NK cells at doses of ≥ 0.05 µg/kg, and transient increases in heart rate and body temperature at doses of ≥ 2 µg/kg, all of which were considered non-adverse. As such, the no observed adverse effect level (NOAEL) is 200 µg/kg/week, the highest dose tested. Additional details are available in the Investigator's Brochure.

Ten percent effect concentration (EC10) values were calculated for each cytokine based upon peak plasma concentrations. IL10 and IP10 had the lowest EC10 values (0.0281 ng/ml and 0.0405 ng/ml, respectively), and the other cytokines had values greater than 0.3 ng/ml. IL10 was the most sensitive pharmacodynamic marker and the EC10 value of 0.0281 ng/ml provided a basis for calculation of the MABEL. The dose of GSK1795091 that was estimated to have a Cmax of 0.0281 ng/ml when administered to cynomolgus monkeys is 3 ng/kg.

The human equivalent dose (HED) of the cynomolgus monkey 3 ng/kg dose was calculated using two methods. The first predicted human concentration-time data by constructing a Dedrick plot based on dose-normalized cynomolgus monkey concentration-time data. The elementary Dedrick plot was generated using fixed-exponent scaling, assuming single animal (monkey) power allometric exponents (1 for volumes and 0.67 for clearance resulting in an exponent of 0.33 for physiological time). Using the predicted human clearance and volume values from this analysis, the human dose needed to obtain a Cmax of 0.0281 ng/ml is approximately 200 ng. The second calculation used the FDA Guidance for Industry for Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Based upon the scaling factor of 3.1, the 3 ng/kg dose in the cynomolgus monkey corresponds to a HED of 1 ng/kg, or 70 ng for a 70 kg subject. The latter approach was adopted for the starting dose calculation since it provides a more conservative value. Thus, the HED of the 3 ng/kg dose in the cynomolgus monkey is 70 ng. The addition of a 10-fold margin for safety provides a starting dose of 7 ng.

4.5.1.2. Rabbit pyrogenicity

Rabbits are commonly used as models for assessing endotoxin contamination of parenteral formulations due to their high sensitivity. Therefore, GSK1795091 was given

to New Zealand white rabbits at single intravenous doses of 0.1, 0.5, 1, 2.5 and 5 ng/kg in 5% dextrose. Rabbits given single doses of 5 ng/kg had increases in body temperature of ≥ 0.5°C 60 minutes post dose and generally returned to baseline temperature by 180 minutes post dose, consistent with the expected pharmacologic actions of GSK1795091. Lower doses were not considered to be pyrogenic. Based on a scaling factor of 3.1, the 2.5 ng/kg dose in the rabbit corresponds to a HED of 0.8 ng/kg, or 56 ng for a 70 kg subject. Thus, the HED of the non-pyrogenic dose in rabbits is 8-fold higher than the proposed starting dose of 7 ng.

4.5.1.3. Comparison to Lipopolysaccharide (LPS)

The typical dose of LPS administered to healthy subjects in clinical trials is 2 to 4 ng/kg (approximately 140 ng to 280 ng). Following administration, subjects experience mild to moderate flu-like symptoms and increases in body temperature and heart rate. To confirm the safety of the proposed 7 ng starting dose of GSK1795091, the potencies of GSK1795091 and *E.coli*-derived LPS (commonly used for clinical trials of healthy subjects) were compared. Human whole blood was incubated *in vitro* with either GSK1795091 or LPS, and cytokines were measured to assess the relative potencies of the two TLR4 agonists. At concentrations of 0.2 ng/ml (approximating the plasma concentration following a 8.6 ng/kg dose of LPS for a 70 kg subject with a 3000 ml plasma volume), LPS was as potent or more potent than GSK1795091 by a factor of 1 to 4 for a panel of 5 measureable cytokines. If GSK1795091 is estimated to be of comparable potency to LPS, the doses of LPS routinely administered to healthy subjects are 20-fold to 40-fold greater than the 7 ng starting dose of GSK1795091. Thus, the planned starting dose is appropriately conservative.

4.5.2. Dose escalation

Dose escalation will proceed as follows:

Cohort Number	Dose (ng)
1	7[1]
2	21[1]
3	60[1]
4	60[2]
5	100
6	150
7	210

1. Cohorts completed under Protocol Amendment 2.
2. Study restart dose following stopping criteria met in one subject under Protocol Amendment 2

The dose increments between cohorts progressively decline from 3-times to 1.4-times the previous dose.

4.5.3. Top dose selection

The original maximum proposed dose level proposed on preclinical data was 630 ng but was revised to 210 ng based on emerging clinical data cohorts 1-3 in Part 1.

Dose escalation may stop at a lower dose level based on criteria in Section 4.2. In the repeat dose intravenous toxicity studies in rats and monkeys, GSK1795091 was associated with expected pharmacologic, pro-inflammatory actions of a TLR4 agonist. Therefore, adverse events that stop dose escalation are likely to include mild to moderate flu-like symptoms and increases in body temperature and heart rate.

The original top dose of 630 ng was selected to accommodate a possible range of potencies of GSK1795091, based on *in vitro* evaluations. *In vitro* data suggest that the relative potency of LPS is 1- to 4-times that of GSK1795091. If GSK1795091 is equipotent with LPS, dose escalation is predicted to stop in accordance with Section 4.2 at 2-4 ng/kg (i.e. 140 to 280 ng). If GSK1795091 is less potent than LPS by a factor of 4, higher dose levels will be required to elicit pharmacologic effects similar to LPS; this possibility is accommodated by the maximum proposed dose level of 630 ng (i.e. approximately 4.5-fold greater than the LPS dose of 2 ng/kg).

Based on the maximum predicted human exposure of approximately Cmax 0.09 ng/mL and AUC 2.6 ng.h/mL at a dose of 630 ng, the margin to the NOAEL dose in rat (15 µg/kg/dose) is approximately 777X (Cmax) and 121X (AUC) and in monkey (200 µg/kg/dose) is approximately 19,100X (Cmax) and 8461X (AUC). Thus, at a dose level of 630 ng, severe or irreversible toxicities are not expected.

Based on emerging clinical data from Part 1, the GSK1795091 is likely to produce effects similar to LPS at a dose between 140 to 280 ng. Therefore, the top dose in the study was revised to 210 ng.

4.5.4. Summary

The starting dose of 7 ng is expected to be safe and appropriately conservative for a healthy subject study. Sensitive assays in a cynomolgus monkey model were used to calculate the MABEL. At the dose defining the MABEL, animals experienced neither adverse events nor histopathological changes. Furthermore, the dose is 8-fold higher than the HED of the non-pyrogenic dose in rabbits. Additionally, clinical experience with TLR4 agonists administered to healthy subjects provides a reference value against which the starting dose of GSK1795091 can be compared. Since GSK1795091 has *in vitro* potency similar to (or lower than) LPS, a starting dose of 7 ng is conservative in comparison to the 20 to 40-fold higher doses of LPS routinely administered to healthy subjects. These estimates provide a high level of confidence that the starting dose of 7 ng can safely be administered to healthy subjects. Dose escalation is expected to stop, at or below 210 ng, based on mild to moderate toxicities that are reversible and readily monitored. The top dose in the study, 210 ng, does not approach the Cmax or AUC at the NOAEL in rats or in monkeys. Thus, dose escalation proceeds from a conservative starting dose to a top dose unlikely to be associated with severe or irreversible effects.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK1795091 can be found in the Investigator's Brochure. The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK 1795091]		
Cytokine-associated symptoms (flu-like symptoms, fever, tachycardia, etc.) or cytokine release syndrome.	TLR agonists (including TLR4 agonists) are known to increase serum cytokine levels based upon extensive evaluations in laboratory animals, healthy subjects and patients.	• Starting dose defined based upon MABEL. • Stopping rules designed to not exceed tolerability precedents from prior TLR4 agonist studies in healthy subjects. • Staggered dosing of subjects. • Study conduct in medical center-based phase I unit with experience administering immune system agonists. • Enrolment of subjects with no prior significant medical conditions. Frequent vital sign measurements and telemetry for 24 hours. • Adverse event management with supportive care and paracetamol. • Reference to management algorithms for managing severe cytokine release syndrome (CRS).
Hypotension	Rare cases of severe hypotension have been described in healthy subjects receiving LPS. Risks for these events may include prior history of syncope and inadequate hydration.	• Exclusion of subjects with history of syncope. • Hydration of subjects prior to study product administration. • Frequent vital sign measurements and telemetry for 24 hours. • Adverse event management with fluids, and if necessary, vasopressor support.
Study Procedures		
Frequent phlebotomy		Overall volume of blood drawn will not exceed 500 ml.

4.6.2. Benefit Assessment

Healthy subjects enrolled in this study will not receive clinical benefit from volunteering.

4.6.3. Overall Benefit:Risk Conclusion

The study is designed to minimize risks to subjects through the use of eligibility criteria, a conservative approach to dosing, guidance for stopping criteria, and rigorous safety monitoring by qualified personnel at a center equipped to conduct FTIH studies. The preclinical data, drug class, and safety precautions summarized in the protocol provide a benefit:risk consistent with standard clinical trials performed in healthy subjects.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in the Investigator's Brochure.

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE
1. Between 18 and 50 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and 12-lead ECG. (A subject with a clinically insignificant abnormality or laboratory parameter(s) may be included only if the Investigator documents that the finding is unlikely to represent a safety risk and will not interfere with the study procedures.)

WEIGHT
3. Body weight 55 - 95 kg and body mass index (BMI) within the range 19 – 30 kg/m2 (inclusive).

SEX
4. Male or Female of non-childbearing potential: **Males**: Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until the follow-up visit. . Acceptable forms of contraception include: a. Vasectomy with documentation of azoospermia. b. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant • Intrauterine device or intrauterine system • Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a] • Contraceptive vaginal ring [Hatcher, 2007a] • Percutaneous contraceptive patches [Hatcher, 2007a] This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g., male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the ICH [ICH, M3 (R2) 2009].” The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception. **Females**: A female subject is eligible to participate if she is not pregnant (as confirmed by a negative serum or urine human chorionic gonadotrophin (hCG) test), not lactating, and at least one of the following conditions applies: a. Non-reproductive potential defined as: • Pre-menopausal females with one of the following: • Documented tubal ligation • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion • Hysterectomy • Documented Bilateral Oophorectomy

Postmenopausal defined as 24 months of spontaneous amenorrhea and follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels).

INFORMED CONSENT
5. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTcF INTERVAL)
1. History of any significant medical condition (e.g. cardiac, pulmonary, metabolic, renal, gastrointestinal, rheumatological, etc.) 2. History of frequent (>1 per week) headache or myalgia. 3. History of asthma. 4. History of syncope. 5. History of liver disease, or known hepatic or biliary abnormalities. 6. AST, ALT, gamma-GT and bilirubin >1.0xULN 7. Creatinine >1.0 x ULN. 8. Vital signs • SBP <90 and > 140 mmHG • DBP<50 and >90 mmHG • Heart Rate <50 and >90 bpm • Temperature >37.5°C 9. Clinically significant ECG abnormality and/or • Heart Rate < 50 and > 90 bpm • PR Interval > 220 msec • QRS Duration > 120 • QTcF > 450 msec Note that if ECG abnormalities are identified, the ECG should be repeated two more times (with 5 minutes between ECG readings) and the average of the 3 values used to determine eligibility.

CONCOMITANT MEDICATIONS
10. Anticipated requirement for any prescription medication during the study.

RELEVANT HABITS
11. History of regular alcohol consumption within 6 months of the study averaging a weekly intake of >14 drinks for males or >7 drinks for females or inability to abstain from alcohol from 1 day prior to the inpatient period of the study until discharge. (One drink is equivalent to 8 g of alcohol: 200ml of beer, 100ml of wine or 1 measure (25 ml) of spirits)
12. Urinary cotinine levels indicative of smoking or history or regular use of tobacco or nicotine-containing products within 2 months prior to screening or inability to abstain from smoking during the study.

CONTRAINDICATIONS
13. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
14. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test (anti-HBc Ab) result at screening or within 3 months prior to first dose of study treatment. Subjects with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA PCR test is obtained.
15. A positive pre-study drug/alcohol screen.
16. A positive test for HIV antibody.
17. Donation of blood or blood products in excess of 500 mL within a 56 day period.
18. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first visit (Day -2) in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
19. Exposure to more than four new chemical entities within 12 months prior to the first visit (Day -2).
20. Exposure to GSK1795091 in a previous cohort of this study.
21. Subject is unable to refrain from taking non-prescription drugs (including vitamins and dietary or herbal supplements), within 7 days prior to the first dose of study medication until completion of the follow-up visit, unless in the opinion of the investigator and sponsor the medication will not interfere with the study.
22. Subject is unable to understand and communicate in German/or native language of the site
23. Subject, or close relative of the subject, is the investigator or a subinvestigator, research assistant, pharmacist, study coordinator, or other staff directly involved with the conduct of the study at that site.
24. Vulnerable subjects (eg subjects kept in detention)

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.3.1.4).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

- The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed 'lost to follow up', the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and if necessary a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up".

A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. Furthermore, any female subject who becomes pregnant while participating will be withdrawn from the study [see Section 12.5.2]. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

5.4.1. Subject withdrawal procedures

Where possible, if a subject decides to withdraw or is withdrawn during a study day, all scheduled assessments will be taken as planned. The investigator must also make every effort to perform the following evaluations if not already scheduled for that day:

- Brief Physical exam
- 12-lead ECG
- Supine vital signs
- Clinical laboratory tests (clinical chemistry, haematology and urinalysis)
- Adverse events and concomitant medications assessment
- Pregnancy test (females only)

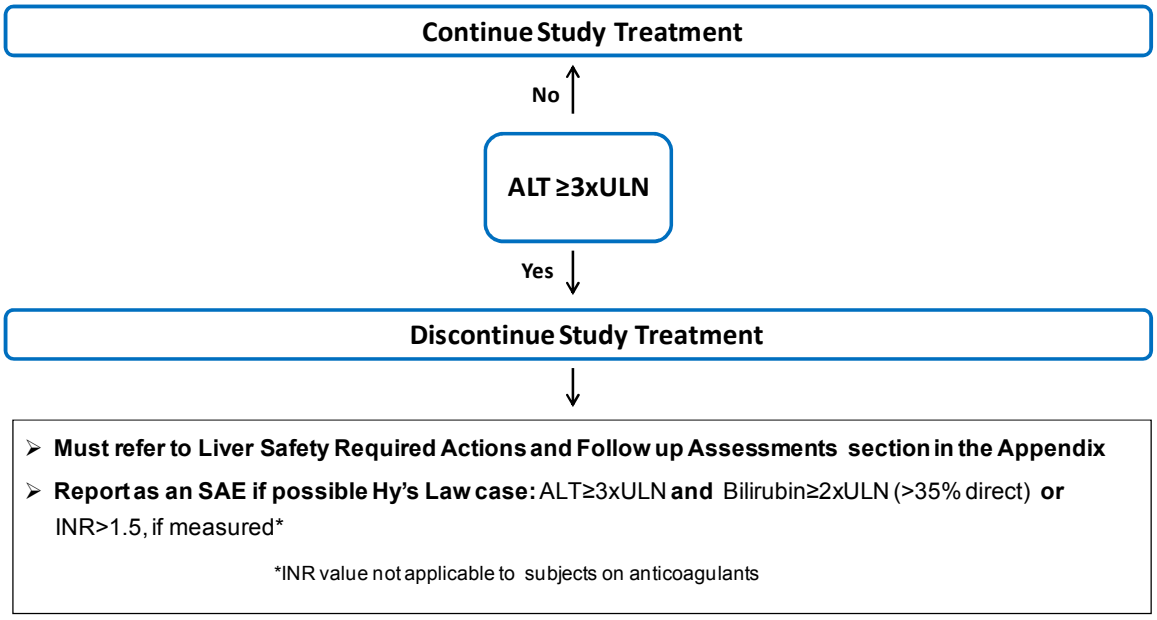
If a subject prematurely withdraws or is withdrawn on a non-study visit day, then the investigator must make every attempt to conduct the above evaluations.

Subjects who withdraw may be replaced following discussions between the investigator and GSK study team. Replacement subjects will be assigned to the same treatment as that of the subject they are replacing.

5.4.2. Liver Chemistry Stopping Criteria

Study treatment will be discontinued **for a subject** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 3.

5.4.3. QTcF Stopping Criteria

- The QTcF should be based on single or averaged QTcF values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion below will be monitored until parameters return to baseline values and will not be eligible to receive additional doses of GSK1795091 (if enrolled in Part 2 of the study).

- QTcF > 500 msec,
- Change from baseline: QTcF >60 msec

5.5. Subject and Study Completion

A completed subject is one who has completed all phases of the study including the follow-up visit or contact.

The end of the study is defined as the last subject's last visit.

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

On Day -1, subjects will be hydrated with approximately 1200 mL glucose/saline (2.5% glucose/0.45% sodium chloride) (e.g. intravenous drip 150 mL/hr). Intravenous hydration (sourced locally) should continue on Day 1, beginning approximately 2 hours prior to and continuing for 6 hours after administration of study product (intravenous drip 150 mL/hr).

Subjects will receive GSK1795091 administered as an intravenous bolus slowly over 2-5 minutes.

	Study Treatment	
Product name:	GSK1795091 Injection, 0.001mg/mL, 5mL vial with 5.2mL fill volume	Placebo Sodium Chloride Injection
Formulation description:	A clear solution in a 5ml vial with a 20mm stopper and overcap.	Commercial saline solution A clear colourless solution containing 0.9% sodium chloride
Dosage form:	Solution for Injection	Solution for Injection
Unit dose strength(s)/Dosage level(s):	0.001mg/mL (1000 nanogram per mL)	N/A
Route of Administration	Intravenous Injection	Intravenous Injection
Dosing instructions:	Administered as an intravenous bolus slowly over 2-5 minutes, followed by an intravenous bolus of 10mL of normal saline	To be administered in an identical manner to GSK1795091
Physical description:	A clear, colourless to slightly coloured solution, free from visible particles in a clear glass vial with grey stopper and aluminium grey brown overseal.	A clear colourless solution

6.2. Treatment Assignment

Subjects will be assigned to a specific treatment group for Part 1 in accordance with the randomization schedule generated by Clinical Statistics or designee using validated software. Subjects will be randomized prior to the first dose of the study treatment (GSK1795091 or placebo), after all screening assessments have been completed and after the Investigator has verified that they are eligible per the study inclusion/exclusion criteria. No subject may receive the study treatment prior to randomization and assignment of a unique subject identification number.

Each subject scheduled to receive the study treatment will receive a treatment allocation number when randomized. The treatment number will indicate if the subject is to receive the scheduled dose of GSK1795091 or placebo.

6.3. Blinding

This study will be conducted in 2 parts.

Part 1

Part 1 will be double-blind, with both the investigator and subject blinded to study treatment. All site personnel with the exception of the pharmacy team will remain blinded to study treatment except as discussed below. The sponsor's team will be unblinded.

The investigator or treating physician may unblind a subject's treatment assignment only in the case of an emergency OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator. Investigators have direct access to the subject's individual study treatment.The unblinded site pharmacist will receive the randomization code and ensure that it is stored in a secure area, accessible by the PI, treating physician or designee, in emergency situations. It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options before unblinding the subject's treatment assignment. If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding. The date and reason for the unblinding must be fully documented in the CRF/eSource.

The GSK medical monitor, statistician, and pharmacokineticist will be unblinded to subject level data for each cohort. Following review of safety data and attribution assessments by the study investigator, the GSK medical monitor may determine that the investigator and GSK study team can be unblinded to study treatment in cases where adverse events in a placebo-treated subject might stop dose escalation. GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with a SAE. Additionally, if a SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may send a copy of the report, identifying the subject's treatment assignment, to investigators in accordance with local regulations and/or GSK policy.

Part 2

Part 2 will be open-label where the sponsor, investigator and subject will be open to study treatment.

6.4. Packaging and Labeling

The contents of the label will be in accordance with all applicable regulatory requirements.

6.5. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for preparation of GSK1795091 will be detailed in a Study Specific Technical Agreement/Memo (TTS) or Pharmacy Manual which will be accompanied by a Quality Agreement.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.6. Compliance with Study Treatment Administration

Subjects will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

GSK1795091 will be intravenously administered to subjects at the site. Administration will be documented in the source documents and reported in the CRF/eSource.

6.7. Management Guidelines for Adverse Events

GSK1795091 has not previously been administered to humans although drugs in this class have generally caused mild to moderate toxicities associated with systemic cytokine

production. In a case report of a subject that received a very high dose (1 mg) of LPS, supportive care was provided in an Intensive Care Unit (ICU). Reported treatment included fluids and vasopressors to maintain hemodynamic stability and diuresis for pulmonary edema (Taveira da Silva, 1993). Recent experiences with adoptive cell therapies in cancer patients have lead to the publication of guidelines for managing cytokine release syndrome (CRS) (Lee, 2014). Patients with mild or moderate adverse events have been managed with supportive care, including fluids, vasopressors, and/or oxygen. Patients with high grade CRS (described by Lee, 2014. as requiring multiple doses of vasopressors, hypoxia requiring >40% oxygen, or organ toxicity) have been managed in an intensive care unit and received immunosuppressive treatment. Anecdotally, tocilizumab (anti-IL6 receptor antibody) has produced rapid and complete correction of CRS associated with cellular therapies although it is not approved for this indication (Maude, 2014). Lee, 2014 recommend administration of tocilizumab 4 mg/kg in adult patients as the first-line treatment of severe CRS with a repeat dose if clinical signs and symptoms do not improve within 24-48 hours.

Based upon these experiences with LPS and with cytokine release syndrome, recommendations for managing adverse events are as follows. Grade 1 and 2 adverse events should be managed with supportive care. Paracetamol may be administered for Grade 1 and 2 adverse events. Grade 3 and higher adverse events should be managed with supportive care, as determined by the investigator. Fluids and vasopressors should be used to support blood pressure. Tocilizumab may be considered if conventional supportive measures are unsuccessful or if transfer to an ICU is imminent.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK1795091 greater than the intended dose will be considered an overdose.

In the event of an overdose the investigator should:

1. contact the Medical Monitor immediately
2. closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities.
3. obtain serial plasma samples for PK and PD analyses over the first 24 hours
4. document the quantity of the excess dose in the CRF/eSource.

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy volunteers are eligible for study participation. Subjects may only participate in one cohort of this study.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Patients are required to consume fluids liberally from the time of admission to the inpatient unit until administration of study product.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) from 24 hours prior to admission to the inpatient unit until discharge
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to admission until after day 7 and 24h prior to the follow up
- Regular use (i.e. more than 3 times per week) of tobacco products is not allowed from 2 months prior to screening until after the final follow-up visit.

6.10.3. Activity

Subjects will abstain from strenuous exercise during the inpatient period of the study. Subjects may participate in light recreational activities (e.g., watch television, read).

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

Occasional paracetamol or NSAIDs, at prescribed doses, are permitted. Other concomitant medication may be considered on a case by case basis by the investigator in consultation with the GSK Medical Monitor.

6.11.2. Prohibited Medications and Non-Drug Therapies

Except as noted above, subjects are not allowed to receive non-study medications.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1.

The following points must be noted:

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:
    1. 12-lead ECG

2. vital signs
3. blood draws.

Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.

- The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamic/biomarker and exploratory biomarkers assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.
- No more than 500 mL of blood per subject will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Time and Events Table

7.1.1. Screening

Procedure	Screening (up to 30 days prior to Day -1)
Informed consent	X
Outpatient visit	X
Inclusion and exclusion criteria	X
Demographics	X
Full physical exam including height and weight	X
Medical history[1]	X
Drug/Alcohol screen	X
Serum/urine pregnancy test (WNCBP)	X
HIV, Hep B and Hep C screen	X
Haem/Clin Chem/PT & PTT/Urinalysis	X
12-lead ECG[2]	X
Vital signs (BP and heart rate)	X
Concomitant medication review	X

1. To include substance usage, family history of premature cardiovascular disease, medication, drug/alcohol history
2. Triplicate ECGs collected 5mins apart

7.1.2. Part 1: In-House Assessments

Procedure	Day -2	Day -1	Treatment Period Day 1														Day 2 (24h)	Day 3 (48h)	Day 4 (72h)	Day 5 (96h)
			Pre dose	0h	5min	0.25h	0.5h	1h	2h	3h	4h	6h	8h	10h	12h	16h				
Admission to the unit	X																			
Overnight stay	X	X	←==========→																	
Serum/urine pregnancy test (WNCBP)	X																			
Full physical exam including height and weight	X																			
Inclusion and exclusion criteria	X	X																		
Haem/Clin Chem/Urinalysis /Coagulation		X	X														X		X	
WBC with differential[1]		X	X					X	X		X		X			X	X	X	X	
Drug/Alcohol test	X																			
12 lead ECG			X						X		X		X				X		X	
Vital signs (Temperature, BP and Heart rate)		X[2]	X					X	X		X	X	X		X	X	X	X	X	X
Telemetry			←==========→																	
Dosing				X																
Intravenous hydration		X	←==========→																	
PK blood sampling[3]			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Procedure	Day -2	Day -1	Treatment Period Day 1													Day 2 (24h)	Day 3 (48h)	Day 4 (72h)	Day 5 (96h)
			Pre dose	0h	5min	0.25h	0.5h	1h	2h	3h	4h	6h	8h	10h	12h	16h				
Cytokine measurements[3]			X					X	X		X		X		X	X	X	X		
Leukocyte phenotyping[3]			X								X						X			
AE/SAE review[4]	X	X	←==========→																	
Concomitant medication review	X	X	←==========→																	
Discharge																				X

1. Where WBC+Differential and Haematology are scheduled for the same time, WBC+Differential may be collected as part of the Haematology assessment
2. Baseline Vital Signs to match Day 1 timepoints
3. PK and Biomarker Sampling: The pre-dose blood samples should be collected within 1 hr before administration of GSK1795091. Every attempt should be made to collect samples within ±5 min on samples up to 2 hrs, ±20 min on samples up to 12 hrs, and ±40 min on samples after 12 hrs. Actual date/time of sample collection and dosing must be recorded. Explanations are required for any deviations of >5 min from the planned time during the first 2 hrs and for deviations of more than 20 min from the planned time for samples collected at 3 hrs up to 10 hrs.
4. Blood samples may be collected to explore biomarkers at the discretion of the Investigator

7.1.3. Part 1 Outpatient Assessments

Procedure	Day 7	Follow-up (23 days ± 2 days post-last visit) or early withdrawal
Outpatient Visit or Contact[1]	X	X
Serum/urine pregnancy test (WNCBP)		X
Full physical exam including height and weight	X	
Haem/Clin Chem/Urinalysis/Coagulation	X	
12 lead ECG	X	
Vital signs (Temperature, BP and Heart rate)	X	
PK blood sampling[2]	X	
Cytokine measurements	X	
Leukocyte phenotyping	X	
AE/SAE review	X	X
Concomitant medication review	X	X

1. Outpatient visit at Day 7; Outpatient visit or Contact at FU. All subjects with (1) new AE or (2) unresolved AEs or abnormal labs at the Day 7 visit should return for a follow-up visit. Female subjects should return for pregnancy testing. For all other subjects a FU contact is acceptable
2. PK Sampling: Blood samples for analysis of GSK1795091 concentrations will be collected following dosing 144 hours (Day 7) after administration

7.1.4. Part 2: In-House Assessments

Procedure	Day -2	Day -1	Treatment Period Day 1[1]														Day 2 (24h)	Day 3 (48h)	Day 4 (72h)	Day 5 (96h)
			Pre dose	0h	0.083h (5min)	0.25h	0.5h	1h	2h	3h	4h	6h	8h	10h	12h	16h				
Admission to the unit	X																			
Overnight stay	X	X	←==========→																	
Serum/urine pregnancy test (WNCBP)	X																			
Full physical exam including height and weight	X																			
Inclusion and exclusion criteria	X	X																		
Haem/Clin Chem/Urinalysis/ Coagulation		X	X														X		X	
WBC with differential[2]		X	X					X	X		X		X		X		X		X	
Drug/Alcohol test	X																			
12 lead ECG[3]			X																X	
Vital signs (Temperature, BP and Heart rate)		X[4]	X					X	X		X	X	X		X	X	X	X	X	X
Dosing				X																
Intravenous hydration		X	←==========→																	
PK blood sampling[5]			X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	
Metabolite urine sampling			X	←==========→																
Cytokine measurements[5]			X					X	X		X		X		X	X	X	X		
Leukocyte phenotyping[5]			X								X						X			
Gene signature analysis from PBMCs[5]			X								X						X			
AE/SAE review[6]			←==========→																	X
Concomitant medication review	X	X	←==========→																	X
Discharge																				X[7]

1. Day 1 and Day 8 C1/ Day 15 C2
2. Where WBC+Differential and Haematology are scheduled for the same time, WBC+Differential may be collected as part of the Haematology assessment
3. Single ECGs all timepoints
4. Baseline Vital Signs to match Day 1 timepoints
5. PK and Biomarker Sampling: The pre-dose blood samples should be collected within 1 hr before administration of GSK1795091. Every attempt should be made to collect samples within ±5 min on samples up to 2 hrs, ±20 min on samples up to 12 hrs, and ±40 min on samples after 12 hrs. Actual date/time of sample collection and dosing must be recorded. Explanations are required for any deviations of >5 min from the planned time during the first 2 hrs and for deviations of more than 20 min from the planned time for samples collected at 3 hrs up to 10 hrs.
6. Blood samples may be collected to explore biomarkers at the discretion of the Investigator
7. Cohort 2 only. Cohort 1 will remain inhouse until discharge on Day 13

7.1.5. Part 2 Assessments

Procedure	7 Days Post Dose[1]	Follow-up[2] (23 days ± 2 days post-last visit) or early withdrawal
Outpatient Visit	X	X
Urine pregnancy test (WNCBP)		X
Full physical exam including height and weight		
Haem/Clin Chem/ Urinalysis/Coagulation		
12 lead ECG		
Vital signs (Temperature, BP and Heart rate)		
PK blood sampling	X	
AE/SAE review	X	X
Concomitant medication review	X	X

1. 7 days post first dose assessments (Cohort 1 D7; Cohort 2 D14) will be conducted in house; 7 days post second dose (Cohort 1 D15; Cohort 2 D22) assessments will be conducted as an outpatient visit
2. Outpatient visit or Contact at FU. All subjects with (1) new AE or (2) unresolved AEs or abnormal labs at the Day 7 visit should return for a follow-up visit. Female subjects should return for pregnancy testing. For all other subjects a FU contact is acceptable

7.2. Screening and Critical Baseline Assessments

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed at screening and day -1 as related to the inclusion/exclusion criteria listed in Section 5.

7.3. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.3.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 4.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.3.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- AEs will be collected from the start of Study Treatment until the follow-up contact (see Section 7.3.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF/eSource.
- All SAEs will be recorded and reported to GSK (or designee) within 24 hours, as indicated in Appendix 4.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Appendix 4.

7.3.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?"

7.3.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up (as defined in Section 5.4). Further information on follow-up procedures is given in Appendix 5.

7.3.1.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK (or designee) of SAEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.2. Pregnancy

- Details of all pregnancies in female partners of male subjects will be collected after the start of dosing and until final study follow-up contact.
- If a pregnancy is reported then the investigator should inform GSK within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Appendix 5.

7.3.3. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height and weight will also be measured and recorded.
- Investigators should pay special attention to clinical signs related to previous serious illnesses

7.3.4. Vital Signs

- Vital signs will be measured in semi-supine position after 5 minutes rest and will include oral temperature, systolic and diastolic blood pressure and heart rate and respiratory rate.
- Triplicate readings, separated by 1 minute or more, will be obtained at screening and at predose. Single readings will be obtained at all other timepoints.
- The grading of heart rate adverse events should be performed according to time-matched baseline measurements from Day -1.

7.3.5. Electrocardiogram (ECG)

ECG's will be measured in the semi-supine position following a 10 minute rest.

- Triplicate 12-lead ECGs, separated by 5 minutes or more, will be obtained at screening, Day 1 and Day 4 in Part 1. Single 12 lead ECGs will be obtained at all other timepoints for both Part 1 and Part 2. An ECG machine will be used that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals. Refer to Section 5.4.3 for QTcF withdrawal criteria and additional QTcF readings that may be necessary.
- Continuous cardiac telemetry will be performed for the first 24 hours for subjects in Part 1. Full disclosures will be reviewed in detail and the review maintained as part of the subject's source documents. As indicated, the investigator may perform unscheduled vital sign and/or ECG collections, based upon telemetry data, for the purpose of reporting adverse events.

7.3.6. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the SRM OR the laboratory manual. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF/eSource.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws. Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**
Haematology	Platelet Count; RBC Count; Hemoglobin; Hematocrit *RBC Indices*: MCV; MCH; MCHC *WBC count with Differential:* Neutrophils; Lymphocytes; Monocytes; Eosinophils; Basophils
Clinical Chemistry [1]	Urea; Potassium; AST (SGOT); Total and direct bilirubin Creatinine; Sodium; ALT (SGPT); Total Protein Glucose; Calcium; Alkaline phosphatise; Albumin CRP
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)
Coagulation Tests	• prothrombin time (or international normalized ratio) • partial thromboplastin time
Other Screening Tests	• HIV • Hepatitis B surface antigen (HBsAg) and core antibody (anti-HBc Ab) • Hepatitis C (Hep C antibody) • FSH and estradiol (as needed in women of non-child bearing potential only) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serum or urine hCG Pregnancy test. • Lipids, Total cholesterol, HDL, LDL • Gamma-GT

NOTES :

1. Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.4.2 and Appendix 3
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

7.3.7. Assessment windows

Procedure	Time	Allowed deviation
ECG and Vitals	Pre-dose	- 60 minutes
	5 min – 30 min	± 3 minutes
	45 min – 4 hours	± 15 minutes
	>4 hours	± 30 minutes
Labs	Pre-dose	- 60 minutes
	24 hours	± 60 minutes

7.4. Pharmacokinetics

7.4.1. Blood Sample Collection

Blood samples (4ml per timepoint) for pharmacokinetic (PK) analysis of GSK1795091 will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Sample collection, processing and storage details will be provided in the Study Reference Manual.

7.4.1.1. Part 1

Blood samples for the determination of GSK1795091 will be obtained on Day 1 through 7 following a single dose at the time points indicated in the Time and Events Table (Section 7.1).

7.4.1.2. Part 2

For Cohort 1 (1 week repeat dose), blood samples for the determination of GSK1795091 will be obtained on Day 1 through 7 following the first dose and on Day 8 through 15 following the second dose at the time points indicated in the Time and Events Table (Section 7.1). For Cohort 2 (2 week repeat dose), blood samples for the determination of GSK1795091 will be obtained on Day 1 through 14 following the first dose and Day 15 through 22 following the second dose at the time points indicated in the Time and Events Table (Section 7.1).

7.4.2. Urine Sample Collection

Urine will be collected following the first dose in Part 2 for GSK1795091 and metabolite identification. Urine will be collected pre-dose and 24 hours following the administration of GSK1975091. A 30 mL aliquot will be obtained from the pre-dose and 24-hour collections.

7.4.3. Sample Analysis

Plasma analysis will be performed under the control of PTS-BIB GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of GSK1795091 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Once the plasma has been analyzed for GSK1795091 any remaining plasma may be analyzed qualitatively for other compound-related metabolites and the results reported under a separate GlaxoSmithKline protocol.

Plasma and urine may be analyzed qualitatively for GSK1795091 and other compound-related metabolites and results reported under a separate GlaxoSmithKline protocol.

7.5. Biomarker(s)/Pharmacodynamic Markers

For exploratory Biomarkers, Blood samples will be collected at time points indicated in Section 7.1 to determine the pharmacodynamic effect of GSK1795091 on plasma cytokines which are, but not limited to, TNF-alpha, IL-10, IL-1beta, IL-1Ra, IFN-gamma, IL-12p70, IL-2, IL-4, IL-5, IL-6, IL-8, GCSF, IP-10, MCP-1, RANTES by multiplex immunoassay, leukocyte populations and their activation status or phenotype by flow cytometry and PBMCs Pan cancer immune gene expression profiles, but not limited to, by nanostring. Details for sample collection, processing, storage, and shipment will be provided in the Study Reference Manual (SRM)

8. DATA MANAGEMENT

- All participant data relating to the study will be recorded on printed CRF/eSources or collected electronically (e.g. eCRF, an eSource system, Laboratory Information Management System/LIMS etc). The investigator is responsible for verifying that site data is accurate and complete by physically or electronically signing the CRF or eSource in the absence of a CRF.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

This study is designed to primarily investigate the safety, and tolerability of GSK1795091 following IV doses in healthy subjects, and to inform the design of the first-in-cancer patient clinical trial.

Part 2 of the study is designed to inform the dosing interval to be evaluated in the first-in-cancer patient trial. Bayesian decision criteria will be used to inform the recommendation regarding two proposed dosing intervals (i.e., Q1W or Q2W). The posterior probability that C-reactive protein (CRP) change from baseline of the second dose is less than that of the first dose will be evaluated at the end of study based on a minimally informative prior distribution describing CRP changes in concert with the observed data of CRP changes in both Part 1 and Part 2 of the study. Tolerance to GSK1795091 will be defined as a 33% decrease in change from baseline in CRP following the second dose of GSK1795091 relative to that of the first dose with a posterior probability greater than 90%.

The pharmacokinetics of GSK1795091 will be investigated as secondary objectives.

No formal statistical hypotheses will be tested. An estimation approach providing point estimates and corresponding confidence intervals will be used, where appropriate.

9.2. Sample Size Considerations

9.2.1. Sample Size Assumptions

As this is the first in human study, sample size for part 1 is largely based on feasibility, i.e., no formal power calculations were performed.

For part 2 of the study, the sample size was estimated based on the assumption that the dose of GSK1795091 administered would produce changes in CRP comparable to a 2 ng/kg dose of LPS, 18 ± 6 mg/L (Dillingh, 2014). The prior distribution of CRP changes from baseline for the first dose and second dose will follow a bivariate normal distribution with a minimal informative prior mean and covariance matrix. This simulation assumes a correlation of 0.5 in the CRP change from baseline between day 1 and day 7. With a sample size of six subjects (n=6) and based on 1000 simulations, the chance of declaring an unacceptable dose interval is 11% when there is no difference in CRP change from baseline between the first dose and second dose. The chance of declaring an unacceptable dosing interval will be 82% when there is a 33% decrease in the CRP following the second dose relative to that of the first dose.

9.2.2. Sample Size Sensitivity

No statistical sample size sensitivity calculations were performed

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation will be performed.

9.3. Data Analysis Considerations

Analysis datasets will be created according to GSK standards within CDISC standards (SDTM IG Version 3.1.3 & AdaM IG Version 1.0].

9.3.1. Analysis Populations

All Subjects Population: All subjects enrolled into the study who have received a dose of study medication (GSK1795091 or placebo) will be included in the safety population. This population will be used in the evaluation of safety/tolerability, subject disposition, and in the demographic summary.

PK Concentration Population: This population will include all subjects in the "All Subjects Population" for whom a pharmacokinetic blood sample was obtained and assayed. This population will be used for listing, summarizing and plotting of plasma concentration-time data.

PK Parameter Population: This population will include all subjects in the "PK Concentration Population" for whom valid and evaluable pharmacokinetic parameters were derived. This population will be used in the assessment and characterization of PK parameters.

PD Population: This population will include all subjects in the "All Subjects Population" for whom valid and evaluable pharmacodynamic parameters were derived. This population will be used in the assessments and characterization of PD parameters.

9.3.2. Interim Analysis

No formal interim analyses are planned for this study except, as described above, for making dose escalation decisions (Section 4).

9.3.3. Final Analysis

Final analysis will be performed after all subjects enrolled have completed the study and after Data Base Freeze (DBF) and unblinding

9.4. Key Elements of Analysis Plan

9.4.1. Primary Analyses

No formal statistical hypothesis testing of safety data will be carried out.

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL) standards.

All subjects enrolled into the study who have received a dose of study medication (GSK1795091 or placebo) will be included in the safety population. This population will be used in the evaluation of safety/tolerability, subject disposition, and in the demographic summary.

The probability of safety events (e.g. liver toxicity defined by an ALT greater than 3xULN, QTcF > 500 msec, change from baseline QTc > 60 msec) will be estimated and with associated 95% corresponding confidence intervals for each cohort using the binomial distribution.

If a certain safety adverse event is not observed at a dose level, it does not mean that the risk associated with this event is very low. The upper limit of 95% confidence interval of the probability of such undesirable events that have not yet occurred in a finite number of subjects is estimated below using exact calculations (i.e. 1 – alpha (1/n)).

Sample Size (n)	3	4	6	8	12	18	20
Upper limit of risk	0.63	0.52	0.39	0.31	0.22	0.15	0.14

So for example with 6 subjects, if no adverse event is seen at a dose level there is reasonably certainty that the rate of events in the population of subjects, at the dose level, will be less than 39%.

9.4.2. Secondary Analyses

9.4.2.1. Pharmacokinetic Analyses (Part 1 and Part 2)

Non-Compartmental PK Analyses

Pharmacokinetic analysis of GSK1795091 concentration-time data will be conducted by non-compartmental methods. The following PK parameters will be determined if data permit:

- Maximum concentration (Cmax)
- Time to maximum concentration (tmax)
- Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration [AUC(0-last)]
- Partial area under the concentration-time curve to time = [AUC(0-t)] (where t may be 24, 168 h or other as appropriate), and/or
- Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time [AUC(0-∞)]
- Terminal half life (t1/2)
- clearance (CL)
- Volume of distribution (Vd)
- Volume of distribution (Vss) (where V = CL/MRTiv)

Statistical Analyses of Pharmacokinetic Data

GSK1795091 concentration-time data will be listed for each subject and summarized by planned time point and dose cohort in Parts 1 and 2.

Except for tmax, pharmacokinetic parameters will be listed and summarized descriptively (mean, standard deviation [SD], median, minimum, maximum, geometric mean, and the SD, CV% and 95% confidence interval [CI] of $\log_e$-transformed parameters) by dose cohort in Parts 1 and 2. Tmax will be listed and summarized descriptively (mean, standard deviation [SD], median, minimum, maximum).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively.

Dose Proportionality

If more than two dose cohorts are completed, dose proportionality of GSK1795091 AUC(0-∞) (or AUC(0-t)) and Cmax following single dose administration and AUC(0- t) and Cmax following repeat dose administration will be evaluated graphically and using the power model as described below:

$$\log_e (\text{PK parameter}) = a + b * \log_e (\text{dose})$$

where a is the intercept and b is the slope

The power model will be fitted by restricted maximum likelihood (REML) using SAS Proc Mixed. Both the intercept and slope will be fitted as fixed effects. If there is sufficient data, the model may also be fit with the intercept and/or slope as random effects depending on the ability of the model to converge and on estimation of variance-covariance matrix. The mean slope and corresponding 90% CI will be estimated from the power model.

9.4.2.2. Pharmacokinetic Analysis for Part 2 Only

Accumulation Ratio

To estimate the extent of accumulation after repeat dosing, the observed accumulation ratio (Ro) will be determined as AUC(0-τ) following the second dose to AUC(0-τ) after single dose, where τ is the dosing interval (1 or 2 weeks). The ratio of AUC (0-τ) at steady-state to AUC (0-∞) for a single dose will be calculated to assess time invariance.

Time Invariance

For the repeat dose phase, an analysis of variance (ANOVA) with terms for subject as random-effect and day as fixed-effect will be presented by dose on the log-transformed AUC values. The time invariance of GSK1795091 will be assessed by comparing AUC(0-∞) following a single dose on Day 1 and AUC(0-τ) following a second dose on either Day 7 (Cohort 1) or 14 (Cohort 2) for each dose and a listing of individual ratios will be presented.

9.4.3. Other Analyses

9.4.3.1. Exploratory Biomarker(s) Analyses

The results of these biomarker investigations may be reported separately from the main clinical study report. All endpoints of interest from all comparisons will be descriptively and/or graphically summarized as appropriate to the data. Additional exploratory analyses may be performed to further characterize these Biomarkers.

9.4.3.2. Pharmacokinetic/Pharmacodynamic Analyses

Exploratory graphical PK/PD analyses may be performed to examine the relationship between GSK1795091 PK and response as measured by vital signs (e.g., body temperature, heart rate, etc.) or PD biomarkers (e.g., cytokines/chemokines) or other endpoints if warranted by the data (e.g., grade, onset and duration of AEs). The relationship(s) between PD endpoints and PK parameters initially will be explored graphically. Plots of PD endpoint versus PK parameters of GSK1795091 will be generated.

Further analysis may be conducted if the initial plots suggest a correlation between the PD and PK endpoints. Models to describe the relationship(s) may include linear models and/or a maximum effect models. Other more complex models may be explored if warranted by the data. Results may be included in the final study report for this study or reported separately.

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

10.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

Prior to initiation of a site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable

- Obtaining signed informed consent
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study

10.3. Quality Control (Study Monitoring)

- In accordance with applicable regulations including GCP, and GSK procedures, monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements.
- When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the CRF/ eSource will serve as the source document.

GSK will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents

10.4. Quality Assurance

- To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.
- In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.

- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.
- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.
- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.
- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.

- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11. REFERENCES

Astiz ME, Rackow EC, Still JG, Scott T, Cato A, Von Eschen K, Ulrich JT, Rudbach JA, McMahon G, Vargas R, Stern W. Pretreatment of normal humans with monophosphoryl lipid A induces tolerance to endotoxin: A prospective, double-blind, randomized, controlled trial. *Critical Care Medicine.* 1995;23:9-17.

Bahador M, Cross AS. From therapy to experimental model: a hundred years of endotoxin administration to human subjects. *Journal of Endotoxin Research.* 2007;13:251-279.

Chen DS, Mellman I. Oncology meets immunology: The cancer- immunity cycle. *Immunity.* 2013;39:July 25.

Clyne B, Olshaker JS. The C-reactive protein. *The Journal of Emergency Medicine.* 1999;17:1019-1025.

de Bono JS, Dalgleish AG, Carmichael J, Diffley, J, Lofts FJ, Fyffe D, Ellard S, Gordon RJ, Brindley CJ, Evans, TRJ. Phase I study of ONO-4007, a synthetic analogue of the lipid A moiety of bacterial lipopolysaccharide. *Clinical Cancer Research.* 2000;6:397-405.

de Vos AF, Pater JM, van den Pangaart PS, de Kruif MD, van't VeerC, van der Poll T. In vivo lipopolysaccharide exposure of human blood leukocytes induces cross-tolerance to multiple TLR ligands. *Journal of Immunology.* 2009;183:533-542.

Dillingh MR, van Poelgeest EP, Malone KE, Kemper EM, Stroes ESG, Moerland M, Burggraaf J. Characterization of inflammation and immune cell modulation induced by low-dose LPS administration to healthy volunteers. *Journal of Inflammation.* 2014;11:28.

Food and Drug Administration (FDA). *Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers.* U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), .; 2005. Pharmacology and Toxicology.

Guha M. Anticancer TLR agonists on the ropes. *Nature Reviews/ Drug Discovery.* 2012;11:503-505.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(a): 24. Table 3-2.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(b): 28.

ICH, M3 (R2), 2009
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf

Isambert N, Fumoleau P, Paul, C, Ferrand C, Zanetta S, Bauer J, Ragot K, Lizard G, Jeanin J-F, Bardou M. Phase I study of OM-174, a lipid A analogue, with assessment of immunological response, in patients with refractory solid tumors. *BMC Cancer.* 2013;13:172.

Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nature Medicine.* 2007;13:552-559.

Lee DW, Gardner R, Porter DL, Louise CU, Ahmed N, Jensen M, Grupp SA, Mackall CL. Current concepts in the diagnosis and management of cytokine release syndrome. Blood, 2014 124: 188-195

Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Sheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL, Grupp SA. Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. *The New England Journal of Medicine.* 2014;371:1507-1517.

Taveira da Silva AM, Kaulbach HC, Chuidian FS, Lambert DR, Suffredini AF, Danner RL. Brief Report: Shock and multiple organ dysfunction after self-administration of salmonella endotoxin. *The New England Journal of Medicine.* 1993;328:1457-1460.

van Eijk LTGJ, Pickkers P, Smits P, Bouw MPWJM, van der Hoeven JG. Severe vagal response after endotoxin administration in humans. *Intensive Care Med.* 2004;30:2279-2281

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
AGP	Aminoalkyl glucosaminide 4-phosphate
ALT	Alanine aminotransferase (SGPT)
ANOVA	Analysis of variance
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
AUC(0-last)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration
AUC (0-t)	Partial area under the concentration-time curve to time = t (where t may be 24, 168 h or other as appropriate),
AUC (0-∞)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
BMI	Body mass index
BP	Blood pressure
BPM	Beat Per Minute
BUN	Blood urea nitrogen
CI	Confidence Interval
CL	Clearance
Cmax	Maximum observed drug concentration
CPK	Creatine phosphokinase
CRF	Case Report Form
CRP	C-reactive protein
CRS	Cytokine release syndrome
CV	Cardiovascular
DBF	Database freeze
DBP	Diastolic blood pressure
DC	Dendritic cell
DNA	Deoxyribonucleic acid
EC10	10% Effect Concentration
ECG	Electrocardiogram
FDA	Food and drug administration
FSH	Follicle Stimulating Hormone
FTIH	First time in humans
GCP	Good Clinical Practice
GCSF	Granulocyte-colony stimulating factor
GCSP	Global Clinical Safety and Pharmacovigilance
GGT	Gamma glutamyltransferase
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HED	Human equivalent dose

HIV	Human Immunodeficiency Virus
h/hr	Hour(s)
HR	Heart rate
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive care unit
IDSL	Integrated Data Standards Library
IEC	Independent Ethics Committee
IFN	Interferon
IgM	Immunoglobulin M
IL	Interleukin
IP	Investigational Product
IRB	Institutional Review Board
IU	International Unit
IV	Intravenous
L	Litre
LFTs	Liver function tests
LPS	Lipopolysaccharide
MABEL	Minimum anticipated biologic effect level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCP	Monocyte chemoattractant protein
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Millilitre
mm	Millimetre
mmHg	Millimetres of mercury
MPL	Monophosphoryl lipid
MSDS	Material Safety Data Sheet
msec	Milliseconds
N	Sample size
NOAEL	No observed adverse effect level
NSAIDs	Non steroidal anti-inflammatory drugs
PAMPs	Pathogen-Associated Molecular Patterns
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PK	Pharmacokinetic(s)
PTS-BIB	Platform Technology and Science, Bioanalysis, Immunoginicity and Biomarkers
PT	Prothrombin time
QTcF	QT duration corrected for heart rate by Fridericia's formula

RANTES	Regulated on Activation, Normal T Expressed and Secreted
RAP	Reporting and Analysis Plan
RBC	Red blood cells
REML	Restricted maximum likelihood
RNA	Ribonucleic acid
Ro	Accumulation ratio
SAE	Serious adverse event(s)
SBP	Systolic blood pressure
SD	Standard deviation
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SOP	Standard Operating Procedure
SRM	Study Reference manual
t	Time of last observed quantifiable concentration
t1/2	Terminal half life
TLR	Toll-like receptor
tmax	Time of occurrence of Cmax
TNF	Tumor necrosis factor
TTS	Study Specific Technical Agreement/Memo
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
UK	United Kingdom
USA	United States
Vd	Volume of distribution
Vss	Volume of distribution
WBC	White blood cells
WNCBP	Women of non child bearing potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Chiron RIBA
SAS
WinNonlin

12.2. Appendix 2: FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials

Taken from the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

The purpose of this appendix is to provide guidance and is to be used in conjunction with the investigator's judgment. Alternative criteria for fever and tachycardia are provided in Section 4.2. The scales for vital signs and laboratory abnormalities are intended to guide the grading of adverse events that are clinically significant and changed from a subject's baseline. Similarly, the scales for systemic (general) events should take into account the normal range of symptom intensity and duration for the setting as well as an individual subject's baseline status, in particular, when evaluating subjective criteria (e.g. "some interference with activity").

Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs *	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia

Vital Signs *	**Mild (Grade 1)**	**Moderate(Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

*Subject should be at rest for all vital sign measurements

**Oral temperature; no recent hot or cold beverages or smoking

***When resting heart rate is between 60-100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes

Systemic (General)	**Mild (Grade 1)**	**Moderate(Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***ULN" is the upper limit of the normal range.

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** "ULN" is the upper limit of the normal range.

Urine *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

12.3. Appendix 3: Liver Safety Required Actions and Follow up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="2">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td>ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >1.5, Report as an SAE.
See additional Actions and Follow Up Assessments listed below</td></tr>
<tr><th colspan="2">Required Actions and Follow up Assessments following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th>Follow Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment Report the event to GSK within 24 hours
• Complete the liver event CRF/eSource, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline
• A specialist or hepatology consultation is</td><td>• Viral hepatitis serology3
• Blood sample for pharmacokinetic (PK) analysis, obtained 7 days post last dose[4]
• Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
• Fractionate bilirubin, if total bilirubin≥2xULN
• Obtain complete blood count with differential to assess eosinophilia
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form
• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications.
• Record alcohol use on the liver event alcohol intake case report form
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	• Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF/ eSource forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that subject if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF/eSource. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

References

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA, Davern TJ, Lee WM. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. Definition of Adverse Events

Adverse Event Definition:
• An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae). • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. • The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

Events NOT meeting definition of an AE include:
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.4.2. Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires hospitalization or prolongation of existing hospitalization NOTE: • In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen

from baseline is not considered an AE.

d. Results in disability/incapacity

NOTE:

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious.
- Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse

g. Is associated with liver injury and impaired liver function defined as:

- ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or**
- ALT ≥ 3xULN and INR** > 1.5.

* Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE.

** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.

- Refer to Appendix 3 for the required liver chemistry follow-up instructions

12.4.3. Recording of AEs and SAEs

AEs and SAE Recording:
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. • The investigator will then record all relevant information regarding an AE/SAE in the CRF/eSource • It is **not** acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE CRF/eSource page. • There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms. • Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study. • Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer. • The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.4.4. Evaluating AEs and SAEs

Assessment of Intensity
The investigator will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories: • Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities • Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. • An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.
- For each AE/SAE the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings, including histopathology.
- New or updated information will be recorded in the originally completed CRF/eSource.
- The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

12.4.5. Reporting of SAEs to GSK

SAE reporting to GSK via paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail • Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.

12.5. Appendix 5: Modified List of Highly Effective Methods for Avoiding Pregnancy in FRP and Collection of Pregnancy Information

12.5.1. Modified List of Highly Effective Methods for Avoiding Pregnancy in Females of Reproductive Potential (FRP)

This list does not apply to FRP with same sex partners, when this is their preferred and usual lifestyle or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis.

- Contraceptive subdermal implant
- Intrauterine device or intrauterine system
- Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a]
- Injectable progestogen [Hatcher, 2007a]
- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]
- Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher, 2007a].
- Male condom plus partner use of one of the contraceptive options below:
    - Contraceptive subdermal implant
    - Intrauterine device or intrauterine system
    - Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a]
    - Injectable progestogen [Hatcher, 2007a]
    - Contraceptive vaginal ring [Hatcher, 2007a]
    - Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all inclusive list of those methods that meet the GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and, correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the ICH [ICH, M3 (R2) 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

12.5.2. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 4. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating:

- will be withdrawn from the study

- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomized to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy
- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.6. Appendix 6: Country Specific Requirements

No country-specific requirements exist.

12.7. Appendix 7: Protocol Changes

12.7.1. AMENDMENT 1

This amendment will apply to all study sites and all countries.

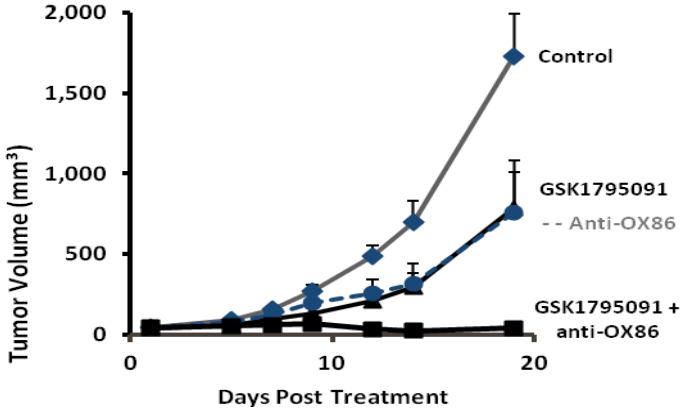
List of specific changes:

SECTION 2.2 Brief Background

TLR biology

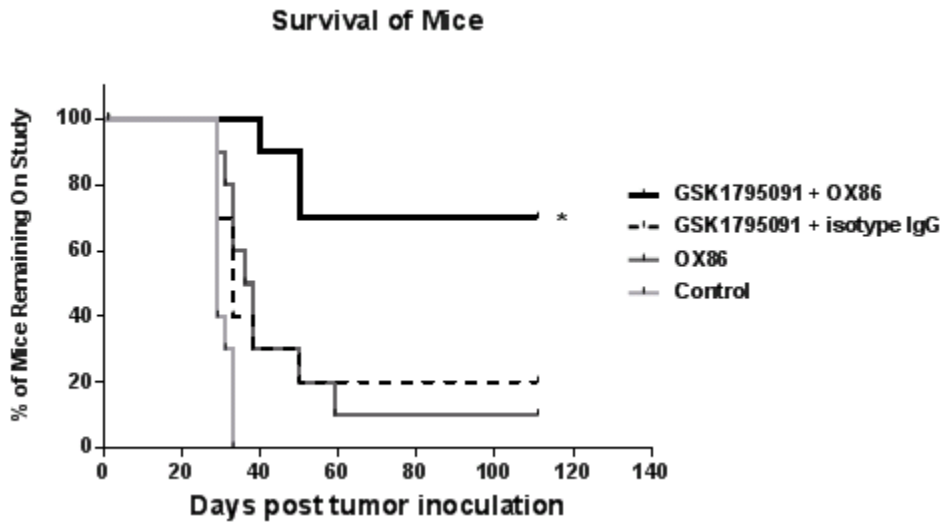
GSK1795091 is an agonist of TLR4 that induces immunologic responses *in vitro* and *in vivo*. GSK1795091, as a single-agent, stimulates cytokine production (*in vitro* and *in vivo*), changes in immune cell populations (*in vivo*) and generates fever response (*in vivo*). When administered by intravenous bolus and combined with the immune checkpoint modulator, OX86, mouse surrogate OX40 agonist antibody, ~~OX86~~ induced a potent immunologic response and significant tumor shrinkage in a syngeneic mouse model of cancer. ~~Indeed, 30-70% of mice were tumor free for at least 2 months after combination treatment of 25 µg GSK1795091 and 25 µg OX86.~~ Four (4) groups of 10 Balb/c mice (with intact immune systems) were implanted with syngeneic CT-26 tumors. Mice received either placebo, GSK1795091, OX86, or the combination of GSK1795091 and OX86. While the monotherapies had modest effects on tumor growth (Figure 1), the combination produced durable responses.

Figure 1. CT26 Tumor Volume



The reduction in tumor volume translated to significant improvement in the survival of animals (Figure 2). Approximately, 70% of animals that received the combination of GSK1795091 and survived more than 100 days. By contrast, only 10-20% of the animals that received either monotherapy survived 100 days.

Figure 2: Survival of mice



This model provides support for the evaluation of this combination in patients with cancer. Additional details are available in the Investigator's Brochure.

Healthy subject experience with TLR4 agonists

Synthetic TLR4 agonists, such as monophosphoryl lipid A (MPL), have similarly been studied in ascending dose studies [Astiz, 1995]. MPL is used as a vaccine adjuvant in Fendrix, a hepatitis B vaccine, and Cervarix, a human papillomavirus vaccine [Kanzler, 2007]. Early in development of MPL, healthy subjects received escalating intravenous doses from 1 to 20 µg/kg. The 20 ~~u~~µg/kg dose was considered safe and biologically active. Systemic adverse events and cytokine increases were observed after doses of 10 ~~u~~µg/kg. At the highest doses tested, 6 of 6 subjects experienced adverse events including fevers, chills, headache, myalgia, and pain at the injection site. Adverse events were mild to moderate in severity. Changes in vital signs, as compared to control, included increases in mean temperature of 2^{o}C and increases in mean heart rate of 30 beats/min. Changes in respiratory rate and blood pressure were not observed. Vital sign changes peaked within 3 to 4 hours and returned to normal within 12 hours. Total white blood cell count transiently increased and total lymphocyte count transiently decreased. Laboratory biomarkers, TNFα, IL6 and IL8 peaked within 2 to 4 hours. Clinical and laboratory parameters returned to baseline in 6 to 24 hours. A second cohort of subjects received 20 ~~u~~µg /kg MPL followed by a dose of endotoxin 24 hours later. Subjects that received endotoxin 24 hours after MPL experienced diminished cytokine release and improved tolerability consistent with drug tolerance which has been described following repeat dosing of TLR agonists [de Vos, 2009]. This study provides an example of a synthetic TLR4 agonist that was safely administered intravenous (IV) in ascending doses to healthy subjects and produced transient changes in clinical and laboratory measurements.

TLR4 agonist development plan

It is possible that immunotherapies acting at different steps in the cycle could have improved therapeutic indices over currently available monotherapies. Thus, a TLR4 agonist may enhance anti-tumor effects of checkpoint modulators by its actions on dendritic cells and antigen presenting cells. This hypothesis is supported by nonclinical data (Figure 1 and Figure 2).

Relevance of a healthy subject study to future development in cancer patients

Part 1 of this study in healthy subjects will provide relevant monotherapy safety, pharmacokinetic and pharmacodynamic information for GSK1795091 to support a subsequent trial in patients with cancer. To ensure the safety of subjects in a FTIH study of an immune system agonist, whether the trial is performed in healthy subjects or in cancer patients, the starting dose is conservative and anticipated to have minimal biological activity. As dose escalation progresses, pharmacologically relevant doses can be identified safely in healthy subjects based on monitorable and reversible, mild to moderate clinical effects (e.g. changes in body temperature, heart rate, and flu-like symptoms, etc.). Subsequently, a pharmacologically active dose could reasonably be explored as a starting dose in a study in cancer patients. Thus, by beginning development of GSK1795091 in healthy subjects, it will be possible to conduct the FTIH study safely while avoiding the exposure of cohorts of cancer patients to pharmacologically inactive doses of the study drug.

The pharmacodynamic markers to be measured in this FTIH study are directly relevant to a subsequent study to be conducted in cancer patients. For example, we will measure IL-1, TNFα, IL-6 cytokines as markers of TLR4 signaling and immune activation; these markers will also be measured in patient studies to confirm TLR4 signaling and to explore the relationship between the markers and clinical activity. In study 204685, we will also measure immune cell activation and immune cell expression of target proteins of interest (e.g., OX40, ICOS, and others) in peripheral blood. This cellular phenotype information will inform our understanding of the pharmacologic effects of GSK1795091 on circulating immune cells and may suggest additional immunotherapy combinations of interest. By conducting study 204685 in healthy subjects, it will enable detailed PK and PD assessments (e.g., multiple time points to characterize time-course) that would be more difficult to obtain from a study in cancers due to blood-drawn volume considerations and the inconvenience.

A primary goal of Part 2 of study 204685 is to optimize the dosing interval for GSK1795091 in future studies. Administration of TLR agonists can be associated with tachyphylaxis when the dosing interval is short. Therefore, two parallel cohorts of subjects will receive 2 doses of GSK1795091, either 1 week apart (Part 2, Cohort 1) or 2 weeks apart (Part 2, Cohort 2). The pharmacodynamic effects of the second dose will be compared to those of the first dose using biomarker endpoints. This part of the study will provide a more robust assessment of the dosing interval for GSK1795091 than would be possible in a cancer patient study.

OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of GSK1795091 when administered to healthy subjects.	• Safety data comprising adverse events, vital signs, laboratory tests, and 12-lead ECGs.
Secondary	
• To evaluate the systemic pharmacokinetics of GSK1795091 following administration of a single intravenous dose to healthy subjects.	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t), AUC(0-last), AUC(0-∞), CL, Vd, and t1/2.
• To evaluate the pharmacodynamic effects of GSK1795091 following administration as a single intravenous dose to healthy subjects.	• Vital signs, cytokine measurements, WBC and differential, immune cell phenotype.
• To evaluate the systemic pharmacokinetics of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose..	• Plasma pharmacokinetic parameters such as Cmax, tmax, AUC(0-t) , AUC(0-last), and AUC(0-τ), CL, Vss, t1/2 and accumulation and time invariance (as possible).
• To evaluate the clinical and pharmacodynamic effects of GSK1795091 following a second intravenous dose administered to healthy subjects 1 week or 2 weeks after the first dose.	• Vital signs, cytokine measurements, WBC and differential, immune cell phenotype, and laboratory tests.
Exploratory	
• To evaluate immune system effects following intravenous administration of GSK1795091.	• Cytokine measurements in plasma and immune cell phenotyping of leukocytes.
• To evaluate the pharmacodynamic effects of intravenous administration of GSK1795091 on gene signature analysis of PBMCs from part 2 healthy subjects.	• Gene signature analysis
• To explore the relationship between pharmacokinetics, pharmacodynamic markers, and adverse events	• Correlation between pharmacokinetics and pharmacodynamic markers, such as vital signs and CRP, and adverse events
• <u>To characterize the metabolic profile of GSK1795091</u>	• <u>Collection of samples to characterize the metabolites in plasma and urine</u>

SECTION 4.1 OVERALL DESIGN

Figure ~~1~~3 Part 1 study schematic

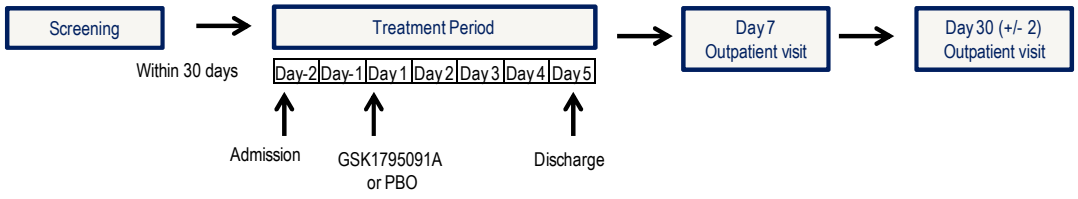
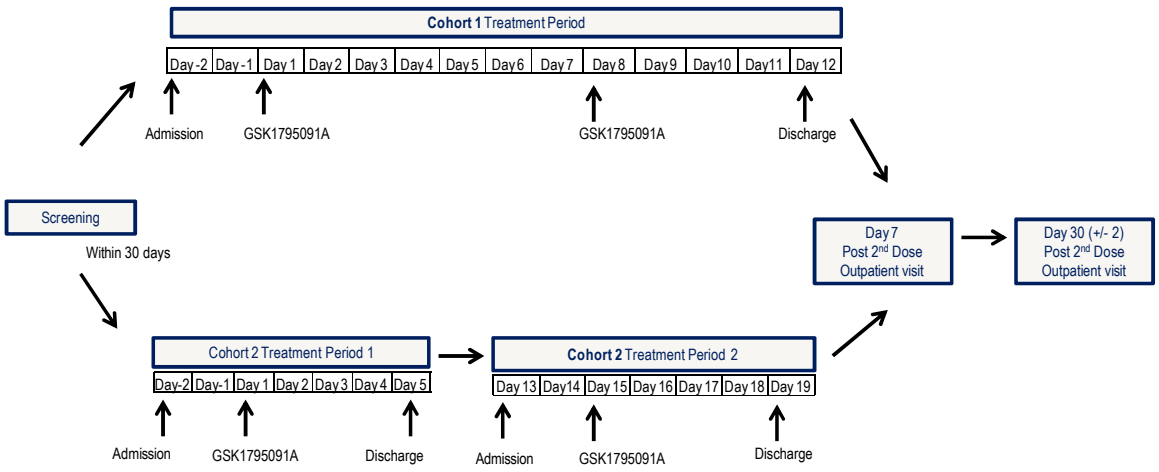


Figure ~~2~~ 4 Part 2 study schematic



SECTION 4.2 TREATMENT ARMS AND DURATION

Part 1

In Part 1, the Principal Investigator should pause dosing of new subjects, within a cohort, for review with the GSK Medical Monitor in the following cases:

- If 1 or more subjects experience a Grade 3 or greater adverse event ~~or a serious adverse event~~ that can reasonably be attributed to GSK1795091 or a serious adverse event or QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline, dosing of additional subjects within the cohort should stop.
- If 1 or more subjects experience a Grade 2 or greater adverse event that lasts more than 24 hours and can reasonably be attributed to GSK1795091, dosing of

additional subjects within a cohort should be held until the adverse event has resolved to Grade 1 or less.

In Part 1, cohorts will be opened sequentially after evaluation of adverse events, vital signs, laboratory tests, and 12-lead ECGs by the Principal Investigator (blinded), GSK Medical Monitor (unblinded), GSK statistician (unblinded). All available safety and tolerability data (adverse events, vital signs, 12-lead ECG and safety laboratory assessments) will be reviewed from ongoing and preceding cohorts. Safety data from 96 hours after administration of study product must be available for a decision to dose-escalate to be taken. Additionally, safety data from at least 5 of the 6 subjects who were randomized to receive GSK1795091 must be available in order for a decision to dose-escalate to be taken. If subjects prematurely discontinue from the study, additional replacement subjects may be enrolled and assigned to the same treatment at the discretion of the Sponsor in consultation with the Investigator. Up to 8 subjects may be replaced during the study.

Progression to the next higher dose level will be stopped if any of the following occur:

- 1 or more subjects experience a serious adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a Grade 3 or greater adverse event ~~or a serious adverse event~~ that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a transaminase increase 3xULN (see Section 5.4.2).
- ~~2~~ 1 or more subjects experience QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.
- 3 or more subjects in a cohort experience Grade 2 adverse events that can reasonably be attributed to GSK1795091.
- Any other event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

Following completion of Part 1, the safety data and proposed dose level for Part 2 will be submitted to the Ethics Committee for review. Once the Ethics Committee vote is received, Part 2 will begin.

Part 2

In Part 2, on Day 1 subjects will receive intravenous study product GSK1795091at a dose determined by results from Part 1.

If dose escalation in Part 1 was stopped for one of the following reasons, GSK1795091 will be administered in Part 2 at a dose one level below the highest dose evaluated in Part 1:

- 1 or more subjects experienced a serious adverse event that can reasonably be attributed to GSK1795091.

- 1 or more subjects experienced a Grade 3 or greater adverse event ~~or a serious adverse event~~ that could reasonably be attributed to GSK1795091.
- 1 or more subjects experienced a transaminase increase 3xULN.
- ~~2~~ 1 or more subjects experience~~d~~ QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.
- Another event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

Subjects will be observed as inpatients for at least 96 hours, after assessments have been performed by the investigator. Subjects in Cohort 1 will remain inpatients until Day 12. Subjects in Cohort 2 will be discharged after completion of the Day 5 (96 hour) assessments and will return to the clinical unit for a second inpatient visit on Day 13 (Part 2, Cohort 2). If AEs attributed to the first dose of GSK1795091 have resolved, subjects will receive a second dose of GSK1795091 on Day 8 (Part 1, Cohort 1) or Day 15 (Part 2, Cohort 2). Subjects will be followed according to the same schedule as after the first dose of GSK1795091. In addition, the Principal Investigator should review all available safety data following the second dose of GSK1795091 and interrupt subsequent dosing (within or between cohorts) if any of the following occur ~~subject experiences a Grade 3 or greater adverse event or a serious adverse event that can reasonably be attributed to GSK1795091. Events that interrupt dosing should be reviewed with the GSK Medical Monitor.~~

- 1 or more subjects experiences a serious adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a Grade 3 or greater adverse event that can reasonably be attributed to GSK1795091.
- 1 or more subjects experience a transaminase increase 3xULN (see Section 5.4.2).
- 1 or more subjects experience QTcF >500 msec or an increase in QTcF by 60 msec or more compared to baseline.
- Any other event deemed to pose an unacceptable risk to subjects by the principal investigator or medical monitor.

In the event that dosing is interrupted, administration of GSK1795091 in Part 2 will only resume after approval by the Ethics Committee and Higher Health Authority following a substantial amendment.

SECTION 4.3 TYPE AND NUMBER OF SUBJECTS

If subjects prematurely discontinue from the study, additional replacement subjects may be enrolled and assigned to the same treatment at the discretion of the Sponsor in consultation with the Investigator. Up to 8 subjects may be replaced during the study.

SECTION 4.5.1 STARTING DOSE SELECTION

~~CALCULATION OF MINIMUM ANTICIPATED BIOLOGICAL EFFECT LEVEL~~

The proposed starting dose for the study was determined after due consideration of all available *in vitro* and *in vivo* preclinical data, in accordance with EMEA and FDA regulatory guidance for first-time-in-human studies.

SECTION 4.5.1.1 CALCULATION OF MINIMUM ANTICIPATED BIOLOGICAL EFFECT LEVEL

The dose is ~~based upon~~ calculated using the *in vivo* Minimum Anticipated Biological Effect Level (MABEL) in the 6-week cynomolgus monkey study, and secondarily, supported by rabbit pyrogenicity studies and *in vitro* potency comparisons to LPS. The cynomolgus monkey was chosen as the *in vivo* toxicology model based upon greatest sensitivity and highest ~~the~~ similarity to humans as determined by cytokine production following exposure of whole blood from several species to GSK1795091 *ex vivo*~~tr~~. Five (5) cytokines ~~selected from a panel for~~ were measured *in vivo;* these cytokines were selected for evaluation based upon high *ex vivo* ~~*in vitro*~~ sensitivity of whole blood to GSK1795091 (IL10 and IP10) or based upon increases following administration of TLR4 agonists to healthy subjects in published clinical trials (TNFa, IL6, and IL8) [Astiz, 1995]. Vehicle or GSK1795091, at doses of 0.05, 2, and 200 ~~u~~µg/kg, was administered to cynomolgus monkeys. IP10 and IL10 were the most sensitive pharmacodynamic markers and were detectable in all evaluable monkeys at 0.05 µg/kg. There were minor decreases in lymphocytes, monocytes, eosinophils, and NK cells at doses of ≥ 0.05 ~~u~~µg/kg, and transient increases in heart rate and body temperature at doses of ≥ 2 ~~u~~µg/kg, all of which were considered non-adverse.

SECTION 4.5.1.2 RABBIT PYROGENICITY

Rabbits are commonly used as models for assessing endotoxin contamination of parenteral formulations due to their high sensitivity. Therefore, GSK1795091 was given to New Zealand white rabbits at single intravenous doses of 0.1, 0.5, 1, 2.5 and 5 ng/kg in 5% dextrose. Rabbits given single doses of 5 ng/kg had increases in body temperature of > 0.5^{o}C 60 minutes post dose and generally returned to baseline temperature by 180 minutes post dose, consistent with the expected pharmacologic actions of GSK1795091. Lower doses were not considered to be pyrogenic. Based on a scaling factor of 3.1, the 2.5 ng/kg dose in the rabbit corresponds to a HED of 0.8 ng/kg, or 56 ng for a 70 kg subject. Thus, the HED of the non-pyrogenic dose in rabbits is 8-fold higher than the proposed starting dose of 7 ng.

SECTION ~~4.5.2~~ 4.5.1.3 COMPARISON TO LIPOPOLYSACCHARIDE (LPS)

The typical dose of LPS administered to healthy subjects in clinical trials is 2 to 4 ng/kg (approximately 140 ng to 280 ng). Following administration, subjects experience mild to moderate flu-like symptoms and increases in body temperature and heart rate. To confirm the safety of the proposed 7 ng starting dose of GSK1795091, the potencies of

GSK1795091 and *E.coli*-derived LPS (commonly used for clinical trials of healthy subjects) were compared. Human whole blood was incubated *in vitro* with either GSK1795091 or LPS, and cytokines were measured to assess the relative potencies of the two TLR4 agonists. At concentrations of 0.2 ng/ml (approximating the plasma concentration following a 8.6 ng/kg dose of LPS for a 70 kg subject with a 3000 ml plasma volume), LPS was as potent or more potent than GSK1795091 by a factor of 1 to 4 for a panel of 5 measureable cytokines. If GSK1795091 is estimated to be of comparable potency to LPS, the doses of LPS routinely administered to healthy subjects are 20-fold to 40-fold greater than the 7 ng starting dose of GSK1795091. Thus, the planned starting dose is appropriately conservative.

If GSK1795091 is estimated to be less potent than LPS by a factor of 4, dose escalation would need to exceed 4-times the 2 ng/kg dose of LPS administered to healthy subjects (i.e. at least 560 ng) to achieve pharmacologic activity similar to LPS. Thus, the top dose is selected to accommodate a possible range of potencies of GSK1795091, based on *in vitro* evaluations.

SECTION 4.5.2 DOSE ESCALATION

Dose escalation will proceed as follows: 7, 21, 60, 120, 180, 280, 420, 630ng. The dose increments between cohorts progressively decline from 3-times to 1.5-times the previous dose.

SECTION 4.5.3 TOP DOSE SELECTION

The maximum proposed dose level is 630 ng. However, dose escalation may stop at a lower dose level based on criteria in Section 4.2. In the repeat dose intravenous toxicity studies in rats and monkeys, GSK1795091 was associated with expected pharmacologic, pro-inflammatory actions of a TLR4 agonist. Therefore, adverse events that stop dose escalation are likely to include mild to moderate flu-like symptoms and increases in body temperature and heart rate.

The top dose is selected to accommodate a possible range of potencies of GSK1795091, based on *in vitro* evaluations. *In vitro* data suggest that the relative potency of LPS is 1- to 4-times that of GSK1795091. If GSK1795091 is equipotent with LPS, dose escalation is predicted to stop in accordance with Section 4.2 at 2-4 ng/kg (i.e. 140 to 280 ng). If GSK1795091 is less potent than LPS by a factor of 4, higher dose levels will be required to elicit pharmacologic effects similar to LPS; this possibility is accommodated by the maximum proposed dose level of 630 ng (i.e. approximately 4.5-fold greater than the LPS dose of 2 ng/kg).

Based on the maximum predicted human exposure of approximately Cmax 0.09 ng/mL and AUC 2.6 ng.h/mL at the highest planned clinical dose of 630 ng, the margin to the NOAEL dose in rat (15 ~~u~~µg g/kg/dose) is approximately 777X (Cmax) and 121X (AUC) and in monkey (200 ~~u~~µg/kg/dose) is approximately 19,100X (Cmax) and 8461X (AUC). Thus, at the maximum proposed dose level, 630 ng, severe or irreversible toxicities are not expected.

SECTION 4.5.4 SUMMARY

The starting dose of 7 ng is expected to be safe and appropriately conservative for a healthy subject study. Sensitive assays in a cynomolgus monkey model were used to calculate the MABEL. At the dose defining the MABEL, animals experienced neither adverse events nor histopathological changes. Furthermore, the dose is 8-fold higher than the HED of the non-pyrogenic dose in rabbits. Additionally, clinical experience with TLR4 agonists administered to healthy subjects provides a reference value against which the starting dose of GSK1795091 can be compared. Since GSK1795091 has *in vitro* potency similar to (or lower than) LPS, a starting dose of 7 ng is conservative in comparison to the 20 to 40-fold higher doses of LPS routinely administered to healthy subjects. These estimates provide a high level of confidence that the starting dose of 7 ng can safely be administered to healthy subjects. Dose escalation is expected to stop, at or below 630 ng, based on mild to moderate toxicities that are reversible and readily monitored. The top dose in the study, 630 ng, does not approach the Cmax or AUC at the NOAEL in rats or in monkeys. Thus, dose escalation proceeds from a conservative starting dose to a top dose unlikely to be associated with severe or irreversible effects.

~~**Dose escalation**~~

~~Dose escalation will proceed as follows:~~

~~7, 21, 60, 120, 180, 280, 420, 630ng~~

SECTION 5.2 EXCLUSION CRITERIA: CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTcF INTERVAL

5. History of liver disease, or known hepatic or biliary abnormalities~~. (with the exception of Gilbert's syndrome).~~

6. AST, ALT, gamma-GT and bilirubin >1.~~1xULN~~ 0xULN ~~(isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).~~

7. Creatinine >1.0 x ULN.

SECTION 5.2 EXCLUSION CRITERIA: DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA

21. Subject is unable to understand and communicate in German/or native language of the site

SECTION 5.4 WITHDRAWAL/STOPPING CRITERIA

A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. Furthermore, any female subject who becomes pregnant while participating will be withdrawn from the study [see Section 12.5.2]. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

SECTION 6.1

	Study Treatment	
Product name:	GSK1795091 Injection, 0.001mg/mL ~~and 0.0001mg/mL~~, 5mL vial with 5.2mL fill volume	Placebo Sodium Chloride Injection
Formulation description:	A clear solution in a 5ml vial with a 20mm stopper and overcap.	Commercial saline solution A clear colourless solution containing 0.9% sodium chloride
Dosage form:	Solution for Injection	Solution for Injection
Unit dose strength(s)/Dosage level(s):	0.001mg/mL (1000 nanogram per mL) ~~0.0001mg/mL (100 nanogram per mL)~~	N/A
Route of Administration	Intravenous Injection	Intravenous Injection
Dosing instructions:	Administered as an intravenous bolus slowly over 2-5 minutes, followed by an intravenous bolus of 10mL of normal saline	To be administered in an identical manner to GSK1795091
Physical description:	A clear, colourless to slightly coloured solution, free from visible particles in a clear glass vial with grey stopper and aluminium grey brown overseal.	A clear colourless solution

SECTION 7.1: TIME AND EVENTS TABLE

SECTION 7.1.2: Part 1: In-House Assessments

Row 7: Haem/Clin Chem/Urinalysis/Coagulation

SECTION 7.1.3: Part 1: Outpatient Assessments
Row 5: Haem/Clin Chem/Urinalysis/Coagulation

SECTION 7.1.4: Part 2: In-House Assessments
Row 7: Haem/Clin Chem/Urinalysis/Coagulation
Row 16: ~~PK~~ Metabolite urine sampling

SECTION 7.1.5: Part 2: Outpatient Assessments
Row 5: Haem/Clin Chem/ Urinalysis/Coagulation

SECTION 7.3.6 CLINICAL SAFETY LABORATORY ASSESSMENTS

Table 2

Coagulation Tests	• prothrombin time (or international normalized ratio) • partial thromboplastin time
Other Screening Tests	• HIV • Hepatitis B surface antigen (HBsAg) and core antibody (anti-HBc Ab) • Hepatitis C (Hep C antibody) • FSH and estradiol (as needed in women of non-child bearing potential only) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serum or urine hCG Pregnancy test. • Lipids, Total cholesterol, HDL, LDL ~~• PT (or INR)/PTT~~

SECTION 7.4 PHARMACOKINETICS

SECTION 7.4.1 Blood Sample Collection
Blood samples (~~2ml~~ 4ml per timepoint) for pharmacokinetic (PK) analysis of GSK1795091 will be collected at the time points indicated in Section 7.1, Time and Events Table.
SECTION 7.4.3 Sample Analysis
Once the plasma has been analyzed for GSK1795091 any remaining plasma may be analyzed qualitatively for other compound-related metabolites and the results reported under a separate ~~PTS-BIB,~~ GlaxoSmithKline protocol.

Plasma and u~~U~~rine may be analyzed qualitatively for GSK1795091 and other compound-related metabolites and results reported under a separate ~~PTS-BIB,~~ GlaxoSmithKline protocol.

SECTION 12.5.2 COLLECTION OF PREGNANCY INFORMATION

Any female subject who becomes pregnant while participating:

- will be withdrawn from the study

12.7.2. AMENDMENT 2

SECTION 5.2 Exclusion criteria

CONTRAINDICATIONS
13. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
~~13.~~ 14. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test (anti-HBc Ab) result at screening or within 3 months prior to first dose of study treatment. Subjects with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA PCR test is obtained.

12.7.3. AMENDMENT 3

This amendment will apply to all study sites and all countries.

Summary of Amendment Changes with Rationale

This protocol amendment is revising Part 1 dose escalations and subject numbers following a halt in dosing after one subject experienced a transient heart rate increase from 68 bpm to 125 bpm, meeting protocol-defined stopping criteria as a grade 3 change.

For Part 1, this protocol amendment is intended to generate additional clinical data while applying the available clinical data to maximize the safety of subjects enrolled in subsequent cohorts. The original protocol included stopping rules that were designed to be more conservative than rules used in vaccine trials to ensure a pause for review of safety data at early signals of pharmacologic activity. Modifying the stopping rules to, now, include an effect threshold as well as an increase from baseline is supported by the good tolerability profile of GSK1795091 in cohorts 1 – 3. Repeating the 60 ng cohort will further characterize the frequency and magnitude of heart rate increases and temperature increases before taking any additional steps. In turn, because clinical signs and symptoms are now being observed, future dose increments are smaller and the potential top dose has been lowered. These modifications will provide additional data that further characterizes safety and dose-effect relationships to facilitate the design of subsequent studies of GSK1795091 in cancer patients.

Minor typographical errors and other sections have been amended for clarification.

List of Specific Changes

TITLE PAGE

PPD has replaced PPD in the list of Authors.

MEDICAL MONITOR/SAE CONTACT INFORMATION**:**

Addition of the secondary medical monitor's email address into the table:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number	Site Address
Secondary Medical Monitor	Dr PPD PPD	PPD			1250 S. Collegeville Road, Collegeville, PA 19426

SECTION 1: PROTOCOL SYNOPSIS FOR STUDY 204685

Cross references in the summary have been deleted. They were included in previous versions in error.

Treatment Arms and Duration – Part 1

Part 1 is planned to enrol up to ~~8~~7 sequential cohorts of 8 subjects per cohort (at protocol Amendment 03, the planned number of cohorts is reduced from 8 to 7).

Treatment Arms and Duration

If AEs attributed to the first dose of GSK1795091 have resolved, subjects will receive a second dose of GSK1795091 on Day 8 (Part 2, Cohort 1) or Day 15 (Part 2, Cohort 2~~)~~; ~~(~~Otherwise, subjects will not receive the second dose and will be followed according to the schedule for Part 1). Subjects will be followed according to the same schedule as after the first dose of GSK1795091.

Part 2

Part 2 is planned to enrol 2 parallel cohorts of 6 subjects per cohort.

Type and Number of Subjects

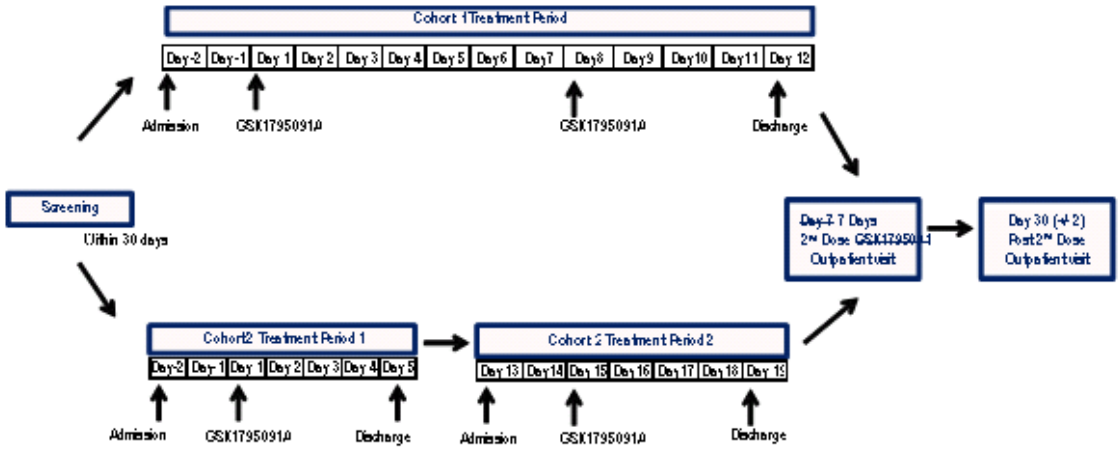
Approximately ~~76~~68 subjects may be enrolled in the study. Between 8 and ~~64~~56 subjects will be enrolled in Part 1. Twelve (12) subjects will be enrolled in Part 2.

Part 1 is planned to enrol up to ~~8~~7 cohorts of 8 subjects per cohort.

Part 2 is planned to enrol 2 cohorts of 6 subjects per cohort.

SECTION 4.1 OVERALL DESIGN

Figure 4 Part 2 study schematic.



SECTION 4.2 TREATMENT ARMS AND DURATION

Overview:

Part 1 is planned to enrol up to ~~8~~7 sequential cohorts of 8 subjects per cohort (at protocol Amendment 03, the planned number of cohorts is reduced, and the dose of GSK1795091 for cohorts 5, 6 and 7 lowered. Cohort 4 is a repeat of the 60 ng dose; refer to Section 4.5.2).

Part 2 is planned to enrol 2 parallel cohorts of 6 subjects per cohort.

Part 1:

The grading of adverse events will be guided by the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Appendix 2) with the exception of fluctuations in white blood cell counts (which are anticipated and may be considered adverse events only after >24 hours) and fever and tachycardia which ~~are graded according to~~ also require a change~~s~~ from time-matched baseline (per table below).

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Temperature ~~(increase from baseline)~~	>0.5~~–1.5~~ °C change AND >38.0oC	>1.5~~–3~~°C change AND >38.5oC	>3°C change (<24 hours) AND >39.0oC	>3°C change (≥24 hours) AND >40oC
Heart rate ~~(increase from baseline)~~	>15~~–30~~ bpm change AND >101 bpm	>30~~–45~~ bpm change AND >116 bpm	>45 bpm change AND >130 bpm	Emergency intervention required

SECTION 4.3 TYPE AND NUMBER OF SUBJECTS

Approximately ~~76~~68 subjects may be enrolled in the study. Between 8 and ~~64~~56 subjects will be enrolled in Part 1. Twelve (12) subjects will be enrolled in Part 2.

Part 1 is planned to enrol up to ~~8~~7 cohorts of 8 subjects per cohort.

Part 2 is planned to enrol 2 cohorts of 6 subjects per cohort.

SECTION 4.5.2 Dose escalation

Cohort Number	**Dose (ng)**
1	7[1]
2	21[1]
3	60[1]
4	60[2]
5	100
6	150
7	210

1. Cohorts completed under Protocol Amendment 2.
2. Study restart dose following stopping criteria met in one subject under Protocol Amendment 2

~~7, 21, 60, 120, 180, 280, 420, 630ng.~~ The dose increments between cohorts progressively decline from 3-times to 1.~~5~~4-times the previous dose.

SECTION 4.5.3 TOP DOSE SELECTION

The original maximum proposed dose level proposed on preclinical data ~~is~~ was 630 ng but was revised to 210 ng based on emerging clinical data cohorts 1-3 in Part 1.

~~However,~~ Dose escalation may stop at a lower dose level based on criteria in Section 4.2. In the repeat dose intravenous toxicity studies in rats and monkeys, GSK1795091 was associated with expected pharmacologic, pro-inflammatory actions of a TLR4 agonist. Therefore, adverse events that stop dose escalation are likely to include mild to moderate flu-like symptoms and increases in body temperature and heart rate.

The original top dose of 630 ng was ~~is~~ selected to accommodate a possible range of potencies of GSK1795091, based on *in vitro* evaluations. *In vitro* data suggest that the relative potency of LPS is 1- to 4-times that of GSK1795091. If GSK1795091 is equipotent with LPS, dose escalation is predicted to stop in accordance with Section 4.2 at 2-4 ng/kg (i.e. 140 to 280 ng). If GSK1795091 is less potent than LPS by a factor of 4, higher dose levels will be required to elicit pharmacologic effects similar to LPS; this possibility is accommodated by the maximum proposed dose level of 630 ng (i.e. approximately 4.5-fold greater than the LPS dose of 2 ng/kg).

Based on the maximum predicted human exposure of approximately Cmax 0.09 ng/mL and AUC 2.6 ng.h/mL at ~~the highest planned clinical dose of~~ a dose of 630 ng, the margin to the NOAEL dose in rat (15 µg/kg/dose) is approximately 777X (Cmax) and 121X (AUC) and in monkey (200 µg/kg/dose) is approximately 19,100X (Cmax) and 8461X (AUC). Thus, at ~~the maximum proposed~~ a dose level~~,~~ of 630 ng, severe or irreversible toxicities are not expected.

Based on emerging clinical data from Part 1, the GSK1795091 is likely to produce effects similar to LPS at a dose between 140 to 280 ng. Therefore, the top dose in the study was revised to 210 ng.

SECTION 4.5.4 SUMMARY

The starting dose of 7 ng is expected to be safe and appropriately conservative for a healthy subject study. Sensitive assays in a cynomolgus monkey model were used to calculate the MABEL. At the dose defining the MABEL, animals experienced neither adverse events nor histopathological changes. Furthermore, the dose is 8-fold higher than the HED of the non-pyrogenic dose in rabbits. Additionally, clinical experience with TLR4 agonists administered to healthy subjects provides a reference value against which the starting dose of GSK1795091 can be compared. Since GSK1795091 has *in vitro* potency similar to (or lower than) LPS, a starting dose of 7 ng is conservative in comparison to the 20 to 40-fold higher doses of LPS routinely administered to healthy subjects. These estimates provide a high level of confidence that the starting dose of 7 ng can safely be administered to healthy subjects. Dose escalation is expected to stop, at or below ~~630~~ 210 ng, based on mild to moderate toxicities that are reversible and readily monitored. The top dose in the study, ~~630~~ 210 ng, does not approach the Cmax or AUC at the NOAEL in rats or in monkeys. Thus, dose escalation proceeds from a conservative starting dose to a top dose unlikely to be associated with severe or irreversible effects.

SECTION 6.1 INVESTIGATIONAL PRODUCT AND OTHER STUDY TREATMENT

On Day -1, subjects will be hydrated with approximately 1200 mL glucose/saline (2.5% glucose/0.45% sodium chloride) (e.g. intravenous drip 150 mL/hr). Intravenous hydration (sourced locally) should continue on Day 1, beginning approximately 2 hours prior to and continuing for 6 hours after administration of study product (intravenous drip 150 mL/hr).

Subjects will receive GSK1795091 administered as an intravenous bolus slowly over 2-5 minutes~~, followed by an intravenous bolus of 10mL of normal saline~~.

SECTION 7.1.5. PART 2 ~~OUTPATIENT~~ ASSESSMENTS

Procedure	~~Day 7~~ 7 Days Post Dose[1]	Follow-up[2] (23 days ± 2 days post-last visit) or early withdrawal
Outpatient Visit	X	X
Urine pregnancy test (WNCBP)		X
Full physical exam including height and weight		
Haem/Clin Chem/ Urinalysis/Coagulation		
12 lead ECG		
Vital signs (Temperature, BP and Heart rate)		
PK blood sampling	X	
AE/SAE review	X	X
Concomitant medication review	X	X

1. ~~Following first dose, Day~~ 7 days post first dose assessments (Cohort 1 D7; Cohort 2 D14) will be conducted in house; ~~following second dose Day~~ 7 days post second dose (Cohort 1 D15; Cohort 2 D22) assessments will be conducted as an outpatient visit
2. Outpatient visit or Contact at FU. All subjects with (1) new AE or (2) unresolved AEs or abnormal labs at the Day 7 visit should return for a follow-up visit. Female subjects should return for pregnancy testing. For all other subjects a FU contact is acceptable

SECTION 7.3.6. CLINICAL SAFETY LABORATORY ASSESSMENTS

Per Inclusion/exclusion criteria addition of "Gamma-GT" into the table for 'other screening tests':

Other Screening Tests	• HIV • Hepatitis B surface antigen (HBsAg) and core antibody (anti-HBc Ab) • Hepatitis C (Hep C antibody) • FSH and estradiol (as needed in women of non-child bearing potential only) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serum or urine hCG Pregnancy test. • Lipids, Total cholesterol, HDL, LDL • Gamma-GT