22 February 2018
EMA/CHMP/781541/2018
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

Entolimod TMC

International non-proprietary name: entolimod

Procedure No. EMEA/H/C/004656/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of Contents

List of abbreviations

Abbreviation	Definition
adjAUC$_{0-24}$	Area under the plasma concentration-time curve from time 0 to 24 hours postdose adjusted for baseline value
AE	Adverse event
ALC	Absolute leukocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ANC24	Fold change in the absolute neutrophil count at 24 hours postdose
ARS	Acute radiation syndrome
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC$_{0-24}$	Area under the plasma concentration-time curve from time 0 to 24 hours postdose
AUC$_{0-\infty}$	Area under the plasma concentration-time curve from time zero to infinity
AUC$_{\infty}$	Area under the curve from time 0 to infinity
AUC$_{t}$	Area under the curve from time 0 to last quantifiable concentration
BCG	Bacillus Calmette-Guérin - a TLR2 and TLR4 agonist
BLA	Biologics License Application
BUN	Blood urea nitrogen
CBC	Complete blood count
CBLB502	Entolimod
CBLI	Cleveland Biolabs, Inc.
CFR	Code of Federal Regulations
CL/F	Apparent clearance
C$_{last}$	Last quantifiable concentration
CLIA	Clinical Laboratory Improvement Amendments
C$_{max}$	Maximum plasma concentration
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient Variation
DAMP	Damage-associated molecular patterns
ECG	electrocardiogram, electrocardiographic
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
Entolimod	Toll-like receptor 5 (TLR5) agonist; also known as CBLB502 (a derivative of the microbial protein, flagellin)
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
GCP	Good Clinical Practices
G-CSF	Granulocyte colony-stimulating factor
GLP	Good Laboratory Practice

HIV	Human immunodeficiency virus
HU-7014	Study CBL-DD-CH-B502-07-014
HU-9001	Study CBL-DD-CH-B502-09-001A
HV	Healthy Volunteer
IBP	Ibuprofen
ICH	International Conference on Harmonisation
IFN	Interferon
IL	Interleukin
IM	Intramuscular or intramuscularly
ITT	Intent to treat
IV	Intravenous or intravenously
$LD_{70/60}$	Dose of irradiation that would result in the death of ~70% of the animals within 60 days
LDH	Lactate dehydrogenase
LLN	Lower limit of normal
LLOQ	Lower limit of quantification
M/F	Males/females
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
MPL	Monophosphoryl lipid A - a TLR4 agonist
MRC	Medical radiation countermeasure
MW	Molecular Weight
NA	Not available
NF-κB	Nuclear factor kappa-B
NHP	Nonhuman primate(s)
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PD	Pharmacodynamics
PK	Pharmacokinetic or pharmacokinetics
PR	Pulse rate
PTT	Partial thromboplastin time
QD	Once per day
QT	A measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTcF	The QT interval corrected heart rate using Fridericia's formula
RBC	Red blood cell
RR	Respiratory rate
Rs-001	Study CBL1.1-N-01-Rs-001
Rs-23	Study CBL1.1-N-01-Rs-23
SAE	Serious adverse event
SC	Subcutaneous, subcutaneously
SD	Standard deviation
SEM	Standard error of the mean
SOC	System organ class
$t_{1/2}$	Terminal elimination half-life
TBI	Total body irradiation
TEAE	Treatment-emergent adverse event

TLR	Toll-like receptor
TLR5	Toll-like receptor 5
$TLR5^{392stop}$	Toll-like receptor 5 stop codon
T_{max}	Time to maximum plasma concentration
TNFα	Tumor necrosis factor-alpha
ULN	Upper limit of normal
US	United States
V_z/F	Apparent volume of distribution
WBC	White blood cell
λ_z	Terminal elimination rate

1. Recommendation

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for Entolimod TMC, an orphan medicinal product to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster:

- is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time.

The major objections precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

Quality:

- The proposed Drug Substance manufacturing process is not considered sufficiently established as to produce a consistent product.
- There is currently uncertainty about the proposed control strategy.
- Process validation for entolimod bulk drug substance (BDS) has not been performed.
- Comparability studies should be presented to assess the impact on quality of the changes introduced during development.
- The characterization of the Entolimod Drug Substance is limited and incomplete.
- The proposed DS specification is considered not appropriate to allow an adequate control of the entolimod DS at release and during shelf life.
- A clear procedure to establish a reference standard has not been provided.
- The data provided by the applicant do not permit to reach a clear conclusion about the stability of Entolimod.
- Process validation for Entolimod Drug Product has not been performed.
- The proposed control of entolimod related substances/impurities/degradation products in the final drug product is inadequate and not in line with ICHQ6B.

Non Clinical:

- The toxicity studies provided by the applicant failed to adequately characterize the toxicological profile of the test compound and to identify the potential target organs of toxicity.

Clinical:

- Lack of justification how the conditions of the pivotal NHP study (supportive care limited) has external validity with respect to a magnitude of benefit in case of full supportive care, and of what realistic conditions of use might be supported by the particular conduct of the pivotal NHP study.
- The formal GLP status of the pivotal non-clinical study Rs-23 in unclear.
- Lack of justification of approval of entolimod based on a single pivotal trial.
- The entolimod dose of 10 ug/kg chosen by the company in the NHPs could not be the optimal dose.
- The currently proposed dose conversion approach is not conclusive.

- Lack of justification that the limited safety database on the use of entolimod is sufficient to characterize the advese effect profile.
- Lack of justification that entolimod is suitable to be used in a radiation accident where supportive care could not be administered, particularly as it causes a profound reduction in blood pressure.

Proposal for questions to be posed to additional experts

A SAG may be requested during the procedure pending the assessment of the responses to the LoQ.

Proposal for inspection

GMP inspection(s)

None

GLP inspection(s)

GLP inspection is not deemed necessary at this stage of the procedure. However, a request of a GLP inspection will be reconsidered during the evaluation.

GCP inspection(s)

As both trials in healthy volunteers in phase I in order to characterise PK, PD, dose-finding and conversion in humans as well as complete safety assessment in humans are deemed insufficient to characterise adequately the safety of entolimod currently no need for inspection is seen.

New active substance status

Based on the review of the data the CHMP considers that the active substance Entolimod TMC contained in the medicinal product entolimod is to be qualified as a new active substance in itself.

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

Entolimod is being developed as a medical radiation countermeasure to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster in adults and paediatric population aged ≥2 years old. Nuclear and radiological events that could expose people (intended or unintended) to significant amounts of ionizing radiation and/or radioactive materials has become a public health concern. Excessive radiation dose can cause short-term health effects including death, and long-term health risks especially cancer. Short-term health effects of radiation exposure are acute radiation syndrome (ARS) and cutaneous radiation injury (CDCa).

ARS is an acute illness caused by exposure of the entire body (or most of the body) to an external high dose of penetrating radiation in a very short period of time (usually a matter of minutes) (CDCb).

2.1.2. Epidemiology

The time course and severity of clinical signs and symptoms of ARS are a function of the overall body volume irradiated, the inhomogeneity of dose exposure, the particle type, the absorbed dose and the dose rate (Macià et al. 2011). It is the absorbed radiation dose, or the amount of radiation, that is the critical issue in determining health consequences.

According to International Atomic Energy Agency (IAEA paper), probability of death by ARS is 0% for absorbed doses of 1-2 Gy, 50-100% for doses in the range of 6-8 Gy, and 100% for doses >8 Gy (see table above). To determine lethal dose in human beings, only data from persons who died as a result of radiation accidents can be used and the total number over the last 50 years is low. After atomic bombs were dropped on Hiroshima and Nagasaki, experiments were carried out on animals to determine the required absorbed radiation dose to kill 50% of the animals in a known time (Leikin et al. 2003). The lethal effect depended on health, age, environment, and health of the various animal species, and varied from species to species. In human beings, LD50/60 is used, that is, lethal dose required to kill 50% of the population within 60 days. Published LD50/60 data for human beings without medical treatment is about 3.5 Gy increasing to about 5–6 Gy with adequate supportive treatment (based on IAEA paper).

TABLE XI. CRITICAL PHASE OF ACUTE RADIATION SYNDROME

	Degree of ARS and approximate dose of acute WBE (Gy)				
	Mild (1–2 Gy)	Moderate (2–4 Gy)	Severe (4–6 Gy)	Very severe (6–8 Gy)	Lethal (>8 Gy)
Onset of symptoms	>30 d	18–28 d	8–18 d	<7 d	<3 d
Lymphocytes (G/L)	0.8–1.5	0.5–0.8	0.3–0.5	0.1–0.3	0–0.1
Platelets (G/L)	60–100 10–25%	30–60 25–40%	25–35 40–80%	15–25 60–80%	<20 80–100%[a]
Clinical manifestations	Fatigue, weakness	Fever, infections, bleeding, weakness, epilation	High fever, infections bleeding, epilation	High fever, diarrhoea, vomiting, dizziness and disorientation, hypotension	High fever, diarrhoea, unconsciousness
Lethality (%)	0	0–50 Onset 6–8 weeks	20–70 Onset 4–8 weeks	50–100 Onset 1–2 weeks	100 1–2 weeks
Medical response	Prophylactic	Special prophylactic treatment from days 14–20; isolation from days 10–20	Special prophylactic treatment from days 7–10; isolation from the beginning	Special treatment from the first day; isolation from the beginning	Symptomatic only

[a] In very severe cases, with a dose >50 Gy, death precedes cytopenia.

Other similar numbers have also been published (FEMA paper), and that many victims receiving radiation doses over 6 Gy would not be expected to survive even with medical care (see table herein below).

Table 1.5: Death from acute radiation exposure as a function of whole-body absorbed doses (for adults), for use in decision making after short-term[a] radiation exposure adapted from NCRP, AFRRI, Goans, IAEA, ICRP and Mettler.[15]

Short-Term Whole-Body Dose [rad (Gy)]	Death[b] from Acute Radiation Without Medical Treatment (%)	Death from Acute Radiation with Medical Treatment (%)	Acute Symptoms (nausea and vomiting within 4 h) (%)
1 (0.01)	0	0	0
10 (0.1)	0	0	0
50 (0.5)	0	0	0
100 (1)	<5	0	5 – 30
150 (1.5)	<5	<5	40
200 (2)	5	<5	60
300 (3)	30 – 50	15 – 30	75
600 (6)	95 – 100	50	100
1,000 (10)	100	>90	100

[a]Short-term refers to the radiation exposure during the initial response to the incident. The acute effects listed are likely to be reduced by about one-half if radiation exposure occurs over weeks.
[b]Acute deaths are likely to occur from 30 to 180 d after exposure and few if any after that time. Estimates are for healthy adults. Individuals with other injuries, and children, will be at greater risk.

Patients with combined injury (physical, thermal and/or chemical injury combined with radiation exposure at a dose sufficient), which is common in a radiation mass casualty event, have a worse overall prognosis than do patients with radiation exposure alone (REMMb).

2.1.3. Biologic features

At present it is believed that normal tissue response to radiation is an integrated response involving cytocidal effects (cell death), indirect effects (eg. production of cytokines and reactive oxygen species) and functional effects (i.e. non-lethal effects on different intracellular and extracellular molecules and changes in gene expression in irradiated cells).

ARS is classically subdivided into three subsyndromes: the hematopoietic, gastrointestinal and neurovascular syndrome. The minimum absorbed dose for which signs or symptoms of ARS are described is 1 Gy (based on IAEA paper). Hematopoietic ARS emerges at the lowest end of the scale of radiation doses that leads to acute injury (Singh et al. 2017). The gastrointestinal occurs at higher doses, and the cerebrovascular at doses higher than 20 Gy (Macià et al. 2011). Contemporaneously, a new syndrome called radiation-induced multi-organ dysfunction (cardiovascular, respiratory, liver and urogenital damage) is believed to be also part of ARS.

The hematopoietic ARS involves pancytopenia, with predisposition to complications such as infection (due to leukopenia) and bleeding (due to thrombocytopenia) that, if sufficiently severe, can result in death (Macià et al. 2011). The gastrointestinal ARS is due to denudation of intestinal mucosa which produces watery diarrhoea, dehydration and electrolyte loss, gastrointestinal bleeding and perforation (which facilitates the entry of bacterias into the bloodstream); death can occur due to sepsis, bleeding, dehydration and multisystem organ failure. Cerebrovascular ARS occurs presumably with endothelial cells damage and vascular leak with edema and consequently an increase in intracranial pressure, and death almost invariably occurs quickly (e.g. generally within 24–48 h). The radiation-induced multi-organ failure is thought to be due to damaged endothelial cells leading to a radiation-induced systemic inflammatory response syndrome mediated by the release of inflammatory cytokines.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

The extent of radiation-induced injury to hematopoietic tissues, gastrointestinal and neurovascular systems, lung, and skin plays a vital role in the diagnosis, treatment and the prognosis of ARS (Singh 2017). It is the absorbed radiation dose, or the amount of radiation, that is the critical issue in determining health consequences in ARS.

Diagnosis of ARS is based on clinical and laboratory data (IAEA paper). The IAEA has published good summary of signs and symptoms of ARS and their correlation with absorbed doses (see table above) (IAEA paper).

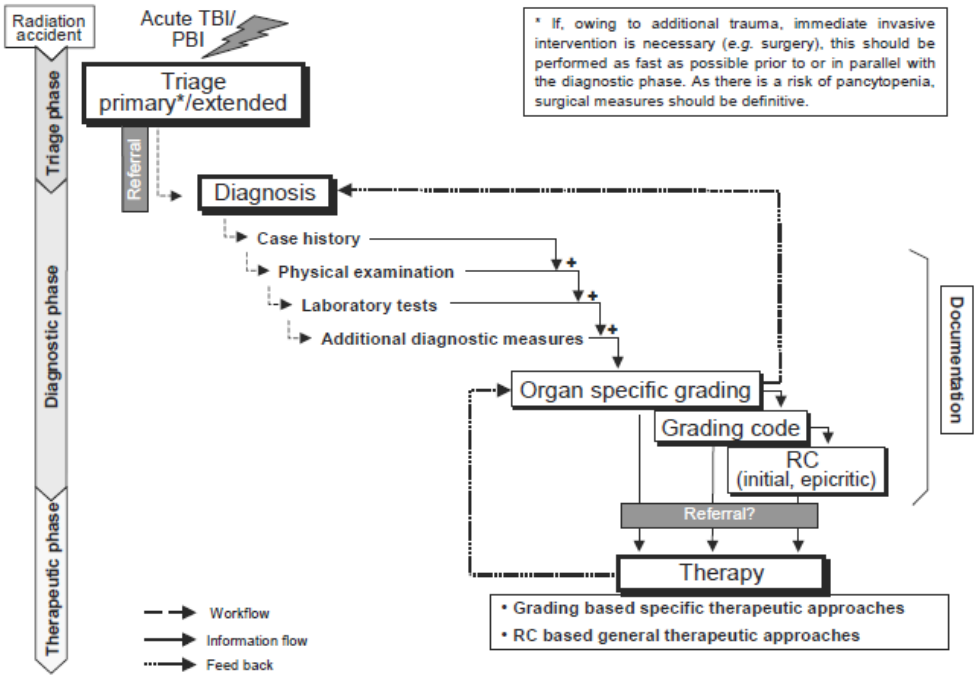
2.1.5. Management

Updated guidance on radiation emergency medical management exists (REMMc). Early response activities are aimed at the preservation of life, and they should focus on medical triage. Whenever it happens, and after performing the lifesaving tasks, the evaluation for contamination and/or exposure is mandatory in order to choose the appropriate algorithm to manage radiation problems in the patient (REMMd). If only contaminated, the patient should be assessed for external contamination and then be thoroughly decontaminated. Next, he/she should be treated of any life- or limb-threatening injuries that might exist, and also rule out whole-body exposure and ARS (REMMe). If only exposed (REMMf), the patient should be evaluated for ARS, looking for signs (at physical examination) and estimating dose from exposure (either using any clinical data available or dose reconstruction by patient location), and managed for ARS if exists. If both contaminated and exposed (REMMg), the patient should go for external decontamination first, subsequently be treated of any life- or limb-threatening injuries that might exist; in this case, if estimated whole-body exposure is higher than 2 Gy, the patient should be assessed for whole-body exposure, assessed and treated for internal contamination, and finally assessed and managed for ARS.

This management might be modified by different factors such as: concomitant burns or trauma, mass casualty, timing of surgery, blood products use, or special needs populations. In case of mass casualty events (REMMh) particular radiation triage systems have been established (REMMi), and usual "standards of care" for medical activities may need to be modified (recently named “crisis standards of care”) to reflect actual conditions and reduced availability of resources.

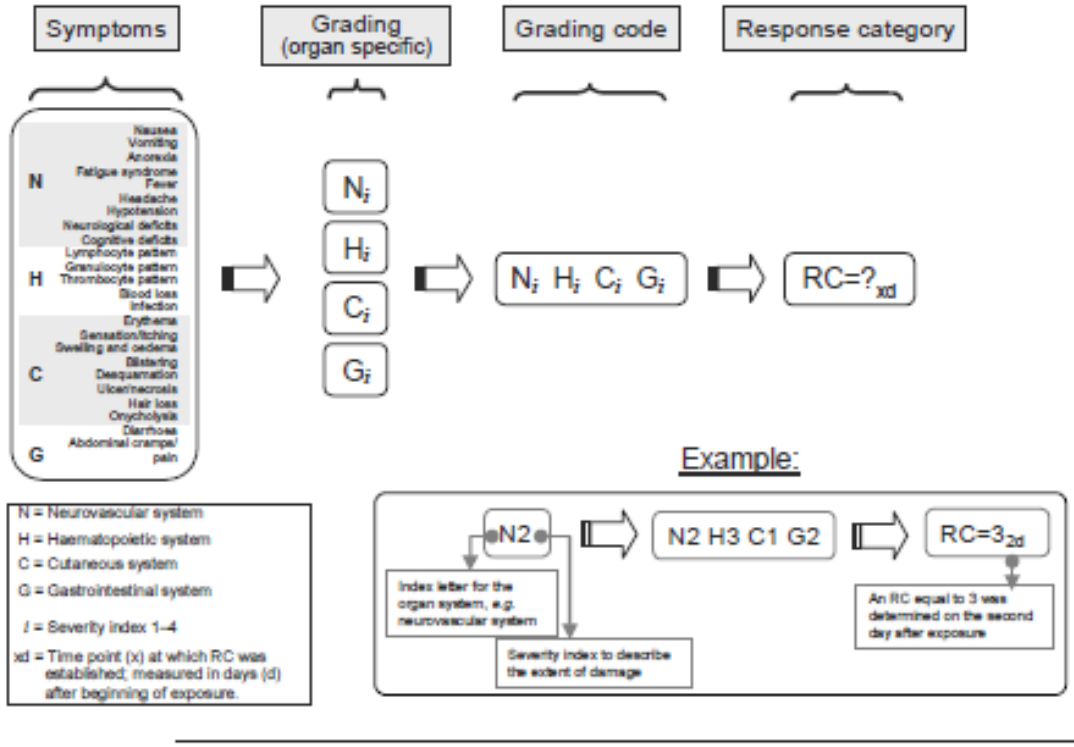
The European approach for managing radiation casualties, called METREPOL (Fliedner et al. 2001), provides tools for diagnosis and triage of persons exposed to radiation in small and medium size radiation accidents (FEMA paper). Patients are assigned a response category (RC), which require specific therapeutic strategies, based on the assessment of the damage to the critical organ systems as a function of time after radiation exposure using indicators of effect, i.e. observable clinical signs and symptoms (Fliedner et al. 2001). This approach is based on medical signs and symptoms and laboratory data, not on dose per se.

The following figure shows the "workflow" that will guide the management of the radiation accident victim.



Terminology

The following figure depicts the terminology used for the RC concept.

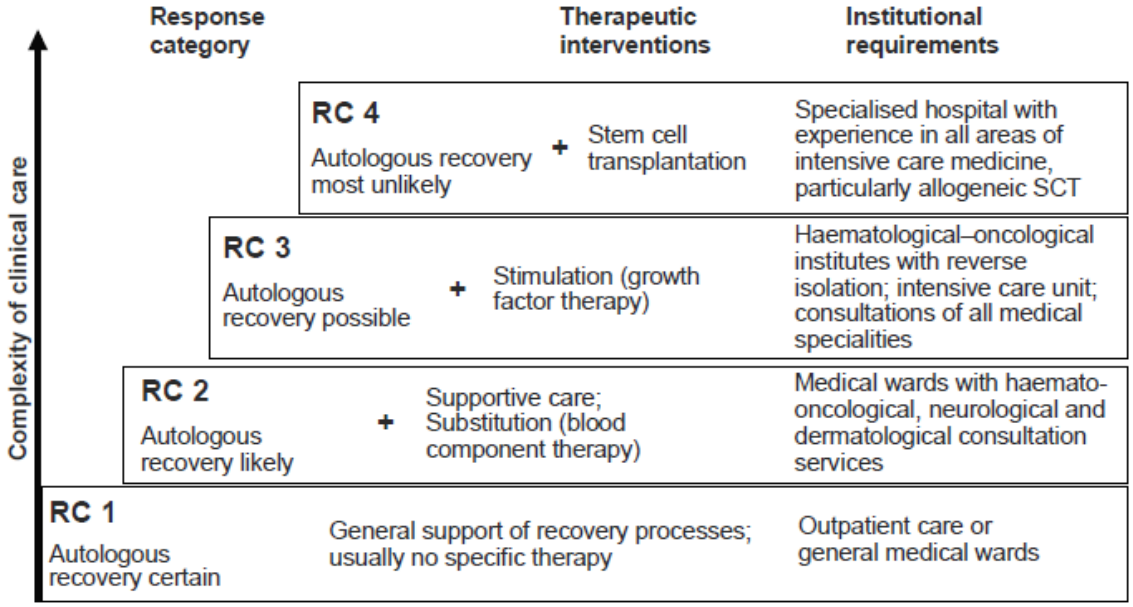


Specific therapeutic strategies depends on the assigned response category as illustrated below. Supportive medical care may consist of antiemetics, analgesics, brain oedema therapy, adapted nutrition, antibiotics, skin treatment, and other medical/surgical approaches (e.g. for oral mucositis,

pain and decontamination, etc.). Substitution (blood component therapy), stimulation (growth factor therapy), stem cell transplantation or surgery are other treatments.

Therapeutic and institutional levels of care

The following figure gives a synopsis of the RC-dependent therapeutic and institutional levels of care for radiation accident victims.



Despite this protocolised approach for managing radiation casualties, no medicine is formally approved in the EU for use as a medical radiation countermeasure. Two granulocyte-colony stimulating factors (G-CSF) products, filgrastim (Neupogen®) and pegfilgrastim (Neulasta®), are approved medical radiation countermeasures by Food and Drug Administration (FDA) for adult and paediatric human use. They are indicated to mitigate hematopoietic ARS, administering the first dose as soon as possible after suspected or confirmed exposure to radiation levels greater than 2 Gy, since they can effectively reduce the duration and severity of neutropenia and increase survival (FDA paper). Efficacy data were derived from studies in nonhuman primates (NHP) conducted in accordance with United States governmental regulations as promulgated under the FDA Animal Rule. The Animal Rule states that for drugs developed to ameliorate or prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic substances, when human efficacy studies are not ethical and field trials are not feasible, FDA may grant marketing approval based on adequate and well-controlled animal efficacy studies when the results of those studies establish that the drug is reasonably likely to produce clinical benefit in humans.

G-CSF are products that stimulate the proliferation and differentiation of normal leukocytes. They have been utilized for the treatment of radiation-induced myelosuppression associated with radiological/ nuclear accidents since 1986, but the number of victims who received them was small for each accident and treatment generally started late after the accident (Dickerson 2013, Singh et al. 2015). Although the data seem to indicate that their use appeared to be beneficial (improve time to white blood cell recovery and for some victims may improve survival), and would have been more effective if given earlier, there is no definitive proof that their administration actually decreases mortality in radiation-exposed humans. Some investigators and researchers suggest however, that G-

CSF treatments may adversely affect blood platelet counts so the IAEA recommends that platelet counts be monitored when G-CSF is being administered.

Entolimod is proposed to address an unmet medical need in Europe with the argument that filgastrim and pegfilgastrim are not adequate for use in a mass-causality radiation catastrophe since intensive supportive care may be limited. When used together with supportive care, both G-CSF demonstrated to reduce the risk of radiation-induced neutropenia in ARS (FDA paper). However, as said in their SmPC approved by FDA, they had minimal direct in vivo or in vitro effects on the production or activity of hematopoietic cell types other than the neutrophil lineage, and therefore they are not expected to materially reduce the risks of radiation-induced thrombocytopenia or haemorrhagic complications. Neither are to act on radiation-induced gastrointestinal injury. Contrarily, entolimod is proposed as having effect on all three hematopoietic cell lineages (neutrophils, platelet, and erythrocytes) and on mitigating the erosive and haemorrhagic gastrointestinal toxicities of ARS.

See section 7 "References"

2.2. About the product

Entolimod is a recombinant biologic drug derived from the *Salmonella typhimurium* protein, FliC flagellin. The company claims that entolimod is a ligand for toll-like receptor 5 (TLR5) and, binding to this receptor, activates nuclear factor kappa-B (NF-κB), a transcriptional enhancer of multiple genes encoding antioxidants, antiapoptotic proteins, antimicrobial peptides, and cytokines.

Entolimod is being developed as a medical radiation countermeasure (MRC) for the following specific indication:

"Entolimod is indicated to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster.

It is intended that the use of entolimod be directed towards children or adults who have received potentially lethal irradiation, defined as ≥2 Gy of whole-body radiation exposure based on radiation exposure history, proximity to the radiation source, symptoms or physical findings of radiation exposure, blood lymphocyte counts (if available), and/or dosimetry (if available)."

The recommended human dose in paediatric population aged 2-18 years, adults and elderly is based on patient body weight, as shown in Table 1. For patients with body weights ≥114 kg, a maximum dose of 50 µg should be used.

Table 1: Recommended Dose by Patient Body Weight

Body Weight Range, kg	Entolimod Dose, µg	Entolimod Dose, µL
1.75-3	1	10
4-5	2	20
6-8	3	30
9-13	5	50
14-20	8	80
21-30	12	120
31-33	13	130

34-50	20	200
51-75	30	300
76-113	45	450
≥114	50	500

A single administration is needed, and therefore the company attempts to demonstrate the safety and efficacy of a single use.

2.3. The development programme/compliance with CHMP guidance/scientific advice

Because humans cannot be lethally irradiated to test the activity and safety of entolimod for the proposed indication, clinical efficacy studies of the drug are not ethical or feasible. For this reason, development of entolimod as a MRC is based on efficacy, pharmacodynamic (PD), safety, and pharmacokinetic (PK) data obtained in lethally irradiated nonhuman primates (NHP) as well as PD, safety, and PK data obtained in non-irradiated NHP and adult humans.

Eight proof-of-efficacy studies and one Good Laboratory Practices (GLP) pivotal efficacy study in irradiated NHP and a GLP pivotal pharmacology study in non-irradiated NHP have established the dose-dependent efficacy and pharmacology of entolimod for reducing the risk of death following exposure to potentially lethal irradiation. The results of all studies are proposed as supportive of entolimod's efficacy and dose selection for its intended purpose.

The applicant has primarily acquired clinical data in healthy human subjects in 2 phase I studies that have characterised the safety, PD, and PK of single-dose, intramuscular (IM) administration of the drug consistent with the proposed schedule and route of administration in a radiation emergency. Entolimod has also been evaluated in 3 small studies in patients with cancer; safety data from these studies supplements the safety database for entolimod.

In the applicant's view the estimation of an effective human dose is supported by these collective efficacy data from irradiated NHP, PD observations from non-irradiated NHP, and PD and safety data in healthy human subjects. Based on the results of these studies a formal statistical dose-conversion analysis was provided as rational for the proposed posology.

Investigational Use Applications

Entolimod has been under development as an MRC against potentially lethal irradiation in the US under Investigational New Drug (IND) application and supported studies in health subjects (CBL-DD-CH-B502-07-014 [HU-7014]) and CBL-DD-CH-B502-09-001A [HU-9001]). Entolimod has also been evaluated for a potential cancer indication under IND and supported an initial study in patients with advanced cancer (I196111-US). Both INDs are currently open.

In the clinical overview it is stated that entolimod was submitted in the US in 2008 for the indication applied for and, additionally, in 2011 for "a potential cancer indication". Both applications are reported to be currently open. The applicant is requested to update information regarding these applications and to report on the current stage of these procedures.

An investigational use application (Permit 584) to support a trial in patients with advanced cancer (I196111-Russia) became effective in the Russian Federation on 23 October 2014. A further investigational use application (Permit 475) to support the neoadjuvant administration of entolimod to

patients undergoing surgery for primary colorectal cancer (BL612-CBLB502) became effective in the Russian Federation on 01 September 2015. Drug administration in these trials has been completed and available data are summarized in this application.

No other investigational-use applications have been submitted anywhere in the world.

Regulatory Designations

For use as an MRC, entolimod received fast-track designation from the FDA on 18 July 2010 and Orphan Drug designation (ODD) on 23 November 2010. Entolimod also received ODD from the European Medicines Agency (EMA) on 13 January 2016.

Marketing Authorisations

There are no approved marketing applications for entolimod anywhere in the world, and entolimod has not been used by any international mutual defence organizations.

In the EU, the drug was the subject of advice from the Committee for Medicinal Products for Human Use (CHMP) on 26 May 2016 (Procedure Number: EMEA/H/SA/3314/1/2016/PA/SME/ III). The applicant also met with EMA rapporteurs in a series of pre-MAA meetings held between 3 March and 13 March 2017.

The applicant believes that the entolimod development program is now sufficiently advanced to support submission a Marketing Authorisation Application (MAA) under exceptional circumstances with the intent of making entolimod available for the benefit of European citizens in case of a radiation emergency.

Scientific Advice

The development plan to support licensure of entolimod in Europe was presented in a scientific advice procedure in 2016 (EMEA/H/SA/3314/1/2016/PA/SME/III), with the following questions (Q):

Q: "In the context of an orphan indication for treatment of acute radiation syndrome (for which no human cases exist or are expected to exist until the time of a radiation disaster), does the CHMP agree that the irradiated NHP is an appropriate model of the human for evaluating drug effects on survival following potentially lethal radiation exposure and that the survival efficacy of entolimod has been adequately investigated to support the review of a marketing authorization application (MAA)?"

The CHMP response confirmed that since no efficacy data could be drawn from human patients, this information could rely on non-clinical data. The rhesus macaque was supported as relevant animal model. With the limited data provided at the time of ScAd request, the CHMP also considered the extent of the study Rs-23 could be sufficient for the evaluation. The dose extrapolation of non-clinical rhesus macaque data to humans was considered a most relevant aspect. It was also considered that only minimal supportive care could be administered in a clinical scenario and this means that the benefit of entolimod cannot be extrapolated to an add-on benefit in humans administered full supportive care. The CHMP also supported the use of IL-6 and G-CSF as relevant biomarkers. It was also considerd that entolimod would be mainly acting as a supportive, rather than radio protective.

Q: "Does the CHMP agree that the strategy used to identify the effective human dose of entolimod, including the choice of biomarkers of survival and statistical methods, is acceptable for MAA purposes?"

The approach of the applicant in deriving a presumably effective human dose was endorsed but taking into account the identification of PD biomarkers instead of the selection of PK parameters.

Q: "CHMP agree that the nonclinical and clinical safety profiles of entolimod have been sufficiently characterized to support a CHMP assessment of benefit/risk in the context of a MAA?"

It was agreed at the time of the ScAd request with the available data at the moment that the non-clinical and clinical safety data would be sufficient to support the benefit-risk assessment in the context of a MAA. It was also concluded that the non-clinical data provided demonstrated efficacy in the context of basic supportive care only and the applicant was asked to consider a single further NHP trial comparing outcome in macaques offered intensive care (IV fluids, IV antibiotics, G-CSF) as Standard of Care against comparator group, SOC with additional entolimod.

2.4. General comments on compliance with GMP, GLP, GCP

GMP

GMP certificates are available for all sites involved in manufacturing.

GLP

The applicant submits a large number of non-clinical studies. Many of them do not claim GLP conformity. Regarding the pivotal non-clinical studies there remain questions regarding GLP status.

The final conclusion on the benefit risk balance strongly depends on non-clinical studies (in particular Rs-001 and Rs-23), the proof of the scientific quality, the reliability and the validity of the studies are paramount. The applicant claims the studies were conducted in accordance with the standards of Good Laboratory Practice (GLP) as required by the major regulatory authorities, but possibly not under formal GLP conditions in a certified facility. Reassurance about the quality and GLP status including considering the value and feasibility of an inspection of the site has been requested.

GCP

Confirmation was given in module 1.9 that the clinical trials CBL-DD-CHB502-07-014 (HU-7014), CBL-DD-CHB502-09-001 (HU-9001), I 196111 (US)*, I 196111 (Russia)*, and BL612-CBLB502 carried out outside the European Union meet the ethical requirements of Directive 2001/20/EC.

2.5. Type of application and other comments on the submitted dossier

- Legal basis

  A centralised procedure (according to Regulation (EC) No 726/2004). Mandatory scope (Article 3(1) of Regulation (EC) No 726/2004). Annex (4) (Orphan designated medicinal product).

- Accelerated procedure

  N/A

- Conditional approval

  N/A

- Exceptional circumstances

  Applied

  Justification:

  a.) INABILITY TO PROVIDE COMPREHENSIVE EFFICACY AND SAFETY DATA DUE TO THE RARITY OF THE INDICATION**:** Given the rarity of the condition and circumstances in which ARS is likely to occur, it is not feasible to conduct clinical efficacy studies because the possibility of recruitment for such studies, including large co-operative multicentre studies, does not exist on practical grounds.

b.) INABILITY TO COLLECT SUCH INFORMATION BECAUSE CONTRARY TO MEDICAL ETHICS**:** ARS has already been described above as not naturally occurring. Therefore, it is conclusive that to expose any human beings to a lethal dose of radiation for the purposes of medical research in order to generate the required human efficacy and safety data would violate the fundamental principles of the Declaration of Helsinki (2013) and, as such, would be considered medically unethical.

- Biosimilar application

  N/A

- CHMP guidelines/Scientific Advice

  EMEA/H/SA/3314/1/2016/PA/SME/III: 26-05-2016

- 1 year data exclusivity

  N/A

- Significance of paediatric studies

  The PDCO adopted on 29 September 2017 an opinion on the modification of the agreed paediatric investigation plan (PIP) as set out in the latest Agency's Decision (P/0104/2017) of 11 April 2017. The new PDCO Opinion (P/0295/2017) on the modified agreed PIP includes a deferral.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

Entolimod Injection is manufactured as an aseptically processed, preservative-free liquid containing 0.10 mg/mL entolimod (CBLB502) protein filled into 2 mL Type 1 DIN 2R glass vials. Each vial is sealed with a sterilized V35 bromo-butyl injection stopper secured to the vial by an aluminum crimp with a white plastic flip-off cap.

Entolimod is available in a liquid formulation to be administered to humans intramuscularly (IM) as a single administration post-irradiation.

3.1.2. Active Substance

General Information

Entolimod (CBLB502) is a recombinant protein encoded by an engineered variant of the flagellin FliC gene of Salmonella enterica serovar Dublin. The natural FliC protein is the major component of Salmonella flagella. FliC is polymeric when present as part of flagella and can also form multimers in solution under certain conditions (e.g., low pH). Entolimod is a deletion variant of FliC flagellin that consists of 329 amino acids, i.e., N-terminal His6-tag and enterokinase cleavage site, and aa 1-176 and 402-505 of the flagellin protein connected by a flexible peptide linker.

Entolimod is a Toll-like receptor 5 (TLR5) agonist that has been developed for use as radioprotectant. Whether the postulated mechanism of action is based on the truncated flagellin only or whether other elements of the protein contribute to the MoA or safety profile of the product has not been addressed.

Manufacture, process controls and characterisation

Manufacturers

Manufacturers and release and stability testing sites have been adequately identified. However, for testing of some residuals, the current sites are no longer available and the applicant is in the process of finding new testing sites. These new testing sites should be provided before marketing authorization.

Description of manufacturing process and process controls

Entolimod is expressed intracellularly in *E. coli*. The manufacturing process of entolimod starts with the thawing of one vial of WCB. After reaching a target OD600 the pre-culture is used for inoculation of a production fermenter. Once the fermentation is terminated the bacterial cells including the protein are harvested. The cell paste is collected and stored. The required amount of cell paste is re-suspended, followed by homogenization, centrifugation, filtration and washing steps. This is followed by a series of chromatography and filtration steps.

A summary of the manufacturing process has been presented in tabulated form followed by a brief description of each step. The applicant should also present a flow chart for an easier understanding of the process. Further process optimisation is planned during process validation. This creates uncertainties about the robustness and consistency of the manufacturing process.

In the summary, a number of Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs) are highlighted. However, these have not been justified and in cases do not even comply with the definitions, e.g. filter integrity testing, OD, or buffer characteristics (pH, conductivity), are listed as CQAs. A list of CPPs and CQAs should be presented (ideally in tabulated format) with a justification for their classification as such.

No courier company has been contracted yet for the transportation. No validation of the transport has been carried out but a validation master plan has been presented, as for the rest of the manufacturing process.

Control of materials

The cell banks used for producing entolimod have progressed from the original laboratory-generated expression vector expressed in an *E. coli* strain to a final production vector expressed in another *E. coli* strain. On this issue, the applicant should confirm whether full sequence and restriction has been confirmed in the final production vector. A rationale is requested to be provided for the chosen amino acid sequence of Entolimod protein.

The current entolimod cell banks are MCB-3 and WCB-2. Information regarding their generation, their characterization, storage, disposition and requalification has been provided for both cell banks. However, further information is requested on the identification system of the MCB and WCB including investigation of translational fidelity and full amino acid sequencing of the recombinant protein. A protocol for generation of future WCB and specifications for MCB and WCB should be provided. The limit for in vitro cell age for production should be defined. Based on data derived from production cells expanded under pilot plant scale or full scale conditions to the proposed in vitro cell age or beyond, plasmid copy number, the correct DNA-sequence of the expression cassette as well as the correct amino acid sequence of the protein should be confirmed.

Sufficient information regarding raw materials and components is provided.

Control of critical steps and intermediates

Critical Process Steps were identified. Although the approach is considered valid, the final classification of the process steps does not correspond to those initially planned. In addition, the results are not fully reflected by the information provided in Section 3.2.S.2.2. Determination of critical quality attributes has not yet performed but is seen as the prerequisite for process development and evaluation. Thus, the proposed CPP classification is questionable.

Regarding the proposed critical IPCs it is noted that certain process steps still need optimisation. Hence, evaluation and classification of PPs and CPPs for these steps is seen with some reservation. Moreover, for certain steps it is currently unknown whether the critical IPC is performed and if so, which method is used and what is the final acceptance criterion. As determination of concentration and purity is key for manufacturing process control, a respective IPC method must be available for the commercial DS manufacturing process.

All the DS manufacturing processes used for manufacture of DS batches preceding a certain DS lot are different in detail and have been subjected to several improvements, optimizations and adaptations. Even for the proposed commercial manufacturing process differences between the small-scale experiments and the full scale process performances are seen, thus extrapolation of small scale results is not demonstrated. Further optimization is planned to be performed during process validation. Thus, the process development is not considered finalised limiting evaluation of criticality of the PP. There is currently no sound experimental evidence for the suggested process control and acceptance criteria.

To summarise, there is currently uncertainty about the proposed control strategy due to the following issues: (i) the proposed manufacturing process is only exemplary and solely used for manufacture of a certain batch and seems not to be finally defined, (ii) the process development is not considered finalized, (iii) determination of critical quality attributes has not yet been performed and (iv) there is currently no developmental basis for the suggested process control. As the manufacturing process intended for commercial production cannot be considered finally determined and as the critical quality attributes have not yet been determined, the proposed control of critical process steps cannot be considered a final one. Rather, it should be considered a preliminary and premature control strategy that should be revised when the CQAs are defined and the manufacturing process for commercial production is finally defined and validated.

Process validation and/or evaluation

Process validation for entolimod bulk drug substance (BDS) has not been performed. The applicant has requested approval under exceptional circumstances and proposes a concurrent validation, for which validation protocols are provided in Module 3.2.R.2 of this dossier. To apply the option of concurrent validation the following pre-requisites are described in the process validation guideline: i) studies performed for process evaluation are appropriate representations of the commercial process, and ii) control strategy will properly verify that the process has performed as intended and that active substance and intermediates comply with pre-defined acceptance criteria.

These prerequisites are not fulfilled. Process evaluation as part of process characterisation is considered to be currently at a preliminary and premature level only. Process knowledge is very limited. Only two DS batches manufactured so far have successfully passed the entire 1100L manufacturing process (one in 2007 and one in 2010). However, representativeness of a given DS batch for the commercial process has not yet been demonstrated.

The control strategy of the proposed commercial manufacturing process is considered not yet finalised. The proposed commercial manufacturing process has been defined by the applicant with reference to the manufacture of a certain DS batch. However, all preceding DS manufacturing process have been performed differently and also for the commercial process further optimisation is planned. Currently,

there is uncertainty about the DS manufacturing process intended for commercial production of Entolimod DS (see Major Objection). Taking together, the proposal for concurrent validation cannot be acceptable at the present time and so the absence of process validation and thus, lack of evidence for a validated DS manufacturing process is considered an issue

Removal of process-related impurities has not been addressed so far should be covered by process validation. Respective reduction capability of the proposed commercial process should be demonstrated. Reuse time of the employed column resin materials remains to be addressed and validated.

Manufacturing process development

Certain steps are proposed to be optimized during process validation. This is not considered acceptable. Process optimisation should be performed and once the manufacturing process is established and characterised process verification runs should confirm the validated steps.

Several manufacturing changes were introduced between the clinical and the proposed commercial process. No comparability studies are presented to assess the impact on quality of any of the changes introduced during development. This is particularly relevant for the changes introduced from the clinical to the commercial processes. The manufacturing processes used to produce the DS material that has been employed in clinical and pre-clinical studies should be directly compared and differences should be outlined. The manufacturing process used to manufacture the DS batch which is the origin of the second reference standard should be included in this comparison. The comparability studies should be evaluated by the applicant taking into consideration ICH Q5E requirements.

Characterization

A summary of entolimod bulk drug substance (BDS) characteristics, the methods used to assess them, the rational to select them, and the general conclusions, as well as links to the corresponding summary reports and sections, have been provided.

Characterization of the Entolimod Drug Substance needs further clarification. No analytical evidence substantiating the postulated amino acid sequence of entolimod DS is presented. The secondary and tertiary structure of entolimod DS have not been studied.

Currently, neither dose/response curves nor EC50 data are presented. Receptor binding remains to be demonstrated.

The impurity profile of entolimod has not been studied in sufficient detail. It is unknown whether and at which amounts low-molecular weight proteins or aggregates of the monomer (dimer, multimer) exist or may exist following forced degradation.No systematic investigation of degraded Entolimod has been performed.

Overall, information provided on characterisation is considered too limited to provide confirmation of the intended protein structure as well as the proposed impurity profile.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Specifications

Release and stability specifications for entolimod (CBLB502) bulk drug substance (BDS) have been proposed.

The provided DS release and stability specification comprises testing of appearance, pH, identity, purity, potency, strength, impurities and microbial safety. Notably, some analytical methods have not been qualified/validated.

Some analytical methods are "not longer in use" as reported by the applicant. Likewise, the analytical testing sites responsible for some tests are no longer in business. It should be unambiguously clarified where each testing included in the specification is performed. For those manufacturing sites confirmation of GMP compliance should be available.

Acceptance criteria for some tests are not provided since limits will be established later, based on the data of process validation. This is not appropriate. A maximum, justified, limit for these impurities should be set.

Taking together all the noted exceptions and restrictions and the lack of sound purity/impurity acceptance criteria (due to the poor characterisation) as well as absence of formal method validation data for most of the proposed analytical procedures, the proposed DS specification with the respective analytical methods is considered exceptionally questionable and not considered suitable to allow an adequate control of the entolimod DS.

Analytical Procedures

The analytical procedures used to test entolimod (CBLB502) bulk drug substance (BDS) are described in the corresponding sections.

Numerous issues are noted with respect to description and validation of the analytical methods applied for control of Entolimod. Only two analytical procedures have undergone formal method validation in accordance with ICHQ2. Moreover, qualification of some methods includes testing of DP batches only.

Overall the proposed potency assay is considered suitable for the intended use.

Batch analyses

Batch data are provided for 11 DS batches. Data from six of the 11 batch data sets are derived from developmental lots. Five of the 11 DS batches have been manufactured with the intended commercial scale. Release data for the commercial scale batches show important differences in the levels of some impurities. In addition, some tests were not performed (or data are not available) in some batches. The applicant should discuss these inconsistencies. With regard to the provided justification of specification a number of issues need clarification.

Acceptance criteria should reflect the batch data (taking into account the manufacturing variability) and the variability of the analytical method used.

Reference standards of materials

Entolimod reference standards (RS) have been manufactured according to entolimod bulk drug substance (BDS) processes in place at the time.

Three different reference standards have been "produced" so far. Although the applicant states that each new RS was tested against the previous RS, the fact is that in the absence of appropriate comparability studies, no conclusion can be drawn on the suitability of every new RS with respect to the previous one. Characterization of the first existing reference standard has not been performed. Specifically the primary structure of this RS may be different to the proposed one due to the applied manufacturing process.

Identity tests were added to the release testing of the current RS. In fact, a lower potency was detected when run in the same assay with the previous RS. This observation triggered a readjustment

of the relative potency results. This approach is difficult to understand and should be further clarified by the applicant.

The applicant states that consequently early toxicology and pharmacology study results were modified. A systematic error is expected. The issues should be clarified and a rationale/justification should be provided for the chosen approach.

Each of the three RS was produced by a different version and/or scale of the manufacturing process so a proper qualification of every new standard against the previous one would be expected. Instead, only release data for each new RS are presented. This is considered insufficient. Thorough characterisation data should be provided for all RS materials. Representativeness of the reference standard material for the material generated by the proposed commercial manufacturing process needs to be confirmed. A protocol for manufacture, qualification and storage of future RS should be provided. Summarising, there are numerous issues that have been identified. The reference standard system is concluded to be not reliable and not suitable and puts into question batch results for certain parameters.

Stability

Three BDS lots and three reference standard lots were analyzed to determine DS stability. Representativeness of the stability batches for the commercial DS has not been demonstrated. GMP BDS lots are placed on formal stability protocols and RS lots are requalified periodically. BDS samples were stored under long term and accelerated conditions.

It needs to be confirmed that the chosen small scale storage container is representative for the proposed commercial DS storage container.

The applicant claims that the methods used for the analysis of stability are "stability indicating" however data demonstrating the ability of these methods to detect changes in Entolimod molecule have not been presented.

For the three BDS Lots, all stability test results at all time-points, and at both storage conditions, were within specification.

Although the stability studies indicate that Entolimod is stable, a lack of consistency along the studies is observed. This is not appropriate and contradicts the requirement for a consistent, reproducible manufacturing process and a sound specification.

Based on the provided data and in the absence of a reliable, stability-indicating analytical method panel, no conclusion on stability can be reached at the present time.

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Description of the product

Entolimod Injection is manufactured as an aseptically processed, preservative-free liquid containing 0.10 mg/mL entolimod (CBLB502) protein filled into glass vials.. The maximum dose for patients with ≥114 kg body weight is 50 µg (=0.5 mL). The selected fill volume should be justified in view of Entolimod maximum dose.

Pharmaceutical Development

Entolimod Injection drug product (DP) is a clear, colorless to slightly yellow solution. The excipients used in the formulation buffer included sodium chloride, potassium chloride, disodium hydrogen phosphate dehydrate and potassium dihydrogen phosphate to ensure a pH of 7.4 as well as polysorbate 80. Only two batches have been manufactured so far representing entolimod DP intended for emergency use. The applicant have been requested to provide a justification of the DS source for one of the the DP lots, since it seems that the DS lot used was expired.

The DP manufacturing process has not been changed during the history of GMP entolimod DP manufacturing. Likewise, the components of the primary packaging system have not been changed across product development. In view of the proposed DP storage temperature the risk of incompatibilities between packaging materials and the entolimod DP formulation such as e.g. leaching is deemed rather low. However, the suitability of the selected primary packaging should be further substantiated by data.

Manufacture of the product and process controls

The DP manufacturing process involves dilution of entolimod bulk drug substance (BDS) with formulation buffer followed by sterilizing filtration, aseptic filling into vials, labelling, visual inspection, secondary packaging and labelling.

The mixing step during product formulation has not been categorised as 'critical'; that needs to be justified.

Based on an analysis of the results of development, engineering and cGMP runs and a general risk assessment, the process parameters that are most likely to influence product quality or result in process failure if not properly controlled were classified as Critical Process Parameters (CPP). For each operation, the severity, occurrence and detection were evaluated based on experience with the process during development, scale-up and cGMP production. A clear presentation of the manufacturing steps classified as critical and non-critical and the justification for the categorisation is requested. Quality by Design summaries identifying in-process controls and assessing the potential impact of CQAs and CPPs on product QTPP were also prepared for the fill and finish process and for the labelling, packaging and shipment processes. In these summaries the results of the assessment of the DP manufacturing process steps are linked with the acceptance ranges of the process parameters. The term QbD is not appropriate in this case as there is no data basis for a QbD approach. In addition, defining acceptance ranges for process parameters is not equivalent to establishing a 'design space. Section CTD P.3.4 needs to be revised.

Final manufacturing process validation for entolimod drug product (DP) has not been performed, although the manufacturing process is fixed and validation protocols are provided in this dossier. The applicant requests authorization under exception circumstances with concurrent validation which is not endorsed since a "strong benefit-risk ratio for the patient" needs to be determined as stated in 30 March 2015 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Annex 15: Qualification and Validation, Sections 5.16 and 5.17. Moreover, very limited batch data of GMP entolimod DP lots (four lots manufactured by use of two entolimod BDS lots; among them only two lots of final composition and protein concentration from the same BDS lot) and the lack of ICH Q5E comparative characterisation studies among all lots produced so far do not provide a basis to have confidence in a robust and consistent process leading to a final product of the defined quality. Therefore, the applicant is requested to present a full validation of the DP manufacturing process.

Product specification, analytical procedures, batch analysis

Product Specifications

The release and stability specification for entolimod (CBLB502) 0.10 mg/mL drug product (DP) have been provided. Revisions of the presented Entolimod DP specification are needed which should reflect the acceptance criteria of future commercial production. Moreover, the proposed control of Entolimod drug product at release and during shelf life is inadequate and not in line with ICH Q6B.

Most analytical methods used for DP release and stability are compendial and therefore description and validation are not provided. The non-compendial methods were validated and the information was provided in the corresponding DS sections.

To date, nine lots of entolimod (CBLB502) drug product (DP) have been manufactured (5 non-GMP lots and 4 GMP lots). Release data for all lots were presented. Due to lack of comparability studies according to ICH Q5E Entolimod DP non-GMP lots cannot be considered representative for the commercial DP lots in view of the considerable differences in protein concentrations, formulation composition, parameters tested and methods applied. Among the four GMP lots manufactured so far only two of them had the proposed commercial protein concentration of 0.1 mg/mL. Only one drug substance lot was utilised for the production of these two batches which is insufficient to demonstrate that DS variability within the approved DS specification results in adequate DP quality. A side-by-side re-analysis of two GMP lots was conducted, but only compliance with release specification was tested. Overall, it is currently not feasible to conclude that the GMP batches used in the clinical studies are representative for Entolimod drug product proposed for commercial use due to lack of comprehensive comparability studies on product characteristics and taking into account changes in the formulation as well as within DS and DP manufacture over the time.

Entolimod DP impurity/product-related substances are not satisfactorily studied.

A summary of the risk assessment on elemental impurities in Entolimod DP according to ICH Q3D is lacking. In addition, reference to historical data to justify certain acceptance ranges is not acceptable without evidence that the non-GMP batches and the first GMP batches are representative for commercial Entolimod DP quality and without proof that the significant changes in the DS manufacture over the time have no impact on entolimod quality.

The same reference standard is used for the testing of both entolimod BDS and entolimod DP. The difference in the formulation is adequately taken into account when the reference standard is used for DP analysis.

Glass vials and stoppers comply with the quality requirements of Ph.Eur. Container closure integrity is tested in accordance with guidance provided in USP <1207>. This method has been qualified for both entolimod DP lots due to the differences in their compositions. The applicant is further requested to perform leachable/extractable studies of the container closure system or justify otherwise.

Stability of the product

Long-term storage conditions, accelerated storage conditions and stressed storage conditions were studied in the stability studies. Information should be provided about the container in which the samples were kept during the stability studies. The formulation of the stability batches is not consistent.

The applicant proposes a shelf life. However the stability data presented are insufficient to support the proposed shelf life, since stability was only proven for one batch representative of the commercial product. For the other batch that is proposed to be marketed only limited data are currently available. Overall, the stability studies initiated and the data presented are not appropriate to satisfactorily demonstrate Entolimod DP stability.

The in-use stability of entolimod DP was studied. The applicant indicates that this study was designed to investigate the stability of Entolimod 0.10 mg/mL DP, under conditions as envisioned in the Entolimod proposed indication for use. However, the statement given in the proposed SmPC is not supported by the stability testing results.

Adventitious agents

No animal or human sourced materials are used in any step of the production of entolimod, except for

a milk-derived analog of galactose that is non-metabolizable and inactivates the lac repressor to induce synthesis of α-galactosidase in *E. coli*. Per EMA/410/01 Revision 03: "In the light of the current scientific knowledge and irrespective of the geographical origin, bovine milk is unlikely to present any risk of TSE contamination."

Therefore, additional adventitious agent testing is not required.

3.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Entolimod (CBLB502) is a recombinant protein encoded by an engineered variant of the flagellin FliC gene of Salmonella enterica serovar Dublin. The natural FliC protein is the major component of Salmonella flagella. FliC is polymeric when present as part of flagella and can also form multimers in solution under certain conditions (e.g., low pH). Entolimod is a deletion variant of FliC flagellin that consists of 329 amino acids, 1-176 and 402-505, which are connected by a flexible peptide linker.

Entolimod is a Toll-like receptor 5 (TLR5) agonist that has been developed for use as radioprotectant.

Ten Major Objections have been identified which currently preclude a Marketing authorisation. In addition a number of other concerns have been identified. The quality dossier is very poor, reflecting the many deficiencies identified in the manufacturing, control, characterization and stability of both Drug Substance and Drug Product. The main deficiencies have been identified as Major Objections, which as summarized below:

> Uncertainty persists on the proposed drug substance manufacturing process and control strategy due to the following issues: (i) the proposed manufacturing process is only exemplary and has only been used for manufacture of one batch and seems not to be finally defined, (ii) the process development is not considered finalized, (iii) determination of critical quality attributes has not yet been performed and (iv) the suggested process control is not substantiated by process development and evaluation. The proposed control strategy is considered preliminary and should be revised when the CQAs are defined and the manufacturing process for commercial production is finally fixed and validated. Process validation for entolimod bulk drug substance (BDS) has not been performed. In fact, the most recent successfully manufactured batch was produced in April 2010. In addition, serious doubts on process consistency and robustness remain. Thus a prospective process validation, as defined in ICH Q11, encompassing the entire manufacturing process is expected to be performed.
>
> Despite significant changes across DS manufacturing process development comparability among the DS lots has not yet been demonstrated. This limits the evaluation of data for the proposed MAA. The manufacturing processes used to produce the drug substance material that has been employed in clinical and pre-clinical studies and has been used for generation of reference substance material remain to be directly compared and differences outlined according to ICH Q5E.

Evidence is missing that the DS batches used to manufacture DP batches for pre-clinical and clinical studies are representative for the intended commercial process.

The characterization of the entolimod Drug Substance is limited and incomplete to sufficiently confirm the intended protein structure as well as the respective impurity profile. DS lot demonstrated to be representative for the commercial manufacturing process should be used for characterisation experiments. Characterisation data should provide analytical confirmation for primary, secondary and tertiary structure. Information about analytical methods used for entolimod characterization is insufficient. Data presented in the dossier is minimal. No risk assessments were performed to determine the impact of the product and process related substances on bioactivity, immunogenicity and safety. The impurity profile of entolimod should be determined. The degradation profile of entolimod DS should be studied.

The proposed DS specification with the respective analytical methods is considered questionable and not appropriate to allow an adequate control of the entolimod drug substance. All purity analytical methods have neither been validated nor qualified for purity/impurity testing. Formal method validation data is lacking for most of the proposed analytical procedures. Product-related substances and impurities are not controlled at DS release and during shelf life due to the lack of respective acceptance criteria. Finally, a number of exceptions and restrictions with regard to the applied methods and testing sites are listed.

The established RS system is not reliable and not appropriate to build the basis especially for potency determination of entolimod DS and DP batches. Thorough characterisation data are missing for all RS materials that have been applied in DS manufacture.

Drug Substance and Drug Product stability data provided by the applicant do not permit to reach a clear conclusion about the stability of Entolimod. Data on DP batches representative of the commercial drug product and derived from different bulk drug substance batches should be generated to a low a final assessment on stability.

Process validation data of a sufficient number of batches representative of the commercial process should be presented to confirm production consistency and effectiveness of Entolimod DP manufacture to reproducibly achieve the intended product quality. Therefore, the applicant is requested to present a full validation of the DP manufacturing process prior to approval.

The proposed control of entolimod related substances/impurities/degradation products in the final drug product is inadequate and not in line with ICH Q6B. Acceptance limits should be established in order to ensure an adequate DP impurity control after manufacture and at the end of shelf life.

A comprehensive analytical comparison between those Entolimod DP batches used for the human safety studies and commercial lots has to be presented to confirm the representativeness of the clinical trial batches for Entolimod DP proposed for emergency-use. Data which solely demonstrate that all batches comply with DP specification is far too limited to allow a conclusion on analytical comparability. The exercises should be conducted in line with the principles as outlined in ICH Q5E.

Overall evidence of appropriate quality is not provided for Entolimod. A number of serious deficiencies with regard to active substance analytical characterization, active substance and drug product manufacture and control were identified during assessment. The manufacturing process and control strategy for Entolimod is considered preliminary and premature. Moreover, comparability of drug substances and drug product batches derived from different stages of manufacturing process and product development is not substantiated. Instead, from the information provided significant differences can be assumed. In consequence, Entolimod batches used in previous human safety studies are not proven to be representative of the batches most recently manufactured and proposed

for emergency-use. Further studies/investigations and development of manufacture and control of the active substance and drug product are needed to ensure appropriate quality of Entolimod for emergency-use.

3.2. Non clinical aspects

3.2.1. Pharmacology

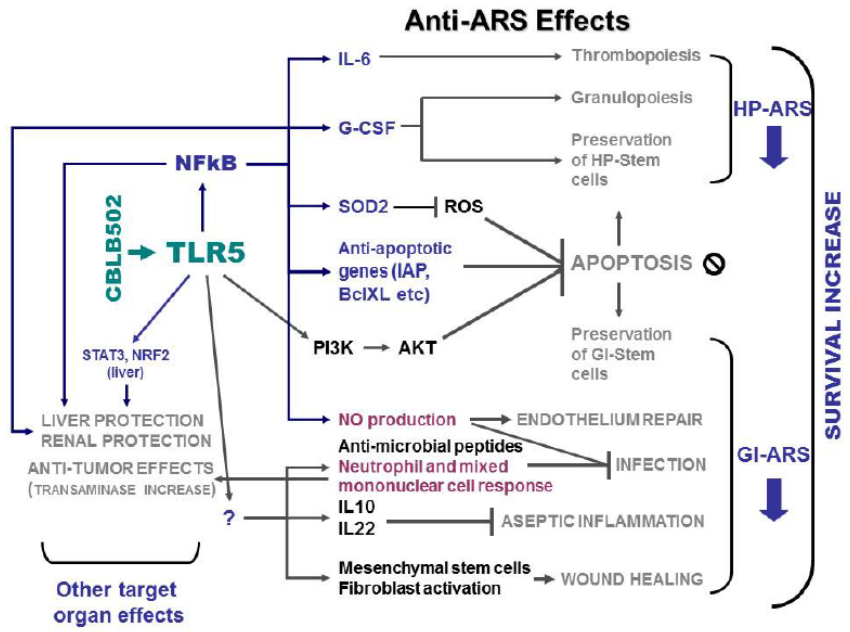
Entolimod is clinically intended to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster. Entolimod is a 35 kDa protein composed of 329 amino acids corresponding mainly to the N- and C-terminal regions of *Salmonella enterica* FliC flagellin, which are believed to be responsible for binding to Toll-like receptor (TLR) 5. Additional 34 N-terminal amino acids of entolimod contain a His6 tag and an enterokinase cleavage site. **The applicant is asked to comment whether the N-terminal 34 amino acid portion of entolimod, which is not part of the sequence of *Salmonella* FliC flagellin, could pose a risk to patients treated with entolimod (e.g. immunogenicity).**

According to Module 2.4 Non-Clinical Overview section 1.6 Proposed Entolimod Therapeutic Regimen "the therapeutic regimen proposed for authorization in the event of a radiation disaster consists of a single entolimod injection administered approximately 25 hours post exposure to a potentially lethal dose of TBI." In the SmPC 4 CLINICAL PARTICULARS; subsection 4.2 Posology and method of administration; Method of administration it is stated "Entolimod TMC should be administered as a single intramuscular injection as soon as possible following radiation exposure." The time point chosen for assessment of entolimod efficacy in the pivotal primary pharmacodynamics study CBL1.1-N-01-Rs-23 in rhesus monkeys was 25 hrs after irradiation. **The applicant is asked to clarify regarding the clinically intended time point of entolimod administration in relation to time point of irradiation and to scientifically justify this time point taking into account the complete set of non-clinical data.**

Regarding pharmacology, the applicant submitted study documentations on 43 studies. The designation of a substantial part of these studies as secondary pharmacodynamics studies as opposed to primary pharmacodynamics studies is not agreed to. **The applicant is requested to submit adequate data on secondary pharmacodynamics.**

The applicant offers the following scheme for the proposed molecular mechanism of entolimod (for literature data, please, see the submission of the applicant).

Proposed Molecular Mechanisms of Entolimod Activity Mediated via Triggering of TLR5 according the Applicant's information



The scheme summarizes data from the following literature references: (67); (68); (69); (70); (71); (72); (73); (74); (75); (76); (77); (78); (79); (80); (81); (82); (83); (84); (85); (86); (87); (88); (89); (90); (91); (92); (93); (94); (95); (96); (97); (98);(99); (100); (101); (102); (103), (104), (105); (106) ; and CBLI studies: CBL1.1-N-04-Rs-01/02, CBL1.1-N-04-Rs-03, CBL1.1-N-01-Rs-04, CBL1.1-N-01-Rs-08, CBL1.1-N-01-Rs-14, CBL1.1-N-01-Rs-09, CBL1.1-N-01-Rs-10, CBL1.1-N-01-Rs-23, CBL-DD-CH-B502-07-014, CBL-DD-CH-B502-09-001.

In-vitro data using reporter cell lines indicate that entolimod in able to activate human, mouse and rhesus monkey TLR5 receptors functionally coupling to NF-κB. NF-κB, which is not a single protein, but a set of several different proteins, which after dimerization affect transcription of a plethora of different protein molecules.

In vitro studies using human TLR5 wild-type and according to literature TLR5 dominant negative peripheral blood mononuclear cells could not demonstrate unequivocally dependence on functional TLR5 signalling. **The applicant is asked to provide evidence for the specificity of the binding of entolimod to TLR5 and to justify scientifically the absence of any data on binding of entolimod to molecular structures other than TLR5 or to provide these experimental *in vitro* data.**

Although the applicant makes reference to other signaling pathways, no relevant discussion was provided in support for those undisclosed potential targets. Consequently the potential effects that activation those may have in the efficacy of the product is currently unknown. NF-κB transcriptionally activates multiple genes encoding factors that enhance survival and regeneration: (1) anti-apoptotic proteins; (2) antioxidant proteins that scavenge reactive oxygen species (ROS); (3) cytokines and growth factors promoting survival and proliferation of hematopoietic (HP) and other stem cells. **According to the applicant, two of the most prominent entolimod responsive cytokines, G-CSF and IL-6 were selected as efficacy biomarkers. The applicant makes reference to activation of other genes, nevertheless the dossier failed to provide justification of those genes and their role on the pharmacology of the product. The claimed gene activation of different genes and their relevance on the mechanism of action has not been adequately addressed or studied, therefore the applicant is requested to produce the relevant information in this regard**.

In a pilot study using luciferase transporter mice (BALB/c- c-IκBα-Luc transgenic mice), allowing detection of luciferase expressing cells *in vivo*, entolimod administration induced strong NF-κB-dependent luciferase activity starting from 1 h, peaking at 4 h and fading by 7.5-23 h after

administration. The luminescence signal was localized to the abdominal area, most likely originating from the liver and the intestine. **Regarding the bright colour of female mouse F5 in a bioluminescence image at 22.75 h after entolimod administration (Figure 1 of study CBL1.3-01-Ms-02) neither a comment nor an explanation is suggested. The applicant is asked to comment.**

The Applicant stated that mice when administered a ligand of Toll-like receptor 2 resulted in no NF-κB activation (CBL1.3-01-Ms-02) . Furthermore, when this ligand was used as a pretreatment of entolimod, NF-κB activation was completely abolished. The mechanism behind this finding is not understood since it is known that TLR2 and TLR5 in addition to TLR7 and TLR9 are all MYD88 dependent TLRs and in consequence, NF-κB activation is expected in the presence of a TLR2 ligand (i.e. O'Neill *et al*. 2013). **The applicant is requested to justify the mechanism behind this unexpected finding and discuss the relevance into humans. In addition the discussion should include the reasons behind the differential response to NF-κB inhibitors mentioned in the study**.

According to the applicants data in the study in mice *in vivo* (CBL1.3-A-01-Ms-01) Entolimod displays higher activity in terms of strength of NF-κB activation when compared to LPS with a differential organ preference for NF-κB activation. The tissues and organs examined did not include bone marrow. This organ has also the potential of NF-κB activation and display a role in the mechanism of action claimed by the Company. **The type of cells responsible of NF-κB activation following entolimod administration is unknown. Both set of data are relevant in order to compile necessary information for establishing the mode of action of the product and therefore an adequate justification of these issues is granted**. It should be noted that the liver is the organ where the data forwarded indicates that NF-κB activation was the highest and interestingly, transaminase increase following entolimod administration has been also described.

Using electrophoretic mobility [super] shift assay (EM[S]SA) or immunohistochemical (IHC) analysis the applicant investigated tissue-and cell-specific activation of NF-κB following IM administration of 10 µg/kg bw entolimod *vs.* vehicle in rhesus macaques. Strong or moderate NF-κB activation was demonstrated in lung, liver, kidney, heart, salivary gland, urinary bladder, adrenal gland, inguinal and tracheobronchial lymph nodes. NF-κB p50/p50 NF-κB homodimer DNA-binding was observed in peripheral blood nuclear cells and bone marrow at 0.5-4 h post-dose. According to the study documentation (CBL1.1-U-01-Rs-20) this homodimer is transcriptionally inactive and represents a classical desensitization loop of NF−κB activating signal. Regarding bone marrow the applicant concludes entolimod-induced NF-κB activation. **The applicant is asked to clarify this apparent controversy and to elaborate in detail on the proposed site(s) of entolimod-dependent generation of cytokines (e.g. G-CSF and IL-6) and the signal pathway leading to entolimod-dependent generation of these cytokines.** In addition, the study revealed that NF-κB activation following entolimod administration was much weaker in the intestine of this species than the signal reported in mice. No justification was put forward to clarify this finding. Since the gastrointestinal acute radiation syndrome is one of the main causes of death in irradiated individuals this issue gains more relevance. **The importance of this finding should be discussed in depth and clarified also with relation to its clinical relevance**.

In a study in Rhesus Macaques (CBL1.1-U-01-Rs-25) it was reported that NF-κB activation in the liver was determined to be from weak to moderate. This data is in contradiction with the previous study in the same species (CBL1.1-U-01-Rs-20) in which the activation was reported to be strong. In addition, in the RS-25 study gradual infiltration of liver parenchyma with neutrophils was also reported, and this may suggest a liver inflammatory process that needs to be discussed. **This finding in addition to the discrepancy in NF-kB activation in both studies needs to be thoroughly justified and if not**

relevant justification can be raised, additional confirmatory studies for the assessment may be granted performed. In the same study, production of a broad range of cytokines of ex vivo cultured peripheral blood (PB) and bone marrow (BM) nucleated cells from the two untreated animals in response to 0.3, 1, 3, or 10 ng/mL entolimod or vehicle in culture medium was determined. For the majority of the investigated cytokines the effect seen at 10 ng/mL entolimod in the incubation medium is smaller than the effects seen at 1 and 3 ng/mL entolimod. **The applicant is asked to elaborate on the proposed underlying molecular mechanism for the fact that for the majority of the investigated cytokines the effect seen at 10 ng/mL entolimod in the incubation medium is smaller than the effects seen at 1 and 3 ng/mL entolimod. In addition the applicant is asked to set the entolimod concentrations investigated in this study in relation to the (pre)clinically achieved ones and to draw the appropriate conclusions. Furthermore the applicant is asked to integrate the results of this study into the proposed mode of action of entolimod on a molecular level.**

In the study in irradiated monkeys CBL1.1-U-01-Rs-25 employing the same methodology for the assessment of NF-κB activation than in the study CBL1.1-U-01-Rs-20, the data reveal NF-κB responses in peripheral blood nuclear cells and bone marrow at 0.5-4 h post-dose, and therefore this discrepancy needs further clarification. In addition to this, IL-6 and G-CSF, selected by the Applicant as biomarkers of activity, were mostly induced in the BM and less in PB (4-15-fold difference in C_{max}). **Despite the large differences reported between BM and PB in cytokine production, no relevant information of differential NF-κB responses was discussed and it is therefore unknown if the differences seen correlate with a dissimilar NF-κB activation profile**. **The applicant is asked to elaborate in detail on the proposed site(s) of entolimod-dependent generation of cytokines (e.g. G-CSF and IL-6) and the signal pathway leading to entolimod-dependent generation of these cytokines.**

The data provided in a study assessing the cytokine response to entolimod induction *in vitro* in human peripheral blood mononuclear cells (PBMC) employing TLR5 homozigotic cells *vs.* TLR5 wt/mut cells is not clear (CBL1.1-12). While TLR5 wt/mut cells induction was reduced in 2/3 samples, in TLR5 wt/wt cells the reduction was seen in 1/4 samples. **The relevance of the data for the assessment is questioned and should be clarified by the applicant**.

In the study carried out in irradiated mice that were TLR5 knock out but transplanted with WT TLR5 BM (RP-100-102), despite the beneficial cytokine production claimed in previous studies, entolimod administration did not result in an increased survival. **The reasons behind this unexpected lack of efficacy of entolimod should be thoroughly discussed taking also into account the mechanism of action that the applicant proposes for this indication**.

The data forwarded a study carried out in lethally irradiated mice indicated that entolimod failed to rescue the IL-6 KO animals (2CBL1.3-A-01-Ms-07). Addition of IL-6+entolimod resulted in a 100% increased survival. **The data questions the role of IL-6 as a principal component of the activity of entolimod and therefore the role of IL-6 as biomarker of activity**.

Although the presence G-CSF and IL-6 appear to be necessary for increasing survival in irradiated mice receiving entolimod (CBL-R11-E1-Ms-12), it is surprising that the administration of an unspecific Ab results in a twofold increased survival in comparison to entolimod. **This needs an in depth justification by the applicant in order to clarity the reasons behind this unexpected increased efficacy**.

It should be noted in the study CBL1.1-N-01-Rs-03 in monkeys that CBLB612 did not increase survival despite data reported levels of measured parameters rather comparable to CBLB502. This raises doubts on the endpoints used for efficacy of the product since survival was increased in CBLB502

compared to CBLB612, TLR5 and TLR2 dependent respectively. All parameters measured including G-CSF, IL-6, IL-8, IL-10 and GM-CSF were largely comparable. **The applicant should explain the selection of efficacy endpoints for the assessment in view of the data reported**.

Even though the applicant claims in study CBL1.1-N-01-Rs-04 that entolimod provides an apparent mitigating effect on the GI tract of irradiated rhesus monkeys, when administered at 1 hour after lethal irradiation (6.5 or 10 Gy), no effect in survival increase was reported. **The applicant is invited to provide justification for the claims regarding GI mitigating effect as this is not clear in the provided data**. Of note, the administered dose was 4 times higher than the dose selected for human administration.

In a study where monkeys were exposed to levels of radiation of 5 Gy, and received a dose of 40 µg/kg (CBL1.1-N-01-Rs-05) it is to be noted that the SD were extremely high and overlapping. The difference in survival is only 20% compared to untreated controls (100% survival in treated groups against 80% in control group). The validity of this study in support for the Applicant claims is questionable. **Although the applicant claims that entolimod was effective in reducing the severity of neutropenia in this study, the data reveals that reduction was not relevant from a statistical point of view, and this raises doubts on the use of this endpoint as biomarker of activity**.

The applicant also claims that beneficial effects elicited by entolimod treatment given at 1 or 16 hours post-irradiation were somewhat more prominent than those seen in groups treated with entolimod at later time points (25 and 48 hr). Even though the study data reveals some difference in the measured potential efficacy parameters the survival rates remain invariable. **The relevance of the parameters and the clinical relevance of those in a clinical scenario should be discussed by the applicant. In addition, the cytokine profile at different time points should be made available**.

In the study CBL1.1-N-01-Rs-09 in irradiated monkeys the survival seen at 6.75 Gy TBI was the highest at 10 µg/kg, although no deviations on the parameters have been provided. The Applicant claims also that entolimod administration in the conditions of the study resulted in diminished the severity and duration of thrombocytopenia and neutropenia and improved recovery of erythrocyte. Nonetheless this is not so clear from provided data, as variability is very high and deviations overlap, therefore the Applicant claims regarding this issue are questionable. **Consequently the applicant should forward a relevant justification for the claims as those do not seem to be supported by relevant data and furthermore provide the deviations in OS reported in this study at the different dose levels tested.** In addition the lack of significant difference in terms of neutropenia reduction was confirmed in this study as well.

In the study CBL1.1-N-01-Rs-1, the 10 µg/kg entolimod dose administered to monkeys at 25 hours after 5.75 Gy irradiation resulted in increased survival rate, from 68.8% in control group to 100%, however, lack of significant difference in terms of neutropenia reduction was reported in this study (CBL1.1-N-01-Rs-10). **The lack of consistency of the safety variables selected for the efficacy assessment should be discussed in depth by the applicant**.

As entolimod can be seen as a derivative of naturally occurring *Salmonella* FliC flagellin, entolimod-naïve animals may present with antibodies reacting with entolimod. **The applicant is asked to comment on the basis for the apparently contradictory finding that in 3 animals (3F01, 3F04, 4F04) used in study CBL1.1-N-01-Rs-10 substantial amounts of neutralizing Abs were found, whereas titers of anti-entolimod Abs were obviously low.**

In several of the studies in irradiated rhesus monkeys ketamine had been administered repeatedly to the animals. The applicant concludes that ketamine negatively affected study outcomes. **The**

applicant is asked to elaborate on the size of entolimod dependent effect and amount (and timing) of ketamine administration in the monkey studies submitted with this application.

In another study conflictive results were seen regarding OS in monkeys receiving entolimod (10 µg/kg), at 6.5 Gy 25 days post TBI in comparison with other data produced by the Applicant (CBL1.1-U-01-Rs-17). **This discrepancy needs to be explained appropriately. Furthermore although improved levels of neutropenia and platelets is claimed by the applicant, data produced is not conclusive, as in other studies**.

Study CBL1.1-N-01-Rs-23 ("Rs-23") is considered the main pivotal non-clinical primary pharmacodynamics study conducted in rhesus monkeys with a TBI of 7.2 Gy and entolimod doses from 0.3 to 120 µg/kg bw IM administered 25 h after irradiation. The last study in non irradiated monkeys (CB5BD-P-Rs-001) ("Rs-001") is included in the clinical dossier for the safety assessment and comparability with irradiated animals at the same doses..

As the final conclusion on the benefit risk balance strongly depends on non-clinical studies (in particular Rs-001 and Rs-23), the proof of the scientific quality, the reliability and the validity of the studies are paramount. The Applicant claims the studies were conducted in accordance with the standards of Good Laboratory Practice (GLP) as required by the major regulatory authorities (but possibly not under formal GLP conditions in a certified facility?). **The applicant is asked to provide reassurance about the quality and GLP status, including considering the value and feasibility of an inspection of the sites.**

In the study CBL1.1-N-01-Rs-23, the Day-60-survival rate increased from 28% (vehicle) to 75% (10 µg/kg bw entolimod). Entolimod induced dose-dependent increases in the 3 laboratory markers analyzed as secondary endpoints (AUC_{0-24} of plasma G-CSF, AUC_{0-24} of plasma IL-6, and fold change of absolute neutrophil blood count (ANC) at 24 h after entolimod administration). **The absolute neutrophil counts in blood peaked at 8 h after entolimod administration.** Entolimod dose levels ≥10 µg/kg bw reduced the magnitude and duration of TBI induced neutropenia, thrombocytopenia, and anemia and improved the proportions of study days that the animals were alive and free of cytopenias and anemia. TBI-dependent necropsy findings predominated in animals receiving doses of entolimod ≤6.6 µg/kg bw relative to animals receiving doses of entolimod ≥10 µg/kg bw.

Of note the figures provided for the assessment of NEU and platelets, with all groups and deviation bars overlapping, did not facilitate the individual group evaluations but present rather clearly in an image the lack of relevant variations among groups. The results from this study did not allow concluding on platelets and neutrophil counts improvement following entolimod administration at different doses starting from 0.3 up to 120 µg /kg although in this study an OS increased at 10 µg /kg was seen. **The reason for this improved OS at this dose compared to other studies is unknown and should be discussed**.

The irradiation doses for assessing Gastrointestinal Acute Radiation Syndrome (GARS) in monkeys (Studies CBL1.1-U-01-Rs-22 and CBL1.1-U-01-Rs-21) are different to the selected for the pivotal efficacy non-clinical study (Rs-23) in which a dose of 7.2 Gy TBI did not result in any GARS, therefore this issue cannot be satisfactorily addressed in the Rs-23 study. **Applicant should put forward a relevant justification to justify GARS protection claimed**.

Regarding study CBL1.1-F-04-Rs-01/02 the results of the anti-entolimod-antibody investigation being part of study (Appendix 6) is apparently missing and the applicant is asked to submit the missing document. Furthermore, regarding the topic anti-entolimod-antibodies, the applicant is asked to comment, whether presence of anti-entolimod ABs

and/or of neutralizing anti-entolimod ABs does have any effect on the pharmacodynamic effects of entolimod in the animal studies taking into account the whole set of non-clinical animals studies, in which presence of anti-entolimod antibodies had been determined and to comment on possible implications to the expected clinical efficacy of entolimod in humans.

The applicant had performed initial primary pharmacology studies in rats and dogs, which had not been included in the dossier, but which are stated to be available upon request. Demonstration of the primary pharmacodynamics effects in possibly a third and a fourth species, besides mice and non-human primates, is considered to add even more to the opinion that the desired beneficial effect of entolimod is likely to be expected in humans as well. **Therefore, the applicant is asked, to submit the non-clinical preliminary primary pharmacodynamics studies performed in rats and dogs.**

The applicant lists 6 studies in non-irradiated mice and 3 studies in non-irradiated rhesus monkeys as safety pharmacology studies. These studies investigate certain aspects, but do not investigate the comprehensively the effect of entolimod on the central nervous system, the cardiovascular system and/or the respiratory system. In mice transiently increased NO levels in serum were seen (roughly 2-fold at 167 µg/kg bw of entolimod with T_{max} of 4 h). No histopathological effects of up to single entolimod doses of 6000 µg/kg bw were seen in the hearts of mice.

With regards of the transaminase level variations of entolimod seen previously in human trials, the applicant indicates that this was fully addressed in the toxicology studies, nevertheless this claim is not endorsed. Consequently a dedicated study for assessment of transaminase elevation in non irradiated monkeys was subsequently carried out. This study revealed a peak of transaminase levels 8 hours post entolimod administration. **This was not seen in toxicology studies since entolimod doses were very low and furthermore the time-points selected to assessment were taken later than the 8 h time point employed in this study, therefore the applicant should be aware of the drawbacks of the toxicology studies previously performed in monkeys. The transaminase increase could be derived of liver damage and this issue needs to be fully explored and clarified by the Company.**

According to guideline ICH S7A the purpose of the safety pharmacology core battery is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory and central nervous systems are usually considered the vital organ systems that should be studied in the core battery. For biotechnology-derived products that achieve highly specific receptor targeting, the guideline states that it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies, and therefore safety pharmacology studies can be reduced or eliminated for these products. Regarding biotechnology-derived products that represent a novel therapeutic class and/or those products that do not achieve highly specific receptor targeting, guideline ICH S7A states that a more extensive evaluation by safety pharmacology studies should be considered. Experimental demonstration of high specificity of entolimod for the TLR5 is at present lacking. Apparently at present no TLR5 agonist is commercially available on the European drug market.

The sponsor has carried out a number of non-GLP studies in mice in order to investigate nitric oxide production to justify the decreases in blood pressure previously seen in humans. Increase in nitric oxide was confirmed in the studies provided in mice. Although the potential role of NO in vasodilatation is not questioned, since the decreases in blood pressure seen in humans are quite relevant, and the studies in monkeys designed to assess this issue, failed to result in blood pressure decrease, the role of NO as responsible of this finding is questioned. The mechanism of blood pressure decrease seen in humans cannot be fully explained with the data provided and other mechanism should be explored for the assessment of this relevant adverse effect. **Tachycardia and QT prolongation were reported along with hypotension in humans, therefore ECG and hERG channel assessment is also**

considered relevant for the evaluation. Taking also into account that in addition to CVS events, the importance of the basis of the Core battery (GLP) for the assessment of the product is deemed relevant in the present scenario. Carrying out studies appropriate to the nature of the product in order to fully elucidate the safety pharmacology should be forwarded by the applicant. In addition effects in renal and GI system cannot be ruled out and therefore needs as well to be fully justified.

It is not endorsed that the potential usefulness of entolimod is restricted to the use in major radiation disasters. The dossier provided did not address specifically this issue and all sort of patients that are susceptible to receive an acute irradiation dose, are represented in the dossier provided. Administration to patients affected in less serious radiation events cannot be discarded. In the latter situation the number of therapeutic drugs and treatments available would not be limited and in any case, a significant number of patients would be administered best standard of care treatment in addition to entolimod administration. Consequently, the applicants justification for not pursuing pharmacodynamic drug interactions is not sustained and an adequate assessment of this issue is requested with all medicinal products deemed to be administrated concomitantly in a excessive radiation exposure scenario, considering in particular analgesics, antibiotics and other medicinal products such as filgastrim. In addition patients can be receiving treatment for an array of diseases (i.e. diabetes, blood pressure related diseases…) and therefore **the applicant should perform a full pharmacodynamic drug interactions evaluation**.

The applicant is requested to fulfill the dossier including available data to address the secondary phamacodynamics of entolimod as well as the pharmacodynamic drug interactions.

It should be noted that the dossier does not test administration of entolimod *vs.* a best standard of care equivalent to the one that human patients would receive in the case of accidental irradiation. In consequence it may not be concluded from a non clinical perspective that entolimod administration may have a differential efficacy to best standard of care. **This issue needs thorough discussion in combination as it is unknown if available treatments may have a better outcome in efficacy and/or safety to entolimod administration**.

3.2.2. Pharmacokinetics

The applicant has submitted 19 bioanalytical studies in support of the current application concerning four different types of bioanalytical assays in module 4: 1) ELISA-based quantitation of entolimod concentration in human and animal serum; 2) ELISA-based detection and quantitation of anti-entolimod antibody titers in human and animal serum; 3) cell-based evaluation of neutralizing activity of the anti-entolimod antibodies formed in human or animal serum, and 4) multiplex quantitation of cytokines in human and animal plasma.

Some of these studies (CBLISOP 1-44, CBLI-SOP1-47 and CBLiSOP1-12) are not validation studies but Standard Operating Procedures (SOPs) for the detection of entolimod in serum /plasma (CBLI-144), detection of anti-entolimod antibodies (CBLI 1-47) and a second method for the detection of entolimod in serum /plasma (CBLI-1-12). The placement within the dossier appears to be questionable. Since these studies are non-GLP compliant and the studies using this method are not GLP compliant either, further actions are therefore not required from the non-clinical point of view.

From the non-clinical point of view a formal validation is required for pivotal GLP-compliant toxicity studies only. Under this aspect a formal validation of the cytokine detection is not required (Millipore/11-045B Rhesus monkey).

Long-Term stability data for studies MBR/PBA07-210, MBR/PBA07-357, MBR/PBA11-219, MBR/PBA07-358MBR/PBA07-211, MBR/PBA07212, MBR/PBA07361, MBR/PBA07-362, MBR/PBA08-112, MBR/PBA09-246, MBR/PBA09-244, MBR/PBA07-354 and Mililipore/11-054B are notified to be incorporated by a study protocol amendment. However the Assessor was not able localize the respective documents. The applicant is therefore asked to specify the respective location.

The notification on GLP compliance for study Mililipore/11-054B could not be located. The applicant is therefore asked to specify the respective location.

Pharmacokinetic investigations are limited to absorption data obtained in NHP (Maccaca mulatta and Maccaca fascicularis) with the exception of toxicokinetic data, which are discussed in the toxicity part of the dossier.

The applicant presents twelve studies in in irradiated and non-irradiated NHP with a rather standardised study design. Briefly, the animals were dosed with various doses of entolimod with or without preliminary TBI and plasma/serum concentration was determined at appropriate time points. Time to maximal plasma/serum concentration (T_{max}), area under the concentration-time curve ($AUC_{0-\infty}$), apparent elimination half-life ($t_{1/2}$), apparent volume of distribution (Vd) and apparent plasma clearance (CL) were determined. These investigations are accompanied by antibody detection in several studies. In several studies the study groups were very small (1-3 animals/ sex) and the results are therefore rather incidental findings than reliable scientific data.

Overall, following i.m. administration in non-radiated and radiated NHP entolimod was rapidly absorbed. Systemic exposure (c_{max} and AUC increased) with increasing doses and was roughly dose-dependent, although some exception have been noticed in radiated monkeys. In radiated animals systemic exposure increased dose proportional with doses up to 10 mg/kg b.w. whereas the increase was somewhat more than dose proportional at higher doses. Of note, even though the same dosing and method of quantification was employed in the studies Rs-23 and Rs-001, the elimination pattern was found different between groups. While elimination in non irradiated NHPs was non linear the pattern of elimination in irradiated animals was essentially linear (i.e. independent of dose). Elimination half life was 0.5-2.0 h in most of the non-radiated and radiated NHP. There were no significant sex differences. The Vd was found to be highly variable with no observed dose dependence.

The applicant does not discuss the influence of antibodies on pharmacokinetic parameters in detail. Pre-existing antibodies against entolimod could be detected in several studies although the animals a generally described as (entolimod) naive. This is a rather unexpected finding in a non-human primate study. The applicant tries to explain this finding by a contact of the animals with flagellin of bacteria in the breeding holding. Considering the high standard of hygienic measures in a conventional laboratory animal breeding holding this explanation appears to be a little questionable although it is acknowledged that a contact with bacteria is not completely restricted. In two studies (CB5BD-P-001, CBL1.1-N-01-Rs-23) animals with pre-existing antibodies were excluded from the studies. The study design can be considered a stratified group sampling from the non-clinical point of view but in other studies the pre-existing antibody titer was not determined.

In study CBL1.1-N-04-Rs-05 the influence of neutralising antibodies on pharmacokinetics was investigated in a very small study population. However it remains unclear how the antibody group was achieved in detail taking into account the small number of animals included in the study.

However, the correlation of antibody titers and pharmacokinetic parameters of the studies CBL1.1_N_04-Rs 05, … RS-10 and Rs-11 that pre-existing entolimod antibodies most likely prolong the elimination of the active substance.

The lack of distribution studies has not been justified. The Assessors consider that such studies would be very informative for addressing the PD and toxicology of the product, since necessary data of important target tissues are lacking for the assessment of efficacy and toxicology of the product (i.e. SNC, kidney, liver, GI). **Consequently the applicant is requested to address this issue forwarding the necessary data for the evaluation or a robust discussion that fully justifies the lack of this data. No milk excretion of entolimod was studied by the applicant**.

The potential usefulness of entolimod is not restricted to the use in major radiation disasters as administration to patients affected in less serious radiation events cannot be discarded. In the latter situation the number of drugs available would not be limited and in any case, a significant number of patients would be administered best standard of care treatment in addition to entolimod administration. **Consequently, the applicants justification for not pursuing pharmacokinetic drug interactions is not sustained and an adequate assessment of this issue is requested with all medicinal products deemed to be administrated concomitantly in a excessive radiation exposure scenario.**

3.2.3. Toxicology

The applicant refers to eight single dose toxicity and two repeated dose toxicity studies. The pivotal toxicity studies (including toxicokinetic evaluation) are claimed to be GLP-compliant. However, for the toxicokinic part of the repeated dose toxicity study in monkeys (CBL-DD-07-T-P-1003) no GLP-compliance is claimed. The notified GLP deviations appear to be overall acceptable. However the applicant is asked to state whether the site(s) of conduct of study were subject to laboratory accreditation with reference to GLP compliance monitoring.

The applicant justifies the species selection for the evaluation of reproductive toxicity since these species are the most commonly used species for this evaluation. However, no binding affinities of rat and rabbit TLR5 ectodomains as well as entolimod potency in eliciting activation of TLR5 molecules of these species with entolimod has been provided and therefore, the relevance of these species for the reproductive toxicity is uncertain. **The applicant is asked to forward binding characteristics data of Entolimod to TLR5 in the animal species used *in vivo* studies for investigation of reproductive toxicity in order to confirm whether these species are relevant or not for the reproductive toxicity assessment**.

Single dose toxicity studies have been performed in mice, rats, rabbits and rhesus macaques. Briefly, mice received subcutaneous (s.c.) entolimod injections of various dosages up to 20,000 µg/kg bodyweight. Animals were observed for signs of toxicity over the study period of 14 days (Study CBL-DD-06-T-M-1002, CBL-DD-07-T-M-1005, CBL1.1-02-MS-01 and CBL1.4-02-Ms-02). Studies were terminated after an observation period of 14 days. In study CBL-DD-07-T-M-1005 clinical pathology and histopathology was performed at day 2 and day 14 in half of the group. Studies CBL1.1-02-MS-01 and CBL1.4-02-Ms-02 had an observation period of 7 days. In study CBL1.1-02-MS-01 TLR5 knock out mice were compared with wild-type mice.

In the single rat acute toxicity study (CBL1.1-N-02-Rt-01) the animals (1sex/ dose group) received vehicle control; 10, 30, 100, 300, 1,000, or 3,000 µg/kg subcutaneously and were subjected to a 14 days observation period.

In the single rabbit acute toxicity study CBL1.1-N-02-Rb-01 (1/sex/group) received a single subcutaneous injection of vehicle control, 5, 15, 50, 150, 500 or 1,500 µg/kg entolimod and were observed for 14 days.

Two studies (CBL-DD-06-T-P-1001 and CBL-DD-07-T-P-1004) have been performed in rhesus monkeys (maccaca mulatta). Entolimod was administered by intramuscular (gluteus maximus/ gluteal region) injection at various dosages up to 600 µg/kg bodyweight.and the animals were observed over a 14 days period. In the study CBL-DD-07-T-P-1004, the highest dose employed was 62.5 µg/kg, which is approximately half the dosing employed in the dose response study RS-23 in NHPs (120 µg/kg).

Although the recommended duration should have been one month in both species (*ICH M3 EMA/CPMP/ICH/286/1995),* repeated dose toxicity was investigated for two weeks in mice (CBL-DD-07-T-1006 and CBL1.4-02-MS-01) and rhesus monkeys (CBL-DD-07-T-P-1003). Overall the deviation appears to be acceptable for the current application.

The doses included in the pivotal studies were well below the dosing range assessed as well tolerated in the dose range finding studies. In mice dosages up to 20,000 µg/kg were well tolerated in the DRF Study CBL-DD-06-T-M-1002, however, the pivotal study (CBL-DD-06-T-M-1005) the highest dose used was 6, 000 µg/kg. In monkeys the DRF study (CBL-DD-06-T-P-1001) suggested doses up to 600 µg/kg were generally well tolerated, however, the pivotal study was designed to only test doses up to 100 µg/kg (CBL-DD-07-T-P-1004). Similarly, in study CBL-DD-TR-B502-07-011 study, an increase in preimplantation loss was observed with a dose of 230 µg/kg/day. While it was noted that this observation would need to be verified in the definitive study, the highest dose level evaluated in the GLP study (CBL-DD-TR-B502-10-001) was 100 µg/kg/day. Generally, in toxicity studies, effects that are potentially clinically relevant can be adequately characterized using doses up to the MTD and this information should be part of the safety assessment to support the conduct of human clinical trials. Therefore, the information of the pivotal studies is very limited and insufficient to assess toxicology and predict potential adverse effects in the human target population. Due to the fast pharmacokinetics of the product it cannot be ruled out that the peak in adverse events occur before the first measured time (24 hours). **The applicant is therefore requested to justify the basis for the low levels of dosing selected for pivotal studies in animals and the selected time points for assessment and the relevance of those for the toxicology assessment of the product.**

The second mice study CBL1.4-02-Ms-01 compares the toxicity of repeated entolimod doses in females of different mice strains (NIH Swiss mice vs. C57BL/6) and the maximal number of entolimod injections that is tolerable. 10 animals of each strain received either 5 µg/kg bodyweight or vehicle control by intraperitoneal injections. Animals were dosed on 5 days per week for 30 days. Animals were observed over the treatment period. Hematologic and histologic evaluations were performed at the end of the experiment. The applicant presents with the second mice study CBL1.4-02-Ms-01 a rather unusual design for a repeated dose toxicity study and the results can hardly be compared with the results of the other studies performed in mice. No toxicokinetic data was obtained, although a different route of application was used and the exposure of the animals cannot be estimated. The animals received rather low doses of entolimod compared to the other mice studies. Nevertheless the animals showed slight but notable differences in the response to entolimod administration. WBC numbers were decreased after single dose administration of. Surprisingly, the decrease was observed in all dosed groups. Since that the applicant stated that the decreased WBC observed is due to the pharmacologic action of the drug the applicant should justify why both WT and KO mice produce this effect, since the mechanism for the appearance of this adverse effect is not understood as KO animals lack of TLR-5 receptor. In the full study included in module 4 of the CTD it is noted that conclusions regarding drug toxicity in this study are problematic due to protocol deviations (cage side observations

were performed only for subset of study groups and pre-specified time points; gross pathology analyzed only in vehicle and ≥8 mg/kg CBLB502 dose groups), as well as by small group sizes (2 animals/sex/dose level/genotype/time point). **Therefore, the applicant is invited to discuss the relevance of the study for the toxicology assessment, as also pharmacology concerns regarding the mode of action are noted**.

The finding of a purulent secretion from the eyes in rats and rabbits including hyperemia and purulent exudate after single dosing of entolimod should be discussed bearing in mind that no binding affinities of rat and rabbit TLR5 ectodomains as well as entolimod potency in eliciting activation of TLR5 molecules of this species with entolimod has been provided. In addition, the applicant is requested to further discuss the clinical relevance of these findings.

In the study in monkeys CBL-DD-06-T-P-1001, increases in ALT, AST and LDH were noted in all groups including control at 24 hours post dosing. The lot of entolimod used was EC2. The applicant should comment about the health of the animals used and the presence of antibodies to entolimod.

A GLP study in mice (CBL-DD-07-T-M-1006) was conducted by Next Century Nanjing Laboratory (China), including both acute (single-dose) and 2-week daily repeated dose phases. In the acute phase, CBLB502 was administered subcutaneously at intended doses of 0, 200 or 2,000 µg/kg. In the 2-week repeat dosing phase, CBLB502 was administered subcutaneously at intended dosages of 0, 30, 100, 300 or 1,000 µg/kg. Formulation assays recalculation revealed the actual dosages administered were 60 and 590 µg/kg in the acute dosing phase and 60, 150, and 480 µg/kg/day at the highest dosage levels in the repeat dosing phase. Drug concentrations were below the limit of assay quantitation for the lowest dosage group. For the acute single dose phase, entolimod at doses up to 590 µg/kg (this dose was initially intended to be 2,000 µg/kg) produced decreases in body weight gain. In addition, lymphocytes were decreased in both sexes at 24 hours after treatment at both doses and remained decreased in both sexes in the high dose group on day 15. For the 2-week repeat dosing phase, no adverse effects were observed result expected taking into account the low doses tested. Besides, it is noted that between 30 and 80% of mice in each group administered entolimod for 2 weeks had antibodies to entolimod, most of them neutralizing antibodies.

In a study conducted by Next Century Nanjing Laboratory (China) (CBL-DD-07-T-P-1003), the plan included both acute (single-dose) and two week daily repeated dose phases in monkeys. In the acute phase, CBLB502 was administered subcutaneously at intended doses of 0, 60 or 600 µg/kg. In the 2-week repeat dosing phase, CBLB502 was administered subcutaneously at intended dosages of 0, 2, 20 and 200 µg/kg. Formulation assays revealed the actual dosages administered were 41 and 396 µg/kg µg/kg in the acute dosing phase and 1.5, 14 and 140 µg/kg/day at the highest dosage levels in the repeat dosing phase. For the acute single dose phase, the high dose of 396 µg/kg, initially intended to be 600 µg/kg, resulted in an adverse observation of emesis, decreased body weight gain, reduced food consumption, changes in several clinical pathology parameters and histopathology observations at the injection site. For the 2-week repeat dosing phase, decreased body weight gain, food consumption, changes in several clinical pathology parameters and histological findings were found. Reported adverse effects were reversible.

Although the repeat dose toxicity studies are claimed to be GLP compliant in many of the studies the actual dose administered differs dramatically from the intended doses. For example in Study CBL-DD-07-T-M-1005 the intended doses were 0, 20, 60, 200, 600, 2,000 and 6,000 µg/kg. However dosing formulation assays carried post dosing revealed actual doses were 67, 303, 1,220 and 4,710 µg/kg for the four highest dose groups with the lower dose groups not quantifiable. A similar issue arises with studies CBL-DD-07-T-P-1004, CBL-DD-07-T-M-1006 and CBL-DD-TR-B502-09-002. Indeed, in several of these studies significant levels of antibodies to Entolimod were observed, many of them neutralising

and therefore further diminishing the exposure levels. **The applicant is asked explain these significant deviations from the intended doses, discuss the effects of ADAs on Entolimod exposure levels and to thoroughly justify these findings.**

There were no ophthalmologic findings in monkeys based on examination in all monkey studies. Electrocardiography did not reveal any abnormalities in monkeys when assessed at 2-4 hours after treatment in single dose studies or at the end of the 14 day treatment in the repeat dose study. In some but not all studies a modest decreases in red cell parameters was noticed. Effects on white blood counts were noted in single dose studies in both mice and monkeys but were generally less apparent in repeat dose studies. While the effects are not consistent across studies and not always attributed to treatment in the reports, the data collectively indicates a drug-related increase in neutrophils sometimes associated with a decrease in lymphocytes. The alterations in white blood cell counts are attributed to the pharmacologic action of the drug.

Overall, the applicant´s conclusion that the observed effects are consistent with cytokine release and that there is no evidence of a specific target organ toxicity is agreed to.

Potentially serious adverse events reported in humans such as QT interval prolongation, tachycardia and hypotension among others were not evaluated in non-clinical toxicology studies. Although the low doses administered and the time points selected would not have allowed a proper assessment, the applicant is requested to justify the lack of such studies.

In some studies (CB1.1-N-01-RS-10 and CBL1.1-N01-RS-11 and toxicity studies CBL-DD-07-T-M1006, CBL-DD-07-T-P-1003) the plasma and serum samples of the control groups were detected to be slightly positive for Entolimod. This unexpected result should be further explained by the applicant with a discussion on the effects on the toxicokinetic profile concluded in those studies. In the study CBL-DD-07-T-P-1003 all 24 animals displayed ADAs following entolimod administration. **The discussion regarding the ADA presence in those animals with the toxicokinetics is deemed insufficient as it does not allow to draw conclusions. The applicant is requested to discuss in depth these issues.**

As stated in ICH S6 It is not expected that these substances would interact directly with DNA or other chromosomal material. The absence of genotoxicity studies is acceptable.

Entolimod is a protein intended for single use. The absence of carcinogenicity studies is acceptable.

No studies were conducted concerning placenta transfer and distribution into milk.

In the CBL-DD-TR-B502-07-011 study an increase in preimplantation loss was observed with a dose of 230 µg/kg/day in wistar rats. While it was noted that this observation would need to be verified in the definitive study, the highest dose level evaluated in the GLP study was 100 µg/kg/day. The absence of toxicologically relevant responses of the test animals underlines the lack of value of this study as it does not allow to assess and confirm the preimplantation loss reported in the DRF study. **The applicant is requested to justify the choice of these doses.**

In another embryofoetal development study, animals in the highest dose (300 µg/kg/day) group, slightly higher postimplantation loss and late resorptions, and a reduction in live fetuses were noted in the high dose group relative to controls in wistar rats (CBL-DD-TR-B502-07-012). **The applicant noted that this observation would need to be verified in the definitive study.**

At 300 µg/kg/day, a relatively higher incidence of incomplete ossifications of parietal, squamosal, and supraoccipital bones was observed which was not statistically significant. The report stated that the lab had insufficient historical control data for the Wistar rat in this GLP study (CBL-DD-TR-B502-09-002).

The lack of historical control data is not understood for this species for which more than sufficient historical data should be available. **The applicant is requested to thoroughly justify this finding.**

Embryo foetal development was also assessed in rabbits. The applicant has carried out 3 studies in this species. The first two were preliminary studied and these where followed by the definite GLP Embryo-Fetal Development study. The highest dose selected for the latter study in the preliminary studies was 150 µg/kg/day. There was a change in dosing in the low group from 15 to 5 µg/kg/day (recalculated doses were 1.39 to 4.36 µg/kg/day), issue that makes difficult the assessment of the relevance of the findings and relation to dose. Of note this dose was selected as NOAEL. Furthermore, there was not relevant data regarding ADA formation. **Consequently, the applicant is asked to discuss the impact of the dosing change in the NOAEL with further assessments on ADA formation.**

Visceral malformations were reported in this species and effect of treatment on these malformations could not be excluded. Mortality was reported in maternal animals, which was attributed to GI disturbances although no proof was forwarded to back up this claim and currently the cause of deaths is unknown.

The rationale exposed by the applicant for not pursuing pre and post natal development studies is endorsed independently of comments that the applicant may have received from other Agencies.

Pivotal single and repeat dose toxicity studies have included juvenile animals equivalent in age to human children and adolescents, therefore further juvenile studies was not considered necessary, nonetheless there is an overarching MO regarding the whole toxicity development carried out.

The ages of the animals included cover the development age from 8 years to adulthood. Since no increased toxicology or differential pharmacokinetics are expected in the paediatric population potentially treated with the product, the human developmental age covered with actual studies includes children from 2 years to adulthood. It should be noted that the ontogeny of TLR5 in younger children is largely unknown therefore a differential PD to entolimod treatment cannot be ruled out.

Although in principle antigenicity is not considered a major issue when only single dose administration is envisaged in humans, in this scenario it cannot be ruled out the presence of preexisting antibodies, neutralizing or not reactive to entolimod. In addition, the presence in the formulation of histidine, a known antigenic product is, has not been discussed or measured. **Consequently the potential antigenic effects of entolimod formulation need further discussion, not only from a safety point of view but also from an efficacy perspective.**

No specific studies of dependence and metabolites are considered relevant for the assessment.

The manufacturing process has changed considerably during the product development. It should be noted that the adverse finding reported in the provided studies are inconsistent. The comparability among lots employed in clinical trials, efficacy and toxicology studies is lacking. This should be appropriately justified by the applicant in order to confirm that the assessed product is comparable among studies to evaluate the relevance of the safety data provided, taking also into account that toxicology data with the product to be marketed is absent.

With regards to the non clinical part of the safety specification in the RMP, since the non-clinical pivotal toxicology studies were not fully predictive of the adverse events reported in humans and have presented serious drawbacks and a mayor objection has been raised to the applicant for clarification. **In consequence, the applicant should forward an adequate Part II: Module SII of the RMP taking into account the above mentioned concerns**.

3.2.4. Ecotoxicity/environmental risk assessment

Entolimod is a protein, as such no ecotoxicity/environmental risk is expected. Specific studies to address the potential ecotoxicity/ERA assessment of entolimod are considered not relevant.

3.2.5. Discussion on non-clinical aspects

Regarding pharmacology, there are fundamental deficits in available data related to the purported mechanism of action of Entolimod. Among these are the following ones:

Evidence to substantiate the role of NFkB induced gene transcription and/or other signalling pathway activation in product efficacy.

Until present the specificity of entolimod for binding and activation of the TLR5 as well as affinity to other receptors has not been demonstrated. In addition, information on the cells and tissues with respond to Entolimod administration is lacking

The choice of the three PD biomarkers (IL-6, G-CSF and ANC24) and their relationship with the overall survival.

Adecuate data on safety pharmacology, secondary pharmacodynamics and potential pharmacodynamic drug interactions.

Comparative data to best supportive care or other treatments.

Details relating to the GLP status of 2 important non-clinical studies should be provided.

Pharmacokinetic data is limited to absorption data in NHP and to toxicokinetics. Basically exposure to and elimination of entolimod was shown. Several specific concerns have been raised regarding non-clinical pharmacokinetics.

The toxicity studies provided by the applicant failed to adequately characterize the toxicological profile of the test compound and to identify the potential target organs of toxicity.

The doses included in the pivotal studies were well below the dosing range assessed in dose range finding studies. DRF data did not report limiting toxicity on the highest doses provided with a manageable toxicity profile.

In addition the toxicology studies did not take into account appropriate time points to observe the potential toxicity. Furthermore, the reliability of the data is questioned as often serious shortcomings were reported.

Although the repeat dose toxicity studies are claimed to be GLP compliant in many of the studies the actual dose administered differs dramatically from the intended doses. Indeed, in several of the studies significant levels of antibodies to Entolimod were observed, many of them neutralising and therefore further diminishing the exposure levels.

Potentially serious adverse events reported in humans such as QT interval prolongation, tachycardia and hypotension among others were not evaluated in non-clinical toxicology studies. In any case, if those would have been included the low doses administered and the time points selected would not allow to reach any conclusion.

The comparability among lots employed in clinical trials, efficacy and toxicology studies is lacking. This should be appropriately justified by the applicant in order to confirm that the assessed product is comparable among studies to evaluate the relevance of the safety data provided, taking also into account that toxicology data with the product to be marketed is absent.

If no satisfactory justification can be put forward, unfortunately the need for additional non-clinical data cannot be ruled out, since due to the nature of the application, the safety of human administration relies heavily of data produced non-clinical studies and the current available data is not considered relevant for an adequate toxicology assessment.

3.2.6. Conclusion on non-clinical aspects

The product is considered as non approvable since a major objection and other concerns to the approval have been identified.

3.3. Clinical aspects

Entolimod is a ligand for toll-like receptor 5 (TLR5). The company claims that, after binding to TLR5, entolimod activates nuclear factor kappa-B (NF-κB), a transcriptional enhancer of multiple genes encoding antioxidants, antiapoptotic proteins, antimicrobial peptides, and cytokines. In animal models of irradiation and sepsis, drug-mediated production of cytokines (in particular, granulocyte colony-stimulating factor [G-CSF] and interleukin [IL]-6) and enhancement of neutrophil mobilization and tissue infiltration promote multiorgan tissue protection and regeneration.

Based on this mechanism of action, entolimod is being developed as a medical radiation countermeasure (MRC) for the following specific indication: *"Entolimod is indicated to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster." It is intended that the use of entolimod be directed towards children or adults who have received potentially lethal irradiation, defined as ≥2 Gy of whole-body radiation exposure based on radiation exposure history, proximity to the radiation source, symptoms or physical findings of radiation exposure, blood lymphocyte counts (if available), and/or dosimetry (if available)."*

A single administration is needed, and therefore the company attempts to demonstrate the safety and efficacy of a single use. The recommended human dose in paediatric population aged 2-18 years, adults and elderly is based on patient body weight, as shown in Table 1. For patients with body weights ≥114 kg, a maximum dose of 50 µg should be used.

Table 1: Recommended Dose by Patient Body Weight

Body Weight Range, kg	***Entolimod Dose, µg***	***Entolimod Dose, µL***
1.75-3	*1*	*10*
4-5	*2*	*20*
6-8	*3*	*30*
9-13	*5*	*50*
14-20	*8*	*80*
21-30	*12*	*120*
31-33	*13*	*130*
34-50	*20*	*200*
51-75	*30*	*300*
76-113	*45*	*450*

≥114	*50*	*500*

Because humans cannot be lethally irradiated to test the activity and safety of entolimod for the proposed indication, clinical efficacy studies of the drug are not ethical or feasible. For this reason, development of entolimod as a MRC is based on efficacy, pharmacodynamic (PD), safety, and pharmacokinetic (PK) data obtained in lethally irradiated nonhuman primates (NHP) as well as PD, safety, and PK data obtained in non-irradiated NHP and adult humans. Thus, no human efficacy data is available for this application.

Five clinical studies were performed with entolimod (see "Tabular overview of clinical studies"). Two GCP clinical studies involving the participation of 150 healthy subjects were performed (studies HU-7014 and HU-9001) and 3 studies in oncological patients (studies I196111-US, I196111-Russia and BL612-CBLB502). They aimed to assess safety of entolimod, its effect on biomarkers for dose conversion and to characterise its PK.

Two randomized, blinded, placebo-controlled, parallel-group, dose-ranging GLP studies of entolimod have been performed in rhesus macaques acting as surrogates for human subjects: Rs-001 (healthy primates) and Rs-23 (irradiated primates). The aim of these studies was the assessment in animal models of the entolimod effects on PK and PD (to obtain the human dose by dose conversion calculations) and on survival (efficacy). Results of efficacy from Rs-23 study in non-human primates (rhesus macaques) are claimed as pivotal for the efficacy in this application under exceptional circumstances as trials in the human population in this setting are neither ethical nor feasible.

- ***Tabular overview of clinical studies***

Study	Primary Purpose	Population	Total Subjects
HU-7014	Clinical safety[a] Biomarkers (ANC, G-CSF, IL-6) for dose conversion	Healthy humans	50
HU-9014	Clinical safety[a] Biomarkers (ANC, G-CSF, IL-6) for dose conversion	Healthy humans	100
I196111-US	Clinical safety[b]	Patients with advanced cancers	26
196111-Russia	Clinical safety[b]	Patients with advanced cancers	7
BL612-CBLB502	Clinical safety[b]	Patients with colorectal cancer	30[c]
		Total receiving entolimod:	**213**

ANC=absolute neutrophil count, G-CSF=granulocyte colony-stimulating factor, IL-6=interleukin-6, MAA=marking authorization application, SAE=serious adverse event

a. Full clinical study reports are available in Module 5.3.31 – HU-7014 and HU-9001.
b. Synoptic clinical study reports are available in Module 2.7.5 – I196111-US, I196111-Russia, BL612-CBLB502
c. Of 40 subjects enrolled, 30 received entolimod and 10 received placebo.

Safety in humans (healthy volunteers)

Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report	Study period

Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report	Study period
CBL-DD-CH-B502-07-014 **(HU-7014)** **Study report:** **26.10.2010**	Primary: Determine safety/ tolerance, and characterize PK of single IM doses of entolimod. Secondary: Evaluate PD of single IM doses.	Single center, open- label, non-randomized, single- dose, dose escalation study in healthy subjects with wild- type TLR5	Entolimod Single dose at 2, 6, 12, 24, 30, 35, 40, or 50 µg IM	50 subjects (32M/18F)	**Healthy subjects**	Single dose	Complete Complete	
CBL-DD-CH-B502-09-001A **(HU-9001)** **Study report:** **04.02.2011**	Primary: Compare PD biomarker levels of 4 entolimod dosing regimens including a regimen with IBP pretreatment, Secondary: Compare safety/tolerability of 4 dosing regimens; compare PK.	Two-center, open-label, randomized, parallel group in healthy subjects	Entolimod Single dose at 25 µg, 35 µg ± IBP, 400 mg pretreatment or Two doses at 30 µg 72 hours apart IM	100 subjects (91M/9F)	**Healthy subjects**	Single dose or two doses 72 hrs apart	Complete Complete	

Study	Primary Purpose	Population	Total Subjects
Rs-23	Clinical efficacy (survival, haematological parameters) Biomarkers (ANC, G-CSF, IL-6) for dose conversion	Irradiated NHP	179
Rs-001	Biomarkers (ANC, G-CSF, IL-6) for dose conversion	Nonirradiated NHP	160

ANC=absolute neutrophil count, G-CSF=granulocyte colony-stimulating factor, IL-6=interleukin-6, NHP=nonhuman primate(s)

3.3.1. Pharmacokinetics

Entolimod is available in a liquid formulation to be administered to humans intramuscularly (IM) as a single administration post-irradiation.

The Drug Product (DP) used in nonclinical studies and in prior clinical trials in healthy subjects and patients was changed in the formulations used in Study I196111-Russia and Study BL612-CBLB502, as well as in the formulations proposed for the MAA authorization. The comparability of the formulation used in the previous pivotal animal and human studies (DP Lot) versus those proposed to be stockpiled in the EU (DP Lots) has only been demonstrated analytically.

A clinical (or possibly non-clinical in the primary efficacy species non-human primates) biocomparability study, with final objectives (PK, PD, immunocomparability) still to be discussed, is considered necessary to establish in vivo biocomparability between the to-be-marketed formulation of entolimod proposed for use as a radiation countermeasure and the formulation used in the earlier pivotal preclinical and clinical studies and to allow extrapolation of the non-clinical efficacy findings and assessment of the safety findings in man.

The clinical pharmacokinetic (PK) profile of Entolimod following IM administration has been evaluated in altogether 150 healthy human subjects in one Phase 1 and one Phase 2 study (HU-7014 and HU-9001, respectively). In addition, PK has been evaluated in the primary efficacy species, Nonhuman Primates (NHP), i.a. in one study in 160 nonirradiated rhesus macaques NHP (Rs-001) and in one study in 179 irradiated rhesus macaques NHP (Rs-023).

There were no Bioavailability (BA) Study Reports, Comparative BA and Bioequivalence (BE) Study Reports, Study Reports dedicated to metabolism and elimination, Patient PK and Initial Tolerability Study Reports, Intrinsic Factor PK Study Reports, Extrinsic Factor PK Study Reports, or Population PK Study Reports.

The method for the quantification of entolimod in human serum and for the detection of anti-CBLB502 antibodies in human serum are based on enzyme linked immunosorbent assay (ELISA) procedures. However, method validation is not fully in accordance with the requirements of the Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**). This pertains mainly to the acceptance criteria applied, concentration of QC samples, as well as missing long-term stability data. Moreover, the sensitivity of the antibody screening assay (718 ng/mL) is also considered insufficient. Bioanalytical reports were provided for study HU-7014, but bioanalysis of study samples in study HU-9001 is missing. In HU-7014 (First-in Man), it was noted that the analytical procedure had been amended several times, with even wider acceptance criteria in place and finally using a different matrix for calibration standards except the top standard. Furthermore, based on an amendment to the bioanalytical report, effective only about 3 months after completion of study sample analysis, Incurred Sample Reanalysis was not performed upon request of the sponsor.

In addition, even with the given low sensitivity, pre-existing antibodies cross-reactive to Entolimod were detected in already 12.7% of the overall study population in HU-7014 and HU-9001. The presence of anti-Entolimod reactive antibodies may induce bias in quantification of Entolimod using ELISA based procedures PBA-09.225, -.1, .-2. In conclusion, validity of serum concentration data and thus PK parameters in both human studies HU-7014 and HU-9001 may have to be questioned.

Moreover, some results or specifications regarding analytical validation need further justification. In MBR/PBA09-225 study not all validation results met with the acceptance criteria (Freeze-thaw stability, 6 cycles, High Control HC: 3.75 ng/mL and Interassay precision DC (50 ng/mL)). In MBR/PBA08-148 study, Acceptance Criteria is higher than the previous study (≤30% CV). In MBR/PBA09-103 Cell-Based Assay for the Detection of Entolimod-Reactive Neutralizing Antibodies in Human Serum (table 9, module 2.7.1) the Acceptance Criteria need further clarifications and justification.

General methods have been applied for PK parameter calculation and summary statistics. Whereas in the non-clinical studies in NHP Rs-001 and Rs-023, actual sampling times were used for PK calculations, in HU-7014 and HU-9001, nominal times were used, allowing pre-specified deviations in HU-7014. Not only for better comparability, use of actual sampling times is recommended. For dose normalised analyses of dose proportionality provided only in the clinical summary, no reports were provided, hence details on subject inclusion, etc. are unknown.

Together the HU-7014 and HU-9001 PK results demonstrated that following a drug dose of 2 µg, entolimod serum concentrations in human only hardly reached the assay limit (LLOQ 0.01 ng/mL). Following drug doses ranging from 6 to 50 µg, entolimod appeared in serum at 0.25 to 4 hours postdose. Mean peak concentrations ranging in both studies in the subnanogram/mL range were achieved between 2 and 6 hours postdose. Entolimod concentrations declined from the peak value with a median elimination half-life of 2 to 7 hours (mean 2.9 to 15.9 hours) and reached values below the LLOQ at 24 hours in the majority of subjects.

In both studies, Cmax and AUC values tended to increase with increasing dose. The between-subject variability in Cmax was large, with coefficients of variation (CV) of up to 91% for mean Cmax in the dose groups following the Day 1 dose and as high as 161% for AUC.

Mean terminal elimination half-life (T½) values showed no dependence on dose. Evaluable population may have been biased since the terminal elimination rate could only be determined in 24/50 subjects in HU-7014, and 80/100 in HU-9001 following day 1, generally because the concentrations dropped below the assay LLOQ too fast to obtain the minimum required set of 3 quantifiable concentrations in the declining phase with the sampling schedule in place.

Mean apparent clearance (CL/F) in HU-7014 ranged from 19 L/hour to 48 L/h. This was regarded as an apparent clearance because it was assumed that the bioavailability of entolimod was <100% from the IM injection site.

There were no distribution studies conducted in humans. Data about distribution have been calculated measuring entolimod concentration but none model has been proposed to explain the pharmacokinetic profile in humans.

Mean apparent volume of distribution (Vz/F) in HU-7014 ranged from 151 L to 384 L. These values are substantially greater than body water or body volume and are consistent with less than complete bioavailability from the IM injection site and/or substantial tissue distribution for entolimod.

The bioavailability of entolimod in humans following single dose IM injection was not studied directly due to the lack of a formulation suitable for IV injection. The bioavailability in healthy subjects has only been estimated by the applicant to be in the range of 1% to 5%, similar to the value estimated in non-irradiated NHP, by relating the actually observed mean Cmax to Cmax under the assumptions that the dose had been 100% bioavailable (assuming a nominal serum volume of 3 L) and that the Vz of ~35 kD entolimod will be mostly restricted to the serum of the systemic circulation. This assumption-based approach is stated to provide a minimum estimate for the bioavailability; if entolimod distributed or the observed Cmax was lower because of lag time, then the bioavailability would be greater than the estimate obtained above.

Since it is neither ethical nor feasible, PK studies in the human target population have not been performed. Effect on irradiation on entolimod exposure is available only from irradiated NHP in study Rs-023 in comparison to non-irradiated NHP in study Rs-001.

In irradiated NHP, the patterns of the serum concentration-time profiles were generally similar to those in non-irradiated NHP, with mean Tmax values of 0.80 to 1.38 hours and elimination phase values half-lives of 1.13 to 3.15 hours. However, values of the exposure parameters of Cmax and AUC after the same µg/kg dose were only somewhat more than half in the irradiated NHP, presumably due to radiation-related compromise of vascular and cellular permeability that promoted the movement of drug molecules from the systemic circulation into tissues.

Non-irradiated NHP showed increases in exposure (Cmax, AUC0-t and AUC0-∞) that were more than dose proportional when the lowest dose and exposure were taken as reference. A subsequent analysis of the dose-exposure relationship using dose-normalized Cmax and AUC over the entire 400-fold dosing range studied indicated however, that both the peak exposure (Cmax) and the total exposures (AUC) were consistent with dose proportionality. Still, the magnitude of the differences in mean terminal elimination t½ and CL/F values were within 3-fold across the CBLB502 dose range, suggesting that the elimination kinetics are nonlinear (i.e., dose dependent).

In irradiated NHP, increases in exposure were slightly greater than dose proportional with increasing entolimod dose when administered by IM injection. The impact of the nonlinearity was minimal within

the range of anticipated interest, ranging from 1% to 15% greater increases in exposure than predicted, assuming linear PK.

Exposures to entolimod were similar in male and female non-irradiated and irradiated NHP.

The influence of intrinsic factors on PK has not been directly investigated in humans.

No information regarding PK in renal impairment, hepatic impairment, elderly and children etc. is available. Increases in serum ALT and AST following recommended entolimod doses may be indicative that patients with pre-existing hepatic disease could be at increased risk of liver injury. Considering the complete lack of knowledge regarding Entolimod's metabolism, elimination pathways and potential for interactions, further data in these areas is considered a prerequisite to be able to assess dosing recommendations for special populations from a PK perspective.

The presence of preexisting antibodies in 19/150 subjects in studies HU-7014 and HU-9001 (likely to be underestimated) diminished PK parameters of Entolimod exposure. However, as detailed above, the analyses may have been confounded by bias in the bioanalysis, respectively insufficient sensitivity.

An assessment of a possible effect of the TLR5 genotype ($TLR5^{392stop}$ vs. wildtype) on CBLB502 pharmacokinetics was not conducted due to insufficient numbers of subjects homo- or heterozygous for $TLR5^{392stop}$) in the two studies. Therefore, the consequences on pharmacodinamic responses/efficacy in subjects with 392-stop codon polymorphism should be discussed by the applicant.

No specific studies evaluating the effect of intrinsic factors gender, weight, BMI, race, ethnicity and age on entolimod PK were performed. Cross-study analyses based on data from both studies HU-7014 and HU-9001 indicated that in humans, subject sex, age, ethnicity, and race did not have any apparent influence on entolimod exposures. However, females were underrepresented in the two trials (18/50 in HU-7014 and 9/100 in HU-9001), in HU-7014 only Hispanics were included, and the evaluated age range was limited to 18-(45)55 years. Exposure showed an inverse relationship with body size (whether expressed as weight or as BMI). However, details of the analyses are unclear and it is unknown whether subjects with pre-existing antibodies were excluded from the dose normalised analyses for the bias that group would introduce. Furthermore, considering the low number of subjects in various subgroups and between subject variability as high as 191% observed in PK parameters in both studies, the in part little informative cut-points (e.g. age- in the absence of subjects > 65 years), the analyses cannot be considered reliable. In addition, PK data is expected to be confounded by analytical issues as detailed above. From the PK perspective it is hence concluded that the effect of intrinsic factors on the PK of entolimod has not been sufficiently addressed.

Pretreatment with IBP in subgroup D of studyHU-9001 led to a reduction in CBLB502 exposure as evidenced from ~15 – 35% lower mean Cmax and AUC parameters. However, due to the huge inter-subject variability observed in the study, no conclusion could be drawn.

The absence of proprietary studies investigating DDI represents a gap in the data package according to the current EU guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 July 2012). However, the requirements for in vivo drug-drug interaction studies, with respect to e.g. cytochrome P450 (CYP) enzymes, are generally lower for therapeutic proteins than for conventional products (CHMP/EWP/89249/2004). According to the applicant, the risk of drug interactions is low because use is planned only as single-dose administration and the drug is rapidly eliminated. This is acknowledged; however, Entolimod may affect e.g. CYP enzyme activity via change in transcription and subsequently alter the systemic exposure of concomitant drugs that are metabolized by the affected CYP enzymes. An example of sustained effects on PK of various CYP substrates after single dose administration of a cytokine modulator observed in a DDI study using a cocktail approach has

been published (J Clin Pharmacol. 2015 Dec;55(12)). Furthermore, examples from literature show that CYPs can directly bind other proteins that also affect CYP function (Chem Res Toxicol. 2014 Sep 15; 27(9): 1474–1486). Moreover, several other agents are known to reduce incorporation of radioisotypes and may interact not only with respect to PK but also with respect to PD. Additionally, the impact of concomitantly administered Ibuprofen is insufficiently evaluated in a small not representative subpopulation of 17 volunteers. This data base is not sufficient to recommend Ibuprofen as a matter of routine. The need for any drug drug interaction studies should be thoroughly discussed by the applicant and absence further justified or data should be provided.

In conclusion, characterization of PK of entolimod in human subjects is incomplete and due to clinical and methodological reasons not sufficient to justify the currently proposed posology. Due to a missing adequate validation and insensitivity of methods which additionally have been changed different times, the reliability of PK data as currently described can be significantly challenged. There are no dedicated studies on bioavailability, distribution, elimination, metabolism, potential for drug interactions, and the influence of extrinsic and intrinsic factors on PK of entolimod. Distribution has also not been specifically studied to explain the systemic bioavailability from the intramuscular injection site. Therefore, the pharmacokinetic profile is not correctly characterized.

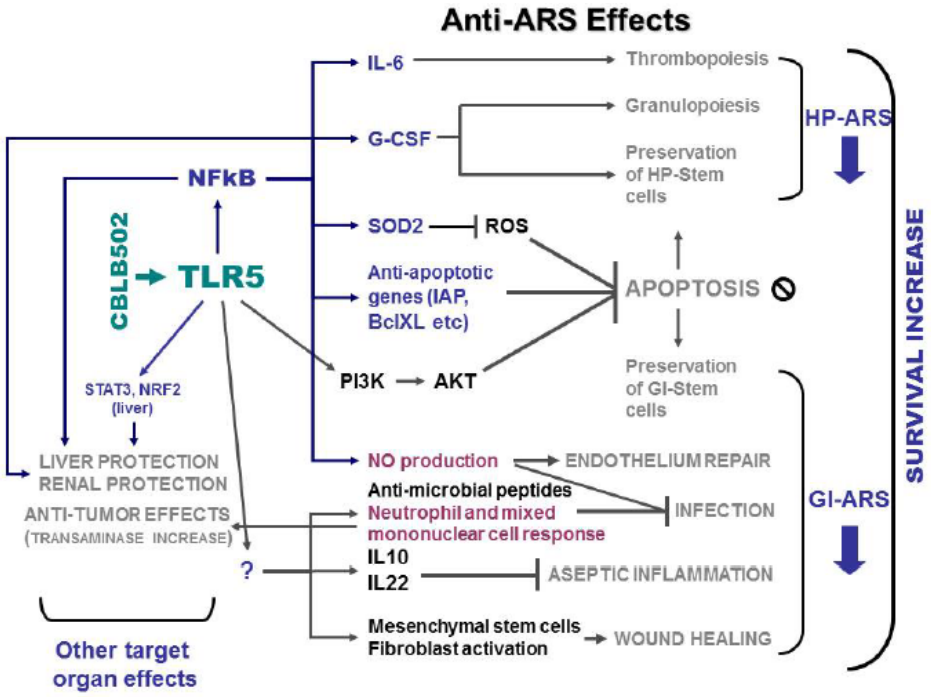
Additionally, the to-be-marketed formulation is not sufficiently defined and was not finally developed. Differences from the formulation used in the clinical and pivotal pre-clinical trials have to be taken in into account and indicate the clear necessity for a biocomparability study. Issues pertaining to the validity of the analytical methods relevant to human PK have been identified, questioning the reliability of the human PK data in general, which may be additionally confounded by the presence of pre-existing anti-CBLB502 antibodies in a subset of human subjects. Because humans are substantially more sensitive to the effects of entolimod than are NHP, animal-to-human dose conversion using only parameters of drug exposure was not considered possible, instead, dose conversion 'for efficacy' was to be made 'predominantly' on the basis of pharmacodynamics parameters.

3.3.2. Pharmacodynamics

Mechanism of action

Entolimod (CBLB502) is a recombinant biologic drug derived from the Salmonella protein, FliC flagellin, and is a ligand for toll-like receptor 5 (TLR5). Entolimod binding to TLR5 activates nuclear factor kappa-B (NF-κB), induces production of cytokines (in particular, granulocyte colony-stimulating factor [G-CSF] and interleukin [IL]-6), and claims to promote multiorgan tissue protection and regeneration.

Figure 1 Proposed Molecular Mechanisms of Entolimod Activity Mediated via Triggering of TLR5 according the applicant's information



The scheme summarizes data from the following literature references: (5); (6); (7); (8); (9); (10); (11); (12); (13); (14); (15); (16); (17); (18); (19); (20); (21); (22); (23); (24); (25); (26); (27); (28); (29); (30); (31); (32); (33); (34); (35); (36);(37); (38); (39); (40); (41), (42), (43); (44) ; and CBLI studies: CBL1.1-N-04-Rs-01/02, CBL1.1-N-04-Rs-03, CBL1.1-N-01-Rs-04, CBL1.1-N-01-Rs-08, CBL1.1-N-01-Rs-14, CBL1.1-N-01-Rs-09, CBL1.1-N-01-Rs-10, CBL1.1-N-01-Rs-23, CBL-DD-CH-B502-07-014, CBL-DD-CH-B502-09-001.

The claimed beneficial effects of entolimod in ARS are presumed to be triggered by the following mechanisms:

1.) NFkB activation leads to conservation of blood organ function with respect to thrombocyto-poiesis, granulocytopoiesis, thereby reducing risk for bleeding and infection (reducing HP-ARS).

2.) Anti-apoptotic gene activation may be associated with preservation of heaematopoietic and gastrointestinal-stem cells.

3.) Activation of mesenchymal stem cells and fibroblasts may induce increased wound healing.

4.) Infection barrier may be conserved by aseptic inflammation and increased (?) availability of anti-microbial peptides.

How these different mechanisms contribute to the observed prolonged survival in the small group of NHPs after potentially lethal radiation remains open at present. From the data presented the applicant has selected three biomarkers (IL-6, G-CSF and ANC) to define primary and secondary pharmacology and to perform dose conversion for the observed survival benefit from NHPs into in the human population.

Primary pharmacology

For demonstration of the proposed mode of action as well as for evaluation of primary pharmacology the applicant argues that mean plasma G-CSF and IL-6 concentrations in NHP and human subjects showed a strong dose-dependent increase with the peak for plasma G-CSF at 4 to 8 hours and for IL-6 at 2 to 4 hours. This cytokine concentrations normalized by ~24 hours for lower dose levels or after 24 hours for higher dose levels of entolimod. According to the known mode of action of G-CSF an increase of this marker may explain the correlated increase (~3- to 5-fold) in mean ANC following entolimod administration. Moreover, it is noted that the changes observed were more pronounced in non-

irradiated NHP compared to humans and irradiated NHP. The time course of the changes may indicate similarity across all 3 groups, but effects are documented only for the first 24 h.

Additionally, increases in IL-8 and IL-10 were also documented in irradiated and non-irradiated NHP and in humans, while levels of tumour necrosis factor-alpha (TNFα) were described to be elevated only humans; it is not clear whether they were also investigated in NHPs. No further interpretation about the impact of IL-8, IL-10 and TNFα for the pharmacodynamics of entolimod was provided.

Levels for the other cytokines – specifically, IL-1α, IL-1β, IL-2, IL-12, IFNα, and IFNγ – were not elevated by entolimod; it is not explicitly stated in which species these measures were performed and results were not shown.

Taking this surrogate marker as evidence for entolimod's PD and primary (as well as secondary) pharmacology, as suggested by the applicant, several important issues remain open.

In general, a dose-depending increase of G-CSF and IL-6 followed by an increase in mean ANC during 24 h after activation of TLR-5 due to administration of entolimod is plausible and can be expected from the mode of action. However, it is deemed hard in general to translate the short-time effects observed regarding the surrogate marker into a convincing concept explaining a better survival rate described in irradiated NHPs and humans.

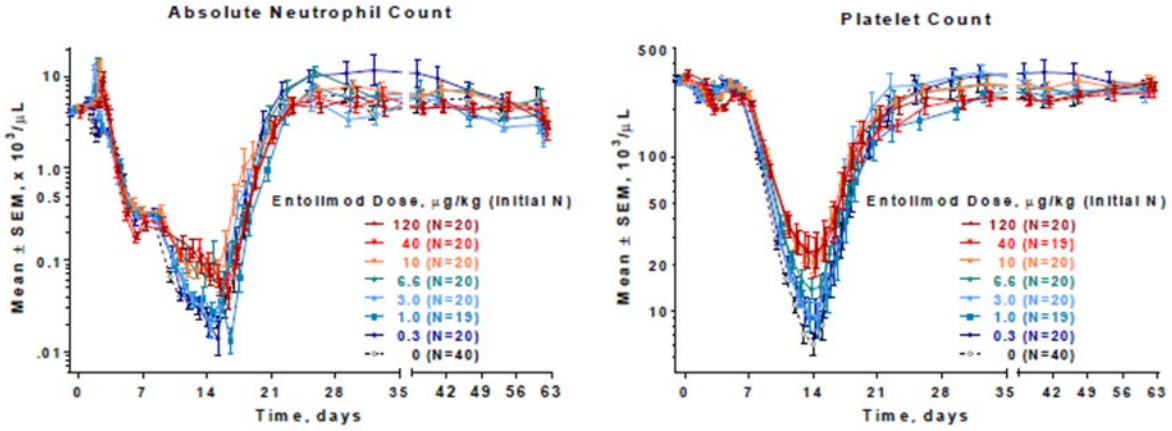
G-CSF is known to regulate the production and release of functional neutrophils from the bone marrow and causes marked increases in peripheral blood neutrophil counts within twenty-four hours, but a protective effect on the HP stem cells is not known. Moreover, G-CSF has no effects on thrombocytopoiesis; in contrast, temporarily thrombocytopenia is often observed when G-CSF is administered therapeutically. G-CSF when given early after exposure to radiation may improve white blood cell counts.

As the serum elimination half-life of G-CSF, e.g. filgrastim, is reported with approximately 3.5 hours, and the clearance rate is approximately 0.6 ml/min/kg, G-CSF administration needs to be daily repeated until full recovery of neutrophils count is measured. Therapeutic benefit for G-CSF in the reduction of duration of neutropenia and the incidence of febrile neutropenia in patients following cytotoxic chemotherapy for malignancy is only demonstrated if G-CSF levels remain increased stable until the expected neutrophil nadir is passed and the neutrophil count has recovered to the normal range. Following termination of filgrastim therapy, circulating neutrophil counts decrease by 50% within 1 to 2 days, and to normal levels within 1 to 7 days. Similarly data available from a publication by Hankey et al. 2015[*] indicate that filgrastim (even pegylated filgrastim) need to be administered over longer times to increase the survival in a comparable sized group of lethally irradiated NHPs. From this data pegylated filgrastim is approved and stockpiled for use in radiation incidents in the US only.

Analysing the details reported from irradiated NHPs in the pivotal trial Rs-23 as shown in the following figure 2 for ANC and platelet count, the initial changes with respect to mean ANCs and platelet counts until day 3 after entolimod administration are probably caused by the applied product.

[*] Hankey KG1, Farese AM1, Blaauw EC2, Gibbs AM1, Smith CP1, Katz BP3, Tong Y3, Prado KL1, MacVittie TJ1. Pegfilgrastim Improves Survival of Lethally Irradiated Nonhuman Primates. Radiat Res. 2015 Jun;183(6):643-55.

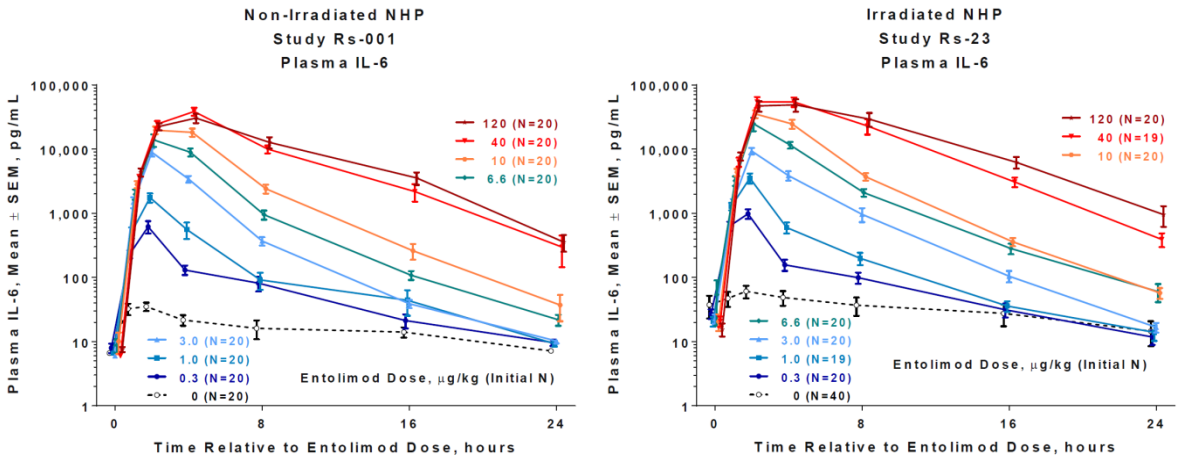
Figure 2 Changes of ANC and Platelet count in irradiated NHPs in trial Rs-23



Higher mean values in ANCs and platelet count seemed to occur during the nadir (between ~day 10 to 18) in doses above 10 µg/kg; however, the bias due to semi logarithmic scale may also contribute to this impression. More information and details regarding the differences noted between the groups are requested to exclude other sources of bias. It needs to be considered that the pivotal trial was initiated already in 2011 and completion of in-life phase is reported in March 2012, but interpretation of the data and finalising the study report takes until February 2015.

IL-6 is an important mediator of fever and of the acute phase response by initiating synthesis of PGE2 in the hypothalamus, thereby changing the body's temperature set-point and is secreted by macrophages in response to specific microbial molecules (PAMPs) binding to TLRs (including TLR5). Moreover, it is also produced in muscles cells and secreted as response also to other factors (including stress hormones like cortisol). IL-6 acts via enhancement of acute protein product and the production of neutrophils in the bone marrow. Some data indicate that it supports also the growth of B cells and is antagonistic to regulatory T cells. However, the half-life time of IL-6 is very short (~ seconds) and data presented in figure below indicate that the significant increase of IL-6 serum levels after entolimod administration is again nearly normalised after 24 h.

Figure 3: Studies Rs-001, Rs-023; Comparison of Plasma IL-6 Concentrations by Species, Irradiation Status, Entolimod Dose, and Time



However, due to the rapid clearance of G-CSF as well as IL-6 levels induced by entolimod it seems not plausible that this surrogate marker has any impact on the later course of the ANCs or recovery of ANCs after radiation.

In conclusion it is not plausible that the observed short elevations of G-CSF and IL-6 should promote a clinical relevant beneficial effect which allows explaining the prolonged survival in NHPs observed. Although some dose depending differences during the ANC and platelet count nadir may have occurred, a convincing and clinical relevant difference in nadir duration or a clinical relevant reduction of neutropenic fever episodes was not demonstrated from the time course displayed from the figures submitted (but claimed in the tables in the efficacy section). Therefore, it seems difficult to correlate the surrogate outcome restricted on the 24 hours after administration reliable with a better survival results as proposed by the applicant. This raises an additional major concern with respect to the mode of action and the primary pharmacology.

Moreover, any difference was only seen in doses of entolimod which are neither investigated nor tolerable for the human population according the safety information available. No dose-finding is possible by comparing the PK characteristics in NHPs and humans.

For this procedure there is a high need to further evaluate the results which currently are presented in the dossier in figures only, while details of the results were not found or not comprehensible for the assessment necessary**.**

Secondary pharmacology

As already mentioned in the non-clinical part of this AR, no specific investigations addressing secondary pharmacology issues were performed for this submission. However, this is essential to evaluate the additional risk added by entolimod to the presumably polymorbid and highly vulnerable target population in the case of potentially lethal irradiation.

Only comparisons of clinical laboratory parameters of serum ALT, AST and phosphate concentrations in non-irradiated NHP in Rs-001 and in non-irradiated human subjects in pooled data from HU-7014 and HU-9001 were presented. This is not sufficient. As this data is mainly claimed for safety assessment also, they are present and discussed in section 4 of this AR.

There is a lack regarding respiratory safety and cardiovascular safety evaluation. Measurements of blood pressure yielded inconclusive results and ECGs performed during toxicity studies were not discussed in a detailed manner allowing to be validly compared with the findings in healthy volunteers.

The limited human safety data indicates a clear trend to QT prolongation and significant decreases in blood pressure after entolimod administration, but hERG channel data were not provided. It needs to be clarified whether hERG channel may interact with the protein, as such interacts have recently be demonstrated to occur also with other comparable large molecules. Moreover, it needs to be excluded that the clinical relevant and impressive decrease in blood pressure is not also at least partially caused or aggravated due to arrhythmic events.

As already elaborated also in the non-clinical assessment the applicant should provide comprehensive data on the safety of entolimod on the major physiological systems (at least on the cardiovascular, respiratory and central nervous system) in humans in order allow assessment of the evident risks added by entolimod treatment. As the target population is highly vulnerable due to the exposure to high radiation, this is essential to conclude whether the toxicity added by entolimod results in more pronounced harm in humans than currently reported from the NHPs as reported from trial Rs-23.

Moreover, the bilirubin values were not adequately provided. As this is essential to excluded occurrence of so-called Hy's law cases indicating a high risk for DILI in humans. The applicant is requested to present this data in a comprehensible manner together with the ALT, AST and Bilirubin levels observed in the human trial population and in NHPs.

Taking into account that flagellin may also act on IL-RN and VCAN and potentially may bind to other receptors (missing information) more evaluation regarding the mechanisms behind effects observed is needed. The same is true with respect to Caveolin-1 which seems to regulate the expression of TLR5 on cells and insofar may also have an impact on entolimod's bioactivity. As an increase of Caveolin-1 expression may be associated with increased resistance against radiation, it needs to be evaluated whether the differences in cytokine increases and entolimod's significantly pronounced toxicities in humans is caused by differences in Caveolin-1.

In conclusion, the concept of entolimod's mode of action in ARS is currently inconclusive. A reliable translation of the findings in NHPs and mice observed with significantly higher doses (20 fold in NHPs up to 4500 fold in mice) is currently not possible.

Pharmacodynamic interactions with other medicinal products or substances

Also from a PD perspective the absence of proprietary studies investigating DDI represents a gap in the data package according to the current EU guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 July 2012). The data presented regarding the impact of ibuprofen on entolimod's PD are deemed inconclusive. The applicant reports that pre-treatment with IBP enhanced entolimod-mediated cytokines concentration, although entolimod-related adverse events or constitutional symptoms decreased. This seems contradictory considering that the cytokine amount is claimed to cause the adverse events and seems also to be not in accordance with the rationale behind the dose conversion proposal.

From the study documents it became not fully clear whether the ibuprofen sub-study was implemented in the trial from the beginning or later amended when higher dose were evaluated and safety events observed in lower doses indicate lower tolerability in humans compared with NPHs. Moreover, no information was provided whether the increase in cytokines measured was already described in the scientific literature in order to conclude on the reliability of the results.

At the end, these data are hardly robust enough to justify the pre-treatment with ibuprofen as currently proposed without further valid investigations, since an further increase in IL-6 may increase the risk for induction of a cytokine release syndrome (CRS)/"cytokine storm".

The need for drug drug-interaction studies should be thoroughly discussed by the applicant and the complete absence needs to be further justified. Even in an emergency situation with insufficient hospital care broad use of antibiotics is expected. Substances to help expel radioactive materials from the gastrointestinal tract may be used (e.g. activated charcoal, antacids, barium sulfate, bisacodyl, and chelating agents). In addition, other substances for internal decontamination (depending on the identification of the radionuclide) will be used and need consideration.

Moreover, the PD effects of pre-existing antibodies against entolimod following previous exposure to bacterial antigens during natural acquired intestinal infections during life needs to be adequately characterised, since these antibodies occur in at least about 15% of the intended target population, but as the method used was assessed as insensitive, the real frequency of antibodies might even be higher. As these naturally occurring antibodies against flagellin are neutralising the PK/PD effects of entolimod completely a significant impact on the drug's efficacy can be reasonably presumed. This is indicated by the exclusion of AB positive animals from the trials.

Genetic differences in PD response

Phase I trial HU-9001 included a secondary objective to describe the effect of TLR5 genotype on entolimod biomarker levels, safety, and PK following IM administration. All enrolled subjects were genotyped for the TLR5 stop codon (TLR5392 stop) mutation. 6/100 subjects enrolled into HU-9001

carried the mutant genotype. Due to the small number of relevant subjects, it was not possible to draw any definitive conclusions regarding the impact on TLR5-genotype on the efficacy or safety profile of entolimod. In order to allow a further assessment on the relevance of TLR-5-genotype for entolimod's PD in humans, the applicant is requested to present the full details of the different cytokine outcome in subjects who carried the TLR5392 stop-mutation. A comparison of levels of response in all PD markers investigated in these subjects and those without this mutation should be provided. Moreover, the adverse events recorded in the 5 patients with TLR5-mutants should be provided together with the entolimod doses administered.

Relationship between plasma concentration and effect

The applicant describes the mean plasma G-CSF and IL-6 concentrations in NHP (non-irradiated and irradiated). Maximum achieved mean plasma G-CSF levels were described to be ~5- to 10-fold higher in humans than in NHP (regardless of the irradiation status), while maximum achieved mean plasma IL-6 levels in non-irradiated and irradiated NHP were generally similar to those in humans at the tested doses. Due to the deficiencies in general PK characterisation (missing information, validity of PK method challenged), it seems difficult to agree with the applicant's view that the PK/PD data indicate substantial similarities between NHPs with humans; similarly safety was observed to be different. Whether this is only explained due to the higher cytokine levels in humans as currently presumed by the applicant remains unknown and not convincingly demonstrated.

3.3.3. Discussion on clinical pharmacology

Entolimod is a first-in-class product. No other Flagellin or comparable TLR5 agonist was approved and there is currently no supportive clinical evidence for any other clinical relevant efficacy of Flagellin in any disease.

Pharmacokinetics

PK of entolimod in human subjects has only been incompletely characterized. There are no dedicated studies on bioavailability, distribution, metabolism , and elimination, as well as regarding entolimod's potential for drug interactions, and the influence of extrinsic and intrinsic factors on the PK of entolimod. Furthermore, in the presented analyses, it is not always clear which subjects were included, presently hampering comparability. Importantly, issues pertaining to the validity of the analytical methods relevant to human PK have been identified, currently questioning the reliability of the human PK data. These may be additionally confounded by the presence of pre-existing anti-CBLB502 antibodies in a subset of human subjects. Moreover, the Drug Product (DP) used in nonclinical studies and in prior clinical trials in healthy subjects and patients was changed in the formulations used in Study I196111-Russia and Study BL612-CBLB502, as well as in the formulations proposed for the MAA authorization. The comparability of the formulation used in the previous pivotal animal and human studies (DP Lot) versus those proposed to be stockpiled in the EU (DP Lots) has only been demonstrated analytically. Therefore, the proposed entolimod formulation for commercialization differs from that used in all pivotal human safety studies and pivotal animal efficacy studies, and no comparability data are presented to assess the impact on clinical PK/PD of the differences between both formulations. (See also the quality assessment report of the Rapporteur for the absence of comparability data assessing the impact on quality of any of the changes in formulation introduced during development). Therefore, the to-be-marketed formulation differs from the formulation used in the clinical and pivotal pre-clinical trials and a biocomparability study is needed.

Apart from that, the proposed entolimod formulation for commercialization is not fit for the proposed intended use (i.e. a single dose based on body weight as soon as possible following radiation exposure)

because two vials would be necessary for subjects weighting over 76 kg (recommended dose in SmPC).

Although entolimod is intended for single administration only, expected to be eliminated mainly by metabolic catabolism, and regression analyses indicated that exposure metric decreased with increasing body size, further data are considered necessary from a PK perspective, especially with respect to metabolism, the potential for drug interactions and the influence of intrinsic and extrinsic factors on PK. Since it is neither ethical nor feasible, studies in the target population have not been performed. Effect of total body irradiation on PK of entolimod is available only from the primary efficacy species rhesus macaques NHP. Because humans are substantially more sensitive to the effects of entolimod than are NHP, animal-to-human dose conversion using only parameters of drug exposure was not possible, instead, dose conversion 'for efficacy' was to be made 'predominantly' on the basis of pharmacodynamics parameters.

Pharmacodynamics

Entolimod's pharmacodynamics is currently not adequately characterised. It is plausible that entolimod binds to TLR5, activates nuclear factor kappa-B (NF-κB) and induces production of cytokines (in particular, granulocyte colony-stimulating factor [G-CSF] and interleukin [IL]-6). However, as the increases of cytokines observed are short time effects and PD effect beyond 24h are not convincingly demonstrated, it remains difficult to justify any multiorgan tissue protection or regeneration with respect to the PD or pharmacology as shown in NPH studies. In particular, taking the significantly higher doses into account used in animal models, but not tolerated by humans.

In the no clinical study (CBL1.1-12), Hawn et al. reported a 392-stop codon polymorphism of human TLR5, which leads to production of a truncated protein; it is carried by about 10% of human population. The consequences on pharmacodinamic responses/efficacy in subjects with 392-stop codon polymorphism should be discussed by the applican.

3.3.4. Conclusions on clinical pharmacology

PK and PD of entolimod in human subjects have only been incompletely characterized and the comparability between the presented analyses is hampered since it is not always clear which subjects were included. Further data are considered necessary from a PK perspective at least regarding metabolism, the potential for drug interactions and the influence of intrinsic and extrinsic factors on PK. The potential in vivo pharmacodynamic drug-drug interactions with a number of treatments used in the foreseen clinical practice or with the most common drugs administered in the most prevalent diseases in the population should have should be discussed.

In addition, a biocomparability study is needed since the to-be-marketed formulation differs from the formulation used in the clinical and pivotal pre-clinical trials. Importantly, issues pertaining to the validity of the analytical methods relevant to human PK have been identified, currently questioning the reliability of the human PK data. These may be additionally confounded by the presence of pre-existing anti-CBLB502 antibodies in a subset of human subjects. Since it is neither ethical nor feasible, studies in the target population have not been performed. Effect of total body irradiation on PK of entolimod is available only from the primary efficacy species rhesus macaques NHP. Because humans are substantially more sensitive to the effects of entolimod than are NHP, animal-to-human dose conversion using only parameters of drug exposure was not possible, instead, dose conversion 'for efficacy' was to be made 'predominantly' on the basis of pharmacodynamics parameters.

Entolimod's primary and secondary pharmacology has not been comprehensively characterized.

3.3.5. Clinical efficacy

Dose-response studies and main clinical studies

Because humans cannot be lethally irradiated to test the activity and safety of entolimod for the proposed indication, clinical efficacy studies of the drug are not ethical or feasible. For this reason, entolimod efficacy data have been derived from studies in NHP conducted in accordance with United States governmental regulations as promulgated under the Food and Drug Administration (FDA) Animal Rule:

- A randomized, blinded, placebo-controlled, dose-ranging trial in irradiated NHP to evaluate the survival benefit of entolimod when administered intramuscularly (IM) after potential lethal irradiation, to determine the dose of entolimod at which significant survival benefits are seen, and to characterize the PD responses of irradiated NHP as measured by the area under the plasma concentration-time curve from time 0 to 24 hours postdose (AUC_{0-24}) for plasma G-CSF and IL-6 and the fold increase in absolute neutrophil count at 24 hours postdose (ANC24) (**pivotal efficacy study, CBL1.1-N-01-Rs-23 [Study Rs-23**]). The trial was performed 2011/2012 in China and is claimed to have been performed GLP conform.
- A randomized, open-label, placebo-controlled dose-ranging trial in nonirradiated NHP to evaluate the PD responses to a single IM injection of entolimod, as measured by AUC_{0-24} for plasma G-CSF and IL-6 and ANC24 (**pivotal dose-conversion biomarker study, CB5BD-P-Rs-001 [Study Rs-001]**). The trial was performed in China.
- Eight completed randomized, controlled supportive trials in irradiated NHP that provide corroboration of entolimod efficacy for the targeted indication: **CBL1.1-N-01-Rs-03 [Study Rs-03], CBL1.1-N-01-Rs-05 [Study Rs-05], CBL1.1-N-01-Rs-06 [Study Rs-06], CBL1.1-N-01-Rs-09 [Study Rs-09], CBL1.1-N-01-Rs-10 [Study Rs-10], CBL1.1-N-01-Rs-11 [Study Rs-11], CBL1.1-N-01-Rs-14 [Study Rs-14] and CBL1.1-U-01-Rs-17 [Study Rs-17]).**

The pivotal efficacy trial (Rs-23) and the dose-conversion trial (Rs-001) were conducted in accordance with Good Laboratory Practices (GLP). The 8 supportive studies of entolimod in NHP were non-GLP studies are only discussed in the non-clinical section of the present report.

Table 1 Overview of Entolimod NHP Pivotal Trials Program

Study ID	No. of Centers Location	Study Status & Dates of Study	Study Design	Study Objectives	Test Product and Route of Dosing	No. of Animals by Entolimod Dose (µg/kg)	Sex (F/M) Median Age (Range)	Duration	Diagnosis & Main Criteria for Inclusion	Endpoints
Rs-23	1 China	Completed **12 Aug 2011 - 08 Mar 2013**	Randomized, blinded, placebo-controlled, parallel-group, dose-ranging study in irradiated (TBI at target dose of 7.2 GY)	To evaluate the dose-dependent effect of entolimod on survival (primary), efficacy biomarkers, and haematology	Placebo or entolimod **1 dose, administered IM**	0	22/18 41 mo (25-68 mo)	Single dose of placebo or entolimod followed by 60-day observation period	Research-naïve, young adult to middle age male or non-pregnant female rhesus macaques weighing 3.5-8 kg; adequate bone marrow, liver, and	**Primary:** Survival **Key Secondary:** AUC0-24 of plasma for G-CSF plasma IL-6,
						0.3	11/9 42 mo (27-54 mo)			
						1	11/8 43 mo (30-56 mo)			

			NHP	parameters		3	11/9 42 mo (27-71 mo)		renal function; no obvious infections, inflammation or co-morbidities; no treatment with immuno-suppressive or anti-inflammatory drugs; no high preexisting entolimod antibody titre	ANC24 Proportion of days alive and free of severe cytopenias
						6.6	11/9 42 mo (27-56 mo)			
						10	11/9 40 mo (27-56 mo)			
						40	11/9 42 (26-70 mo)			
						120	11/9 37 mo (26-54 mo)			
Rs-001	1 China	Completed 04 Apr 2013 - 30 Jan 2014	Randomized, open-label, placebo-controlled, parallel-group, study in nonirradiated NHP	Evaluate PK/PD response to entolimod	Placebo or entolimod **1 dose, administered IM**	0	11/9 40 mo (38-63 mo)	Single dose of placebo or entolimod, followed by 3-day observation period	Healthy research-naïve male or nonpregnant female rhesus macaques aged ~3-7 years; no obvious infections, inflammation or co-morbidities; no treatment with immuno-suppressive or anti-inflammatory drugs; no high preexisting entolimod antibody titre	**Primary:** AUC0-24 of plasma G-CSF, AUC0-24 of plasma IL-6; fold increase of ANC at 24 h postdose **Secondary:** PK
						0.3	11/9 40 mo (39-73 mo)			
						1	10/10 40 mo (39-62 mo)			
						3	10/10 41 mo (38-52 mo)			
						6.6	10/10 45 mo (39-51 mo)			
						10	10/10 40 mo (38-63 mo)			
						40	10/10 40 mo (39-62 mo)			
						120	10/10 40 mo (38-73 mo)			

Summary of main efficacy results

Study Rs-23

Results from trial Rs-23 in non-human primates (rhesus macaques) are claimed as pivotal for the efficacy in this application under exceptional circumstances as trials in the human population in this setting are neither ethical nor feasible.

Study Rs-23 was a randomized, blinded, placebo-controlled, parallel-group, GLP, dose-ranging study in NHP (rhesus macaques).

Methods

- **Study participants**

Entolimod was evaluated in entolimod-naïve and irradiation-naïve, young adult to middle age male or nonpregnant female rhesus macaques weighting 3.5-8 kg; adequate bone marrow, liver, and renal function; no obvious infections, inflammation or co-morbidities; no treatment with immuno-suppressive or anti-inflammatory drugs; no high preexisting entolimod antibody titre. Inclusion as well as exclusion criteria are acceptable.

- **Treatments**

NHP were exposed to in-air total body irradiation (TBI) with 7.2 Gy of gamma ray. The company, based on the survival results from previous studies Rs-09, -10, -11 and -14, considers that an in-air TBI dose of 7.2 Gy would result in the death of ~70% of the animals within 60 days ($LD_{70/60}$).

For logistical reasons, animals were acquired, treated, and evaluated in 4 sequential phases. In all phases animals were managed as shown in fig.1. Within each phase, cohorts of 9 animals were sedated and placed into restraining chairs in a shielded irradiation chamber. The cohorts were stratified by sex, body weight, and body width.

In the discussion section of Qualification final report for the Long Quan Cobald-60 Irradiator in Chengdu (SR-Rs-23 –Section 16.1.8.2) it is reported that the radiation shelter had 10 seats on the right and 10 seats on the left side. It is noted that dose rates were determined to be most uniform between the chair positions L1 to L7 and R1 to R7. However, in the study report it is described that the animals were irradiated in groups of 9 animals. The applicant is requested:

a) To provide the details how radiation was finally performed in trial Rs-23

b) To provide a list which allows to identify the seat position during radiation for all animals according the entolimod treatment group and

c) To provide the dosimetry results from the nanoDot dosimetry for each animal. The results from the ionization chamber dosimetry should also be provided for the group.

Two types of dosimetry were performed during the irradiation procedure in each study phase:

- Measurement of radiation dose in air using an ionization chamber for each irradiation cohort to confirm the prespecified TBI dose
- Individual dosimetry with nanodot dosimeters used to confirm individual exposure levels

The applicant stated in the discussion of the Qualification final report for the Long Quan Cobald-60 Irradiator in Chengdu (SR-Rs-23 –Section 16.1.8.2) that the nanoDot dosimeters were evaluated for their utility for providing dosimetry measurements during the in vivo studies, since ionization chambers cannot be placed with the animals. However, in the study protocol it is mentioned that measurement of radiation dose in air was performed using an ionization chamber for each irradiation cohort to confirm the pre-specified TBI dose. The applicant is requested to explain which description is correct and provide a comparison for both methods described. For each of the animals in the vehicle/placebo arm and those in the 10µg/kg arm the results of dosimetry should be provided in order to better understand what was finally performed.

Following TBI, animals were again randomly assigned to 1 of the 8 treatment groups (0 [placebo control], or 0.3, 1.0, 3.0, 6.6, 10, 40 or 120 µg/kg of entolimod) in a ratio of 2:1:1:1:1:1:1:1. Selection of the entolimod treatment dose levels was based on previous studies in irradiated (Rs-03, Rs-05, Rs-06, Rs-09, Rs-10, Rs-11, Rs-14, and Rs-17) rhesus macaques. The chosen route of administration is the one intended for human use. Each animal received a single IM dose of placebo or

entolimod at 25 hours after completion of TBI and were then monitored over a 60-day follow-up period or until death.

Minimal supportive care (e.g., analgesia, topical wound care, and water-soaked biscuits for extra hydration) could be provided, but intensive supportive care (e.g., transfusions, systemic antibiotics, IV fluids, HP growth factors) was avoided to replicate therapy in a mass human radiation disaster that would preclude large-scale provision of intensive supportive care to large numbers of irradiated patients.

Certain medications such as anti-inflammatory drugs, ketamine, and natural or synthetic opiates known to affect the immune system were not to be used after study drug administration. The synthetic opioid, fentanyl, and the semi-synthetic opiate, buprenorphine, were allowed to alleviate perceived pain and suffering because they are not immunosuppressive. To alleviate potential pain or distress, all animals were treated with fentanyl patches (12.5-25 µg/h) with continuous release on Days 4 to 25 postirradiation. Discretionary medical treatment was to be carried out based upon consensus agreement between the study director and the Frontier study veterinarian.

The selected concomitant therapy and analgesics as proposed in the study protocol is not critical for the study outcome. However, a comprehensible analysis of differences with respect to concomitant medication and analgesics was not found. The applicant is requested to provide this analysis in order to assess potential differences between the groups.

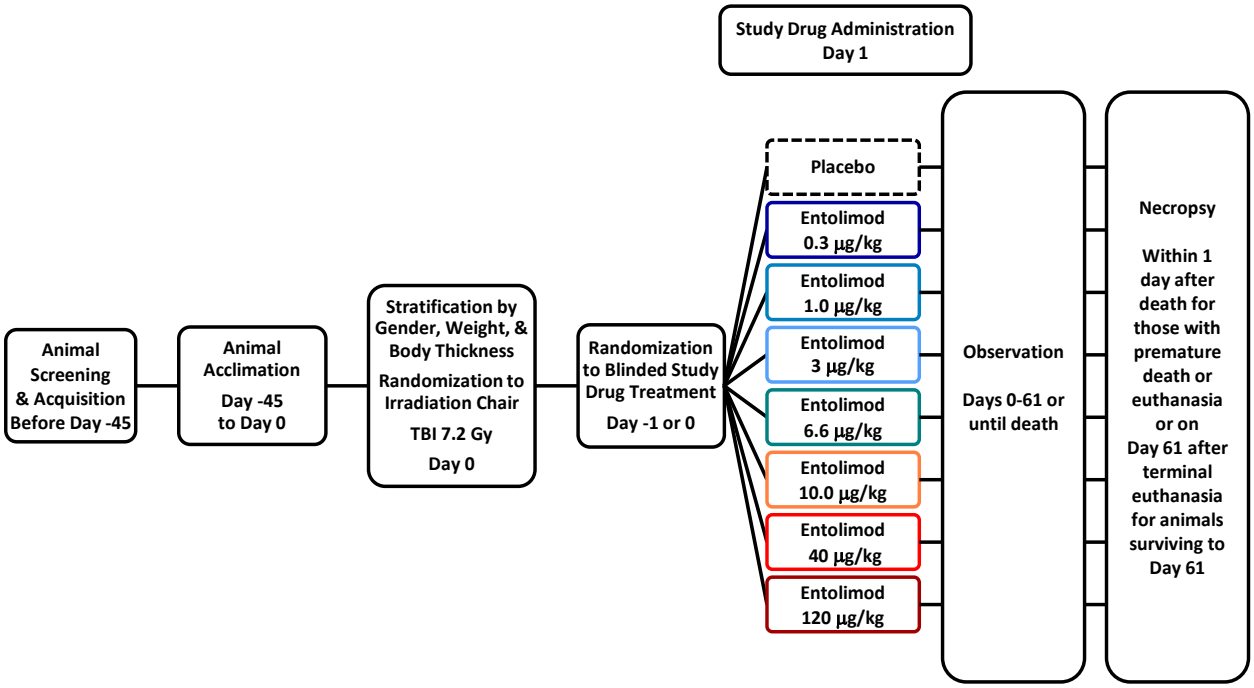
Selection of 25 hours as the interval between TBI and administration of study drug was intended to simulate the time required from the occurrence of a radiation catastrophe to deployment of field medical personnel and triage of patients for entolimod administration. The company should discuss if expected efficacy in humans would be similar when administering entolimod at 25 hours after total body irradiation than earlier.

Animals that died during the 60-day observation period underwent necropsy on the day of death or within 1 day after the day of death. Animals that survived to Day 60 were euthanized on Day 61 and underwent necropsy on that day. The study veterinarian could allow euthanasia for distress not relieved by planned supportive measures before planned euthanasia on day 61 (i.e. unscheduled euthanasia) or could use judgment in extending observation in stressed animals if recovery seemed possible.

The applicant should provide sufficient information to allow a comprehensible assessment of potential source of bias due to euthanasia in trial Rs-23. The applicant is requested to explain the approach leading to decision for euthanasia in the animals and should provide plausible evidence for all animals sacrificed before day 60. Results should be presented according study group and the trial phase should be assessable.

For all animals, a routine necropsy was to be conducted. Gross pathology was examined by a qualified animal pathologist who was to establish the cause of death for all animals that were found dead or euthanized for moribundity.

Figure 1: Study Design – Study Rs-23



- **Objectives**

The primary objective of the study was to evaluate the dose-dependent effect of entolimod on survival.

Secondary objectives:

- To evaluate the pharmacodynamic (PD) effects of entolimod on neutrophil counts and cytokine induction.
- To evaluate the relationship between effect on cytokine biomarkers and the effect on overall survival.
- To evaluate hematology parameters as a reflection of major ARS morbidities.

The study objectives are agreed and seemed adequate for a phase II trial aiming to establish a dose depending response of a drug. Moreover, in this context it makes sense to assess also additional PK/PD in order to evaluate effects of radiation on PK and PD. Moreover, these objectives in principle may be helpful to prepare a dose finding/dose conversion approach.

- **Outcomes/endpoints**

The primary efficacy endpoint was survival at 60 days after total body irradiation. Study personnel monitored animals over a 60-day follow-up period.

The secondary efficacy endpoints were:

- AUC_{0-24} of plasma G-CSF
- AUC_{0-24} of plasma IL-6
- ANC24
- Proportion of days alive and free of severe neutropenia (<0.5 x 10^3 neutrophils/□L of blood)

- Proportion of days alive and free of clinically relevant neutropenia (<1.0 x 10^3 neutrophils/□L of blood)
- Proportion of days alive and free of severe thrombocytopenia (<20 x 10^3 platelets/□L of blood)
- Proportion of days alive and free of clinically relevant thrombocytopenia <50 x 10^3 platelets/□L of blood)

Haematological parameters were assessed over time.

The exploratory PD, hematological, and exposure endpoints analysed in this study were:

- Maximum concentration (Cmax) and area under the curve from 0 to 48 hours after entolimod administration (AUC0-48) of plasma G-CSF and IL-6
- Fold increase of ANC at 8 and 16 hours after entolimod administration
- Cmax, AUC0-24, and AUC0-48 of plasma IL-8 and IL-10
- Proportions of days free of cytopenias while alive
- Nadirs of hematologic parameters (specified as a post-hoc analysis)
- Entolimod exposures in serum after injection (expressed as Cmax, area under the curve from 0 to time t [AUC0-t], and AUC0-24)

Schedule of study assessments is shown in table 9.

Table 9. Schedule of study assessments in study Rs-23.

Procedure	Screening (before Day -45)[a]	Day -17	Day -10	Day -3	Day -1	Day 0	Day 1									Day 2		Day 3	Day 4	Days 5-60	Day 61
Hour relative to study drug	-	-	-	-	-	-	**0**	**0.25**	**0.5**	**0.75**	**1**	**2**	**4**	**8**	**16**	**24**	**36**	**48**	**72**	-	-
Physical examination	X	-	X	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Body thickness			X																		
Specific pathogen testing[b]	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Entolimod-reactive antibodies	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plasma IL-8	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clinical chemistry[c]	X	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coagulation[d]	X	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ECG[e]	X	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Body thickness measurement	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Blood pressure and pulse[f]	X	-	-	X	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-	-	-
Body temperature[g]	-	-	X	X	-	-	X	-	-	-	-	-	-	-	-	-	-	-	X	X	-
TBI[h]	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Study drug administration[i]	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cageside observations[j]	-	X	X	X	X	X	X	-	-	-	X	X	X	X	-	X	X	X	X	X	X
Body weight[k]	X	X	X	X	-	-	X	-	-	-	-	-	-	-	-	-	-	-	X	X	X
Fruit and other food consumption[l]	-	X	X	X	-	X	X	-	-	-	-	-	-	-	-	X	-	X	X	X	-
CBC[m]	X	X	X	X	X	-	X	-	-	-	-	-	-	X	X	X		X	X	X	X
Plasma for cytokines[n]	-	-	-	-	X	-	X	-	-	-	X	X	X	X	X	X	X	X	X	-	-
Serum for entolimod PK	-	-	-	-	X	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-
Terminal euthanasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X

a. Screening was performed prior to the study quarantine at the animal supplier, usually before animal arrival at the testing facility.
b. Specific pathogen testing was performed to evaluate for SIV, STLV–1, SRV, Herpes B, and tuberculosis.
c. Clinical chemistry parameters included BUN, creatinine, AST, ALT, LDH, ALP, albumin, and glucose.
d. Coagulation parameters included PT and aPTT.

e. An ECG was performed on Day -10; a second ECG was performed on Day -3 if the first ECG indicated abnormalities.
f. For Phase B, blood pressure and pulse were measured on Day -31 and were not measured on Day -3.
g. Body temperature was monitored prestudy and 2 times weekly on Days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 40, 46, 53 and 60 or more frequently per the study veterinarian.
h. TBI, 7.2 Gy, was generally administered between 0800 and 1200 hours on Day 0.
i. Study drug (placebo or entolimod at doses of 0.3, 1, 3, 6.6, 10, 40 and 120 µg/kg) was administered as a single IM injection on Day 1 at 25 hours after irradiation.
j. Cageside observations were obtained prestudy, at approximately 1, 2, 4, and 8 hours postirradiation on Day 0, at approximately 1, 2, 4, and 8 hours postdose on Day 1; two times per day on Days 2-4; three to four times per day (at least 6 hours apart) on Days 5-30; two times per day on Days 31-60; and on Day 61.
k. Body weight was monitored 2 times weekly on Days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 40, 46, 53 and 60 or more frequently per the study veterinarian. Body weight was not measured on Day -17 for Phase B.
l. Fruit and other food consumption was monitored prestudy and daily using a qualitative scale. In Phase B, fruit and other food consumption was not recorded on Day -17. For Phase B, fruit and other food consumption was recorded on Day 0 in addition to the other time points noted.
m. CBC included total and differential WBC, RBC, hematocrit, hemoglobin, leukocyte, reticulocyte, platelet count, mean corpuscular hemoglobin concentration, and mean corpuscular volume. CBCs were not measured prior to Day -45 or on Day -17 for Phase B. For all phases, CBCs were obtained on Day -1 at approximately 12 hours before irradiation; on Day 1 pre-dose and at 8 and 16 hours postdose; and on Days 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, 25, 29, 32, 36, 40, 46, 53, 60, and 61.

Plasma cytokines included G-CSF, IL-6, IL-8, and IL-10.

- **Sample size**

Planned: 210 (105 per sex) NHP to be screened, and 180 animals (90 per sex) were to be treated.

Sample size and power were determined for the comparison of survival in the entolimod treatment groups versus survival in the placebo control group. Three states of nature were defined to describe monotonically increasing survival with increasing dose. In all of them, the survival rate was assumed to be 30% in the placebo control group and 70% in the 40-µg/kg group. They differed by the entolimod dose (7.5, 10 or 20 µg/kg) at which the survival rate was 63% (ie, 10% less than the survival rate at 40 µg/kg). In all 3 states of nature, a sample size of 20 animals in each entolimod treatment group and 40 animals in the placebo group provided at least 90% power to find the 40-µg/kg treatment group different from placebo control with respect to survival.

- **Blinding (masking)**

Randomization to treatment assignment and entolimod dose was blinded to NHP staff involved in study activities and to the sponsor personnel. Until unblinding, the independent statistician who generated the treatment randomization list had knowledge of the random order of treatments but not the animal ID numbers that were written next to the treatment assignments. Only the analytical chemist of the NHP center was unblinded and had knowledge of which animal received which treatment. The unblinded analytical chemist prepared vials containing the assigned treatments and marked them with animal numbers. These vials were transferred to blinded staff for dosing the corresponding animals.

In order to provide preliminary results to the sponsor, the study was partially unblinded (ie, to sponsor but not to the center where NHP were treated) upon completion of the in-life phase of the study and when animal survival has been followed for 60 days in all 4 phases of the study. After the breaking of the blind on 11 August 2012, the independent statistician, who had prepared the treatment randomization list, became the study statistician for the final analysis of the trial.

The company should discuss how the tasks performed unblinded might have interfered with the results of the study Rs-23.

- **Statistical methods**

The following 2 analysis populations were defined for this study:

- Intent-to-treat (ITT) population: comprised all animals randomized to study treatment; this population was used in the primary analysis of survival and in other efficacy and safety analyses, as appropriate
- As-treated population: comprised all animals that received study drug; this population was used for relevant safety analyses and other analyses, as appropriate

For the analysis of survival, the null hypothesis was that the 60-day survival rates in the vehicle control group and entolimod dose groups of 0.3, 1.0, 3.0, 6.6, 10, and 40 µg/kg were equal; the alternative hypothesis was that the survival rates were monotonically non-decreasing with increasing dose and ≥1 treatment group had a greater 60-day survival rate than that of the vehicle control group.

For the analyses of secondary efficacy endpoints (AUC0-24 of G-CSF, AUC0-24 of IL-6, and fold increase in ANC at 24 hours) and the analyses of the secondary hematological endpoints (proportions of days alive and free of severe neutropenia, clinically relevant neutropenia, severe thrombocytopenia,

and clinically relevant thrombocytopenia), the null hypotheses were that the mean responses in the vehicle control group and entolimod dose groups of 0.3, 1.0, 3.0, 6.6, 10, and 40 µg/kg were equal; the alternative hypotheses were that the mean responses were monotonically non-decreasing with increasing dose and ≥1 treatment group had a mean response greater than that of the vehicle control group.

The entolimod dose of 120 µg/kg had not been previously studied and had an undefined safety profile. Consequently, it was pre-specified as exploratory.

Statistical inference and modeling of survival was based on the vehicle control group and the groups dosed through 40 µg/kg. Trend tests using logistic regression were the basis for the statistical inference on survival. For the regression model, the independent variable was the logarithm of dose and the dependent variable was the survival outcome (alive or dead). The vehicle group dose of zero was scored as log (0.1), a half-log unit less than the log of the lowest non-zero dose of 0.3 µg/kg.

Statistical inference on the after entolimod administration) was performed using trend testing in the context of analysis of variance. The trend test for each biomarker was performed at the alpha/4 = 0.00625 level of significance to control the Type I error rate.

.

The relationships between survival and were evaluated using Spearman rank correlation coefficients based on, entolimod-effect pairs.

Haematological endpoints: The durations and severities of cytopenias were calculated based on serial CBC determinations from the 60-day, post-TBI follow-up period. Since CBCs were not collected daily, blood counts on days not sampled were imputed within each animal by linear interpolation over time between actual blood counts, or using last observation carried forward between the days of last available blood count and the day of death. The durations of cytopenias were based on the imputed values. Nadir values were based on the actual lowest value observed. Statistical inference on each proportion was performed using trend testing in the context of analysis of variance, where the dependent variables were logit transforms of the proportions of days with the designated severity of cytopenia. To control the family-wise error rate, each test was performed at the alpha level prespecified in the SAP until the first non-significant test.

The family-wise error rate, alpha, was 0.025, one-sided, for the study hypotheses related to survival and to the secondary endpoints. A sequence of logistic regression analyses were performed, with the first analysis using all doses through 40 µg/kg at the 0.025 level and each successive analysis stepping down to lower doses by retaining all but the highest dose used in the previous analysis at the alpha/4 = 0.00625 level of significance.

The statistical methods are generally considered appropriate. The trend tests for testing the null hypothesis for survival and the three biomarkers make assumptions on the functional form of the relationship between the dependent variable (i.e. survival or biomarker) and dose (e.g. a linear relationship between log odds of survival and log of dose). Only if the assumption is fulfilled, rejection of the null hypothesis of the trend test allows accepting the alternative hypothesis that the survival rates (or biomarker) were monotonically non-decreasing with increasing dose. In particular, a trend test allows concluding that the highest dose that is retained being efficacious in a confirmatory sense only under the assumption of the trend test. However, for survival, it needs to be taken into consideration that Fisher's exact test leads to the same conclusion when dose groups are compared separately to the vehicle group.

Results

- **Participant flow**

A total of 179 animals were irradiated in 20 irradiation cohorts. All 179 irradiated animals were randomized to treatment with placebo (n=40) or entolimod (n=139). However, 1 animal in the 40-µg/kg dose group died between irradiation and the time of randomization and, therefore, never received the dose of entolimod.

Thus, the intent-to-treat (ITT) population (used in the analysis of the primary endpoint of survival) included all 179 randomized animals, whereas the as-treated population (used in the analysis of the secondary efficacy endpoints) included the 178 animals that actually received the single dose of study drug.

- **Conduct of the study**

The study protocol was amended 10 times; nearly all of the changes performed may be explain due to technical feasibility reasons and are overall not deemed critical for the conduct of the trial. A listing of potential protocol violations occurred during the trial was not found. The applicant is requested to report whether protocol violations were recorded and should provide this information.

The trial had to be performed sequentially and was complicated due to a protozoan infection in some of the animals, which delayed the trial performance. Currently, it needs confirmation that the NHPs study population remains genetically and otherwise homogenous during the complete study to exclude differences regarding radiation sensitivity between treatment arms.

- **Baseline data**

The treatment groups were comparable in demographic characteristics (age, sex, body width, and weight). The mean (SD) age of the animals was 41.0 (9.1) months, and both animal sexes were almost equally represented in all groups. Animals ranged in age from 25 months to 71 months; these age ranges correspond to human ages to 8-24 years. The mean (SD) weight was 5.20 (1.30) kg for males and 4.69 (0.84) kg for females. The mean (SD) body thickness was 8.75 (0.85) cm for females and 9.27 (1.06) cm for males.

The treatment groups were comparable in screening (i.e., before irradiation) and baseline (after irradiation and before administration of study drug) laboratory parameters. All animals had low pre-existing entolimod reactive antibody titers.

Radiation exposure was comparable among the treatment groups as documented by uniformity of radiation doses in air and by individual dosimetry, as well as by universal and uniform induction of radiation-related lymphopenia within 24 hours after TBI.

- **Numbers analysed**

179 animals were irradiated and randomized and included in the ITT population: placebo (n=40) or 0.3 µg/kg (n=20), 1.0 µg/kg (n=19), 3.0 µg/kg (n=20), 6.6 µg/kg (n=20), 10 µg/kg (n=20), 40 µg (n=20), or 120 µg/kg (n=20) of entolimod. Of these, 1 animal randomized to the 40-µg/kg entolimod group died shortly after TBI and before being treated with entolimod, resulting in an as-treated population of 178 animals.

- **Outcomes and estimation**

SURVIVAL

No deaths occurred at any dose level in the 4 days after administration of study drug. Thereafter, increasing numbers of deaths were seen; the majority occurred in the third week of observation, consistent with the expected pattern of deaths from TBI. Deaths during the 60-day observation period (before the scheduled terminal euthanasia on Day 61 of the study) were more common in the control group and in the lower-dose entolimod groups (Table 11). By contrast, the proportions of animals surviving to Day 61 were greater at the higher entolimod dose levels.

Table 11. Terminal disposition by treatment group – ITT population

Study Period	Study Day	Entolimod Dose, µg/kg (N)																	
		0 (N=40)		0.3 (N=20)		1.0 (N=19)		3.0 (N=20)		6.6 (N=20)		10 (N=20)		40 (N=20)		120 (N=20)		Total (N=179)	
		Died	Alive	Died	Alive	Died	Alive	Died	Alive	Died	Alive	Died	Alive	Died	Alive	Died	Alive	Died	Alive
Pre-dose	0	0	40	0	20	0	19	0	20	0	20	0	20	1	19	0	20	1	178
On study	5	0	40	0	20	1	18	0	20	1	19	0	20	0	19	0	20	2	176
	9	0	40	1	19	0	18	0	20	0	19	0	20	0	19	0	20	1	175
	10	0	40	0	19	0	18	1	19	0	19	0	20	1	18	0	20	2	173
	11	1	39	0	19	0	18	0	19	0	19	0	20	0	18	0	20	1	172
	12	1	38	1	18	1	17	0	19	0	19	0	20	0	18	0	20	3	169
	13	1	37	1	17	0	17	1	18	0	19	0	20	0	18	0	20	3	166
	14	5	32	3	14	2	15	2	16	1	18	1	19	1	17	0	20	15	151
	15	9	23	2	12	2	13	4	12	2	16	0	19	2	15	2[a]	18	23	128
	16	3	20	2	10	2	11	1	11	0	16	1	18	0	15	1	17	10	118
	17	1	19	1	9	2	9	1	10	0	16	1	17	1	14	1	16	8	110
	18	3	16	2	7	0	9	2	8	2	14	1	16	0	14	0	16	10	100
	20	1	15	1	6	0	9	0	8	0	14	0	16	0	14	0	16	2	98
	21	2	13	0	6	0	9	0	8	1	13	0	16	0	14	1	15	4	94
	22	0	13	0	6	1	8	1	7	1	12	0	16	0	14	0	15	3	91
	24	0	13	0	6	0	8	0	7	0	12	0	16	0	14	1	14	1	90
	25	0	13	0	6	1	7	0	7	0	12	1[a]	15	0	14	0	14	2	88
	26	0	13	1	5	0	7	0	7	0	12	0	15	0	14	0	14	1	87
	27	1	12	0	5	0	7	0	7	0	12	0	15	0	14	0	14	1	86
	30	0	12	0	5	0	7	0	7	1	11	0	15	0	14	0	14	1	85
	44	1	11	0	5	0	7	1	6	0	11	0	15	0	14	0	14	2	83
	47	0	11	0	5	0	7	0	6	1	10	0	15	0	14	0	14	1	82
	51	0	11	0	5	0	7	1	5	0	10	0	15	0	14	0	14	1	81
	58	0	11	0	5	0	7	0	5	1	9	0	15	0	14	0	14	1	80
	60	0	11	0	5	0	7	0	5	0	9	0	15	0	14	0	14	0	80
Total dead and alive	60	29	11	15	5	12	7	15	5	11	9	5	15	6	14	6	14	99	80
Scheduled euthanasia	61	11	0	5	0	7	0	5	0	9	0	15	0	14	0	14	0	80	0

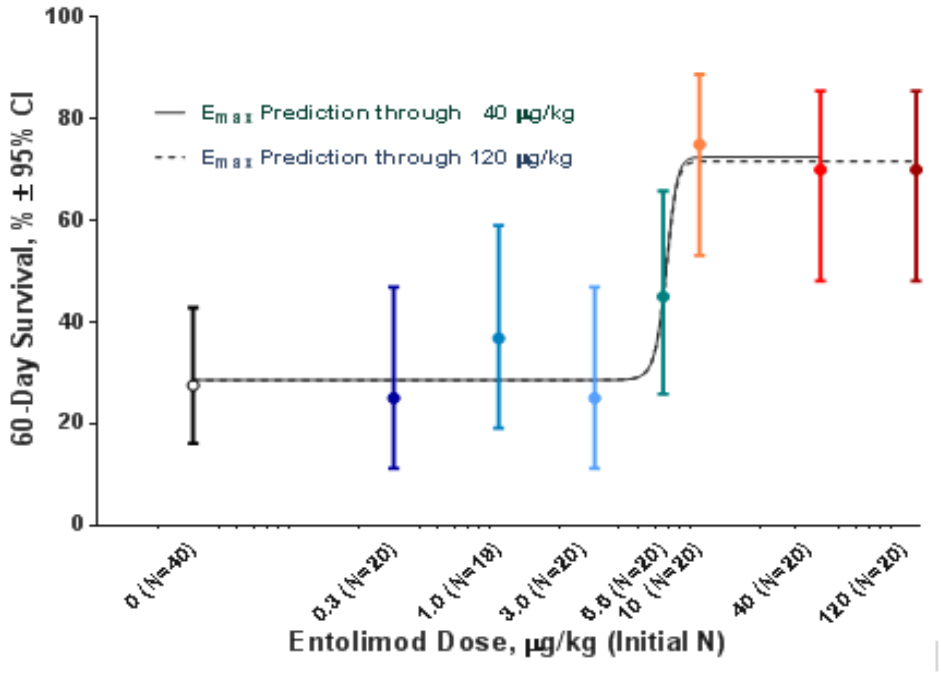
Note: Data show number of deaths occurring during study day (not the total number of deaths) and number of animals remaining alive at the end of study day.

a. Two animals were euthanized for moribundity prior to the scheduled terminal euthanasia on Day 61: one animal (ID Number 200242, female, 120 µg/kg entolimod) was euthanized on Day 15 and one animal (ID Number 200121, male, 10 µg/kg entolimod) was euthanized on Day 25.

Source: T0304.DOC, 21 FEBRUARY 2014

The TBI dose that was used in Study Rs-23 was intended to be an LD70/60, ie, a radiation dose that would induce death in ~70% of the control animals over a 60-day post-radiation period. The 60-day mortality rate was 72.5% (29 of 40) in the placebo control group confirms that the intended radiation exposure was achieved.

The entolimod effect on survival showed a sigmoidal dose-benefit curve. Among entolimod-treated animals, an improvement in survival was observed starting from a dose of 6.6 mg/kg and showed maximal effect at a dose of 10 µg/kg, with survival benefit plateauing across doses of 10, 40, and 120 µg/kg (fig. 19).



Abbreviation: CI=confidence interval, E_{max}=maximum effect attributable to the drug.
Note: Survival was modeled as a function of entolimod dose using a 4-parameter E_{max} model, with maximum likelihood as the fitting criterion.
Source: Report Rs-23, Section 11.4.1.

Figure 19. Survival proportions on day 60 in study Rs-23 by entolimod dose (ITT population)

The stable effect as shown in the plateau for survival for the three highest dose groups is a clear strength of this application. This seems to exclude chance findings as reason for the survival benefit observed and may indicate a really promising benefit for the target population.

The applicant should provide a new survival curve for placebo, 10 and 40 µg/kg entolimod doses specifying the number of exposed subjects at every time point.

The applicant claims that survival at Day 60 was statistical significantly greater in the 10-, 40-, and 120-µg/kg dose groups than in the placebo group (trend tests; adjusted p=0.0021, adjusted p<0.0001, and nominal unadjusted p<0.0001, respectively) as illustrated in the following Table 12. In the placebo control group, 72.5% of the animals died versus only 25% in the 10-µg/kg group, resulting in 47.5% absolute increase in survival and a survival odds ratio of 7.9 in favor of entolimod.

Table 12: Survival and Odds Ratios for Survival to Day 60 in Study Rs-23 by Entolimod Dose (Intent-to-Treat Population)

Parameter/Statistic	Entolimod Dose (µg/kg)							
	0.0 (N=40)	**0.3 (N=20)**	**1.0 (N=19)**	**3.0 (N=20)**	**6.6 (N=20)**	**10.0 (N=20)**	**40.0 (N=20)**	**120.0 (N=20)**
Survival, n (%)	11 (27.5%)	5 (25.0%)	7 (36.8%)	5 (25.0%)	9 (45.0%)	15 (75.0%)	14 (70.0%)	14 (70.0%)
95% CI lower bound	16.1%	11.2%	19.1%	11.2%	25.8%	53.1%	48.1%	48.1%
95% CI upper bound	42.8%	46.9%	59.0%	46.9%	65.8%	88.8%	85.5%	85.5%
Unadjusted p-value[a]	n/a	0.5817	0.2580	0.4473	0.1439	0.0021	<0.0001	<0.0001
Adjusted p-value[b]	n/a	0.5817	0.4473	0.4473	0.1439	0.0021	<0.0001	n/a
Survival odds ratio[c]	n/a	0.9	1.5	0.9	2.2	7.9	6.2	6.2
95% CI lower bound	n/a	0.2	0.4	0.2	0.6	2.0	1.6	1.6
95% CI upper bound	n/a	3.4	5.6	3.4	7.6	33.6	24.2	24.2

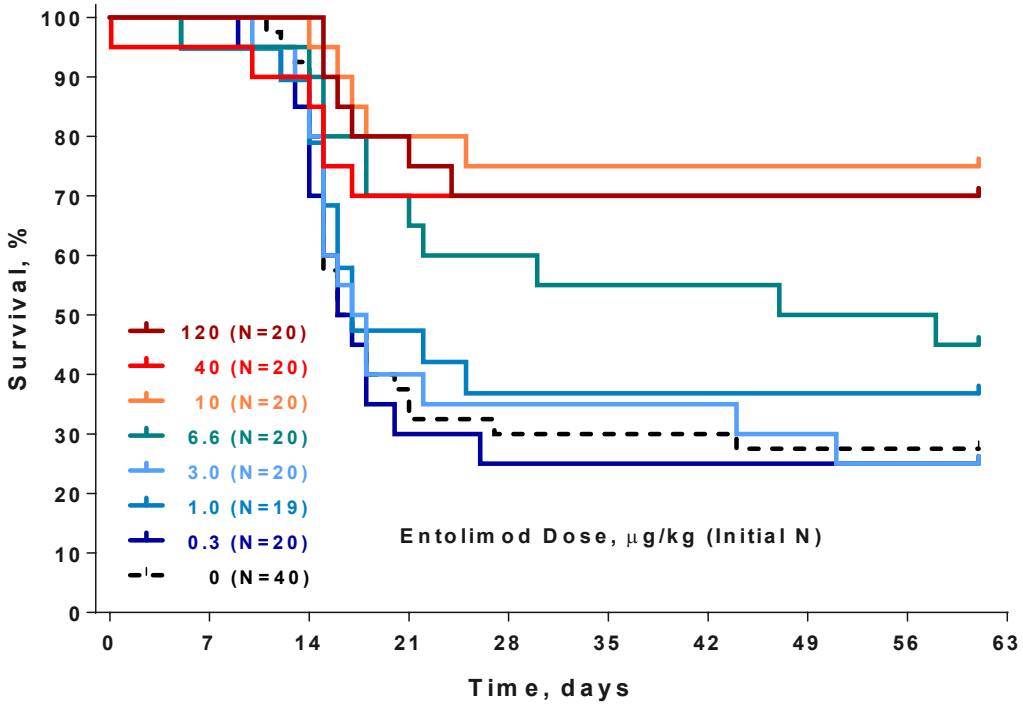
Abbreviations: CI=confidence interval; n/a=not applicable.

a. Unadjusted p-values are one-sided and are derived from trend tests of survival involving doses from placebo (0.0-µg/kg entolimod dose) through the dose of the column header.
b. Adjusted p-values were obtained by treating the trend tests in a hierarchal sequence, starting with all doses through 40 µg/kg and then progressively eliminating doses until only placebo and 0.3 µg/kg dose groups were compared. The exploratory 120-µg/kg dose group was not part of this hierarchy; only its unadjusted p-value is reported, which should be interpreted as a descriptive statistic.)
c. Odds ratios describe the comparison of treatment group odds of survival to placebo group odds. Confidence limits on survival are based on the Wilson method.

Source: Report Rs-23, Section 11.4.1.

Based on Kaplan-Meier analysis, the majority of deaths occurred during the 3rd to 4th week of observation in all of the treatment groups (Figure 0). Median survival ranged from 16.5 to 17.5 days in the animals that received placebo or the 0.3-, 1.0-, or 3.0-µg/kg doses of entolimod and was 52.5 days in the animals that received the 6.6-µg/kg dose of entolimod and >60 days in the animals that received the 10-, 40-, or 120-µg/kg dose of entolimod. No deaths occurred after Day 25 in the animals that were treated at doses of ≥10 µg/kg of entolimod.

Figure 20: Kaplan-Meier Plot of Survival in Study Rs-23 by Entolimod Dose and Time (Intent-to-Treat Population).



Source: Report Rs-23, Section 11.4.1.

Survival was evaluated for covariate effects by sex (male/female), body weight (less than the median/greater than or equal to median), body width (less than the median/greater than or equal to median), entolimod-reactive antibody titre (less than the median/greater than or equal to median), radiation dose (less than the median/greater than or equal to median), and study phase (A, B, C, D). None of the covariates analysed demonstrated either an independent or an interactive effect on the study primary outcome of survival. The treatment effect that was seen in the primary analysis was maintained independent of sex, body weight, body width, entolimod-reactive antibody titre at baseline, individual animal radiation dose, or study phase.

Moreover, with respect to the reliability of the difference between statistically insignificant and highly significant increase in survival of NHPs it should be noted that the difference claiming the trial successful is driven by few (probably 2 or 3) animals per group only. This is obvious from a comparison of the number of animals survived in the different treatment groups as illustrated in the Table 47:

Table 47: Number (%) of animals surviving to day 60 with unadjusted and adjusted p-values and survival odds ratio

CBLB502 Dose	Overall Survival	Unadjusted P-value	Adjusted P-value	Survival odds ratio (vs vehicle)
0.0	11/40 (27.5)	-	n/a	-
0.3	5/20 (25.0)	0.5817	0.5817	0.9
1.0	7/19 (36.8)	0.2580	0.4473	1.5
3.0	5/20 (25.0)	0.4473	0.4473	0.9
6.6	9/20 (45.0)	0.1439	0.1439	2.2
10.0	15/20 (75.0)	0.0021	0.0021*	7.9
40.0	14/20 (70.0)	<.0001	<.0001*	6.2
120.0	14/20 (70.0)	<.0001	n/a	6.2

Source: Study CBL1.1-N-01-Rs-23

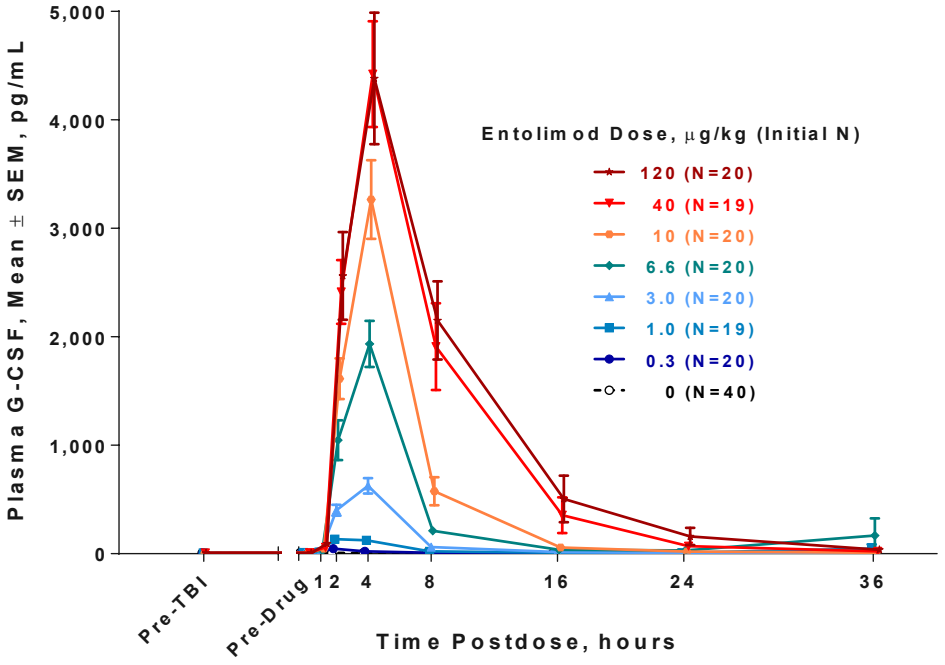
In order to fully understand the impact of number of animals on the level of statistical significance in the pivotal trial Rs-23, the applicant is requested to provide a table which allows to assess the impact of the number of animals survived on the unadjusted p-value, the adjusted p-value and the survival odds ratios as reported in Table 47 of CBL1.1-N-01-Rs-23 study report.

Whether the broad claim for efficacy in a broad human population can be already sufficiently justified with the increased survival observed in total 10 or 12 animals allotted into three groups, can be challenged. It should be noted that the general rationale behind the approach of trial Rs-23 with inclusion of many dose finding groups is fully adequate for an exploratory trial. However, it seems hard to accept pivotal claims from such a trial design. In particular, as the evidence for efficacy is restricted to one pivotal trial in NHPs.

AUC0-24 of plasma G-CSF

Mean plasma concentrations of G-CSF increased rapidly after administration of entolimod, generally peaking at approximately 4 hours postdose and returning to baseline values by 4 to 36 hours postdose, depending on dose level (Figure).

Figure 21: Plasma G-CSF Concentrations in Study Rs-23 by Entolimod Dose and Time (As-Treated Population)



Abbreviations: G-CSF=granulocyte colony-stimulating factor, SEM=standard error of the mean, TBI=total body irradiation.
Source: Report Rs-23, Section 11.4.4.1.

The adjusted AUC_{0-24} (adjAUC_{0-24}) of plasma G-CSF increased with increasing entolimod dose, plateauing across doses of 40 to 120 µg/kg (Figure 2).

Figure 2: AdjAUC_{0-24} for Plasma G-CSF in Study Rs-23 by Entolimod Dose (As-Treated Population)

The values for adjAUC_{0-24} of plasma G-CSF were significantly greater for each of the entolimod doses than for placebo (trend tests $p<0.0001$ for all comparisons) (Table).

Table 13: AdjAUC$_{0-24}$ of Plasma G-CSF (h·pg/mL) in Study Rs-23 by Entolimod Dose (As-Treated Population)

Statistic	Entolimod Dose (µg/kg)							
	0.0 (N=40)	**0.3 (N=20)**	**1.0 (N=19)**	**3.0 (N=20)**	**6.6 (N=20)**	**10.0 (N=20)**	**40.0 (N=19)**	**120.0 (N=20)**
N	37[a]	20	19	20	20	20	19	20
Mean	9	132	674	2,872	8,918	16,078	31,302	34,526
Median	0	81	549	2,908	8,704	14,869	19,852	31,881
Adjusted Geometric Mean[b]	1	79	511	2,450	7,860	13,629	24,915	26,704
SEM	3	27	129	317	997	2,048	5,084	6,205
Minimum	0	5	143	610	3,125	3,781	6,661	3,388
Maximum	90	373	2,415	5,669	19,024	37,405	80,690	136,917
P-value[c]	n/a	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

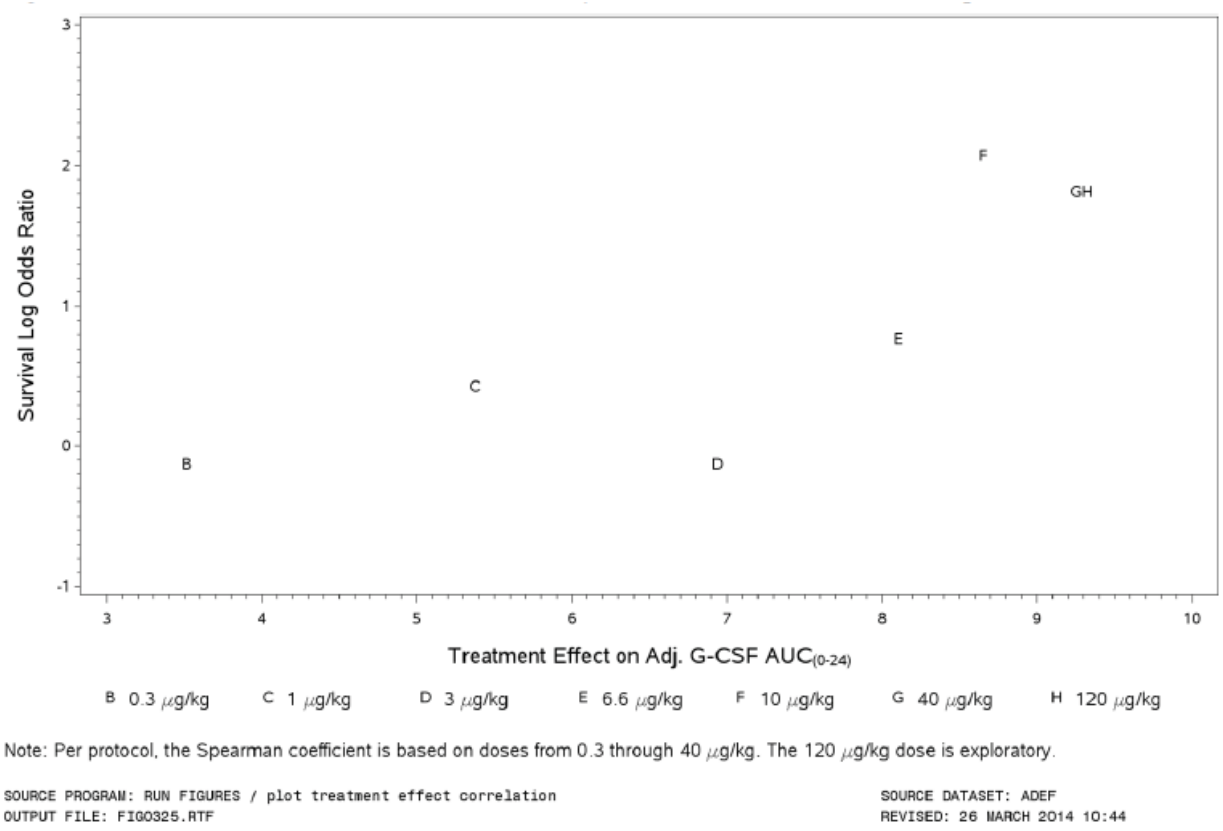
Abbreviations: AUC=area under the curve, adjAUC$_{0-24}$=adjusted area under the plasma concentration-time curve from time 0 to 24 hours postdose, G-CSF=granulocyte colony-stimulating factor, SEM=standard error of the mean.

a. The adjAUC$_{0-24}$ was not calculable for 3 animals in the placebo group because they have a pattern of background-adjusted concentrations (a positive value at time zero with the remaining values zero) for which the WinNonlin software was designed not to calculate the AUC.
b. The adjusted geometric mean is a variant of the geometric mean for skewed data which may include values of zero. It is defined as the exponential of the mean of the natural log of 1 plus the data value, then minus 1.
c. P-values are one-sided, based on a trend test within analysis of variance and encompassing all doses through each column dose. Statistical significance for the trend test of AUC$_{0-24}$ versus placebo through an entolimod dose of 40 µg/kg was evaluated relative to a pre-specified 0.00625 significance level. All other p-values are considered descriptive statistics.

Source: Report Rs-23, Section 11.4.4.1.

The Spearman rank correlation of AUC_{0-24} of plasma G-CSF value and survival was 0.84 (fig. 23).

Figure 23. Treatment effect correlation: survival by AUC_{0-24} of G-CSF – As-Treated Population



Note: Per protocol, the Spearman coefficient is based on doses from 0.3 through 40 µg/kg. The 120 µg/kg dose is exploratory.

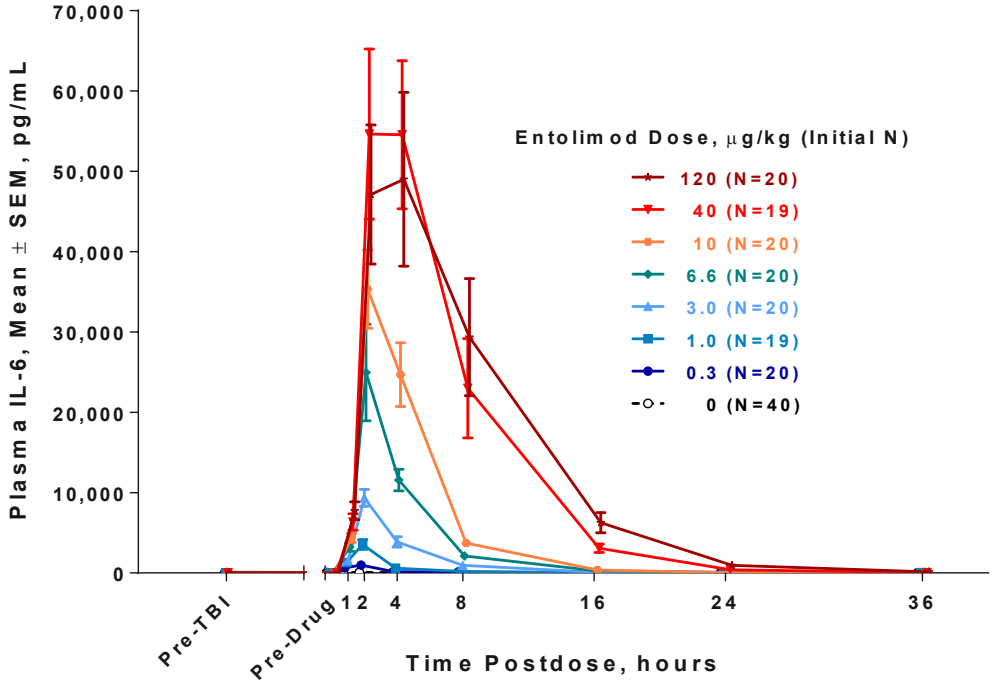
SOURCE PROGRAM: RUN FIGURES / plot treatment effect correlation
OUTPUT FILE: FIGO325.RTF

SOURCE DATASET: ADEF
REVISED: 26 MARCH 2014 10:44

AUC0-24 of plasma IL-6

Mean plasma concentrations of IL-6 increased rapidly after entolimod administration, generally peaking at ~2 hours postdose (Figure 3).

Figure 3: Plasma IL-6 Concentrations in Study Rs-23 by Entolimod Dose and Time (As-Treated Population)



Abbreviations: IL-6=interleukin-6, SEM=standard error of the mean, TBI=total body irradiation.
Source: Report Rs-23, Section 11.4.4.2.

$AdjAUC_{0-24}$ values for plasma IL-6 increased with increasing entolimod dose, plateauing across the doses of 40 and 120 µg/kg (Figure).

Figure 25: $AdjAUC_{0-24}$ for Plasma IL-6 in Study Rs-23 by Entolimod Dose (As-Treated Population)

The values for $adjAUC_{0-24}$ of plasma IL-6 were significantly greater for each of the entolimod doses than for placebo (trend tests $p<0.0001$ for all comparisons) (Table 14).

Table 14: $AdjAUC_{0-24}$ of Plasma IL-6 (h·pg/mL) in Study Rs-23 by Entolimod Dose (As-Treated Population)

Statistic	Entolimod Dose (µg/kg)							
	0.0 (N=40)	**0.3 (N=20)**	**1.0 (N=19)**	**3.0 (N=20)**	**6.6 (N=20)**	**10.0 (N=20)**	**40.0 (N=19)**	**120.0 (N=20)**
N	40	20	19	20	20	20	19	20
Mean	484	3,225	9,695	33,555	90,035	156,389	415,398	454,790
Median	277	2,400	6,965	27,977	88,378	114,748	265,978	324,712
Adjusted Geometric Mean [a]	300	2,465	7,904	28,368	77,889	133,953	315,705	317,181
SEM	106	535	1,445	4,379	11,374	20,427	76,507	94,342
Minimum	47	723	2,239	10,287	28,002	49,249	111,257	26,335
Maximum	3,978	8,484	23,039	67,513	253,107	332,653	1,347,421	1,756,479
P-value[b]	n/a	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001[b]	<0.0001

Abbreviations: adjAUC_{0-24}=adjusted area under the plasma concentration-time curve from time 0 to 24 hours postdose, IL-6=interleukin-6, n/a=not applicable, SEM=standard error of the mean.

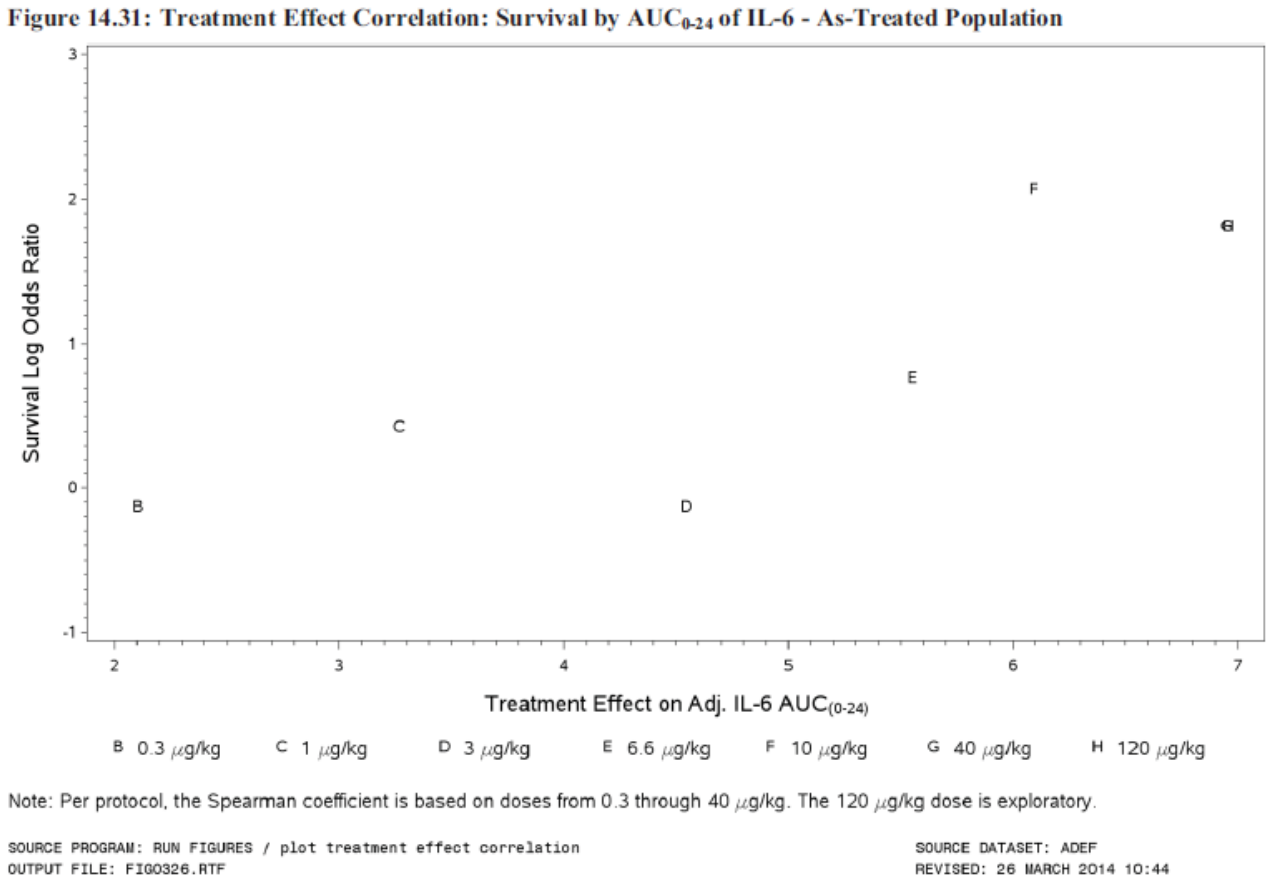
[a]. The adjusted geometric mean is a variant of the geometric mean for skewed data which may include values of zero. It is defined as the exponential of the mean of the natural log of 1 plus the data value, then minus 1

b. P-values are one-sided, based on a trend test within analysis of variance and encompassing all doses through each column dose. Statistical significance for the trend test of AUC_{0-24} versus placebo through an entolimod dose of 40 µg/kg was evaluated relative to a pre-specified 0.00625 significance level. All other p-values are considered descriptive statistics.

Source: Report Rs-23, Section 11.4.4.2.

The Spearman rank correlation of AUC_{0-24} of plasma IL-6 value and survival was 0.84 (Fig. 26).

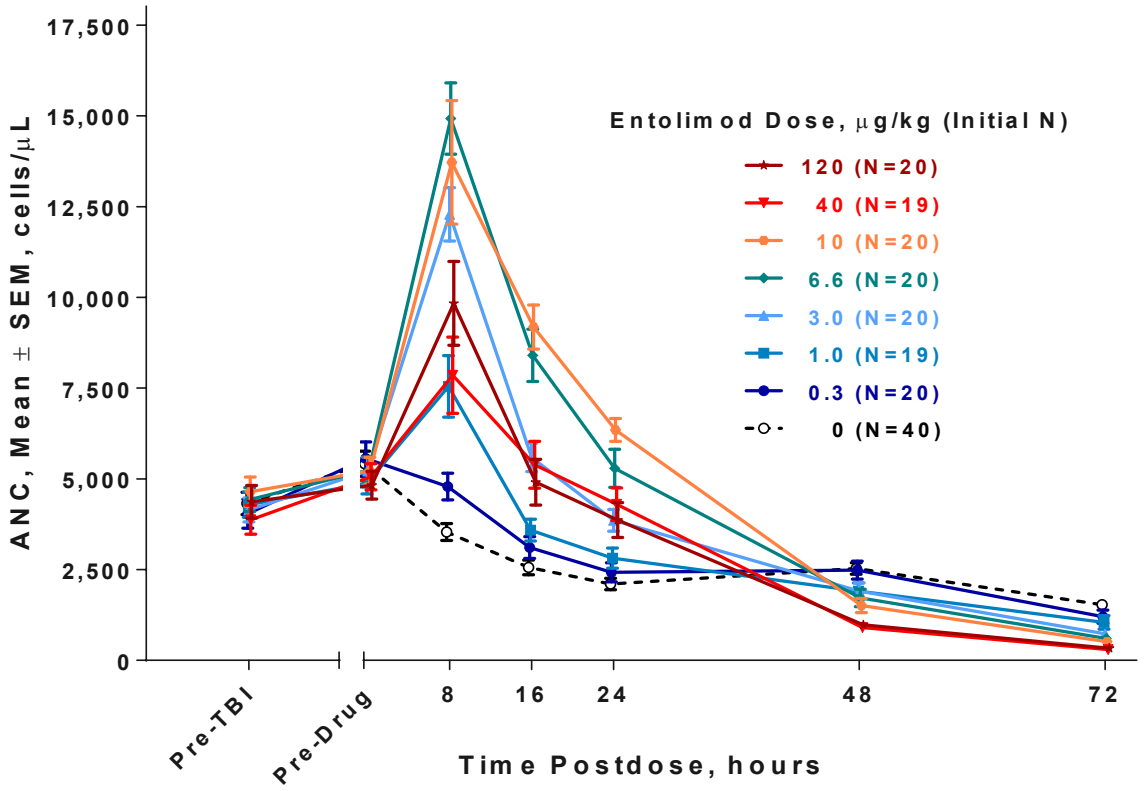
Figure 26. Treatment effect correlation: survival by AUC_{0-24} of IL-6 – As-Treated Population



ANC24

Absolute neutrophil count increased rapidly after administration of entolimod, peaking at ~8 hours post-dose and falling below predose values by 16 to 48 hours postdose, depending on dose levels (Figure 24).

Figure 24: ANC in Study Rs-23 by Entolimod Dose and Time (As-Treated Population)



Abbreviations: ANC=absolute neutrophil count, SEM=standard error of the mean, TBI=total body irradiation.
Source: Report Rs-23, Section 11.4.4.3.

There was a trend for increasing fold change in ANC at 24 hours post-dose through a dose of 6.6 µg/kg, with plateauing of the effect at higher doses (Figure 25).

Figure 25: ANC24 in Study Rs-23 at 24 Hours by Entolimod Dose (As-Treated Population)

The fold increase in ANC at 8, 16, and 24 hours postdose was greater at entolimod doses of ≥3 µg/kg than in the placebo group (trend tests p<0.0001 for all comparison) (Table 1).

Table 1: Fold Change in ANC in Study Rs-23 through 24 Hours Postdose by Entolimod Dose (As-Treated Population)

Parameter/Statistic	Entolimod Dose, µg/kg (N)							
	0.0 (N=40)	0.3 (N=20)	1.0 (N=19)	3.0 (N=20)	6.6 (N=20)	10.0 (N=20)	40.0 (N=19)	120.0 (N=20)
Fold change in ANC at 24 hours postdose								
N	40	20	19	20	20	20	19	20
Mean	0.41	0.44	0.60	0.78	1.04	1.36	0.91	0.86
Median	0.39	0.41	0.63	0.76	1.16	1.23	0.86	0.73
SEM	0.02	0.03	0.06	0.07	0.08	0.13	0.12	0.10
Minimum	0.18	0.18	0.15	0.38	0.13	0.31	0.29	0.16
Maximum	0.72	0.72	1.31	1.56	1.55	2.91	2.50	1.95
P-value[a]	n/a	0.2330	0.0057	<0.0001	<0.0001	<0.0001	<0.0001 [a]	<0.0001
Fold change in ANC at 16 hours postdose								
N	40	20	19	20	20	20	19	20
Mean	0.50	0.57	0.77	1.14	1.66	1.92	1.15	1.08
Median	0.46	0.55	0.74	1.19	1.81	1.80	0.87	0.98
SEM	0.04	0.04	0.07	0.09	0.12	0.17	0.16	0.14
Minimum	0.24	0.31	0.30	0.47	0.63	0.56	0.39	0.21
Maximum	1.46	0.97	1.24	2.48	2.50	3.78	2.89	2.76
P-value[a]	n/a	0.0668	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
Fold change in ANC at 8 hours postdose								
N	40	20	19	20	20	20	19	20
Mean	0.70	0.90	1.57	2.53	3.01	2.86	1.73	2.26
Median	0.66	0.88	1.66	2.41	2.82	2.48	1.47	2.26
SEM	0.06	0.06	0.16	0.22	0.21	0.33	0.29	0.27
Minimum	0.26	0.45	0.60	1.35	1.39	0.75	0.29	0.35
Maximum	2.91	1.69	2.95	5.81	5.03	6.23	4.77	4.67
P-value[a]	n/a	0.0142	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001

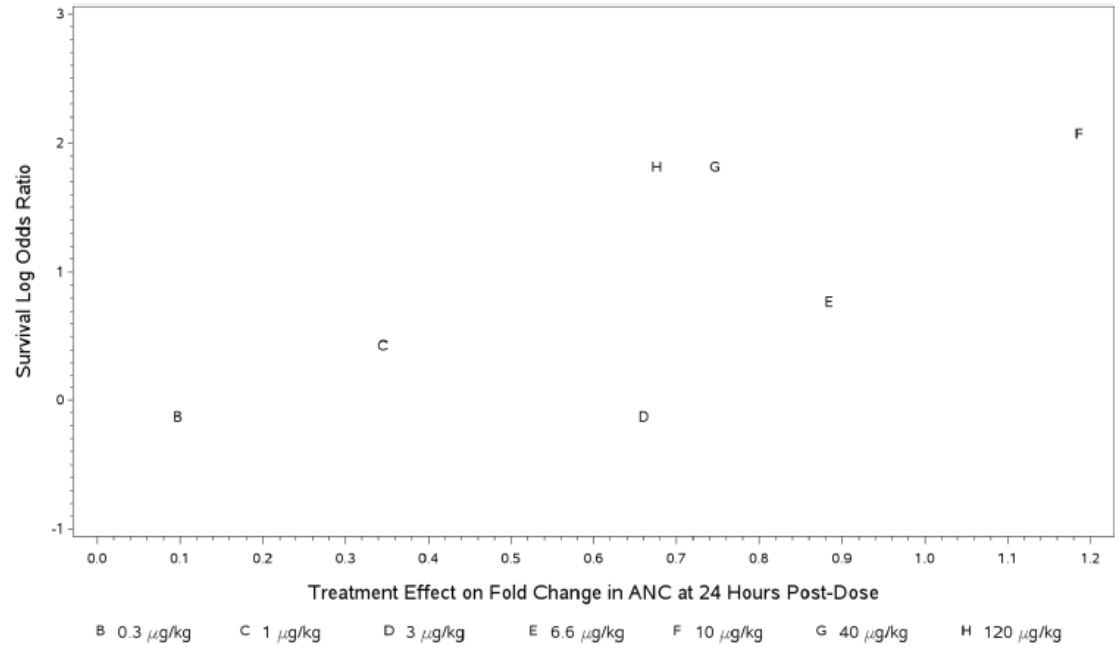
Abbreviations: ANC=absolute neutrophil count, SEM=standard error of the mean.

a. P-values are one-sided, based on a trend test within analysis of variance and encompassing all doses through each column dose. Statistical significance for the trend test of fold change at 24 hours postdose versus placebo through an entolimod dose of 40 µg/kg was evaluated relative to a pre-specified 0.00625 significance level. All other p-values are considered descriptive statistics.

b. Source: Report Rs-23, Section 11.4.4.2.

The Spearman rank correlation of ANC24 value and survival was 0.84 (Fig. 29).

Figure 29. Treatment effect correlation: survival by fold change in ANC at 24 hours postdose – As-Treated Population



Note: Per protocol, the Spearman coefficient is based on doses from 0.3 through 40 μg/kg. The 120 μg/kg dose is exploratory.

SOURCE PROGRAM: RUN FIGURES / plot treatment effect correlation
OUTPUT FILE: FIG0327.RTF

SOURCE DATASET: ADEF
REVISED: 26 MARCH 2014 10:44

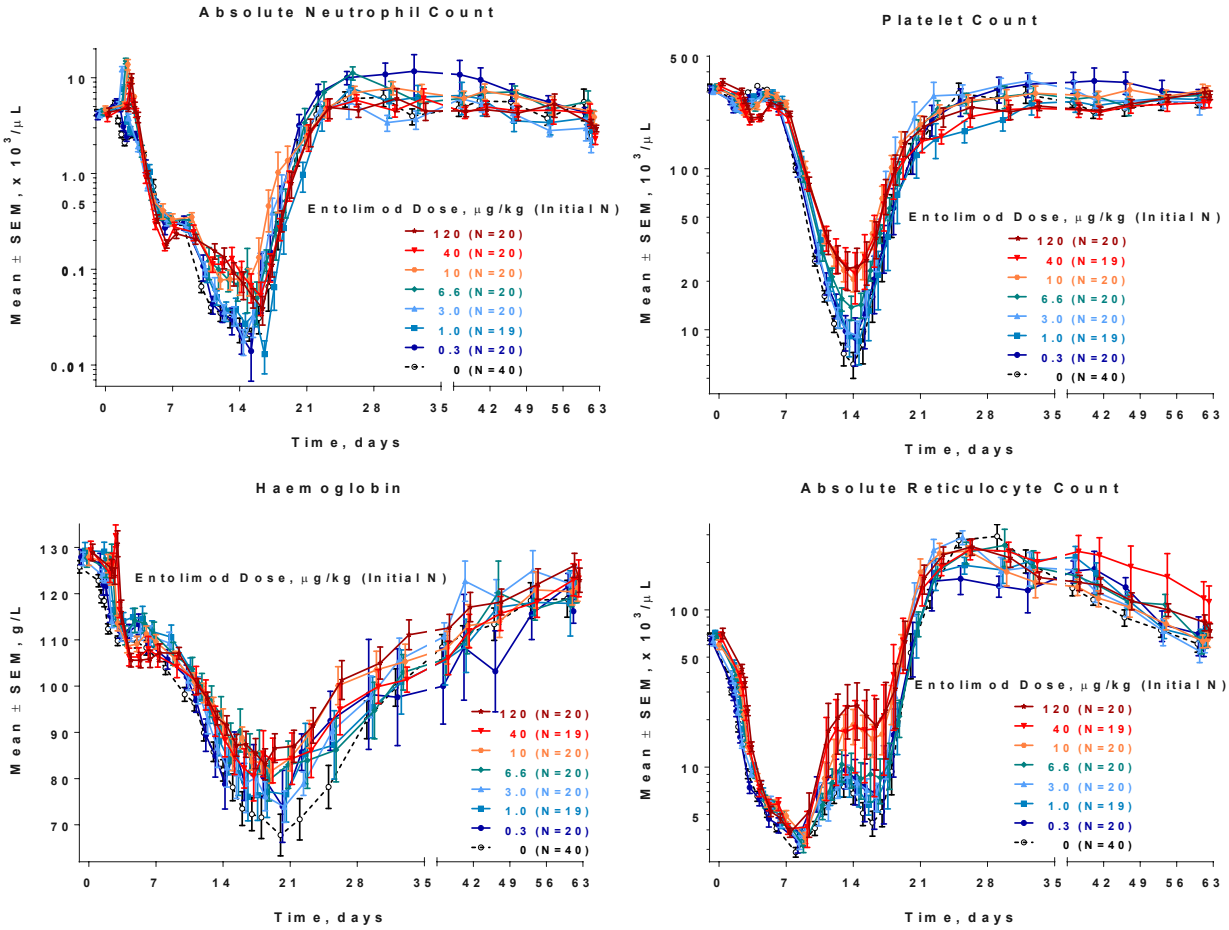
Although the outcome of the secondary endpoints entolimod has shown that in irradiated animals significant dose-dependent increases for the biomarkers AUC0-24 of plasma G-CSF, AUC0-24 of plasma IL-6 occurred and ANC24 occurred as expected, the link between the measured PD marker and overall survival at day 60 remains difficult to understand. The applicant should provide the survival curve showing the AdjAUC0-24h for ANC in order to have a complete demonstration of the effect of all entolimod doses in ANC.

The applicant should provide AdjAUC0-24, and AdjAUC0-48 for plasma ANC by entolimod Dose for the As-Treated Population.

HAEMATOLOGY PARAMETERS

The dose of TBI that was administered in Study Rs-23 produced haematological toxicity, resulting in neutropenia, thrombocytopenia, and anaemia in all treatment groups (Figure 30). In the placebo group, average neutrophil and platelet nadirs occurred on Days 14 to 15, with many animals having undetectable circulating neutrophils or platelets. TBI also provoked an average ~60-g/L decrease in haemoglobin in controls. Entolimod improved neutrophil, platelet, and haemoglobin nadir values and reticulocytosis in a dose-dependent manner.

Figure 6: Haematology Parameters in Study Rs-23 by Entolimod Dose and Time (As-Treated Population)



Abbreviation: SEM=standard error of the mean.
Source: Report Rs-23, Section 11.4.6.

Entolimod doses ≥10 µg/kg significantly improved the proportions of days that animals were both alive and free of severe neutropenia, thrombocytopenia, and anaemia (table 16).

Table 16: Proportions of Days Alive and Free of Cytopenias in Study Rs-23 by Severity and Entolimod Dose (As-Treated Population)

Parameter/Statistic	Entolimod Dose (µg/kg)							
	0.0 (N=40)	0.3 (N=20)	1.0 (N=19)	3.0 (N=20)	6.6 (N=20)	10.0 (N=20)	40.0 (N=19)	120.0 (N=20)
Severe neutropenia (ANC <500 cells/µL), % of days								
Mean	29	27	34	31	46	62	58	56
Median	9	8	10	12	60	78	75	75
Standard deviation	32	32	35	32	34	32	32	33
Range	5-85	5-83	3-82	3-82	3-83	7-88	5-82	3-82
p-value[a]							<0.0001	<0.0001
Clinically relevant neutropenia (ANC <1,000 cells/µL), % of days								
Median	26	24	30	28	43	58	55	52
Median	7	7	7	7	57	73	72	71
Standard deviation	30	31	33	31	34	31	31	32
Range	3-80	3-80	3-77	3-77	2-82	3-83	3-78	2-78
p-value[a]							<0.0001	<0.0001
Severe thrombocytopenia (platelet count $<20 \times 10^3$ cells/µL), %								
Mean	39	37	46	43	62	78	75	74
Median	17	17	20	19	84	95	93	95
Standard deviation	34	36	39	35	39	32	36	36
Range	15-100	13-100	8-100	15-100	8-100	15-100	15-100	15-100
p-value[a]							<0.0001	<0.0001
Clinically relevant thrombocytopenia (platelet count $<50 \times 10^3$ cells/µL), %								
Mean	36	33	41	39	55	73	69	69
Median	15	15	15	16	73	91	88	88
Standard deviation	32	33	35	34	35	33	34	35
Range	12-93	12-95	8-93	12-93	8-92	15-100	12-100	12-100
p-value[a]							<0.0001	<0.0001
Severe anaemia (haemoglobin <80 g/L), %								
Mean	40	39	45	44	60	75	71	74
Median	24	24	23	27	67	90	80	92
Standard deviation	30	29	35	31	35	31	33	33
Range	17-100	13-100	8-100	17-100	8-100	20-100	17-100	20-100
p-value[a,b]								

Clinically relevant anaemia (haemoglobin <100 g/L), %								
Mean	28	25	37	32	44	57	53	53
Median	17	18	20	22	37	68	52	63
Standard deviation	24	21	30	22	29	27	32	30
Range	3-90	7-88	8-97	8-83	8-95	17-88	7-100	8-100
p-value[a,b]								

Abbreviation: ANC=absolute neutrophil count.

a. P-values are one-sided, based on a trend test within analysis of variance and encompassing all doses through 40.0 µg/kg or 120.0 µg/kg.

b. An analysis of anaemia was not prespecified; thus, trend testing was not performed for this parameter.

Source: Report Rs-23, Section 11.4.6.

It remains not plausible that the short time effects documented are a valid explanation for the findings observed. Confirmed by the published results by Farese et al (2013 and 2015), irradiated NHPs G-CSF needs to remain increased for significant longer time as 24 h as indicated by administration of filgrastim 10µg/kg daily until nadirs >1000 ANCs or pegfilgrastim 300µg/kg on day 1 and 8 in irradiated NHPs to be of benefit in this species. Moreover, the extensive clinical experience with the therapeutic use of G-CSF indicates that sufficient long duration of increase of levels of G-CSF is essential for recovery of the haematopoietic system after different sources of toxicity. Insofar, it seems rational to task the changes in IL-6 for the effects observed in nadir. But again due to the short half-life of this cytokine, sustainable effects are rather implausible. As IL-6 in addition has an important impact on safety events and the increase probably indicates a high risk for uncontrolled cytokine storm (please refer to the safety section), the impact of the changes observed needs to be further assessed.

The applicant is requested to show data which allows concluding on febrile neutropenia events during the nadir period in the animals. The applicant is requested to analyse differences observed in the cageside observation during the time of nadir.

In order to fully clarify the level of supportive care administered in trial Rs-23 the applicant is requested to provided more information regarding the supportive care the animals received during the nadir of clinical relevant neutropenia in those animals who survived and those who died in order to exclude bias. The applicant is requested to analyse differences observed in the cage side observation during the time of nadir.

NECROPSY FINDINGS

Considering all animals, 99 (55.3%) died prematurely, and 80 (44.7%) underwent planned euthanasia on Day 61. The majority of animals receiving doses of entolimod ≤6.6 had early deaths (n=82) while only a minority of animals receiving doses of entolimod ≥10 µg/kg (n=17) died early.

Irradiation-related hemorrhagic or septic phenomena were most commonly implicated in the cause of death among animals dying prematurely. In multiple animals, severe gastrointestinal lesions were judged to have contributed to the cause of death.

Considering all animals, the highest incidences of necropsy findings were those associated with the gastrointestinal system (79.2%). In particular, hemorrhagic lesions (60.7%) and hyperemia (29.8%) of the bowel were observed. In general, gastrointestinal findings appeared more frequently in animals receiving placebo or entolimod doses ≤6.6 than in those receiving entolimod doses ≥10 µg/kg. In the respiratory system, hemorrhagic (62.4%) and septic lesions (23.0%) of the lung were often described. Incidences of such findings were generally higher among animals treated with placebo or entolimod doses ≤6.6 µg/kg than among those treated with entolimod doses ≥10 µg/kg. In the urinary system, hemorrhagic lesions of the bladder (34.8%) were most commonly observed and showed a dose-dependent pattern favoring higher entolimod doses. Hemorrhagic lesions of the kidney (24.7%) were fairly uniformly present across all treatment groups. Skin findings were frequent, comprising primarily hemorrhagic (40.4%) or erosive lesions (18.5%) with. Many animals had cardiac hemorrhagic lesions (39.3%) or pericardial effusions (25.8%). In the lymphoid system, the dominant observations were lymph node hemorrhages (25.8%). Fewer such findings in animals receiving entolimod doses ≥10 µg/kg. Findings in the brain, reproductive, and endocrine systems were infrequent, typically comprising infectious or hemorrhagies.

The applicant should provide a table summarising the incidence and type of necropsy findings by organ system in study Rs-23 for placebo and 10 and 40 µg/kg doses for those animals who were euthanized at day 61 and for those who died before day 60. The applicant is also asked to discuss whether the

necropsy findings in the Rs-23 study can support any entolimod non-G-CSF mechanism of action (e.g. a possible reduction in GI toxicity.

Several mortality periods were noted. During Days 0 through 5, only 3 animals died (none had been treated with doses of entolimod ≥10 µg/kg). From Days 9 to 13, ten animals died, primarily with hemorrhagic complications of ARS, and only 1 had been treated with doses of entolimod ≥10 µg/kg. The substantial majority of animals dying prematurely (66 of 99), died on Days 14 through 18 with deaths attributable to severe combined hemorrhagic and septic complications, bowel intussusception, and pulmonary edema. Only 12 of the 66 animals that died during this time period that had been treated with doses of entolimod ≥10 µg/kg. During Days 19 through 30, there were 15 deaths; in these animals, generalized sepsis appeared to predominate, with several animals also having hemorrhagic lesions in the lung, pulmonary edema, or severe bowel intussusceptions. Of the 15 animals, only 3 had been treated with doses of entolimod ≥10 µg/kg. After a hiatus in deaths from Days 31-43, 5 animals (that had been administered study drug at dose levels of 0, 3, 6.6, 3, and 6.6 µg/kg, respectively) died between Days 44 and 58. All of these animals had apparently recovered from ARS. At necropsy, 4 of 5 animals had generalized hemorrhagic syndrome with death likely occurring from gastrointestinal bleeding. One animal was not subjected to necropsy due to suspicions of contagious infection.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 4. Summary of efficacy for trial Rs-23 in $LD_{70/60}$ irradiated NHPs

Title:	**A Randomized, Blinded, Placebo-Controlled Single-Center Study of Dose-dependent Effect of CBLB502 on Survival and Biomarker Induction in Rhesus Macaques (Maccaca mulatta) Exposed to 7.2 Gy (LD70/60) Total Body Irradiation when Administered Intramuscularly at 25 hours after Radiation Exposure**		
Study identifier	CBL1.1-N-01-**Rs-23**		
Design	For logistical reasons, animals were acquired, treated, and evaluated in 4 sequential phases (Phase A followed chronologically by Phase B, Phase C, and then Phase D). In all phases, animals first underwent a minimum 45-day acclimation process at the test facility. During each study phase, several cohorts of 9 animals were placed in restraining chairs in a shielded room and exposed to total body irradiation (TBI) at a target dose level of 7.2 Gy by bilateral exposure to gamma rays from the radioactive isotope, Cobalt-60. Animals were stratified by gender, body weight, and body width into cohorts of 9.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		60 days 45-day acclimation process at the test facility N/A (necropsy in all animals)
Hypothesis		Superiority for overall survival and Exploratory: dose finding	

Treatments groups		Animals were randomized to 1 of 8 treatment groups (0 [placebo control], or 0.3, 1.0, 3.0, 6.6, 10, 40, or 120 g/kg of entolimod) in a ratio of 2:1:1:1:1:1:1:1, and each received a single IM dose of placebo control or entolimod at 25 hours after completion of TBI. Each treatment group includes 20 animals; while the placebo group includes 40 animals	
Endpoints and definitions	Primary endpoint	Survival within 60 days after TBI <free text>	
	Secondary endpoint		Area under the curve from 0 to 24 hours after entolimod administration (AUC0-24) of plasma granulocyte colony-stimulating factor (G-CSF)
	Secondary endpoint		AUC0-24 of plasma interleukin-6 (IL-6)
	Secondary endpoint		Fold increase of absolute neutrophil count (ANC) at 24 hours after entolimod administration
	Secondary endpoint		Proportion of days alive and free of severe neutropenia (<500 neutrophils/µL blood)
	Secondary endpoint		Proportion of days alive and free of clinically relevant neutropenia (<1,000 neutrophils/µL blood)
	Secondary endpoint		Proportion of days alive and free of severe thrombocytopenia (<20 x 103 platelets/µL blood)
	Secondary endpoint		Proportion of days alive and free of clinically relevant thrombocytopenia (<50 x 103 platelets/µL blood)
Database lock	8 March 2012 (Completion of in-life phase Date of the report**:** 11 June 2014; Revised 19 August 2014		

Results and Analysis

Analysis description		**Primary Analysis**			
Analysis population and time point description		Intent to treat			
Descriptive statistics and estimate variability	Entolimod dose administered at 25 h after TBI	0 µg/kg	6,6 µg/kg	10µg/kg	40µg/kg
	Number of subject	40	20	20	20
	Primary endpoint: Survival within 60 days after TBI	11/40 (27.5%)	9/20 (45%)	15/20 (75%)	14/20 (70%)
	95% CI	16.1%-42.8%	25.8%-65.8%	53.1%-88.8%	48.1%-85.5%
	Unadjusted p-value	n/a	0.1439	0.0021	<0.0001
Effect estimate per comparison	**Secondary endpoint:** Area under the	Mean 9	Mean 8,918	Mean 16,078	Mean 31,302

	curve from 0 to 24 hours after entolimod administration (AUC0-24) of plasma G-CSF) AdjAUC0-24, [h·pg/mL]	Median 0	Median 8,704	Median 14,869	Median 19,853
		SD 21	SD 4,459	SD 9,159	SD 22,159
	Secondary endpoint: AUC0-24 of plasma interleukin-6 (IL-6) after entolimod administration [h·pg/mL]	Mean 484	Mean 90,035	Mean 156,389	Mean 415,3989
		Median 277	Median 88,378	Median 114,748	Median 265,978
		SEM 106	SEM 11,374	SEM 20,427	SEM 76,507
	Secondary endpoint: Fold increase of absolute neutrophil count (ANC) at 24 hours after entolimod administration	Mean 0,41	Mean 1,04	Mean 1,36	Mean 0,91
		Median 0,39	Median 1,16	Median 1,23	Median 0,86
		SD 0,23	SD 0,35	SD 0,58	SD 0,52
	Proportion of days alive and free of severe neutropenia (<500 neutrophils/µL blood)	Mean 29	Mean 66	Mean 52	Mean 48
		Median 9	Median 60	Median 68	Median 75
		SD 32	SD 34	SD 32	SD 32
	Proportion of days alive and free of clinically relevant neutropenia (<1,000 neutrophils/µL blood)	Mean 26	Mean 43	Mean 58	Mean 55
		Median 7	Median 57	Median 73	Median 72
		SD 30	SD 34	SD 31	SD 31
	Proportion of days alive and free of severe thrombocytopenia (<20 x 103 platelets/µL blood)	Mean 39	Mean 62	Mean 68	Mean 75
		Median 17	Median 84	Median 95	Median 93
		SD 34	SD 39	SD 32	SD 36

	Proportion of days alive and free of clinically relevant thrombocytopenia (<50 x 103 platelets/µL blood)	Mean 36	Mean 55	Mean 73	Mean 79
		Median 15	Median 73	Median 91	Median 88
		SD 32	SD 35	SD 33	SD 34
Analysis description		**The applicant claims that survival at Day 60 was statistical significantly greater in the 10-, 40-, and 120-µg/kg dose groups than in the placebo group (trend tests; adjusted p=0.0021, adjusted p<0.0001, and nominal unadjusted p<0.0001, respectively)**			

STUDY Rs-001 (dose conversion study)

In this application under exceptional circumstances for a product intended to be used in the case of a radiation disaster, efficacy evaluation is based on animal efficacy data only due to ethical and feasibility reasons. In such a situation a convincing dose conversion approach is essential to reason that efficacy and safety observed for a certain dose in animals may be translated in an equivalent human posology in order to presume that benefit- risk balance in both populations might be also comparable.

Clinical studies in special populations

No clinical studies in special population were performed.

NHPs included in pivotal study Rs-23 had an age equivalent to 8-24 years in humans. Therefore no pivotal efficacy data of entolimod in NHPs is available for extrapolation to humans aged less than 8 years or elderly.

The use of entolimod in NHPs with renal or hepatic impairment was not particularly studied.

Analysis performed across trials (pooled analyses AND meta-analysis)

Due to the different entolimod dosing and radiation concepts used in the NPHs trial as well as probably the fact that the quality of the applied product in different batches during the development was not adequately characterised (which might have had a significant impact on the product's bioactivity and efficacy), no pooled efficacy data were provided. This is agreed.

Supportive study(ies)

No clinical studies were performed. Eight non-clinical studies in NHP were presented as supportive but they were non-GLP studies and only discussed in the non-clinical section of the present report.

3.3.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Entolimod is being developed as a medical radiation countermeasure for the following specific indication:

"Entolimod is indicated to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster.

It is intended that the use of entolimod be directed towards children or adults who have received potentially lethal irradiation, defined as ≥2 Gy of whole-body radiation exposure based on radiation exposure history, proximity to the radiation source, symptoms or physical findings of radiation exposure, blood lymphocyte counts (if available), and/or dosimetry (if available).

Entolimod is indicated in adults (≥18 years), adolescents (12 to 17 years), and children (2 to 11 years)"

Because humans cannot be irradiated to test the activity and safety of entolimod for the proposed indication, clinical efficacy studies of the drug are not ethical or feasible. For this reason, entolimod efficacy data have been derived from studies in NHP conducted in accordance with United States governmental regulations as promulgated under the Food and Drug Administration (FDA) Animal Rule. While there is no formal 'animal rule' framework in the EU this does not preclude the possibility that animal models data could play a critical role in the assessment of medicinal products for humans in such very specific situations provided that the results of those well controlled studies establish that the drug is reasonably likely to produce clinical benefit in humans. In case of approval, the fact that entolimod efficacy is based on animal studies alone should be clearly reflected in the SmPC.

Given that all efficacy data is necessarily extrapolated from a non-human animal model, albeit the most appropriate one, the data cannot be regarded as comprehensive, therefore the applicant requested a MAA under exceptional circumstances. As such, in case of approval, the applicant should consider what measures they would plan to put in place so that relevant human safety and efficacy data could be collected in the event of product use in an emergency radiation exposure situation.

Entolimod efficacy data have been derived from a single pivotal study in lethally irradiated NHPs (study Rs-23). The pathophysiological mechanism of injury after acute total body irradiation at high dose rate in humans is well-understood. The NHP model most closely reproduces the clinical, histopathological, and pathophysiological aspects of radiation injury in humans, although NHPs tend to exhibit greater innate radioresistance to acute total body irradiation than most likely humans (Singh et al. 2017). It is noted that NHPs as Rhesus macaques are frequently used in animal studies investigation impact on radiation. A NPH model has been also used for other radiation countermeasures tested for efficacy in the USA (the G-CSFs compounds filgrastim and pegfilgrastim). As on the level of molecular conservation of TLR5 itself, the rhesus macaque shows 95% similarity to the human receptor and therefore the rational to use this model is understood and accepted in principle. Nevertheless, the sensitivity of Rhesus macaque and other NHPs should be compared in more detail in order to exclude that other NHPs are more suitable. In addition, a comparison between radiation sensitivity of humans and NHPs should be included in the discussion.

The single pivotal trial Rs-23 was performed in 170 non-human primates (NHPs; Maccaca mulatta of the Western Sichuan subspecies). Although the planned sample size is large, Rs-23 was a dose escalation study and therefore few animals (n=20) were included in each of the 7 different entolimod dose groups. According the applicant's statement in study report trial (Section 9.4.5.2/p38 SR) there was sufficient data from trials CBL1.1-N-01-Rs-09 and CBL1.1-N-01-Rs-14 to conclude that dose plateau for post-irradiation rescue of Rhesus macaques was efficacious between 10 and 40 µg/kg of entolimod. Moreover, it is noted that a significant higher dose of 120 µg/kg was selected for exploratory reasons only. Taking these considerations into account it seems not understood why the study design again investigates the lower doses between 0.3 and 10 µg/kg. As demonstrated by the results of the 80 NHPs in these 4 groups they were treated, although no adequate benefit could be expected. On the other side, the study groups in which efficacy could be expected were underpowered due to the small number of only 20 animals per group. Overall, this approach may be justified in an explorative phase II trial, but seems inadequate for a pivotal trial. Therefore, the applicant needs to explain why this phase II approach should be sufficient to claim already pivotal value.

This study was conducted in China. The applicant claims that the "study was conducted in accordance with Frontier standard operating procedures (SOPs), which comply with the standards of Good Laboratory Practice (GLP) as required by the major regulatory authorities." However, the GLP-compliance statement of the applicant regarding this study is not very precise and the applicant needs to verify in detail that study Rs-23 was conducted in compliance and following the principles of GLP and whether the site(s) of conduct of study were subject to laboratory accreditation with reference to GLP compliance monitoring. This is essential for this application as any suspect for insufficient compliance with GLP standards would need to be seen as a further major objection due to the clinical relevance of the data generated.

Study Rs-23 was a randomized, blinded, placebo-controlled, parallel-group, GLP, dose-dependence study in male and female radiation- and entolimod-naïve rhesus macaques that were not receiving immunosuppressive or anti-inflammatory drugs and that did not have high titres of pre-existing entolimod-reactive antibodies. The primary objective of the study was to demonstrate an entolimod survival benefit. Major secondary objectives focused on documenting drug-mediated amelioration of radiation-related morbidity while providing dose-dependent PD data in support of selection of a human

dose. Although additional clarification concerning some issues regarding the animals included is needed, the studies objectives and eligibility criteria for the selected population is agreed.

For logistical reasons, animals were acquired, treated, and evaluated in 4 sequential phases (Phase A followed chronologically by Phase B, Phase C, and then Phase D). In all phases, animals underwent a minimum 45-day acclimation period to select suitable animals for the study. Within each phase, cohorts of 9 animals were sedated and placed into restraining chairs in a shielded irradiation chamber. The cohorts were stratified by sex, body weight, and body width to achieve balance among the groups. The trial had to be performed sequentially and was complicated due to a protozoan infection in some of the animals, which delayed the trial performance. Currently, it needs confirmation that the NHPs study population remains genetically and otherwise homogenous during the complete study to exclude differences regarding radiation sensitivity between treatment arms.

The animals in each cohort were then randomized to chair position and exposed to TBI at a target dose level of 7.2 Gy by bilateral exposure to gamma rays from the radioactive isotope Cobalt-60. This absorbed dose is expected to induce death due to ARS in approximately 70% of animals within 60 days ($LD_{70/60}$). It is foreseen that the type (i.e. gamma) and dose (i.e. 7.2 Gy) of radiation used in NHPs in this study may not correspond with those to which humans might be exposed after a nuclear/radioactive accident (which otherwise are not possible to predict). Mixed beam external radiation in addition to incorporation is likely. Due to the mixture of different forms of radiation and incorporation of radioactive isotopes, radiation toxicity may be different. This adds further uncertainty to the extrapolation from data in NHP to the human target population. In particular, the possible effect of a combination of external radiation, entolimod treatment and continued radiation exposure due to contamination (and/or internal decon procedures) need to be considered. Additionally information regarding the radiation procedure itself and the dosimetry performed is requested.

Following TBI, animals were again randomized in a 2:1:1:1:1:1:1:1 ratio to 1 of 8 treatment groups: 0 µg/kg (placebo control) or 0.3, 1.0, 3.0, 6.6, 10, 40, or 120 µg/kg of entolimod. From a clinical perspective it seems necessary to reassure that randomisation was not affected by the changes performed in conduct due to the outage of animals before Phase D. Each animal received a single IM dose of placebo (5% dextrose in water) or entolimod at 25 hours after completion of TBI and were then monitored over a 60-day follow-up period. The company has not justified that timeframe of 25 hours and has not clarified that expected efficacy in humans would be similar when administering entolimod at 25 hours after total body irradiation than earlier. Not all radiological/nuclear accident would imply such long time period for a medical countermeasure to be administered to the population (as for example a small-scale one). Potential users for medical countermeasures request that they are available for administration within the critical period of the first 24 hours after irradiation. The SmPC should specify at which timeperiod after total body irradiation entolimod should be administered to humans.

Analgesics could be administered, but administration of other supportive care (e.g., transfusions, systemic antibiotics, intravenous fluids, haematopoietic growth factors) was not allowed. Imbalances in concomitant treatment and other supportive care need to be excluded between the different arms.

The applicant argues that the development program does not include assessment of an add-on benefit of entolimod to full supportive care because the availability of such supportive care might be restricted in the setting of disasters causing exposure of the population to potentially lethal irradiation. However, this argument is not supported. Not all radiation disasters would imply restriction of the availability of supportive care, as for example a small-scale nuclear/radiological accidents. But even in case of a mass casualty accident, in which both the number of people who will need medical attention will undoubtedly be large and the usual "standards of care" for medical activities may need to be modified

to reflect actual conditions and reduced availability of resources, some "crisis standard of care" might be available as replacement resources are brought to the affected area.

The lack of best supportive care used as comparator is an important shortcoming of this dossier. The applicant argues that the development program does not include assessment of an add-on benefit of entolimod to full supportive care because the availability of such supportive care might be restricted in the setting of disasters causing exposure of the population to potentially lethal irradiation. Not all radiation disasters would imply restriction of the availability of supportive care, as for example a small-scale nuclear/radiological accidents. But even in case of a mass casualty accident, in which both the number of people who will need medical attention will undoubtedly be large and the usual "standards of care" for medical activities may need to be modified to reflect actual conditions and reduced availability of resources, some "crisis standard of care" might be available as replacement resources are brought to the affected area.

Nevertheless, the proposed use of entolimod includes both situations where supportive care is fully available, and where this is limited. With regard to the former situation, the applicant should justify how the conditions of the pivotal NHP study (supportive care limited) has external validity with respect to a magnitude of benefit in case of full supportive care, including hematological growth factors. Conversely, the applicant should describe what conditions of use might be supported by the particular conduct of the pivotal NHP study, and justify that such conditions are foreseen to be realistic.

Investigational drugs should be evaluated within the context that reflects anticipated clinical use. The protocolised management of radiation causalities established in Europe (i.e. METREPOL) clearly establishes different therapeutic measures, which are adapted to the extent of damage to different organs and organ systems by radiation exposure (termed "response category") (Friedler et al. 2001). Treatment consists of supportive care and blood component therapy for a patient assigned a response category 2, and growth factor therapy or stem cell transplantation for patients in the response category 3 or 4, respectively. The absence of recommended supportive care in study Rs-23 makes it impossible to ascertain the efficacy and safety of entolimod in the context where is expected to be used as well as its place in the management of radiation casualties where full supportive care is known to increase survival. Although no G-CSF is approved in Europe for ARS, these drugs would be likely to be used in an accident as established by METREPOL. The inclusion of an arm treated with G-CSF as an internal control in study Rs-23 would have provided useful information, although no formal statistical comparison had been performed.

It is to be noted that entolimod was tested irradiating NHP using 60-day survival as primary endpoint in study Rs-23. Given the lethal potential of TBI for NHPs and humans, the animal study endpoint (i.e. enhancement of survival) is clearly related to the desired benefit in humans. Although clinical manifestations of ARS are usually present between 21 and 60 days from the exposure the follow-up period of 60 days can be insufficient to generate full efficacy data particularly in the long-term.

Assessments during the follow-up period included cage-side observations of morbidity and mortality and assessments of vital signs, food consumption, plasma cytokines (G-CSF, IL-6, IL-8, and IL-10; evaluated through 48 hours post-injection), ANC (evaluated through 48 hours postinjection), parameters of myelosuppression and haematopoietic recovery (as determined by complete blood count [CBC] throughout the 60-day treatment period), and body weight (assessed throughout the 60-day treatment period). Pharmacokinetic sampling over 36 hours post-dose was used to assess exposure to entolimod. It seems interesting that IL-8, and IL-10 were also measured. This seems rational in order to justify further PD claims on the result; however, the result from these cytokines were not adequately reported and not further discussed; neither in the PK nor in the efficacy documents submitted. The applicant is requested to explain this approach and to present an analysis of the results

for these parameters.Moreover, it should be explained why these parameters were not included in the dose conversion approach, although increases may contribute to the products efficacy.

Clinically relevant endpoints related to the recovery of neutrophil cells like incidence and duration of febrile neutropenia, frequency and type of infections have not been collected undermining the importance of the secondary endpoints. PD biomarkers, and haematological endpoints were assessed as exploratory endpoints and no statistical comparison versus placebo is available. Therefore, the selected variables would allow to partially assessing the efficacy of entolimod in the haematological subsyndrome of ARS but not in other body system or other ARS subsyndrome asides the haematological one.

Entolimod is a derivative of Salmonella typhimurium protein, FliCflagellin. Like its parental protein, entolimod it may act as a ligand of toll-like receptor 5 (TLR5). Activation of TLR5 may results in a broad signalling program, in which transcription factor NF-kB regulates both cell autonomous survival pathways and production of numerous secreted factors as hematopoietic and anti-inflammatory cytokines as well as antimicrobial peptides. Although the claim indication is to increase survival in patients presenting with ARS after a nuclear/radiological accident, in case of approval, such use would need to be restricted to the treatment of the subsyndrome in which efficacy of entolimod can be demonstrated in the NHP model. The applicant claims that some of the cytokines released may have also a maintenance effect on the function of the gastrointestinal tract, however, no convincing proof for this hypothesis was currently provided. Whether entolimod acts also on other receptors is currently not sufficiently assessable.

It has not been convincingly shown that entolimod exerts any favourable effects other than what possibly can be achieved with G-CSF and perhaps IL6 which raises the question whether entolimod is mostly or exclusively exerting its clinical effect through G-CSF release. The applicant is asked to further discuss any differences in favourable effects observed with entolimod compared with G-CSF, with respect to any clinical effects which might be mediated in other ways than through G-CSF.

Moreover, as the management of radiation casualties is internationally protocolised, it is not the place of the SmPC of entolimod, as proposed by the applicant, to establish the exact conditions in which people should be medically treated after acute whole-body irradiation at a high dose rate. These conditions, which already appear in the protocols, will depend on the presence of specific clinical signs or symptoms (established in METREPOL) and/or on a specific estimated/known absorbed radiation dose.

Animals that died during the 60-day observation period underwent necropsy on the day of death or within 1 day after the day of death, and findings potentially implicated in the death of the animal were assessed. Animals that survived to Day 60 were euthanized on Day 61 and underwent necropsy on that day. Gross pathology was evaluated for all necropsied animals. Although it is acknowledged that the applicant has made the attempt to exclude bias due to euthanasia in trial Rs-23, differences in the necropsy results between animals who had profit and those who died before day 60 is not sufficiently evaluable.

(With respect to the non-clinical aspects of the pivotal trial please refer to the non-clinical part of the assessment report)

Efficacy data and additional analyses

In trial Rs-23 entolimod has shown a statistical significant survival benefit in dose-depending groups of 20 irradiated NHPs (Rhesus macaques) in ITT, when administered with doses ≥ 10 µg/kg intramuscular

at 25 hours after TBI with a radiation dose of 7.2 Gy (intended to be an LD70/60 radiation dose). The 60-day mortality rate for the control arm was 72.5%, as expected in case of supportive care not being given.

The probability of survival at Day 60 increased compared to placebo starting at a dose of 6.6 µg/kg with maximal effect at a dose of 10 µg/kg. The survival benefit compared to placebo plateaued across entolimod doses of 10 µg/kg (n=15/20; adjusted p=0.0021), 40 µg/kg (n=14/20; adjusted p<0.0001) and 120 µg/kg (n=14/20; nominal unadjusted p<0.0001) compared to survival of n=11/40 in the placebo arm.

When comparing survival, 27.5% of the animals in the placebo group survived to Day 60 while 75% of animals in the 10-µg/kg entolimod group survived to Day 60 (and 70% for the 40-µg/kg and 120-µg/kg groups), resulting in an impressive survival odds ratio of 7.9 (6.2 for the 40-µg/kg and 120-µg/kg groups) and an absolute survival benefit of 47.5% (and 42.5% for the 40-µg/kg and 120-µg/kg groups) in favour of entolimod. Such differences are statistically and clinically relevant. The validity of the findings is increased by the demonstration of a stable plateau in efficacy above a dose of >10µg/kg.

Although the observed difference in survival for the mentioned doses is clearly clinically relevant survival curve data are incomplete. The applicant should provide a new survival curve for placebo, 10 and 40 µg/kg entolimod doses specifying the number of exposed subjects at every time point.

The main issue regarding trial Rs-23 remains that the study design reflects only a typical phase II setting. This seems overall not acceptable for pivotal claims. Due to the dose-finding and similar PK/PD evaluation approach animals were randomised on 8 different treatment groups. This resulted in very limited numbers per group (n=20). In consequence the difference classifying a treatment as statistically non-significant or highly significant with respect to survival is driven by few animals only as illustrated by the following table:

Table 47: Number (%) of animals surviving to day 60 with unadjusted and adjusted p-values and survival odds ratio

CBLB502 Dose	Overall Survival	Unadjusted P-value	Adjusted P-value	Survival odds ratio (vs vehicle)
0.0	11/40 (27.5)	-	n/a	-
0.3	5/20 (25.0)	0.5817	0.5817	0.9
1.0	7/19 (36.8)	0.2580	0.4473	1.5
3.0	5/20 (25.0)	0.4473	0.4473	0.9
6.6	9/20 (45.0)	0.1439	0.1439	2.2
10.0	15/20 (75.0)	0.0021	0.0021*	7.9
40.0	14/20 (70.0)	<.0001	<.0001*	6.2
120.0	14/20 (70.0)	<.0001	n/a	6.2

Source: Study CBL1.1-N-01-Rs-23

The same is true with respect to the survival odds ratios vs vehicle reported.

Although it is acknowledged that the three groups using doses ≥10 µg/kg are statistically significant, the applicant should justify the hypothesis that these results are sufficiently valid for pivotal purpose. From a clinical perspective it seems bold to claim pivotal evidence from one trial only. In a situation where animal data have to substitute for human data, the resulting uncertainties of interspecies extrapolation increase the need for robust and convincing evidence. Clinical studies in the intended target population marketing authorisations based on one rather than two pivotal trials are the

exception, not the rule. The applicant is asked to justify approval based on a single pivotal trial, or to consider how additional evidence for efficacy in the indication applied for could be provided.

Moreover, as comparability of drug substances and drug product batches derived from different stages of manufacturing process and product development is not substantiated the applicant is requested to justify that consistency regarding the bioactivity of the batches used in the trial Rs-23 and previous trials (including those in the human volunteer population) can be assumed.

The applicant has submitted data showing the increase in mean plasma concentrations of G-CSF, IL-6 and ANC after 24 hours of entolimod administration by dose. While they have provided the adjAUC0-24h for plasma G-CSF and IL-6 by entolimod dose, only a table containing the fold change in ANC through 24 h has been given. The applicant should provide the curve showing the AdjAUC0-24h for ANC in order to have a complete demonstration of the effect of all entolimod doses in ANC. The correlation between these biomarkers and survival was assessed with the Spearman rank correlation coefficient that was 0.84 for each of them.

In the 60 days of follow-up, TBI produced expected haematological toxicity, resulting in cytopenias (neutropenia, thrombocytopenia, and anaemia) both after placebo and entolimod.

In the placebo group, average neutrophil nadirs occurred around day 15 with recovery around day 22 for all doses and placebo. However, the figures illustrating the effect of entolimod on neutrophil count lack clarity and are not very useful. Values for the different entolimod doses and their variability overlap what makes it difficult to see the behaviour of the 10 and 40 µg/kg doses versus placebo. The applicant is requested to provide additional figures (and 95% CI) for absolute neutrophil count for placebo, and 10 and 40 µg/kg doses together with a table with the numerical data. As previously mentioned these secondary endpoints were exploratory endpoints and, therefore, no statistical comparisons are available.

The applicant has presented the mean percentage of days that animals on placebo were free of severe neutropenia (ANC<500 cells/µL), that was 62% versus 29% in those on entolimod 10 µg/kg. For clinically relevant neutropenia (ANC<1,000 cells/µL) the mean percentage of days was 26% for placebo and 58% for entolimod. In general, dose dependent improvements in the percentage of post-treatment study days alive and free of these levels of neutropenia were seen with entolimod treatment. However, other relevant information in terms of efficacy have not been provided. The applicant should provide a table with the following data, for placebo, 10 and 40 µg/kg doses of entolimod: the mean duration of severe neutropenia (ANC<500 cells/µL), the mean duration of clinically relevant neutropenia (ANC<1,000 cells/µL), time to recovery from neutropenia (with ANC≥500 cells/µL and with ANC ≥1,000 cells/µL), incidence of febrile neutropenia (defined as a rise in temperature above 38.5 centigrade while an absolute neutrophil count < 0.5×10^9/L) and days of febrile neutropenia.

The applicant has also provided the fold change in ANC at 24 hours post dose to show that entolimod doses >3 µg achieve greater increases than placebo. This is, however, little informative. The applicant should provide AdjAUC0-24, and AdjAUC0-48 for plasma ANC by Entolimod Dose for the As-Treated Population.

In the placebo group, average and platelet nadirs seem to occur on days 14 and returned to normality around day 22. In general, there seems to be a trend in favour to a less pronounced thrombocytopenia with nadir platelet counts of 4.5 versus 18.2 cells/µL (placebo and entolimod 10 µg/kg respectively). On average, animals in the placebo group were alive and without severe thrombocytopenia (<20,000 cells/µL) 39% of the post-treatment study days versus 78% in entolimod 10 µg/kg group, and without

clinically relevant thrombocytopenia (<50,000 cell/µL) in 36% of NPH with placebo versus 73% in entolimod 10 µg/kg. This data, however, are not very informative. As for neutrophil count, the figures provided are not very useful and the applicant is requested to provide additional figures only for placebo, 10 and 40 µg/kg for platelet counts (and 95% CI) as well as a table with the numerical data. In order to have more helpful information of the effect of entolimod in platelet recovery, the applicant should also provide with data, for placebo, 10 and 40 µg/kg of entolimod, the mean duration of severe thrombocytopenia (<20,000 cells/µL), time to recovery from severe thrombocytopenia, and first day of severe thrombocytopenia.

In the placebo group, mean haemoglobin values seem to fall to nadirs at approximately day 20 before gradually returning to near-baseline levels by the end of the study. As for neutrophil and platelet counts, the applicant is requested to provide an additional figure for haemoglobin (and 95% IC) for placebo, 10 and 40 µg/kg arms as well as a table with the numerical results. There seems to be a trend for higher mean haemoglobin nadirs with entolimod therapy but differences are very small and no clinical consequences are expected from them. The applicant should be provided a table with the time to recovery to normal haemoglobin values for placebo, 10 and 40 µg/kg doses.

In principle it was shown that entolimod at doses of ≥10 µg/kg decreased the magnitude and duration of radiation-induced neutropenia, thrombocytopenia, and anaemia in NHPs which would be in line with reports from the necropsy that septic, haemorrhagic, and mucosal erosive effects of irradiation were less frequent in animals that received doses of entolimod ≥10 µg/kg, reflecting greater recovery before necropsy among the entolimod-treated animals as well as the salutary radiomitigation effects of entolimod. However, the details are not really transparent from the submitted figures and tables and need to be reassessed in order to fully understand the clinical relevance of the effects described.

Summarised data on body weight, cage-side observations (eg, hypoactivity/fatigue, inappetence/anorexia, emesis/vomiting, diarrhea) and food consumption of surviving primates have not been provided. The applicant should provide tabulated information of these observations (pre-dose and at Day 60) for surviving NHP at Day 60 for placebo, 10 and 40 µg/kg doses and discuss if these findings correlate with survival, efficacy endpoints and necropsy data for the most important organs.

The applicant is asked to present clinical observations other than haematological in the Rs-23 study in the placebo group compared to the entolimod dose cohorts, e.g. days of severe diarrhoea. A tabulated form is preferred.

Although the applicant proposes the 10 µg/kg dose as the optimal dose in NHPs, this is not clearly supported by the data on survival and haematological endpoints provided. The data requested on haematological parameters may help elucidate both entolimod efficacy and confirm the dose selection. The latter is crucial as the dose in NHPs has been used to predict the dose to be given in humans. The risk of estimating a subtherapeutic dose for humans when there is no chance of confirmation in the target population is of concern.

After total body irradiation there were 80 NHPs that survived for 60 days, 11 after placebo and 69 after entolimod (5, 7, 5, 9, 15, 14, 14 for the corresponding doses of 0.3, 1, 3, 6.6, 10, 40 and 120 µg/kg of entolimod). The company stated that NHP surviving at day 60 had fully recovered or showed almost full recovery by the end of this period, as documented by clinical observations, clinical pathology data, and necropsy data obtained on Day 61. However, summarised data on body weight, cage-side observations, and food consumption of surviving primates have not been provided. The applicant should provided tabulated information of these observations (pre-dose and at Day 60) for surviving NHP at Day 60 for placebo, 10 and 40 µg/kg doses and discuss if these findings correlate with survival, efficacy endpoints and necropsy data for the most important organs.

For those 99 animals dying prematurely in study Rs-23, irradiation-related hemorrhagic or septic phenomena were most commonly implicated in the cause of death. Many of these animals had widespread haemorrhaging in multiple organ systems. Septic lesions leading to death included pneumonia, purulent pleural or pericardial effusions, and abscesses in multiple organ systems. In multiple animals, severe gastrointestinal lesions, including bowel obstruction, intussusception, erosive ulcers, or perforations were judged to have contributed to the cause of death. Pulmonary edema was also observed.

Considering all 179 NHPs included, the highest incidences of necropsy findings were those associated with the gastrointestinal system (79.2%), particularly hemorrhagic lesions (60.7%) and hyperemia (29.8%) of the bowel. In the respiratory system, hemorrhagic (62.4%) and septic lesions (23.0%) of the lung were often described, as were adhesions within the thoracic cavity (26.4%). Incidences of such findings were generally higher among animals treated with placebo or entolimod doses ≤6.6 µg/kg than among those treated with entolimod doses ≥10 µg/kg. There were some findings described in the urinary system, skin, heart and lymphoid system in the form of haemorrhages that were most commonly observed at placebo and doses of entolimod ≤6.6 µg/kg. The applicant should provide a table summarising the incidence and type of necropsy findings by organ system for placebo and 10 and 40 µg/kg doses for those animals who were euthanized at day 61.

The identification of an effective human dose was done by a dose conversion study (Rs-001) from Rhesus macaques. It is acknowledged that dose conversion between animals and human population may be sometimes difficult in general. However, for an application under exceptional circumstances in which data on efficacy were evaluated in animals only, reliable dose conversion for the human population is essential and a prerequisite for the conclusion of a positive benefit-risk balance.

For entolimod, it is not sufficiently demonstrated that the proposed dose conversion approach from NHP to humans leads to a human dose which can be reliably assessed as similar efficacious and safe like that used in NHPs. The proposed approach for dose conversion based on not sufficiently validated biomarkers is not acceptable.

From a methodological and clinical point of view the proposed dose conversion approach is not acceptable. The attempt to use correlation between treatment effects on survival and a biomarker in a dose-ranging study is clearly not sufficient evidence that a therapy-induced increase in the biomarker is predictive for a treatment's efficacy and safety, in particular regarding an effect on survival. The proposed indication of entolimod includes children aged 2 years and older in section 4.1. However, in section 4.2 specific dosing recommendations are proposed for children below 2 years of age given that very likely these children will be treated if needed. This seems acceptable. However, the rationale for the specific dosing recommendations in this group is missing (see clinical safety section).

The proposed indication in children is the same as that proposed in adults. No paediatric clinical study is planned as entolimod cannot be studied in healthy children. This is acceptable. As mentioned in the PIP summary report of entolimod "*the applicant does not expect differences between adults and children since the mechanism of action of entolimod is mediated via the innate immune system, which is considered mostly mature from birth. However, while TLR expression in neonates seems equivalent to the adult level, the neonatal response to TLR stimulation seems to be qualitatively different. An efficacy-by age analysis in study Rs-23 showed similar survival benefits for NHP of ages equivalent to human age categories of children between 8 and 18 years and adults and revealed no important differences for PD or PK among these age categories. NHP and humans show comparable patterns of PD (i.e. increases in the levels of circulating neutrophils in the blood and in the concentrations of specific plasma cytokines, in particular, granulocyte colony-stimulating factor [G-CSF] and interleukin [IL]-6) and toxic responses to the drug (i.e. animals show signs of hypoactivity, lethargy, inappetence, and emesis, humans show "flu-like" syndrome, both species show elevations of serum ALT and AST*

levels and decreases in serum phosphate concentrations and decreases in blood pressure). An analysis of PD and PK by age in irradiated and non-irradiated NHP revealed no important differences. From a safety point of view, it is deemed acceptable to extrapolate the data obtained in NHPs from 24 months of age down to children from 2 to less than 8 years."

Nevertheless, in section 4.2 specific dosing recommendations are proposed for children below 2 years of age given that very likely these children will be treated if needed but the rationale for such recommendation is missing. TLR expression in neonates seems equivalent to the adult level but the neonatal response to TLR stimulation seems to be qualitatively different and therefore an approach based only on pharmacokinetic data seems not adequate across all age groups below 2 years of age. The applicant should justify the dosing recommendations for children below 2 years of age taking into account the potential differences in pharmacokinetics.

3.3.7. Conclusions on clinical efficacy

Although no formal 'Animal Rule' framework exists in Europe, this does not preclude the possibility that animal models data could play a critical role in the assessment of medicinal products for humans in the specific situations in which studies in humans are neither ethical nor feasible. This would be acceptable provided that the results of those well controlled studies in animals establish that the drug is reasonably likely to produce clinical benefit in humans.

Study Rs-23 was performed in the NHP model that it is validated in this clinical situation. Data from this study show that the highest doses of entolimod achieve survival rates that are statistically significant and clinically relevant compared to placebo. When comparing survival, 27.5% of the animals in the placebo group and 75% of animals in the 10-µg/kg entolimod group survived to Day 60, resulting in a survival odds ratio of 7.9 and an absolute survival benefit of 47.5% in favour of entolimod. Although results from trial Rs-23 are highly promising, several important issues remain open and need clarification. The following issues are most important:

The study design of trial Rs-23 reflects a typical phase II dose-finding setting, but is as a single trial doubtful if acceptable for pivotal claims even in an application under exceptional circumstances. It seems bold to claim sufficient evidence for a dramatic benefit from one trial/study only. In a situation where animal data have to substitute for human data, the resulting uncertainties of interspecies extrapolation increase the need for robust and convincing evidence. It is fully acknowledged that the study ended with demonstration of a highly statistical significant effect indicating increased survival which was in addition stable for doses above 10 µg/kg. However, the size of the 8 different treatment groups was very limited (n=20 per group). The robustness and reliability of the findings can therefore be challenged, considering that the difference classifying a treatment group as statistical highly significant or non-significant depends from the outcome in very few animals only. The impact of chance in such small groups is much higher than in larger groups. Clinical studies in the intended target population marketing authorisations based on one rather than two pivotal trials are the exception, not the rule.

The guideline EMA document "POINTS TO CONSIDER ON APPLICATION WITH 1. META-ANALYSES; 2. ONE PIVOTAL STUDY" (CPMP/EWP/2330/99) stated correctly "*that minimum requirement is generally one controlled study with statistically compelling and clinically relevant results. However there are many reasons why it is usually prudent to plan for more than one study in the phase III program.*

Moreover, the same document recommends even two pivotal trials in the case of a "*lack of pharmacological rationale (unknown mechanism of action), new pharmacological principle or in the case phase I and phase II data are limited or unconvincing."*

In the exceptional event of a submission with only one pivotal study, this *has to be particularly compelling with respect to internal and external validity, clinical relevance, statistical significance, data quality, and internal consistency*.

As the pharmacological principle is new and insufficiently characterised, no other medicinal product has proven clinical relevant efficacy or safety of TLR5 activation and a gap of plausibility remains with respect to the claimed mode of action and the data, even the conditions in which two pivotal trials are needed are clearly seen.

Although it is acknowledged that recently CHMP has granted approval sometime with phase II data only, it needs to be considered that this was mainly in oncology indications and in situations in which phase III trials are still ongoing, but a high medical need was identified to allow patients an early access to new treatment options. Moreover, for all of these products post-marketing data after approval allows assessing whether the benefit-risk balance conclusion at the time of approval was correct or needs to be reconsidered.

Both conditions are not fulfilled for entolimod's application. No additional data are planned to be submitted. Therefore, it is rather likely that no confirmative clinical data in humans will ever become available in the intended indication and target population.

Considering all these arguments, the applicant is asked to justify approval based on a single pivotal trial, or to consider how additional evidence for efficacy in the indication applied for could be provided.

The proposed use of entolimod includes both situations where supportive care is fully available, and where this is limited. With regard to the former situation, the applicant should justify how the conditions of the pivotal NHP study (supportive care limited) has external validity with respect to a magnitude of benefit in case of full supportive care, including hematological growth factors. Conversely, the applicant should describe what conditions of use might be supported by the particular conduct of the pivotal NHP study, and justify that such conditions are foreseen to be realistic.

The application strongly depends on the results of RS-23. The (formal) GLP status of the study is unclear. The applicant needs to verify in detail if or to what extent study Rs-23 was conducted in compliance and following the principles of GLP.

The entolimod dose of 10 ug/kg chosen by the company in the NHPs may not be the optimal dose. No clear differences have been observed between the highest entolimod doses for survival and the haematological parameters in study Rs-23. The adequacy of dose selection in NHP is critical for the estimation of the dose to be used in humans and 10 µg/kg dose could be subtherapeutic. The applicant should justify the selected dose in NHP based on survival and/or on other parameters (haematological related to the effect on other organs, etc).

In addition, there are doubts about the prediction of the dose to be used in humans what is critical as entolimod would be given to humans without having a single data in the target population.

3.3.8. Clinical safety

Because humans cannot be lethally irradiated to test the safety of entolimod under the specific conditions of use, the applicant has primarily acquired safety data in healthy human subjects in 2 open-label studies (HU-7014 and HU-9001) after single-dose, intramuscular administration of the drug.

Entolimod has also been evaluated in 2 open-label studies (I196111-US and I196111-Russia) in patients with advanced solid tumours, and in 1 placebo-controlled study (BL612-CBLB502) in patients

with colorectal cancer who had never received antitumor treatment and receiving entolimod as neoadjuvant therapy before surgical resection of the primary tumour. The I19611 – US study has been conducted in USA and the other two studies have been conducted in the Russian Federation.

The applicant has also included in the dossier preclinical studies with some data relevant for the safety assessment of the product. Two studies in Rhesus macaques were carried out. Those studies were performed in non irradiated Rhesus macaques (Rs-001) and irradiated animals (Rs-23). As commented above, they were planned to assess efficacy although some safety exploratory endpoints were included in the studies design.

Class Effects

Native TLRs are a class of proteins that play a key role in the innate immune system, protecting the host from invasion by microorganisms and viruses. No agonist of TLR5 has been approved in the EU. Agonists of other TLRs have received regulatory approval for use in humans, including:

- Bacillus Calmette-Guérin (BCG) is a TLR2 and TLR4 agonist. BCG Vaccine SSI (67) is administered percutaneously and is indicated for the prevention of tuberculosis.
- Monophosphoryl lipid A (MPL) is a TLR4 agonist that serves as an immune adjuvant in the formulation for the recombinant human papillomavirus vaccine (Cervarix)
- Imiquimod (Aldara) (71) is a TLR7 agonist that is applied as a topical cream and that is indicated for the treatment of actinic keratoses, primary superficial basal cell carcinoma, and external genital and perianal warts/condyloma acuminata.

Each of these products typically induces local inflammatory effects at the site of administration (eg, bladder irritability in the case of intravesical BCG administration; and pain, swelling, erythema, and pruritus of the skin for the other products). BCG vaccination may cause regional lymphadenopathy. Systemic AEs are common with any of these products and comprise a transient "flu-like" syndrome evidenced by events such as fatigue, fever, headache, anorexia, nausea, diarrhoea, myalgia, arthralgia, and neuralgia. The symptoms typically abate within 1 to 2 days of administration. Administration of Cervarix has been associated with syncope that is usually transient and typically responds to placing the patient in a supine or Trendelenburg position.

In clinical studies of entolimod conducted in humans, potential AEs that were prospectively anticipated were local effects and systemic constitutional and gastrointestinal symptoms comparable to those observed in the flu-like syndrome associated with other TLR agonists. Consequently, the safety evaluation plan for entolimod in the 2 completed healthy subject clinical trials included assessment of standard safety outcomes: AE frequency and severity, safety laboratory parameters (haematology, coagulation, serum chemistry, and urinalysis), vital signs, physical examinations, and electrocardiograms (ECGs). Further, in accordance with its mechanism of action, assessments of cytokine responses to entolimod were evaluated in the clinical studies of entolimod in healthy subjects (HU-7014 and HU-9001).

Measurements of plasma G-CSF, IL-6, IL-8, IL-10, IL-12, and IL-1β, and tumour necrosis factor-alpha (TNFα) were performed in both studies. Serum IL-12 and IL-1β were evaluated in the 1st clinical study but, because of a lack of induction at any dose in this 1st study, were not evaluated in the 2nd study. In addition, based on entolimod's protein nature and flagellin derivation, the generation of host-derived, anti-entolimod neutralizing antibodies was assessed.

Patient exposure

Study HU-7014:

A Phase 1 trial, single-centre, open-label, non randomized, single-dose, dose-escalation study that evaluated the PK and PD of IM injections of entolimod in 50 healthy human subjects (32 male and 18 female, 19-45 years and 18.4-31.6 BMI) with wild-type Toll-like receptor 5 (TLR5).

A single IM dose of entolimod of 2 µg (6 HV), 6 µg (6 HV), 12 µg (6 HV), 24 µg (6 HV), 30 µg (12 HV), 35 µg (6 HV), 40 µg (5 HV), or 50 µg (3 HV) were administered on a dose-per-subject basis in fast conditions. Subjects were observed for ~36 hours following entolimod administration, discharged and required to return to the clinic up to Day 28 for follow-up.

Study HU-9001

It was a Phase 2 trial, multicenter (2 centers) open-label, randomized, parallel-group study that evaluated the PK and PD of IM injections (multiple injections) of entolimod in 100 healthy subjects (91 males and 9 females, 18-55 years and 19-32.2 BMI). Entolimod doses of 25 µg (25 subjects), 30 µg (24 subjects, 2 doses administered 72 hours apart), 35 µg (25 subjects), and 35 µg plus ibuprofen (IBP) (26 subjects, 400 mg orally 30 minutes before IM administration of entolimod to determine if the severity of the "flu-like syndrome" AEs observed in the Phase 1 HU-7014 trial could be reduced without altering the cytokine profile of CBLB502 following intramuscular (IM) administration) were evaluated. In the main criteria for inclusion there was not specified Toll-like receptor 5 (TLR5) genotype but it was determined in each subject.

The subjects (in no fast conditions and required to ingest fluids) were randomized in a ratio of 1:1:1:1 to one of four treatment groups. Following entolimod administration, subjects were observed for ~8 hours, discharged and returned to the clinical for follow-up until day 6 or 9 depending on the dose group.

In both healthy studies, the formulation of the product used was different that the one proposed for marketing. The trials HU-7014/HU9001 in humans were performed before the trial Rs-23 in NHPs was conducted. It seems not clear whether the analytical approaches used for evaluation of PK and PD response are identical or similar to that implemented several years later in trial Rs-23.

The exposure by absolute dose group and body-weight-adjusted dose groups for combined studies HU-7014/HU-9001 is detailed in table 17.

Table 27: Exposure by Absolute Dose Group within Body-Weight-Adjusted Dose Groups for Combined Studies HU-7014/HU-9001 – All Subjects						
Entolimod Absolute Dose Group, µg	All Subjects (N=150)			By IBP Pretreatment 0.40-0.60 µg/kg (N=51)		2nd Dose[a]
	<0.40 µg/kg (N=92)	0.40-0.60 µg/kg (N=51)	>0.60 µg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 µg/kg (N=13)
	N	n	N	n	N	N
2 (N=6)	6	0	0	0	0	0
6 (N=6)	6	0	0	0	0	0
12 (N=6)	6	0	0	0	0	0
24-25 (N=31)	30	1	0	1	0	0
30 (N=36)	28	8	0	8	0	13
35, without IBP (N=31)	7	23	1	23	0	0
35 with IBP (N=26)	9	17	0	0	17	0
40 (N=5)	0	2	3	2	0	0
50 (N=3)	0	0	3	0	0	0

IBP=ibuprofen
a The 2nd-dose group was a subset of the 1st-dose group.
Source: Program-T_TABLE3, Date Produced: 04FEB2015

With respect to demographic and other characteristics of the HU-7014 and HU-9001 trial population the applicant stated that demographic characteristics were as far as possible balanced across the dose groups. In the combined HU-7014/HU-9001 healthy subject population, subjects are reported to range in age from 18 to 55 years and were primarily Caucasian men of Hispanic ethnicity; however, women and African Americans were also represented. Screening body weights ranged from 46.0 kg to 109.5 kg. Although it is acknowledged that the applicant may have tried to reduce imbalances between the dose groups, it is obvious from the trial design that the very small dose groups do not allow an adequate safety assessment. Imbalances in demographic and baseline characteristics can be reasonably presumed. Although in principle new safety data in healthy volunteers would be useful, it has to be also considered that exposing healthy volunteers to the risk of inducing a cytokine storm seems critical.

The Committee considers that clinical experience with entolimod in humans is limited. Although the applicant claims that 150 subjects in HU-7014/HU-9001 were included in the phase I trials, only 58 of these healthy volunteers had a relevant single dose exposure in the recommended target range for

adults between 0.40 and 0.60 µg/kg (N=51). This is explained by the dose escalation approach implemented in both phase I trials as tolerability testing was the main aim of this "First in Man" trial. However, even this population is not homogeneous. Only 34 of these 51 subjects received entolimod alone, while safety data for the remaining subjects was generated after pre-administration of 400 mg of IBP in order to increase tolerance of the product.

In conclusion entolimod's safety for the applied posology can only be assessed adequately from 51 or 34 healthy volunteers. It is obvious that this marginal human safety data allows (as initially planned) only a first impression of tolerability, but is deemed rather insufficient to characterise safety for this product, even (or in particular) in an application under exceptional circumstances. Additional safety data in humans be helpful; however, the low tolerability in humans and the potential risk for a cytokine release syndrome (CRS)/ cytokine storm has also to be considered and questions the feasibility of additional studies in healthy volunteers.

I196111-US:

It was a single-centre, open-label, non-randomized, sequential dose-escalation study conducted in the US that evaluated the safety and tolerability of entolimod. The study was planned to include up to 66 subjects but 26 subjects were enrolled in total and treated with absolute doses of 5 µg (N=4), 10 µg (N=3), 15 µg (N=3), and 20 µg (N=4) were administered SC QD for 5 days on a dose-per-subject basis. Following completion of the 20-µg dose group with this schedule, the protocol was amended to support administration of 4 SC doses QD on Days 1, 4, 8, and 11. The study continued using the revised dosing regimen for entolimod doses of 30 µg (N=5) and 40 µg (N=7). Of the 26 subjects, 19 subjects received body-weight-adjusted doses of <0.40 µg/kg, 4 subjects received body-weight-adjusted doses of 0.40 to 0.60 µg/kg, and 3 subjects received body-weight-adjusted doses of >0.60 µg/kg. Subjects were observed for 9 hours after the first entolimod injection and followed up to 43 days postdose.

They presented with histologically or cytologically confirmed, locally advanced, inoperable or metastatic solid tumor for which no effective therapy existed. Patients aged from 42 to 82 years and were primarily male and Caucasian. Body weights ranged from 40.7 kg to 117.3 kg. Considering the small sample sizes in some of the dose groups, demographic characteristics of age, sex and race/ethnicity were balanced as far as possible across the dose groups; however, imbalances are obvious and not avoidable due to the very limited number of patients included. Mean body weights in the <0.40-µg/kg and 0.40-0.60-µg/kg groups were ~80 and ~62.5 kg, respectively, while mean body weight was lower in the >60-µg/kg group at ~56 kg. Subjects had a variety of cancer types; colorectal cancer and lung cancer were most commonly represented.

I196111-Russia

It was a 2-centre, open-label, nonrandomized, sequential dose-ranging study that evaluated the safety and tolerability of entolimod.

The study was performed in the Russian Federation as a companion study to I196111-US and was designed to build on the data that were obtained from the first 5 dose levels (5, 10, 15, 20, and 30 µg/dose) of the US study. Thus, enrolment in I196111-Russia began at the 5th dose level (30 µg/dose) of entolimod with subjects receiving an IM dose of 30 µg of entolimod on Day 1 and SC injections of 30 µg entolimod once daily on Days 4, 8, and 11 (total of 4 injections). Subjects were to be observed for at least 36 hours after each entolimod injection (on Days 1, 4, 8, and 11 or Days 1 and 4), as well as at follow-up visits on Days 15, 22, and 43. Subjects were to be evaluated every 3 weeks after Day 43 until disease progression or another discontinuation criterion was met. The study included a 30-day safety follow-up period after the last image that showed progression of disease or until resolution of any drug-related toxicity.

The study was planned to include up to 40 subjects. Enrolment to the study was interrupted for administrative reasons after 7 subjects had been accrued to Dose Level 5 (30 µg/dose). Patients presented with histologically or cytologically confirmed, locally advanced, inoperable or metastatic solid tumor for which no effective therapy existed.

Patients aged from 27 to 65 years and weighted from 54 to 89 kg. Subjects were primarily female (6 females, 1 male) and Caucasian. Body weights ranged from 54 kg to 89 kg. Five subjects had colorectal cancer, 1 had lung cancer, and 1 had breast cancer.

By dose groups, the subjects received ≥1 dose of entolimod as follows: <0.40 µg/kg (N=2), 0.40-0.60 µg/kg (N=5), and >0.60 µg/kg (N=0).

BL612-CBLB502

It was a 3-centre, randomized, placebo-controlled, single-blind study conducted in the Russian Federation to assess the safety, PD, and preliminary efficacy of entolimod in patients with colorectal cancer who had never received antitumor treatment and who were eligible for scheduled surgical resection of the primary tumour.

Subjects received SC injections of the study drug, comprising entolimod (at either a dose of 0.35 µg/kg or 0.45 µg/kg) or placebo, administered as either 1 injection or as 2 injections separated by 3 (± 1) days. The last injection was to be administered 4 (± 2) days before surgical resection of the primary tumour. To mitigate the flu-like syndrome that can associated with entolimod administration, subjects were to be premedicated with 400 mg of ibuprofen orally 30 (± 5) minutes prior to each study drug injection.

The study was conducted in five sequential treatment cohorts, each enrolling 8 subjects (with a 3:1 randomization such that 6 subjects received entolimod and 2 subjects received placebo):

• Group 1: Single injection of entolimod, 0.35 µg/kg (total dose not to exceed 30 µg/injection) or placebo, 4 (± 2) days prior to surgery – Day 1

• Group 2: Single injection of entolimod, 0.45 µg/kg (total dose not to exceed 40 µg/injection) or placebo, 4 (± 2) days prior to surgery – Day 1

• Group 3: Two injections of entolimod, 0.35 µg/kg (total dose not to exceed 30 µg/injection) or placebo, separated by 3 (± 1) days, 4 (± 2) days prior to surgery – Day 1 and Day 4

• Group 4: Two injections of entolimod, 0.45 µg/kg (total dose not to exceed 40 µg/injection) or placebo, separated by 3 (± 1) days, 4 (± 2) days prior to surgery – Day 1 and Day 4

• Group 5: Two injections of entolimod, 0.45 µg/kg (total dose not to exceed 40 µg/injection) or placebo, separated by 3 (± 1) days, 4 (± 2) days prior to surgery – Day 1 and Day 4

Because of the sequential nature of cohort assignment, both study personnel and study subjects were aware of the dose level and dosing regimen. With the single-blind approach used during randomization within a cohort, study personnel were aware of entolimod or placebo assignment, but study subjects were not.

After accrual and follow-up of Groups 1 to 4, an interim data review was performed to determine the regimen that safely provided the greatest pharmacodynamic effects. Based on this review, subjects were then enrolled to Group 5.

To mitigate the flu-like syndrome that can associated with entolimod administration, subjects were to be premedicated with 400 mg of ibuprofen orally 30 (± 5) minutes prior to each study drug injection. Overall, 40 subjects were enrolled, 30 to receive entolimod and 10 to receive placebo.

The ages of subjects ranged from 33 to 84 years but only 30 of them received entolimod. Thirty-nine subjects were Caucasian and 1 subject was Asian. Thirty-seven subjects were of Russian ethnicity, 2 subjects were of Tatar ethnicity and 1 subject was of Kalmyk ethnicity. Screening body weights ranged from 43.0 kg to 107.2 kg. Thirty-eight (95%) of subjects had colorectal cancer. One subject (2.5%) in the placebo group had an extranodal B-cell lymphoma of the cecum. For 1 subject (2.5%), the diagnosis of cancer was not confirmed. Baseline comorbidities were reported the study population. Collectively, comorbidities were reported in 32 (80.0 %) subjects, and included vascular disorders in 22 (55.0%) subjects, gastrointestinal disorders in 9 (22.5%) subjects, and cardiac disorders.

Study Rs-001

This study was a GLP, randomized, placebo-controlled, parallel-group, dose-ranging, PK/PD study in 160 healthy, research-naïve, non-irradiated NHP (rhesus macaques). The study evaluated the PK and PD of entolimod in animals receiving a single IM injection of the product. Single IM doses were administered including groups of placebo or 0.3, 1, 3, 6.6, 10, 40, or 120 µg/kg of entolimod were administered. Each group included between 9-10 males and 10-11 females. The age of the animals at the start of dosing ranged between 3 to 7 years. Body weight at the day of dosing ranged in males from 3.36 to 7.76 kg and in females from 3.42 to 5.84 kg.

Study Rs-23

This study was a GLP, randomized, blinded, placebo-controlled, parallel-group, dose-ranging study in 179 healthy, research-naïve NHP (rhesus macaques). The study evaluated the dose dependent effects of entolimod administration in animals receiving a single IM injection of the product after a 7.2-Gy dose of TBI. This dose was expected to result in a $LD_{70/60}$ on animal survival. Single IM doses of placebo or 0.3, 1, 3, 6.6, 10, 40, or 120 µg/kg of entolimod were administered. Ten animals per sex and group were allocated to each dose administered while in the placebo group 20/20 animals per sex were included. The age of the animals at the start of dosing ranged between. Body weight at the day of dosing ranged in males from 3.5 – 8 kg, pooling both sexes. The age of the animals was not provided.

No information regarding long term safety outcome is available, neither from the human studies nor from the animal studies as the NHPs were sacrificed at the end.

Adverse events

Healthy-subject studies (HU-7014 and HU-9001)

Adverse events including laboratory abnormalities from these limited population are summarized in the following Table below with subjects grouped by body-weight-adjusted dose level considering 3 dose categories (<0.4 µg/kg; 0.4-0.6 µg/kg; and >0.6 µg/kg); the dose category of 0.4-0.6 µg/kg is within the recommended body-weight-adjusted entolimod dosing paradigm described. Findings are listed in descending order of frequency within the group that received 0.4-0.6 µg/kg of entolimod. Data regarding laboratory abnormalities and vital sign abnormalities are derived from actual measured values.

Table 18: Number (%) of Subjects with TEAEs in ☐15 Subjects Overall by Body-Weight-Adjusted Dose Group, SOC and Preferred Term for Combined Studies HU-7014/HU-9001 – All Subjects

MedDRA SOC Preferred Term	Entolimod IM Body-Weight-Adjusted Dose Group					
	All Subjects (N=150)			0.40-0.60 μg/kg (N=51)		2nd Dose[a]
	<0.40 μg/kg (N=92)	0.40-0.60 μg/kg (N=51)	>0.60 μg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 μg/kg (N=13)
TOTAL N (%) OF SUBJECTS WITH TEAEs	**87 (94.6%)**	**51 (100.0%)**	**7 (100.0%)**	**34 (100.0%)**	**17 (100.0%)**	**12 (92.3%)**
General Disorders and Administration Site Conditions	**77 (83.7%)**	**46 (90.2%)**	**6 (85.7%)**	**32 (94.1%)**	**14 (82.4%)**	**10 (76.9%)**
Chills	56 (60.9%)	40 (78.4%)	4 (57.1%)	29 (85.3%)	11 (64.7%)	7 (53.8%)
Pyrexia	21 (22.8%)	20 (39.2%)	5 (71.4%)	17 (50.0%)	3 (17.6%)	2 (15.4%)
Injection site pain	35 (38.0%)	9 (17.6%)	0 (0.0%)	3 (8.8%)	6 (35.3%)	6 (46.2%)
Asthenia	14 (15.2%)	11 (21.6%)	1 (14.3%)	5 (14.7%)	6 (35.3%)	2 (15.4%)
Nervous System Disorders	**68 (73.9%)**	**44 (86.3%)**	**7 (100.0%)**	**29 (85.3%)**	**15 (88.2%)**	**10 (76.9%)**
Headache	62 (67.4%)	39 (76.5%)	7 (100.0%)	28 (82.4%)	11 (64.7%)	8 (61.5%)
Dizziness	23 (25.0%)	17 (33.3%)	3 (42.9%)	11 (32.4%)	6 (35.3%)	3 (23.1%)
Gastrointestinal Disorders	**51 (55.4%)**	**33 (64.7%)**	**7 (100.0%)**	**21 (61.8%)**	**12 (70.6%)**	**5 (38.5%)**
Nausea	44 (47.8%)	25 (49.0%)	6 (85.7%)	16 (47.1%)	9 (52.9%)	2 (15.4%)
Vomiting	24 (26.1%)	24 (47.1%)	5 (71.4%)	15 (44.1%)	9 (52.9%)	3 (23.1%)
Cardiac Disorders	**36 (39.1%)**	**21 (41.2%)**	**6 (85.7%)**	**16 (47.1%)**	**5 (29.4%)**	**2 (15.4%)**
Tachycardia	34 (37.0%)	21 (41.2%)	6 (85.7%)	16 (47.1%)	5 (29.4%)	2 (15.4%)

Table 18: Number (%) of Subjects with TEAEs in ≥15 Subjects Overall by Body-Weight-Adjusted Dose Group, SOC and Preferred Term for Combined Studies HU-7014/HU-9001 – All Subjects

MedDRA SOC Preferred Term	Entolimod IM Body-Weight-Adjusted Dose Group					
	All Subjects (N=150)			0.40-0.60 μg/kg (N=51)		2nd Dose[a]
	<0.40 μg/kg (N=92)	0.40-0.60 μg/kg (N=51)	>0.60 μg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 μg/kg (N=13)
Musculoskeletal and Connective Tissue Disorders	**33 (35.9%)**	**23 (45.1%)**	**4 (57.1%)**	**15 (44.1%)**	**8 (47.1%)**	**5 (38.5%)**
Back pain	16 (17.4%)	12 (23.5%)	1 (14.3%)	7 (20.6%)	5 (29.4%)	2 (15.4%)
Myalgia	15 (16.3%)	8 (15.7%)	1 (14.3%)	7 (20.6%)	1 (5.9%)	2 (15.4%)
Investigations	**30 (32.6%)**	**22 (43.1%)**	**5 (71.4%)**	**20 (58.8%)**	**2 (11.8%)**	**1 (7.7%)**
Leucocytosis	21 (22.8%)	10 (19.6%)	4 (57.1%)	10 (29.4%)	0 (0.0%)	0 (0.0%)
Neutrophil count increased	17 (18.5%)	9 (17.6%)	5 (71.4%)	9 (26.5%)	0 (0.0%)	0 (0.0%)
C-reactive protein increased	18 (19.6%)	7 (13.7%)	5 (71.4%)	7 (20.6%)	0 (0.0%)	0 (0.0%)
Transaminases increased	11 (12.0%)	14 (27.5%)	2 (28.6%)	12 (35.3%)	2 (11.8%)	1 (7.7%)
Vascular Disorders	**23 (25.0%)**	**19 (37.3%)**	**4 (57.1%)**	**13 (38.2%)**	**6 (35.3%)**	**0 (0.0%)**
Hypotension	19 (20.7%)	16 (31.4%)	4 (57.1%)	12 (35.3%)	4 (23.5%)	0 (0.0%)
Respiratory, Thoracic and Mediastinal Disorders	**10 (10.9%)**	**5 (9.8%)**	**0 (0.0%)**	**5 (14.7%)**	**0 (0.0%)**	**0 (0.0%)**

IBP=ibuprofen, IM=intramuscular, MedDRA=Medical Dictionary for Regulatory Activities, SOC=system organ class,
TEAE=treatment-emergent adverse event
aThe 2nd-dose group was a subset of the 1st-dose group.
Note: Subjects who have the same event more than once are counted only once for the preferred term. Subjects who have more than one AE within a SOC are counted only once in that SOC.
Note: The table is ordered by decreasing SOC and preferred term based on the total across all dose groups.
Source: Appendix **Error! Reference source not found.**

Table 18: Number (%) of Subjects with TEAEs in ☐15 Subjects Overall by Body-Weight-Adjusted Dose Group, SOC and Preferred Term for Combined Studies HU-7014/HU-9001 – All Subjects

	Entolimod IM Body-Weight-Adjusted Dose Group					
	All Subjects (N=150)			0.40-0.60 μg/kg (N=51)		2nd Dose[a]
MedDRA SOC Preferred Term	<0.40 μg/kg (N=92)	0.40-0.60 μg/kg (N=51)	>0.60 μg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 μg/kg (N=13)

Nearly 100% of subjects experienced a treatment-emergent adverse events (TEAEs) (see data below – table 18). With the exception of injection site pain, the most commonly experienced TEAEs were symptoms of the flu-like syndrome.

Observations following the 2nd dose in the 13 of the 24(?) subjects who not discontinued and were administered with a 2nd dose of 30 mg adverse events were similar to those following the 1st dose in the 36 subjects who received 30 μg of entolimod, but generally at a reduced frequency. The applicant interprets this as "consistent with a tachyphylaxis to the effects of the drug upon repeated administration"; it is not reported whether neutralising antibodies were involved in tachyphylaxis.

Pre-treatment with ibuprofen is claimed to be associated with lower frequencies of constitutional events and vital abnormalities associated with the flu-like syndrome considering subjects receiving 0.40-0.60 mg/kg of entolimod alone vs those taking 400 mg of ibuprofen before 0.40-0.60 mg/kg of entolimod; there were respective differences in the frequencies of chills (85.3% vs 64.7%), headache (82.4% vs 64.7%), and myalgia (20.6% vs 5.9%), as well as tachycardia (70.6% vs 47.1%) and fever (67.6% vs 41.2%).

Most of the TEAEs were mild (Grade 1) or moderate (Grade 2) in intensity (table 19). However, the findings that TEAEs categorized by the CTCAE as being severe (Grade 3) or potentially life-threatening (Grade 4) occurred in 28/150 (18.7%) subjects.

Table 19: Overall Summary of Number (%) of Subjects with TEAEs by Body-Weight-Adjusted Dose Group and Maximum CTCAE Grade for Combined Studies HU-7014/HU-9001 – All Subjects

	Entolimod IM Body-Weight-Adjusted Dose Group					
	All Subjects (N=150)			0.40-0.60 μg/kg (N=51)		2nd Dose
CTCAE Grade	<0.40 μg/kg (N=92)	0.40-0.60 μg/kg (N=51)	>0.60 μg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 μg/kg (N=13)
TOTAL N (%) OF SUBJECTS WITH TEAEs	**87 (94.6%)**	**51 (100.0%)**	**7 (100.0%)**	**34 (100.0%)**	**17 (100.0%)**	**12 (92.3%)**
Grade 1	35 (38.0%)	18 (35.3%)	0 (0.0%)	12 (35.3%)	6 (35.3%)	7 (53.8%)
Grade 2	40 (43.5%)	19 (37.3%)	5 (71.4%)	9 (26.5%)	10 (58.8%)	5 (38.5%)
Grade 3	11 (12.0%)	10 (19.6%)	2 (28.6%)	9 (26.5%)	1 (5.9%)	0 (0.0%)
Grade 4	1 (1.1%)	4 (7.8%)	0 (0.0%)	4 (11.8%)	0 (0.0%)	0 (0.0%)

CTCAE=Common Terminology Criteria for Adverse Events, IBP=ibuprofen,

Table 18: Number (%) of Subjects with TEAEs in ≥15 Subjects Overall by Body-Weight-Adjusted Dose Group, SOC and Preferred Term for Combined Studies HU-7014/HU-9001 – All Subjects

	Entolimod IM Body-Weight-Adjusted Dose Group					
	All Subjects (N=150)			0.40-0.60 μg/kg (N=51)		2nd Dose[a]
MedDRA SOC Preferred Term	<0.40 μg/kg (N=92)	0.40-0.60 μg/kg (N=51)	>0.60 μg/kg (N=7)	Without IBP (N=34)	With IBP (N=17)	<0.40 μg/kg (N=13)

IM=intramuscular, TEAE=treatment-emergent adverse event t
a The 2nd-dose group was a subset of the 1st-dose group
Note: A subject may have ≥1 CTCAE grade reported for an event. Only the highest grade is counted.
Source: Appendix **Error! Reference source not found.**

The frequency of TEAEs of Grade ≥3 was also lower in the group that received 0.40-0.60-mg/kg entolimod with IBP (5.9%) than in the group receiving 0.40-0.60-mg/kg of entolimod without IBP (38.2%); however as only 17 subjects received ibuprofen these differences may be chance findings.

Dose dependence of adverse events is demonstrated by the fact that the frequency of TEAEs of Grade ≥3 appeared to be lower in the ≤0.4-μg/kg dose group (13%) relative to the frequencies in the 0.40-0.60-μg/kg (27.5%) or in the >0.60μg/kg (28.6%) dose groups.

Study I196111-US

50% of subjects (13 of 26) experienced TEAEs that were Grade 1 or 2 in maximum severity. Grade 3 TEAEs were reported in 8 (20.8%) and Grade 4 TEAEs were reported in 5 (19.2%) of the 26 subjects. Decreases in lymphocytes, anaemia, hypotension, and hypokalaemia were the most commonly reported Grade 3 and 4 TEAEs (ie, reported in more than 1 subject). Grade 4 events were infrequent and included decreased lymphocyte count (4 subjects) and hypoxia due to pneumonia (unrelated to entolimod) (1 subject).

The commonly reported TEAEs at each dose level were consistent with the entolimod-related flu-like syndrome and included chills, pyrexia, nausea, and vomiting. Headache and dizziness was also described in a minority of subjects at higher absolute dose levels.

Hypotension and tachycardia were reported across all dose levels. Hypotension was mostly asymptomatic; while dizziness was reported among some subjects, no cases of syncope or overt drug-related ischemia were reported. Blood pressure normalized spontaneously or with fluid challenge. QT prolongation was also reported across all dose levels without an apparent relationship to dose. QT prolongation was asymptomatic, and there were no occurrences of ventricular arrhythmia.

Anaemia was a commonly reported hematologic abnormality in all dose groups. Lymphopenia was reported and appeared dose-dependent. Lymphopenia is an expected pharmacodynamic effect of entolimod and was not considered an adverse finding.

Serum chemistry abnormalities included hypoalbuminemia, increases in serum ALT or AST levels, hyperglycaemia, and hypophosphatemia. The increases in serum ALT and AST levels were observed sporadically across the dose levels but were accompanied by an increase (Grade 2) in serum total bilirubin level in only 1 subject (30-μg/<0.40-μg/kg dose group).

Study I196111-Russia

TEAEs were primarily Grade 2 in severity. Grade 3 TEAEs were reported in 2 of the 7 subjects (28.6%) and included lymphopenia (n=1) and another subject presented with dyspnoea, weakness, pain in the right infrascapular region, leucocytosis, and increases in serum AST, ALT, alkaline phosphatase, and magnesium levels. No Grade 4 events were reported.

The flu-like syndrome was the most commonly reported clinical adverse events: chills (6 of 7, 85.7%), fever (6 of 7, 85.7%), headache (3 of 7, 42.9%), and nausea (2 of 6, 28.6%). Sinus tachycardia was reported in 2 of the 6 subjects (28.6%), but there were no reports of hypotension. Dizziness was reported in only 1 subject (14.3%); no cases of syncope were reported.

Anaemia (3 of 7, 42.9%) and lymphopenia (3 of 7, 42.9%) were the most commonly reported hematologic abnormalities. Lymphopenia is an expected pharmacodynamic effect of entolimod and was not considered an adverse finding.

Serum chemistry findings included increases in serum alkaline phosphatase (4 of 7, 57.1%), AST (3 of 7, 42.9%), and ALT (2 of 7, 28.6%) levels. Increases in serum alkaline phosphatase and AST levels were accompanied by increases in serum direct bilirubin in 2 of the subjects. One subject each had hyperglycaemia and hypophosphatemia, effects which are consistent with the known safety profile of entolimod.

Study BL612-CBLB502

Grade 3 AEs were largely laboratory abnormalities, including hematologic events (lymphopenia, neutrophilia), hepatic abnormalities (increases in serum ALT, AST, GGT, and/or bilirubin), pancreatic abnormalities (lipase increased), and metabolism abnormalities (hyperglycaemia, hypomagnesemia, hypophosphatemia). Single instances of Grade 3 cardiovascular events were described, including hypotension, acute myocardial infarction, atrial fibrillation, and extrasystoles. Grade 4 AE comprised exclusively laboratory findings of lymphopenia, AST increased, lipase increased, and hypophosphatemia.

Flu-like syndrome was commonly reported, with a combined rate of 14/30 (46.7%) in entolimod-treated groups and 0/10 (0%) in the placebo-treated group. Nausea and/or vomiting were observed at a rate of 3/30 (10% among entolimod-treated subjects and 0/10 (0%) among placebo-treated subjects. Seven of 30 (23.3%) of entolimod-treated subjects and 0/10 (0%) of placebo-treated subjects had injection site erythema/reaction.

Considering TEAEs related to laboratory parameters, lymphopenia, which is an expected pharmacodynamic effect of entolimod, was the most common haematological abnormality, being reported among 15/30 (50.0%) of subjects in the entolimod groups and among 1/10 (10.0%) of subjects in the placebo group. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increases were respectively reported in 5/30 (16.7%) and 7/30 (23.3%) entolimod-treated subjects. A serum bilirubin increase was reported in 2/30 (6.7%) of entolimod-treated subjects. By contrast, ALT, AST, or bilirubin elevations were reported in 0/10 (0%) of placebo-treated subjects. Lipase elevations were described among 9/30 (30%) of those in the entolimod-treated group and among 2/10 (20%) of those in the placebo-treated group. Hypophosphatemia was noted in 6/30 (20%) of subjects given entolimod and among 1/10 (10%) of subjects given placebo. Hyperglycaemia was reported in 2/30 (6.7%) of subjects given entolimod and among 0/10 (10%) of subjects given placebo. Other less frequently reported serum chemistry findings (typically reported in 1 subject each) included anaemia, leucocytosis, leucopoenia, neutropenia, neutrophilia, alkaline phosphatase increased, creatinine increased, gamma glutamyl transferase (GGT) increased, hypercalcemia, and hypomagnesemia.

Hypotension was reported as an AE in 5/30 (16.7%) of subjects receiving entolimod and among 1/10 (10%) of subjects receiving placebo. Two (6.7%) of subjects treated with entolimod described dizziness; this AE was not reported for placebo-treated subjects. Cardiac events included single instances of acute myocardial infarction, atrial fibrillation, extrasystoles, and tachycardia, all occurring in entolimod-treated subjects.

Study Rs-001

In non irradiated Rhesus macaques the most significant findings reported included transient elevations of liver transaminases (ALT and/or AST) in many individual animals treated with entolimod. There were decreases in serum phosphorus values. There were ANC increases and absolute lymphocyte counts decreased. No blood pressure or ECG data, necropsy and histopathology were assessed.

Study Rs-23

In irradiated rhesus monkeys entolimod administration resulted in relevant decreases in systolic and diastolic pressure in addition to increase in pulse rates in animals receiving entolimod doses ≥1.0 µg/kg (See section of laboratory findings below). Necropsy data were only related to TBI and it is not possible to draw conclusions related to entolimod treatment since sacrifice was performed well after entolimod potential related toxicity was recovered (Day 60).

Serious adverse events and deaths

There were no deaths among healthy subjects participating in HU-7014/HU-9001. Nor were within 30 days of last study drug administration for any patients with cancer participating in I196111-US, I196111-Russia, or BL612-CBLB502. No information regarding long term safety outcome is available, neither from the human studies nor from the animal studies as the NHPs were sacrificed at the end.

Regarding serious adverse event (SAE):

- **1. Healthy-subject studies (HU-7014 and HU-9001)**: Four (2.6%) subjects experienced a SAE. Three of these subjects were in the 35-µg group (without IBP): Grade 2 acute cholecystitis (subject 02-081), Grade 4 aspartate aminotransferase [AST] increase (subject 180-103), and Grade 2 hypotension (subject 02-012). A further subject was in the 35-µg group (with IBP) and presented Grade 1 glucose intolerance (subject 02-086). Instead, considering body-weight-adjusted dose groups, 1 subject was in the <0.40-µg/kg group (Grade 2 hypotension) and the 3 remaining subjects were in the 0.40-0.60-µg/kg group.

An additional subject (02-085) had a TEAE that was considered not related to entolimod but it might be medically relevant. From the clinical safety summary is only known that the patient received 35 µg of entolimod (0.40-0.60 µg/kg) and presented with serum transaminase elevations. Narratives of this patient has not been provided (other concern).

- **Study I196111-US:** Five SAEs were reported but only 2 were considered related to entolimod: hypotension at 30 µg and at 40 µg. Narratives of the patients presented with related-entolimod SAE have not been provided.
- **Study I196111-Russia:** One SAE of dyspnoea was reported but judged to be unrelated to entolimod.
- **Study BL612-CBLB502**: 15 SAEs were reported in 10 study subjects. Eleven of them were considered related to entolimod: Grade 4 serum AST elevation (n=2), grade 3 atrial fibrillation (n=1), grade 4 lymphopenia (n=2), grade 4 serum lipase elevation (n=2), grade 4 hypophosphatemia (n=1), grade 3 extrasystoles (n=1) and acute myocardial infarction in a subject

with preexisting cardiac ischemia (n=1). Narratives of the patients presented with related-entolimod SAE have not been provided.

The high number of related SAEs in the small number of healthy volunteers receiving posology recommended doses of entolimod indicate a considerable higher toxicity for entolimod than reported from the NHPs. The observation that 15 SAEs were reported among 10 of the 40 cancer patients who participated in BL612-CBLB502 is alarming and raises significant concerns, whether the product's toxicity can be tolerated really by the intended target population, even if considering the difficulties in assessing causality in cancer patients.

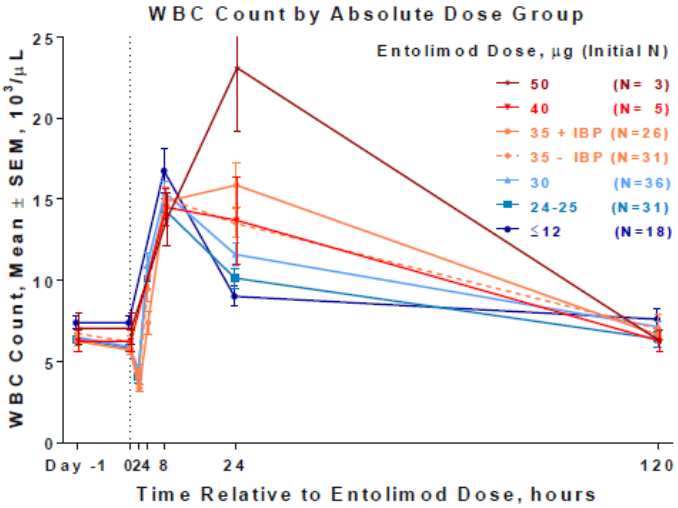
Laboratory findings

For combined HU-7001/HU-9001:

Hematological parameters

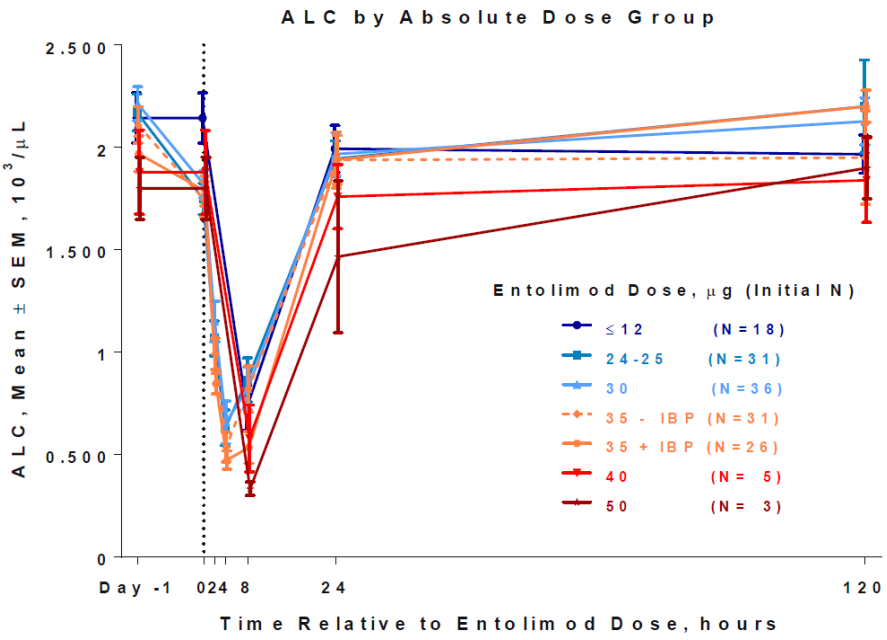
Transient modest decreases followed by substantial increases in mean WBC counts were observed in the human population after administration of entolimod as shown in Figure. Increases in mean WBC counts due to an increase in ANC persisted through 24 hours post dose, particularly at entolimod doses of ≥30 mg absolute or ≥40 µg/kg body-weight-adjusted, and then returned to pre-treatment levels by 120 hours after injection. Pre-treatment with IBP seems not to substantially alter changes in mean WBC counts.

Figure 38 : WBC Counts by Time and Absolute Dose Group for Combined Studies HU-7014/HU-9001 – All Subjects (1stDose)



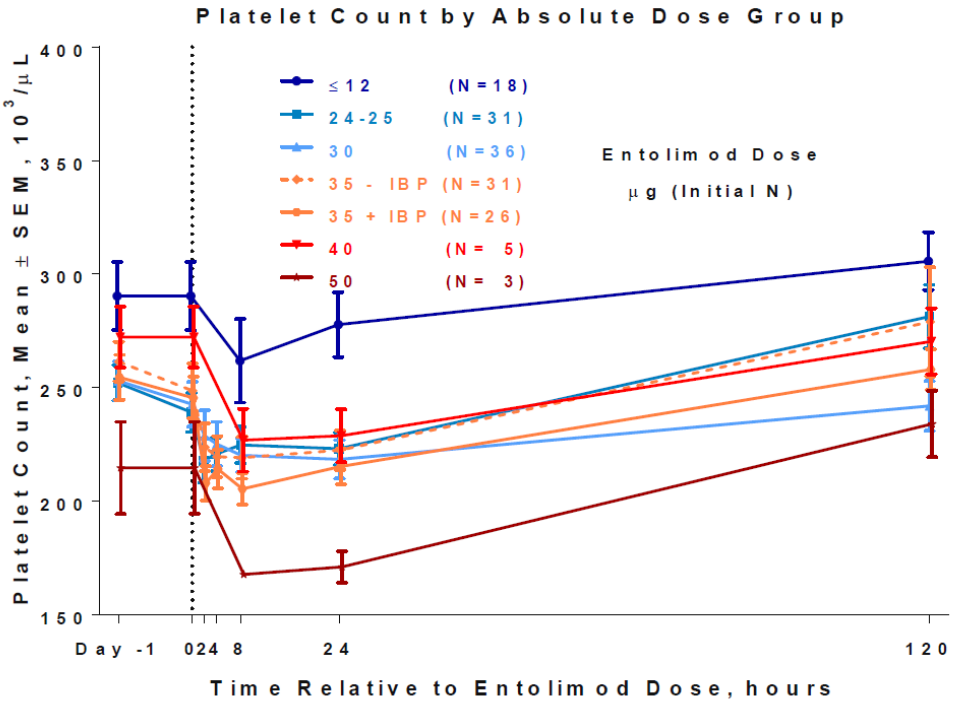
As shown in Figure 3, decreases in ALC were observed after administration of entolimod, with mean values declining below the lower limit of normal (LLN) (0.7 x 103 cells/µL) at 4 to 8 hours postdose and generally recovering by 24 hours postdose. Doses ≥40 mg absolute or >60 µg/kg body-weight-adjusted were associated with more persistent decrements in mean ALC. Pretreatment with IBP did not substantially alter changes in mean ALC.

Figure 39: ALC by Time and Absolute Dose Group for Combined Studies HU-7014/HU-9001 – All Subjects (1stDose)



As illustrated in Figure 40, modest reductions in mean platelet count within the normal range were observed in response to entolimod administration and showed some dose dependency at the extremes of the dosing range. Again recovery from the effect occurred by 120 hours postdose and pretreatment with IBP did not meaningfully alter changes in mean platelet count.

Figure 40: Platelet Counts by Time and Absolute Dose Group for Combined Studies HU-7014/HU-9001 – All Subjects (1stDose)



Differences in mean haemoglobin values among dose groups were also similarly transient mean and increases in haemoglobin from Day -1 to predose followed by decreases from predose to 8 hours postdose with persistence through 120 hours postdose. It is acknowledged that such minor alterations

in mean haemoglobin values were not clinically meaningful and may be explained most likely due to changes in hydration status (overnight fast prior to entolimod administration followed by encouraged hydration following drug administration) in the healthy volunteers.

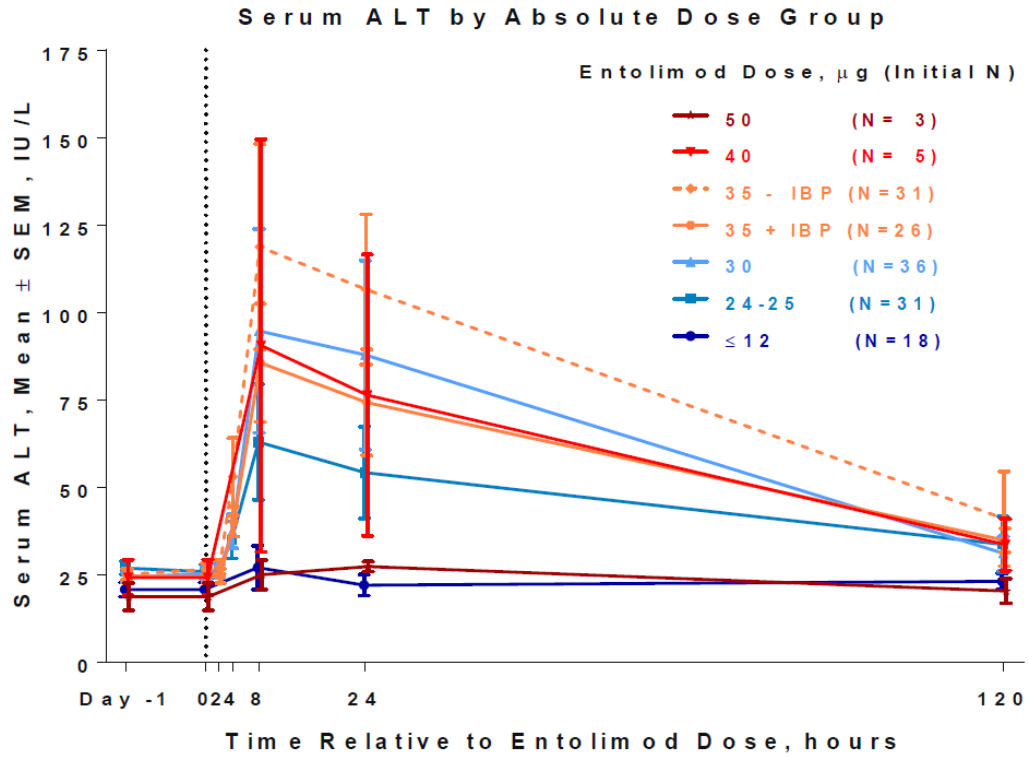
As expected from TLR5 activation transient dose-depending haematological changes as increases in white blood cell count (WBC), increases in ANC, decreases in platelet count, and decreases in ALC were seen in response to entolimod administration and are in accordance with the response observed in the non-clinical trials. However, these changes were considered to be components of the expected mechanism-related activity of entolimod and are not likely to have clinical sequelae and were consequently not considered as adverse events. Nevertheless, at least the dose-depending decrease of platelet count may also indicate disseminated intravascular coagulation (DIC) and the decreases seen for hemoglobin from predose to 8 hours postdose with persistence through 120 hours postdose seems to be best explained as consequences of capillary leakage, because the changes observed during this short period and prolonged recovery during 120 hours are not likely to be caused from blood lost or impaired regeneration.

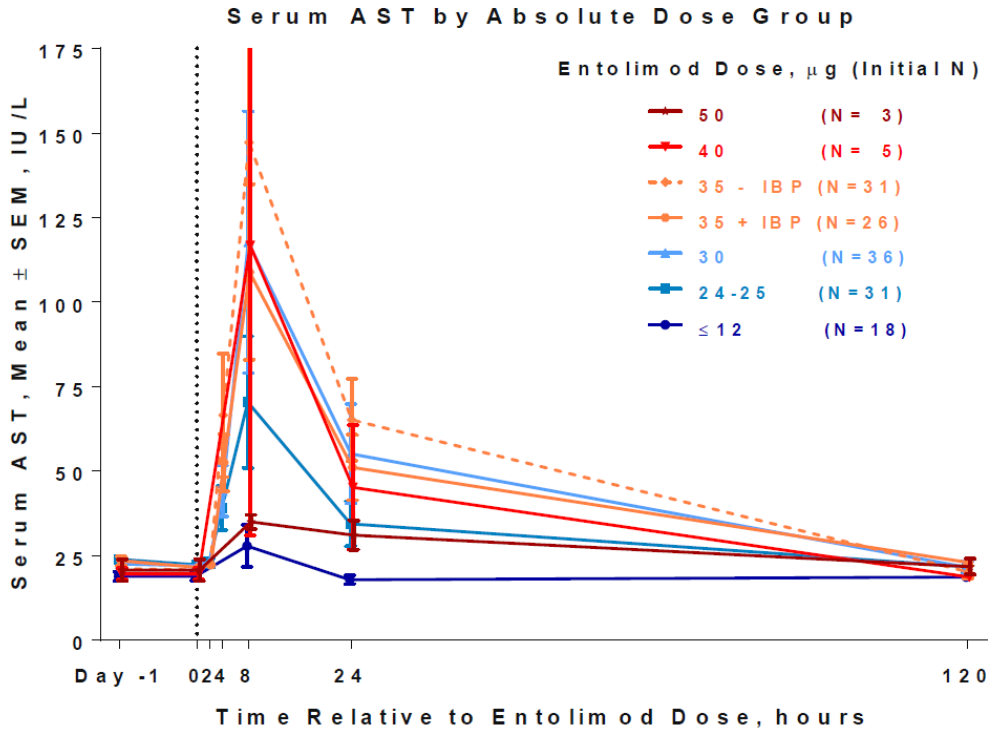
Chemistry laboratory parameters

It is reported that Administration of entolimod was associated with transient increases in serum levels of ALT and AST, without changes in serum levels of ALP or bilirubin. However, the mode of presenting the data is not comprehensible to assess the liver enzyme outcome at present and to exclude Hy's law cases adequately.

As displayed in Figure, rapid transient increases in serum ALT and AST levels were observed after administration of entolimod, particularly at entolimod absolute doses of 30, 35, and 40 µg or body-weight-adjusted doses of 0.40-0.60 µg/kg.

Figure 41: Mean Serum ALT and AST Values by Time and Absolute Dose Group for Combined Studies HU-7014/HU-9001 – All Subjects (1stDose)





The magnitudes of the mean increases and the pattern of changes by dose group were similar between the 2 parameters. By 24 hours postdose, serum AST showed more rapid recovery than did serum ALT, perhaps reflecting the relative T½ values for these enzymes (~18 hours for AST versus ~36 hours for ALT in humans) (21), with the pattern indicating no ongoing hepatic injury.

Since entolimod is a protein composed of natural amino acids, its disposition is likely via proteases present in serum; thus, classical metabolic hepatic toxicity is not a consideration.

Other laboratory abnormalities associated with entolimod administration were transient hyperglycaemia and transient hypophosphatemia. Such effects have been seen following administration of IL-6 in patients with cancer and, thus, likely indicate secondary consequences of entolimod induction of this cytokine.

No clinically relevant changes in serum creatinine or blood urea nitrogen (BUN) were observed.

Cytokine laboratory parameters

Changes in plasma concentrations of G-CSF, IL-6, IL-8, IL-10, TNFα, IL-1α, IL-1β, IL-2, IL-12, IFNα, and IFNγ where assessed in the healthy subjects who participated in HU-7014 and HU-9001.

Entolimod provoked substantial, generally dose-dependent changes in the magnitude and persistence of plasma G-CSF and IL-6. These 2 cytokines (along with ANC24) were pre-specified as efficacy biomarkers. Mean peak concentrations of plasma G-CSF and IL-6 were observed during the first 4 to 8 hours postdose. Resolution toward baseline values occurred by 24 hours, particularly for plasma IL-6.

Entolimod also induced generally dose-dependent changes in the magnitude and persistence of IL-8, IL-10, and TNFα. Peak mean concentrations cytokines were observed during the first 2 to 4 hours postdose and were ~5- to 10-fold less than those for plasma G-CSF and IL-6. Resolution to mean baseline values occurred in most dose groups by 24 hours postdose. The applicant is requested to explain why these parameters were not selected as additional biomarkers in order to clarify dose depending effects and providing a better modelling of entolimod's PK/PD data base.

The types and patterns of induction of cytokines over time that were observed after administration of the first dose of entolimod were also observed after administration of a second dose of entolimod among those randomized to receive two 30-µg (<0.40-µg/kg) doses of study drug in HU-9001. However, the peak values and durations of effect were markedly lower than those after the first dose of entolimod (HU-9001). These observations confirm that the drug induces a tachyphylaxis to its own effects with repeated administration.

Notably, entolimod did not increase concentrations of IL-1α, IL-1β, IL-2, IL-12, IFNα, or IFNγ Entolimod induction of plasma cytokines such as IL-6 and TNFα are likely the cause of the flu-like syndrome and hypotension associated with the drug, based on existing knowledge regarding the AE profile of these cytokines when administrated pharmacologically.

However, when considered collectively, the data regarding cytokine induction by entolimod in the applicant's view does not show a pattern that is consistent with "cytokine storm." The plasma concentrations of cytokines like IL-6 and TNFα, which are generated by entolimod, are substantially lower than those observed with a drug like TGN1412 that has caused cytokine storm. Most importantly, it is claimed that entolimod induces neither IL-2 nor IL-1α, IL-1β, IL-12, or IFNγ that are components of the cytokine profile in patients with cytokine storm.

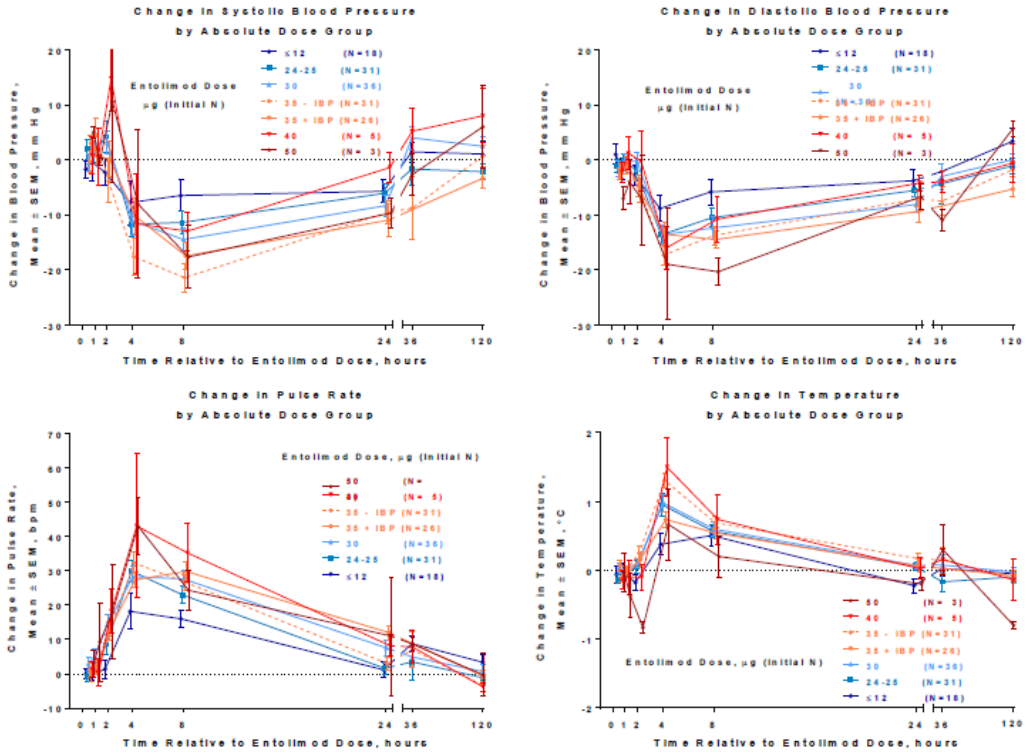
The applicant further argues that these findings are consistent with the knowledge that entolimod TLR5 agonism promotes NF-κB activation mainly in non-immune cells (ie, in epithelial, endothelial, stromal, and hepatic compartments) rather than in immunocytes. TLR5-responsive cells cannot express cytokine-storm-inducing cytokines, which contrasts with the TGN1412 agonism of T-cells that are involved in cytokine response amplification through IL-2. Moreover, changes in IL-6 and TNFα show shorter times to peak effects and resolution than were observed in patients with cytokine storm.

Blood pressure

Table 38: Summary of Vital Sign Parameters: Frequency of Abnormalities, Maximum Changes, Duration of Time Outside Normal Ranges, and Proportion of Subjects Recovering by Absolute Dose Group for Combined Studies HU-7014/HU-9001 - All Subjects (1stDose)

	Entolimod doses						
	≤12 µg (N=18)	**24-25 µg (N=13)**	**30 µg (N=36)**	**35 µg without IBP (N=31)**	**35µg with IBP (N=26)**	**40 µg (N=5)**	**50 µg (N=3)**
Systolic Blood Pressure (decrease)							
Subjects below normal, n (%)	3 (16.7)	5 (16.1)	9 (25.0)	12 (38.7)	6 (23.1)	1 (20.0)	2 (66.7)
Mean lowest observed value, mm Hg	101.8	103.5	96.8	96.4	98.9	92.4	73.3
Mean maximum decrease from baseline, mm Hg	-19.2	-20.9	-25.3	-30.4	-29.2	-16.8	-29.0
Mean duration below normal range, hours	0.347	0.622	1.665	1.834	0.206	1.600	16.311
Subjects below normal who returned to normal, n/N (%)	3/3 (100.0)	5/5 (100.0)	9/9 (100.0)	12/12 (100.0)	6/6 (100.0)	1/1 (100.0)	2/2 (100.0)
Diastolic Blood Pressure (decrease)							
Subjects below normal, n (%)	2 (11.1)	6 (19.4)	10 (27.8)	7 (22.6)	6 (23.1)	1 (20.0)	2 (66.7)

Mean lowest observed value, mm Hg	60.2	61.9	57.4	57.2	59.0	56.2	41.0
Mean maximum decrease from baseline, mm Hg	-15.8	-20.3	-22.2	-23.8	-23.3	-18.2	-29.0
Mean duration below normal range, hours	0.181	0.330	0.464	1.401	1.832	4.000	6.644
Subjects below normal who returned to normal, n/N (%)	2/2 (100.0)	6/6 (100.0)	10/10 (100.0)	7/7 (100.0)	6/6 (100.0)	1/1 (100.0)	2/2 (100.0)
Pulse (increase)							
Subjects above normal, n (%)	4 (22.2)	21 (67.7)	23 (66.7)	21 (67.7)	15 (61.5)	4 (80.0)	3 (100.0)
Mean highest observed value, bpm	9.3	107.3	108.3	108.8	104.5	122.0	124.0
Mean maximum increase from baseline, bpm	26.1	35.2	38.8	38.1	38.2	56.6	47.7
Mean duration above normal range, hours	1.222	50.703	39.274	5.721	8.915	6.600	19.656
Subjects above normal who returned to normal, n/N (%)	4/4 (100.0)	19/21 (90.50	23/24 (95.8)	21/21 (100.0)	15/15 (100.0)	4/4 (100.0)	3/3 (100.0)
Temperature (increase)							
Subjects above normal, n (%)	5 (27.8)	15 (48.4)	23 (63.9)	24 (77.4)	13 (50.0)	3 (60.0)	3 (100.0)
Mean highest observed value, °C	37.28	37.70	37.78	37.94	37.60	38.08	38.30
Mean maximum increase from baseline, °C	0.76	1.22	1.35	1.54	1.05	1.50	1.37
Mean duration above normal range, hours	2.657	2.561	12.786	2.556	3.060	3.800	48.333
Subjects above normal who returned to normal, n/N (%)	5/5 (100.0)	15/15 (100.0)	23/23 (100.0)	24/24 (100.0)	13/13 (100.0)	3/3 (100.0)	3/3 (100.0)



IBP=ibuprofen, SEM=standard error of the mean

Mean values for systolic blood pressure typically rose during the first 2 hours after entolimod administration with diastolic blood pressure being generally stable. Thereafter, mean values for systolic and diastolic blood pressure declined through 4 to 8 hours postdose in all dose groups before returning toward normal by 24 hours. Within the variability of the data, there is a suggestion of a dose response.

Among subjects in the target entolimod dose range of 0.40-0.60 µg/kg, the mean maximum decreases were -28.8 mm Hg and -22.8 mm Hg for diastolic and systolic blood pressure, respectively. While declines in blood pressure were common, nadir values were in the normal ranges for most subjects; thus, among those in the target entolimod dose range of 0.40-0.60 µg/kg, 16/51 (31.4%) and 13/51 (25.5%) subject had values less than the lower limit of normal for systolic and diastolic blood pressure, respectively.

Among those in the 0.40-0.60-µg/kg dose group who had abnormalities in systolic or diastolic blood pressure, mean durations of time below the lower limit of normal were <1.5 hours for both parameters and 51/51 (100%) had normal blood pressure values at recovery.

Coincident with decreases in systolic and diastolic blood pressure, increases in mean pulse rate were observed after administration of entolimod, with values peaking at 4 hours postdose and largely recovering by 24 hours. Small increases in mean temperature were observed in all dose groups at 4 to 8 hours after administration of entolimod, with return to normal values by 24 hours postdose. The changes suggested a dose-related effect on pulse. Among those in the dose range of 0.40-0.60 µg/kg, mean maximum increases in pulse rate were 38.1 bpm and in temperature were 1.52oC. For those receiving entolimod doses of 0.40-0.60 µg/kg, 32/51 (65.7%) and 30/51 (58.8%) had values above the ULN for pulse rate and temperature. Mean recovery times in the 0.40-0.60-µg/kg dose group were <8 hours and any tachycardia or fever fully resolved in 51/51 (100%) of these subjects.

Pretreatment with IBP did not consistently and substantially alter blood pressure measures in these analyses, although body temperature is normalized.

Vital sign changes following administration of the second dose of study drug in the dose group receiving two 30-mg doses of entolimod is documented in the study report for HU-9001. With the second dose, a decrease was again observed for both systolic and diastolic blood pressure, although to a lesser degree, consistent with the attenuation of entolimod-related AEs, PD effects, and laboratory abnormalities following repeated dosing.

Consequently, such changes were addressed inconsistently, with neither the minimum absolute blood pressure nor the maximum decrease from baseline necessarily prompting an investigator decision to administrator IV fluids. As described in study reports for HU-7014 and HU-9001, 13 of 150 subjects (8.7%) received IV fluids and **in one subject (0.6%) IV dopamine was given.**

For entolimod labelling, the applicant proposes to include text recommending that patients should be made aware of the potential for hypotension and should be advised to rest in bed, maintain abundant oral hydration, and proceed cautiously and incrementally when rising from a supine position. IV fluids or other support may be offered if such resources are available, particularly if patients are symptomatic due to hypotension.

Electrocardiography

In **HU-7014**, the mean values for heart rate and for respiratory rate (RR), QT, and QT corrected heart rate using Fridericia's formula (QTcF) during the post treatment period were not markedly different from those that were observed before administration of entolimod. No cases of Grade 3 prolonged QTcF (>501 msec) were reported. The maximum change in QTcF at 3 hours after administration of the entolimod dose was ≥60 msec in only 1 of the 50 subjects (2.0%).

In **HU-9001**, no clinically relevant changes in PR or RR interval were observed. One case of Grade 3 prolonged QTcF (>501 msec) was reported in the 35-µg dose group (Subject 02-086) and represented the maximum QTcF value that was observed during the study. This subject had a QTcF value of 532 msec at 4 hours after entolimod administration. The diagnosis for this subject based on ECG results was sinus tachycardia with T wave abnormality and possible anterolateral ischemia. The observed tachycardia was reported as a Grade 1 AE that was considered related to administration of entolimod and from which the subject totally recovered. No other ECG results suggested an ischemic pattern. The only changes from baseline in QT or QTcF were observed for 1 (4.0%) and 2 (8.0%) subjects, respectively, in the 35-µg dose group.

For STUDY I196111-US

Anemia was a commonly reported hematologic abnormality in all dose groups. Lymphopenia was reported at dose levels ≥15 µg/dose with 4 cases of grade 4 at the 30 and 40 µg/dose levels.

Serum chemistry abnormalities included hypoalbuminemia, elevated serum alkaline phosphatase, increases in serum ALT or AST levels, hyperglycemia and hypophosphatemia. No clinically relevant adverse effects of entolimod were observed on renal (serum creatinine, serum BUN) or acid-base (serum bicarbonate) laboratory parameters.

Administration of entolimod was associated with transient decreases in blood pressure and increases in pulse rate and oral temperature.

Treatment-emergent increases in QTcB were observed in approximately two-thirds of subjects after administration of entolimod, with no apparent relationship to dose. Increases from baseline resulted in Grade 1 values (>450 to ≤480 msec) for QTcB, with only 1 instance of Grade 2 prolongation (>480 to ≤500 msec) and 1 instance of Grade 3 prolongation (>500 msec) observed. The Grade 3 value

(514 msec) was observed in a subject in the 40-µg/dose group and, along with Grade 3 pyrexia, was considered DLT by the investigator.

For STUDY I196111-RUSSIAN

Anemia (3 of 7, 42.9%) and lymphopenia (3 of 7, 42.9%) were the most commonly reported hematologic abnormalities. Serum chemistry findings included increases in serum alkaline phosphatase (4 of 7, 57.1%), AST (3 of 7, 42.9%), and ALT (2 of 7, 28.6%) levels, which were accompanied by increases in serum direct bilirubin in 2 of the subjects. One subject each had hyperglycemia and hypophosphatemia. Other less frequently reported serum chemistry findings (reported in 1 subject each) included decreases in serum albumin, magnesium, and sodium levels and increases in serum lactate dehydrogenase (LDH), lipase, and uric acid levels.

For STUDY BL612-CBLB502

Changes in hematological parameters observed in the entolimod treatment groups but not in the placebo treatment group included decreases in lymphocytes (in 50%), and increases in leukocytes and neutrophils.

Among serum hepatic chemistry changes that were observed in the entolimod treatment groups but not in the placebo treatment group, findings included increases in mean values for ALT, AST, alkaline phosphate, GGT and lactate dehydrogenase (LDH), but not total bilirubin. Other changes occurring just in the entolimod treatment groups included increases in serum glucose and decreases in values for serum phosphate, sodium, and magnesium. Mean serum creatine increased more in entolimod groups than in the placebo group but blood-urea nitrogen (BUN) values did not shift in any of the groups. Serum lipase values increased inconsistently across the groups. No entolimod-exclusive changes were noted for mean serum values for potassium, calcium, chloride, creatine kinase (CK), or uric acid.

There were no clinically significant shifts in the mean systolic or diastolic blood pressure, pulse, body temperature, or respiratory rate values reported during the study in all treatment groups.

Hypotension was reported as an AE in 5/30 (16.7%) of subjects receiving entolimod and among 1/10 (10%) of subjects receiving placebo. Two (6.7%) of subjects treated with entolimod described dizziness; this AE was not reported for placebo-treated subjects. Cardiac events included single instances of acute myocardial infarction, atrial fibrillation, extrasystoles, and tachycardia, all occurring in entolimod-treated subjects.

Grade 3 AEs were largely laboratory abnormalities, including hematologic events (lymphopenia, neutrophilia), hepatic abnormalities (increases in serum ALT, AST, GGT, and/or bilirubin), pancreatic abnormalities (lipase increased), metabolism abnormalities (hyperglycemia, hypomagnesemia, hypophosphatemia). Single instances of Grade 3 cardiovascular events were described, including hypotension, acute myocardial infarction, atrial fibrillation, and extrasystoles. Grade 4 AE comprised exclusively laboratory findings of lymphopenia, AST increased, lipase increased, and hypophosphatemia.

ECGs showed no clinically relevant changes in PR interval or QRS interval. All study subjects had normal QTc interval values at screening. The mean change in the QTc interval 8 hours after administration of the study medication was different depending on the dose group. ECG abnormalities were identified in 5 subjects during the study that were reported as AES, including SAEs: Grade 1

tachycardia, Grade 1 QT interval prolongation, Grade 3 atrial fibrillation, Grade 3 ventricular extrasystoles and Grade 3 myocardial infarction.

Study Rs-001

In non irradiated Rhesus macaques the most significant findings reported included transient elevations of liver transaminases (ALT and/or AST) in many individual animals treated with entolimod. The elevations were higher in males, peaking at 8 hours post dosing and generally started to decline by 24 hours. In most cases, the values returned to normal within 48 hours. Elevation reached values in some animals of up to 6 -8 fold.

There were decreases in serum phosphorus values, starting at 24 hours post-dose, both in males and females treated with 3 µg/kg CBLB502 and higher. The values returned to normal by 72 hrs postdose. The entolimod dose-dependent pattern and magnitude of the ANC increases at 16 hours (the exploratory neutrophil endpoint) was similar to that of ANC increases at 24 hours.

Absolute lymphocyte counts decreased at 8 hours post-dose at doses 0.3 µg/kg and higher (i.e. Groups 3 to 8), both in males and females. The decreases were most pronounced at 40 µg/kg and 120 µg/kg. These values returned to pre-dose levels by 72 hours post-dose.

No blood pressure or ECG data, necropsy and histopathology were assessed.

Study Rs-23

In irradiated rhesus monkeys entolimod administration resulted in relevant decreases in systolic and diastolic pressure in addition to increase in pulse rates in animals receiving entolimod doses ≥1.0 µg/kg. Necropsy data were only related to TBI and it is not possible to draw conclusions related to entolimod treatment since sacrifice was performed well after entolimod potential related toxicity was recovered (Day 60).

Safety in special populations

Entolimod's safety in special populations is not characterised. The applicant presents only some hypotheses regarding dosing of the product in general according age, renal and hepatic function as well as for the treatment of patients with increased cardiac risks; however, no data is available to evaluate the correctness of the conclusions and proposal made.

With respect to risk from overdose the limited information available in humans clearly indicates a narrow therapeutic range and a high sensitivity to overdosing as indicated by the recommendation for a dose maximum of 50 µg in the product information. In this context the applicants statement that to date, animals in general toxicology studies have received very high single doses of entolimod without mortality or organ damage is seen as misleading.

Elderly Population

The applicant stated that data regarding the safety of entolimod in patients as old as 84 years of age suggest entolimod can reasonably be administered to geriatric patients. Dosing of entolimod in people ≥65 years of age is the same as for people <65 years of age and is based on body weight.

Renal Dysfunction

There are no clinical data on the use of entolimod in people with pre-existing renal impairment.

The applicant states that based on the available clinical trial data, entolimod does not appear to induce kidney injury in healthy humans and, as a protein, is most likely degraded (e g, by proteolysis, nonspecific endocytosis or target-mediated clearance) and is not directly excreted. Insofar, it might be

unlikely that a dose adjustment is needed for patients with renal insufficiency because of concerns regarding drug disposition.

Dosing of entolimod in people with renal impairment is proposed to be the same as for people without renal impairment and is based on body weight.

Hepatic Dysfunction

There are no clinical data on the use of entolimod in people with pre-existing hepatic impairment.

In clinical trial participants receiving recommended entolimod doses, increases in serum ALT and AST have been observed. This may indicate increased risks from entolimod for patients with impaired liver function, although these events are reported to have been asymptomatic, transient and were not associated with obvious hepatic dysfunction (eg, increases in bilirubin, decreases in albumin, or prolongation of coagulation times). All clinical trial subjects recovered.

Because patients with pre-existing hepatic disease have not been systematically studied, the extent of the risk in this population is unknown. Patients with pre-existing hepatic disease (eg, non-alcoholic fatty liver disease, alcoholic liver disease, or chronic viral hepatitis) could be at increased risk of liver injury and will be frequent in the target population.

The applicant argues that entolimod is a protein that is most likely degraded (eg, by proteolysis, nonspecific endocytosis or target-mediated clearance) and is not directly excreted, it is unlikely that a dose adjustment is needed for patients with hepatic insufficiency based on concerns regarding drug disposition.

In the event of a radiation disaster, the decision to administer entolimod to people with pre-existing hepatic disorders should consider the risk of death associated with the estimated radiation exposure and the severity of the underlying hepatic disorder. Dosing of entolimod in people with hepatic impairment is the same as for people without hepatic impairment and is based on body weight. Patients should be advised to avoid the use of known hepatotoxic substances (eg, alcohol) in conjunction with entolimod administration.

Vascular Disorders or Low Cardiac Output

Administration of entolimod can be associated with decreases in arterial blood pressure and increases in heart rate that may persist for several hours postdose. Patients who are dehydrated or have traumatic injuries resulting in loss of blood could be at increased risk of developing hypotension following the administration of entolimod. Patients taking medications that acutely decrease vascular tone (eg, nitrates, narcotics) or suppress heart rate might (eg, β-blockers) might also be at increased risk of hypotension.

Paediatric Population

The PK, PD, and safety of entolimod have been evaluated in adults but have not been studied in the paediatric population. No data are available for this subgroup.

The extrapolation of the effects of entolimod to human paediatric patients is based on safety studies in nonhuman primates in age equivalent to human age of 8-24 years.Proposed dosing in paediatric patients is the same as for adult patients and is based on body weight starting at 1,75 Kg.

The safety of entolimod in children from birth to 23 months have not yet been established in human or juvenile animals. The SmPC mentions that the safety of Entolimod in children from birth to 23 months have not yet been established and no data are available. It is noted that the decision to administer entolimod to neonates, infants, or toddlers, should take into account the risk of death associated with

the estimated radiation exposure and the absence of information on the effects of entolimod in these paediatric subgroups.

TLR5 Mutations

HU-9001 included a secondary objective to describe the effect of TLR5 genotype on entolimod biomarker levels, safety, and PK following IM administration. All enrolled subjects were genotyped for the TLR5 stop codon (TLR5392 stop) mutation. The TLR5392 stop mutation is reported to occur as a dominant point mutation in 10% of people, leaving them with increased susceptibility to certain diseases (eg, Legionnaires' disease). Only 6 subjects enrolled into HU-9001 carried the mutant genotype. One subject was homozygous for the mutation, whereas 5 subjects were heterozygous for the same mutation. While 1 subject homozygous for TLR5392 stop and 1 heterozygous subject had almost no PD response to entolimod, 2 of the heterozygotes appeared to have moderate responses, and 2 of the heterozygotes had wild-type TLR5 responses. Due to the small number of relevant subjects, it was not possible to draw any definitive conclusions regarding the impact on the efficacy or safety profile of entolimod.

Use in Pregnancy and Lactation

For studies evaluating the maternal and foetal effects of entolimod when administered to pregnant animals (see the non-clinical report of the Rapporteur).

There are no adequate and well-controlled studies in pregnant women. The specific effects of entolimod on pregnant women or on human embryogenesis, foetal development, or post-natal development are unknown. The company proposes, as SmPC mentions, that the decision to administer entolimod to a pregnant woman should consider the lack of knowledge regarding drug effects during pregnancy relative to the potential benefits, realizing that exposure to high doses of irradiation may be potentially lethal to the pregnant woman and may also have adverse developmental or fatal effects on a foetus. If entolimod is used during pregnancy, the patient should be apprised of the potential for hazards to herself or to the foetus. Pregnant women who receive entolimod in the context of a radiation exposure should be advised to seek obstetrical follow-up as soon as possible after drug administration to determine appropriate surveillance or intervention relating to the pregnancy.

It is not known if entolimod is excreted in human breast milk or whether ingestion of entolimod in breast milk could pose a potential medical risk to a nursing child. The company proposes, as SmPC mentions, that administration of entolimod to a breastfeeding woman should consider the extent of the radiation exposure to both the woman and child, whether the child will also receive entolimod, and whether breastfeeding can be interrupted to provide alternative sources of nutrition for the child

Overdose

The limited information available in humans clearly indicates a narrow therapeutic range and a high sensitivity to overdosing as indicated by the recommendation for a dose maximum of 50 µg in the product information. In this context the applicants statement that to date, animals in general toxicology studies have received very high single doses of entolimod without mortality or organ damage is seen as misleading.

Drug Abuse

Entolimod does not represent a drug with abuse potential as its administration causes patient discomfort due to constitutional symptoms. Moreover, repeated administration induces tachyphylaxis to the drug's pharmacological effects due to development of neutralizing antibodies. Additionally,

entolimod will only be available in the event of a radiation emergency and will be administered by a health care professional.

Withdrawal and Rebound

Because entolimod is administered as a single injection, withdrawals from its effects are not anticipated. There is no evidence of a rebound pharmacological effect associated with single doses of entolimod.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Ability

Constitutional and gastrointestinal symptoms are expected after entolimod administration and may cause patient inattention. Patients may have postural hypotension. Therefore, patients who have received entolimod should refrain from driving or operating machinery until they have recovered from the symptomatic adverse effects of the drug.

Immunological events

Because entolimod is derived from bacterial flagellin protein, humans may have pre-existing cross-reactive antibodies to entolimod due to prior bacterial infections. Preexisting entolimod-reactive neutralizing antibodies have been shown to reduce entolimod exposure (see section of Clinical Pharmacology) and might have the potential to influence entolimod effects. Thus, immunogenicity analyses were performed in HU-7014 and HU-9001 and in I196111-US to investigate the frequency of preexisting entolimod-reactive antibodies and the development of antibodies in response to entolimod administration.

At the proposed dose, 8/51 (15.7%) subjects participating in HU-7014 and HU-9001 had entolimod-reactive antibodies in serum prior to study drug administration, and in 5 out of those 8 subjects (62.5%) those antibodies were considered to be neutralizing. By the last follow-up visit after entolimod administration, 50/51 (98%) of subjects had developed postdose entolimod-reactive antibodies, and 49 out of those 50 (98%) had neutralizing antibodies.

In conclusion, due to the high immunogenicity entolimod can be only administered once. Therefore, the product is not suitable for any repeated use, which seems to limit it clinical usefulness.

Moreover, pre-existing cross-reactive antibodies were reported from 12.7% of the US population investigated, while the frequency of antibodies in the European population is unknown. As in presence of these neutralizing antibodies no efficacy of entolimod can be expected and the data is generated in the US only the applicant is requested to provide information about the incidence of pre-existing cross-reactive antibodies in the EU to further assess the proportion of patients in whom no efficacy of entolimod can be expected.

In study I96111-Russian, all subjects developed high levels of neutralizing antibodies to entolimod after administration of entolimod, regardless of dose or dosing regimen. Neutralizing antibodies emerged between Day 5 and Day 15 for the 5-, 10-, 15-, and 20-µg/dose groups and by Day 8 for the 30- and 40-µg/dose groups. Neutralizing antibodies remained detectable for several months after treatment.

Safety related to drug-drug interactions and other interactions

No drug interaction studies have been performed with entolimod

Although the applicant might be correct with his assumption that the overall impact of entolimod on DDI can be low, this is critical for a substance like entolimod, which is intended for emergency use in a

catastrophic scenario only and in which a remaining complete lack for post marketing evaluation can be reasonably presumed.

Several substances are likely to be co-administered in the intended target population following the scenario of ARS [including Potassium iodide, trisodium calcium diethylenetriaminepentaacetate (Ca-DTPA), Trisodium zinc diethylene triamine pentaacetate (Zn-DTPA), and Prussian Blue (ferric hexacyanoferrate) as chelators]. These agents are well-known to block or reduce body burdens of internalized radioisotopes following isotope exposures. It is justified to expect that they will be used concomitantly with entolimod in the case of acute radiation scenarios. Similarly, anti-emetic agents like granisetron, ondansetron and others as well as antibiotics and anaesthetics including opioids will be administered in the target population. Therefore, a high medical need is seen for the evaluation of entolimod's DDI with these agents.

Discontinuation due to AES

Due to the single-dose nature of the regimen evaluated in **HU-7014**, premature discontinuation of therapy was not relevant and there were no premature discontinuations of study participation due to AEs in this study.

However, in trial **HU-9001**, 12/100 subjects (12.0%) were withdrawn from the study prematurely, 10 because of TEAEs and 2 because they withdrew consent.

Nine subjects, who were randomized to receive 2 doses of 30 mg of entolimod administered 72 hours apart were given the first dose of entolimod but then did not receive the second dose due to a TEAE. Seven of these subjects (4 in the <0.40 µg/kg dose group and 3 in the 0.40-0.60-µg/kg dose group) had elevations in serum transaminases that were all assessed as Grade 1 or 2 in intensity for 6 subjects and Grade 4 in intensity for 1 subject. One subject (<0.40-µg/kg dose group) was noted to have a Grade 1 ventricular extrasystoles and another subject (<0.40-µg/kg dose group) had Grade 2 asthenia.

One additional subject who had been randomized to receive a single entolimod dose of 35 mg (0.40-0.60-mg/kg dose group [plus IBP]) was withdrawn from study after entolimod administration due to a Grade 2 SAE of acute cholecystitis.

Two subjects who were both randomized to receive 2 doses of 30 mg of entolimod administered 72 hours apart (both in the <0.40-µg/kg dose group), were administered the first dose but then did not receive the second dose because they withdrew consent. At the time of withdrawing consent, both had a flu-like syndrome. Comments in study records indicate that consent to receive a second dose of entolimod was likely withdrawn due to the occurrence of these TEAEs following the first dose of study drug.

From the small trial **I196111-US** 6 of 26 patients discontinued prior to the completion of all planned entolimod administrations. Four of these subjects were discontinued due to AEs, all between the 1st and 2nd injection of entolimod. Two of these subjects (1 in the <0.40-µg/kg dose group and 1 in the >60-µg/kg dose group) had Grade 3 hypotension, 1 subject (in the 0.40-0.60-µg/kg dose group) had Grade 3 fever and Grade 3 QT prolongation, and 1 subject (in the >60-µg/kg dose group) had Grade 4 hypoxia due to an intercurrent pneumonia. Two of these events were reported as SAEs. Two subjects started entolimod administration but then did not receive subsequent entolimod doses for reasons that were not specifically described in available study records. One of these subjects (in the <0.40-µg/kg dose group), despite having only Grade 1 TEAEs, did not return for repeated treatment after the 1st dose. The other subject (in the 0.40-0.60-µg/kg dose group) developed Grade 3 hypotension and a Grade 3 increase in AST and was not administered entolimod injections after the 2nd dose.

No of the 6 subjects participating in **I196111-Russia** discontinued entolimod prematurely.

Discontinuations due to AEs occurred in 2 subjects participating in **BL612-CBLB502**. Both subjects had a history of ischemic cardiac disease and both were allocated to receive 2 entolimod injections of 0.45 µg/kg. After the 1st administration of study drug, one of these subjects was found to have bigeminal ventricular extrasystoles and the other experienced an acute myocardial infarction. Thus, they were not given the planned 2nd entolimod dose. Both of these events were reported as SAEs.

Information on discontinuation due to AES is in line with the reported safety events discussed above. The discontinuation rate of 12 % in healthy volunteers and higher rates with in patients (with lower doses) confirm a significant toxicity of the drug. Which needs additionally to be better defined. It may be reasonably presumed that for the dose range now applied for, toxicity is even worse than currently illustrated by the data available.

3.3.9. Discussion on clinical safety

Although studies to assess safety of entolimod when used as indicated (i.e. in the ARS setting) would be needed, such studies are neither feasible nor ethical. The absence of safety data in the target population, although understood, is an important shortcoming of this dossier since the safety profile of entolimod in the subjects of interest cannot be characterised.

The applicant has presented short-term safety data after the administration of entolimod mainly in healthy volunteers. Such data are considered relevant in the absence of safety information in the target population but the limitations of this information are obvious considering the complexity and severity of the clinical picture of the patients who are exposed to high doses of total body irradiation and that a number of concomitant medicines are expected to be concomitantly used as part of the recommended supportive care. It seems rather likely that many subjects of the target population may be polytraumatised or have other injuries/ pre-existing conditions (e.g. dehydration, burnings and others traumas) which are neither reflected by healthy volunteer data nor by the NPH data in irradiated NHPs. In addition, the number of patients exposed is considered very limited taking into account that this medicinal product belongs to a novel therapeutic class (first TLR5).

AEs in healthy volunteers were collected after 28 days of entolimod administration. Although only one dose of entolimod is proposed the applicant claims that durable efficacy on clinical parameters is expected. So durable impact of entolimod on safety should not be ruled out. For the time being the safety profile of entolimod in healthy humans beyond 28 days is unknown.

Safety data in oncological patients in 3 studies (I196111-US, I196111-Russia and BL612-CBLB502) were presented as supplementary. Unless in study BL612-CBLB502 in which patients with colorectal cancer had never received antitumor treatment, patients in the other two studies might be receiving or may had received chemotherapy for cancer, treatment features that confound the overall safety profile. The clinical study reports for these studies have not been provided but only limited demographic and safety data are available. Anyway, oncological patients present immunological changes due to their disease and therefore comparison with people who would be exposed to potentially lethal irradiation (who are likely healthy just prior to that exposure) can be questioned.

Nonclinical pivotal toxicology studies were not fully predictive of the adverse events reported in humans since they presented serious drawbacks and a mayor objection has been raised to the applicant for clarification (see non-clinical report). In both nonclinical efficacy studies, in non-irradiated monkeys (conversion study Rs-001) and in irradiated monkeys (the pivotal efficacy trial Rs-23), animals received the same irradiation levels and dosing, nevertheless the safety evaluation plan was largely not comparable. Necropsy was carried out only in irradiated animals (study Rs-23)

therefore not allowing to draw direct histopathology conclusions. Interestingly animals with predose reactive antibodies to entolimod, titers >600 fold the baseline levels were excluded from the assessment, therefore precluding to evaluate the potential effects of entolimod administration in human patients that present reactive antibodies against the product.

The total animals treated with entolimod in studies Rs-001 and Rs-23 accounted to 140 animals non irradiated and 139 irradiated (7.2-Gy TBI), respectively. The actual number of animals that received the optimally efficacious dose selected to be administered in humans was however low, accounting to only 40 animals of which only 20 monkeys were irradiated.

Contrarily, no safety data of entolimod is presented from patients undergoing total body irradiation as part of their cancer therapy. Although such data might be considered supportive of its use in ARS, it would be difficult to draw definitive conclusions based on the differences in the irradiation procedure in cancer radiotherapy versus a nuclear/radiological accident.

No clinical data are available in the target population and only short-term safety data have been provided. The lack of safety data is relevant as entolimod is a new pharmacotherapeutic class (TLR5) whose safety profile is unknown.

With respect to the exposure data in humans presented, clinical experience with entolimod is limited on 150 subjects in phase I HU-7014/HU-9001 studies and 73 oncological patients. However, due to the dose escalation approach implemented in both phase I trials only 51 of these healthy volunteers had a relevant single dose exposure in the recommended target range for adults between 0.40 and 0.60 µg/kg (N=51), while most of the subjects included received significantly lower doses. Moreover, even the adequately exposed population of 51 is not homogeneous; only 34 of these 51 subjects received entolimod alone, while safety data for the remaining 17 subjects was generated after pre-administration of 400 mg of IBP in order to increase tolerance of the product. This means that entolimod's safety for the applied posology in this applicant can only be assessed correctly from 51 or 34 healthy volunteers. It is obvious that this marginal human safety data allows only a first impression of tolerability; whether it can be assessed as sufficient to characterise safety for this product is highly questionable in the Committee´s view.

It is recognized that safety data in the target population cannot systematically be generated. The applicant should provide a justification that the submitted safety database is sufficient to characterise the adverse effect profile of a single injection of entolimod at the proposed dose, including the impact of the known safety concerns (e.g. hypotension) in patients vulnerable to such effects.

Both trials in healthy volunteers were initiated to establish PK/PD, testing of tolerability and are also used for safety characterisation. Due to the "First-in-Man" and dose-finding character of these trials only a minority of the patients received the dose range now relevant for adult patients in the target population. Moreover, due to the short-time increases of PD markers probably indicating TLR5 activation a need for a repeated use of entolimod was seen at that time. Therefore, trial HU9001 evaluated also repeated use of entolimod in a limited number of the trial subjects, since the development of antibodies in nearly all of the subjects was obviously not known at that time (2010).

Although it is acknowledged that the applicant may have tried to reduce imbalances between the dose groups, it is obvious from the trial design that the very small dose groups do not allow any adequate safety assessment in any subgroups, particularly, as age of inclusion was restricted from 18 to 47/55 years. Insofar, safety of patient above 55 years remains not assessable for this application.

Healthy adult subjects in HU-7014 and HU-9001 studies were exposed to different dose regimens of entolimod by IM injection (n=150). It is important to remind that the formulation of the product used in both heathy studies was different that the one proposed for marketing. The company assessed

safety of entolimod using what they call "absolute dose", which is the total drug amount per subject as given in the clinical studies (i.e. 2 µg, 6 µg, 12 µg, 24-25 µg, 30 µg, 35 µg, 40 µg and 50 µg) and only for the first dose. They also assessed safety by using a different format, with the dose adjusted to the subject's baseline body weight (called "body-weight-adjusted dose") in 3 groups as <0.40 µg/kg, 0.40-0.60 µg/kg and >0.60 µg/kg. Using this latter format, the groups will then include subjects pertaining to different absolute dose groups. In this sense, from the 51 subjects who received entolimod at doses between 0.40 µg/kg and 0.60 µg/kg, 40 (78,4%) received 35 µg (either with or without ibuprofen pretreatment) but there was 1 subject receiving 24-25 µg, 8 subjects receiving 30 µg and 2 subjects received 40 µg. Even if the body-weight-adjusted dose is not similar for all weight ranges, it is mostly 0.4-0.6 ug/kg body weight.

Patients exposed to in the 3 oncological studies received repeated injections of entolimod but subcutaneously or by a combination IM-subcutaneous. Only 5 in study I196111-Russia and none in study BL612-CBLB502 received the dose proposed in the SmPC by intramuscular injection. In study I196111-US the number of patients who received the proposed dose was 4, but it is unknown how many as intramuscular or subcutaneous injection. All oncological patients received more than one doses of entolimod. The number of recruited patients in studies I196111-US and I196111-Russia is far from the planned number and the company should justify this difference.

In the studies in healthy volunteers at the proposed entolimod range dose of 0.4-0.6 µg/kg all subjects presented with TEAEs, which seems to allow concluding that entolimod's toxicity in humans is not trivial as in NHPs. This assumption is confirmed by the fact that TEAEs categorized by the CTCAE as being severe (Grade 3) or potentially life-threatening (Grade 4) occurred in 27.5% of subjects. However, details regarding these events are needed to decide whether these events are clinically relevant also in the target population in the case of a radiation disaster. The percentage ≥3 Grade TEAEs rate is more than doubled (38.2%) in those 34 healthy volunteers who were exposed with the indicated posology, Such percentage clearly indicates that tolerability in the human target population is significantly lower than that observed in NHPs.

For the total 150 healthy volunteers, the most frequent adverse events categorised according CTCAE were chills (85.3%), headache (82.4%),serum glucose increase (88.2%), tachycardia (70.6%), fever (67.6%), ALT/AST increases (40,0%/47.1%), nausea (47.1%, vomiting (44.1%). Most important about one third of the patient had significant decreases in blood pressure (systolic hypotension 32.4%, diastolic hypotension 26.5%) associated with tachycardia. In order to better assess the impact of arterial hypotension observed the circulatory shock index (SI) of all healthy volunteers included in trial HU-7014 and HU-9001 at 8 hours postdose need to be provided.

With respect to the symptoms categorised according CTCAE the most frequent adverse events at the proposed dose were chills (78.4%), headache (76.5%), nausea (49.0%), vomiting (47.1%), tachycardia (41.2%), pyrexia (39.2%), hypotension (31.4%) and increase of transaminases (27.5%). A dose-response relationship was observed for the occurrence of AEs after entolimod administration that is apparent for pyrexia, vomiting, SNC and gastrointestinal symptoms, laboratory parameters and hypotension. The percentages of patients reporting headache, myalgia and pyrexia were lower when patients were on ibuprofen to control the flu-like syndrome, as expected. Although according to the applicant most TEAEs were mild to moderate, 27.5% of them were considered Grade 3 or 4 (severe or life-threatening TEAEs). The applicant should clarify if these Grade 3 or 4 AEs are those referred below as SAEs. If not, the differences should be clarified and provide a table with the reported Grade 3 or 4 TEAEs by Preferred Term and SOC for each dose and total.

For studies in oncological patients very limited data have been provided. The applicant should submit a table with the TEAEs by SOC and Preferred Terms for in the three studies as well a discussion of the TEAEs that were considered entolimod-related by the investigator.

In non irradiated Rhesus macaques (study Rs-001) the most significant findings reported included transient elevations of liver transaminases (ALT and/or AST) in many individual animals treated with entolimod. There were decreases in serum phosphorus values. There were ANC increases and absolute lymphocyte counts decreased. No blood pressure or ECG data, necropsy and histopathology were assessed.

In irradiated rhesus monkeys (study Rs-23) entolimod administration resulted in relevant decreases in systolic and diastolic pressure in addition to increase in pulse rates in animals receiving entolimod doses ≥1.0 µg/kg. Necropsy data were only related to TBI and it is not possible to draw conclusions related to entolimod treatment since sacrifice was performed well after entolimod potential related toxicity was recovered (Day 60).

The applicant stated that in the clinical overview "that it was determined when NHP responses to entolimod administration are compared with those of humans at equivalent time points also with respect to safety; this comparison was not found". Therefore, the applicant is requested to provide a comparison at equivalent time points for the claimed PD biomarkers (e.g., plasma G-CSF, IL-6, and ANC) and evaluable safety events, e.g. clinical laboratory parameters (e.g., ALT, AST, and phosphorus levels), cardiovascular marker (blood pressure, pulse rates, fever, shock index) and gastrointestinal events (diarrhoea or vomiting) in order to evaluate at which dose a comparable safety can be assumed.

No deaths were reported during the human safety studies.

Four healthy volunteers had SAEs: one Grade 2 acute cholecystitis, one Grade 4 aspartate aminotransferase [AST] increase, one Grade 1 glucose intolerance and one Grade 2 hypotension. An additional healthy volunteer (02-085) had a TEAE (serum transaminase elevations) considered not related to entolimod that might be medically significant but no narrative of this patient has not been provided.

For oncological patients conflicting information seems to be given for Grade 3 and 4 TEAEs, under "Adverse events" and "Serious adverse events and deaths" that should be clarified by the applicant. In any case, in these patients hypotension (n=2), serum AST elevation (n=3), atrial fibrillation (n=1), lymphopenia (n=2), serum lipase elevation (n=2), hypophosphatemia (n=1), extrasystoles and acute myocardial infarction in a subject with preexisting cardiac ischemia (n=2) were reported. Extrasystoles and acute myocardial infarction were considered as related to treatment by the investigator (see "Discontinuations due to Adverse events" section). For the rest of AEs and given that patients were receiving other medicinal products the Applicant should clarify those SAEs that were considered related to entolimod by the investigator.

The hematological parameters of healthy subjects transiently changed after entolimod administration at the proposed dose of 0.4-0.6 µg/kg and were dose-dependent in all cases. The applicant states that these changes are components of the expected mechanism-related activity of entolimod. This is agreed. Increases in ANC and severe decreases in ALC were reported and values were outside the normal range in almost all subjects (92.2%, 98%, 92.2%, respectively). A reduction in platelet count and in haemoglobin were seen, and values were outside the normal range in 6% and 31% of subjects, respectively. This reduction in platelets and lymphocytes raises concern about the use of entolimod in the clinical situation in which entolimod is proposed to be used (i.e. subjects with haematological and gastrointestinal ARS). G-CSF and GM-CSF treatments may also adversely affect blood platelet counts

so the IAEA recommends that platelet counts be monitored when G-CSF is being administered (Singh et al. 2015). The applicant should discuss if a similar recommendation should be done for entolimod.

Administration of entolimod at the proposed dose was associated also with rapid increase of serum AST and ALT levels in healthy humans. At the proposed dose of 0.40-0.60 μg/kg, 50% of subjects presented with values higher than the upper limit of normality, and about 20% of healthy subjects with grade ≥3 severity. This is consistent with hepatocellular injury. Although in most cases values returned to normal range by 120 h postdose (in 96% of subjects for ALT and 100% of subjects for AST) the applicant should provide information about the clinical course of patients whose ALT values maintained high after that time point. The applicant should also provide further information about the number and percentage of patients with elevations of 2, 3, 5 and 8XULN. In addition, increases in alkaline phosphatase and GGT have been reported in the oncological studies suggesting cholestatic injury. The mechanism for production of the hepatocellular injury is for the time being unknown.

At the proposed dose of entolimod, hyperglycaemia and hypophosphatemia were observed in most healthy subjects (86.3% and 74.5%, respectively), peaking at 8 hours and recovered within 24 hours in all them. How the administration of entolimod can affect the management of diabetic patients should be discussed by the applicant. Hypophosphatemia was grade ≥3 in 60.8% of the healthy subjects at the proposed dose. The applicant should provide data on the duration of hypophosphatemia, discuss the mechanism behind this reduction of phosphates in plasma as well as the potential clinical consequences.

Decreases in systolic and diastolic blood pressure, increases in mean pulse rate were observed after administration of entolimod, with values peaking at 4 hours postdose and largely recovering by 24 hours. Comparing the graphics and tables provided for the analyses of the decreases in blood pressure and the increases in pulse rate, it seems that several healthy volunteers may have had episodes with clinical relevant tachycardia events. However, in the table presented the absolute minimum and maximums observed in the groups were not provided. The applicant is requested to add this information.

In order to assess further the clinical impact of the blood pressure decreases observed the applicant is requested to provide comprehensible information about the circulatory shock index (SI) of all healthy volunteers included in trial HU-7014 and HU-9001 at 4 hours postdose. Data should be sorted according the groups the patients were included in order to assess the impact of dose.

QT or QTcF were observed for 1 (4.0%) and 2 (8.0%) subjects, respectively, in the 35-μg dose group in trial HU-7014. As a significant QTcF prolongation of ≥60 msec 3 hours after administration was observed and also the diastolic and systolic blood pressure decreases significantly at this time in all dose groups, tachycardia or arrhythmia-related events have to be discussed as a potential reason for these observations. The applicant is requested to provide hERG channel data in order to better assess the risks for dangerous cardiac arrhythmias. In particular, as also macromolecules like antibodies and other proteins have recently been described to induce hERG channel blockade as well as smaller molecules.

As said entolimod infusion causes a profound reduction in blood pressure, with mean maximal changes in diastolic pressure in the range of more than 20 mmHg. Furthermore, the duration of hypotension is dose dependent. The applicant is proposing the use of entolimod in a disaster situation, based on a pivotal animal study mimicking a situation where supportive care, including the use of i.v. fluids was not administered. It is presumed that the proposed indication includes situation where supportive medical care is not available. The applicant should justify that entolimod is suitable for such use, notwithstanding the hemodynamic effects of the drug.

Taking into account that the observed need for administration of catecholamine in a healthy volunteer population in order to balance entolimod's cardiovascular adverse events, it seems questionable whether the safety profile currently demonstrated really allows administering the drug as proposed under out-patient care conditions as necessary in the intended catastrophe. Untreated cardiovascular shock clearly increases mortality significantly even in non-irradiated humans. Insofar, the cardiovascular events observed may be a significant risk. It seems that the impact of this risk is currently not adequately considered in the applicant's calculation of potential survival benefits claimed with respect to the NHPs data.

Preliminary data in the 17 subjects receiving concomitantly ibuprofen may indicate that the flu-like symptoms probably caused by cytokines can be partially suppressed. However, no effect of ibuprofen on blood pressure decreases was demonstrated. In general, the relevance of the difference reported with and without ibuprofen may be challenged due to the small imbalanced number of subjects investigated. At least the current data do not allow confirming this effect as reliable or robustly demonstrated.

Furthermore, it should be noted that clinical symptoms reported and interpreted as flu-like symptoms and probably correctly attributed as side effects mediated by IL-6 release are the same associated with cytokine-release syndrome (CRS) also known as "cytokine storm" and first reported from a phase I trial (TGN1412) in 2006. Similarly the dose-depending drop-down of platelet count may be explained due to the G-CSF release or also as DIC associated platelet consumption probably in consequence of the lower cardiac output which is demonstrated by the significant effects on blood pressure and pulse rate. As DIC may be associated with impaired microcirculation in the liver, the dose depending increase in ALT and AST can reflect hypoxic damage of the liver too. Moreover, the decreases seen for haemoglobin from predose to 8 hours postdose with persistence through 120 hours postdose seems to be best explained as consequences of capillary leakage, because the changes observed during this short period and prolonged recovery during 120 hours are not likely to be caused from blood lost or impaired regeneration. Although it is noted that both effects were observed to be self-limited, it should be noted that the same effects are again part of "cytokine release syndrome" also known as "cytokine storm".

The applicant has provided several interesting arguments justifying that entolimod is not able to induce a cytokine release syndrome (CRS) called "cytokine storm". Although several of these arguments may be correct, the applicant's argumentation does not mention sufficiently that currently IL-6 is seen as the central mediator of toxicity in CRS. Lee and colleagues (2014) have demonstrated that IL-6 is a pleiotropic cytokine with anti-inflammatory and proinflammatory properties. IL-6 signaling requires binding to cell-associated gp130 (CD130), which is broadly expressed and present on macrophages, neutrophils and also hepatocytes and mediates classic signaling, which predominates when IL-6 levels are low. However, when IL-6 levels are elevated, soluble IL-6R can also initiate trans-signaling, which occurs on a much wider array of cells. Current models hold that anti-inflammatory properties of IL-6 are likely mediated via classic signaling, whereas proinflammatory responses occur as a result of trans-signaling. High levels of IL-6 insofar are likely initiating the proinflammatory IL-6-mediated signaling cascade. Taking into account that IL-6 increases are seen as a relevant biomarker of entolimod's pharmacodynamics occurrence of CRS cannot be excluded. Moreover, as nearly all symptoms of CRS were reported as "cytokine related adverse events" for entolimod and the same difference regarding very low toxicity in animals and significantly higher toxicity in man became obvious during the development program, it seems likely that entolimod in principle may induce a CRS similar to TGN1413 and several other drugs. Moreover, a proinflammatory effect of entolimod may have contributed to the occurrence of a cholecystitis event in a healthy volunteer and a grade 4 aggravation of pneumonia (with need to ventilation) in one of the sparse patients exposed. Particularly in elderly patients such a

proinflammatory may increase mortality in general and may affect overall survival in the emergency situation the product applies for.

Taking into account the observed high SAEs and discontinuation rates for a second injection, the Il-6 mediated disease-mechanism as well as the dose dependence of CRS events also evident from the safety data of entolimod, it seems likely that entolimod may lead to a cytokine-storm as observed with TGN1412 in particular in high doses. Therefore, the safety results available indicate a narrow therapeutic range and a significant risk for any overdose. Whether this can adequately be handled in the scenario following a radiation disaster can be challenged.

No clinically significant changes were observed on renal (serum creatinine, serum BUN) or acid-base (serum bicarbonate) laboratory parameters on healthy volunteers.

Selected cytokines were assessed in healthy volunteers (G-CSF, IL-6, IL-8, IL-10, TNFα, IL-12, and IL-1βf). Increased levels of G-CSF and IL-6 were observed when entolimod was administered at the proposed dose, peaking during the first 4-8 hours, and resolved by 24 hours. Other plasma cytokines (IL-8, IL-10 and TNFα) showed lower increases than G-CSF and IL-6, peaking and resolving at about the same time periods. Additional analyses in plasma levels of IFNα, IFNgamma, IL-1α and IL-2 showed that they were not affected by entolimod. For comments about the changes of the assessed cytokine levels, see section Pharmacodynamics.

At the proposed dose, hypotension (diastolic and systolic), occurred in healthy subjects with nadir values below the lower limit of normality in 31.4% and 25.5% of subjects, for less than 1.5 hours, and fully recovered. Also tachycardia and fever was present, in 65.7% and 58.8% of subjects with values above the upper normal limit, respectively, for few hours and fully recovered. Hypotension and tachycardia may lead to ischemic events. QTcB prolongation was observed in two-thirds of subjects after administration of entolimod with no clear dose-response relationship. In the absence of a dedicated QT prolongation study that clarifies the effect of entolimod in HERG channels these safety data are of concern QT prolongation should be considered an important potential risk in the RMP.

In oncological patients the abnormal findings seem to replicate those seen in healthy subjects (anemia, lymphopenia, increases in serum ALT or AST levels, serum alkaline phosphatase, hyperglycemia and hypophosphatemia, hypotension, tachycardia and fever) although some others were also described. The company should discuss the abnormal laboratory findings in the 3 oncological studies in the context of the concomitant treatments and the underlying disease.

In animal studies, the abnormal findings seem to replicate those seen in humans. In non irradiated Rhesus macaques increases in serum ALT or AST levels and in ANC were seen together with decreases in serum phosphorus values and absolute lymphocyte counts. In irradiated rhesus monkeys entolimod administration resulted in relevant decreases in systolic and diastolic pressure in addition to increase in pulse rates in animals receiving entolimod doses ≥1.0 µg/kg. However, no assessment of blood pressure or ECG, necropsy and histopathology was performed in non-irradiated NHP, and necropsy data in irradiated NHP was only related to TBI since sacrifice was performed well after entolimod potential related toxicity was recovered (Day 60).

Eleven healthy subjects (8%) discontinued treatment prematurely due to adverse reaction, 7 due to increase in transaminases, 2 due to flu-like syndrome and 1 to asthenia. The three patients receiving entolimod at 0.40-0.60 µg/kg dose discontinued due to hypertransaminasemia. Only in trial HU-9001 treatment discontinuation was an option as in trial HU-7014 only a single dose was planned. The applicant reported that 12 of 100 subjects (12.0%) were withdrawn from the study prematurely, 10 because of TEAEs and 2 because they withdrew consent. Discontinuation of treatment is seen as an important marker for tolerability.

In oncological studies, the reason for discontinuation were severe hypotension, fever and QT prolongation, a Grade 3 increase in AST, bigeminal ventricular extrasystoles and acute myocardial infarction. Oncological patients may be receiving other concomitant drugs, these serious AEs are of concern particularly by the fact that all of them were also observed in healthy volunteers. Due to the seriousness of these AEs narratives of these patients should be presented.

The company proposes the use of entolimod in the elderly with a similar dose than in adults, based on the safety data in clinical studies in humans aged up to 84 years. Studies in healthy volunteers did not include elderly population, only studies I196111-US and BI612-CBLB502 recruited a few patients (n=25). It is known that bone marrow reserve diminishes with age (and consequently the predisposition to anemia, infections and hemorrhages) (REMM – at risk/special need populations 2017). They might have increased risk of bleeding due to medications and increasing number of co-existing diseases and conditions that may be associated with inability to tolerate certain drugs particularly aggressive salvage treatments as the stem cell transplantation for treatment of ARS. The lack of safety data in the elderly is relevant and this should be reflected in the SmPC. The elderly should be considered as missing information in the RMP. In order to further assess the data base behind these proposals, the applicant is requested to report about

a. how many subjects in the safety population with an age of ≥65 years, ≥70 years and ≥80 year received the SmPC recommended dose according their body weight and

b. to provide -based on table 1 in the SmPC- an overview about the size of data base available to assess safety for the range of body weights claimed.

There are no clinical data on the use of entolimod in humans with pre-existing renal impairment, the applicant proposes the use of entolimod in this special population without any dose adjustment arguing that the product does not appear to be directly excreted. The lack of studies in renal insufficiency should be reflected in the SmPC.

People with pre-existing hepatic impairment may be at increased risk of liver injury after administration of entolimod since increases in serum ALT and AST levels were detected in healthy subjects. Also total changes in bilirubin and alkaline phosphatase were reported in a few patients although they were not related to treatment according to the applicant. The company recommends the use of this product in such special population without dose adjustment from the dose in adults. The SmPC specifies that entolimod in this population should be administered after careful consideration of the risk of death associated with the estimated radiation exposure and the severity of the underlying hepatic disorder although no dose adjustment is recommended. This is considered reasonable. The SmPC also states that patients should be advised to avoid the use of known hepatotoxic substances (eg, alcohol) in conjunction with entolimod administration. A reference to hepatotoxic medication rather than alcohol consumption would be expected as consumption of alcohol is unlikely in the context of ARS. The applicant should discuss if a recommendation for monitoring of hepatic enzymes during entolimod treatment should be included in section 4.4. of the SmPC. Hepatotoxicity should be considered an important potential risk in the RMP.

The company has not considered immune-suppressed people as a special population and no particular efficacy and safety data has been provided for this group. It is known that TBI will exacerbate pre-existing diminished immune functions, that medical countermeasures to enhance blood cell production may be less effective, affected individuals are more subjective to infection, and that they may not be able to tolerate radiation-induced prolonged pancytopenia or stem cell transplantation due to the underlying disease (REMM – at risk/special need populations 2017). The lack of safety data in immune-suppressed subjects should be stated in the SmPC.

People with pre-existing vascular disease or low cardiac output may be at increased risk of hypotension after administration of entolimod, even though no data in such special population exists, but hypotension was detected in healthy subjects and in oncological patients of the clinical studies. The company recommends the use of this product in such special population after careful consideration of the risk of death associated with the estimated radiation exposure and the severity of the underlying vascular disorder. This is supported. Ischemic events should be considered an important potential risk in the RMP.

The proposed indication in paediatric population aged 2-18 years is the same as that proposed in adults. Children have not been exposed to entolimod in clinical trials. The main preclinical studies (Rs-01 and Rs-23) were carried out in NHP with an equivalent to 8-24 years in humans but data in younger animals are not available. Therefore, the use of entolimod in children should be considered missing information, as proposed by the applicant. The SmPC should state that the use of this product is limited to paediatric population of 2-18 years.

Entolimod in children from birth to 23 months have not yet been established either in human or juvenile animals. The proposed indication of entolimod includes children aged 2 years and older in section 4.1. However, in section 4.2 specific dosing recommendations are proposed for children below 2 years of age given that very likely these children will be treated if needed. This seems acceptable. However, the rationale for the specific dosing recommendations in this group is missing. From a pharmacodynamic point of view, no differences between adults and children are expected since the mechanism of action of entolimod is mediated via the innate immune system, which is considered mostly mature from birth. However, while TLR expression in neonates seems equivalent to the adult level, the neonatal response to TLR stimulation seems to be qualitatively different and therefore an approach based only on pharmacokinetic data seems not adequate across all age groups below 2 years of age. In the studies in healthy volunteers urine was collected. However, entolimod was not measured.

The impact of TLR5 mutations on entolimod safety cannot be concluded for the few healthy subjects analysed (n=6). The applicant should comment on it.

As genetic differences of TLR5 may additionally be associated with difference in response to entolimod increased frequency of SAEs can be reasonably expected also from individual differences in sensitivity of the TLR5 receptor. This can only be assessed in a larger population. The narrow therapeutic range remains especially a problem in the also applied paediatric population. Although no data was submitted, a higher sensitivity to entolimod can be presumed. This would mean that the risk for CRS is probably higher in the most vulnerable paediatric population which precludes any administration in the age below 18 without more safety data.

All subjects participate in phase I trial HU-9001 were tested for the presence of TLR5 mutation (TLR5392 stop). This dominant mutation occurs in about 10% of the humans and seems to indicate non-response to entolimod, at least in homozygotes. The applicant is requested to explain whether the NHPs in trials Rs-23 were also tested for this mutation. Taken the small number of animals in the different groups into account this is deemed essential to assess potential imbalances at baseline between the groups.

The adverse events recorded in the 5 patients who carried the TLR5392 stop mutation in HU-9001 should be provided in order to analyse the impact of this mutation on safety profile of entolimod observed.

In the 10% of the human population TLR5392 stop-mutation make patients insensitive to entolimod. This means that no benefit in 10 % of the population can be expected. The applicant should discuss how to select the target population in the case of emergency.

The applicant is requested to clarify the impact of other mutations of TLR5 beside the stop mutations. Are TLR5 or other TLR mutations known which have an increased sensitivity for agonists?

Although there are no clinical data on the use of entolimod in pregnant women, the company recommends the use of this product in such special population after careful consideration of the risk of death associated with the estimated radiation exposure for the pregnant woman and also the risk of adverse developmental or fatal effects on a foetus. Pregnant women should be advised to seek obstetrical follow-up as soon as possible after drug administration to determine appropriate surveillance or intervention relating to the pregnancy. Given the absence of data in this population, pregnancy should be considered as missing information in the RMP.

The applicant recommends the use of this product in breastfeeding women after careful consideration of the extent of the radiation exposure to both the woman and child, whether the child will also receive entolimod, and whether breastfeeding can be interrupted to provide alternative sources of nutrition for the child. However, there are no data on entolimod excretion in human breast milk. The company should clarify the time period for which breastfeeding should be interrupted if the administration of entolimod is considered necessary. Given the absence of data in this population, lactation should be considered as missing information in the RMP.

Healthy humans may have pre-existing cross-reactive antibodies to entolimod due to prior bacterial infections (15.7% reported at the proposed dose group in healthy-subject studies being neutralizing antibodies in 10% of subjects). Such preexisting entolimod-reactive neutralizing antibodies can reduce entolimod efficacy. Other specific adverse effects are not anticipated as a result of pre-existing entolimod-reactive antibodies. After entolimod administration, 98% of healthy subjects had developed postdose entolimod-reactive antibodies, being neutralizing antibodies in 98% of them. This should be properly reflected in the SmPC.

Currently it is not clearly evaluable in which non-clinical trials of the development programme testing for antibodies against entolimod/flagellin were performed. As toxicity in mice and NHPs was very low in comparison to that observed in humans, the applicant is requested to provide a list in which trial animals were tested for antibodies and neutralising activity in order to conclude regarding the reliability of the data, in particular with respect to toxicology.

The methods for testing of antibodies against entolimod are described as "using a validated bioanalytical procedure", but the method validation was not fully comprehensible. Therefore, the applicant is requested to clarify which methods were used for immunogenicity evaluation in which trial, to summarise the analytical validation and to prove finally that the methods' accuracy was suitable to conclude.

As in the presence of pre-existing neutralizing antibodies no efficacy of entolimod can be expected, but the incidence of antibodies was generated in the US only, the applicant is requested to provide information about the incidence of pre-existing cross-reactive antibodies in the EU to further assess the proportion of patients in whom no efficacy of entolimod can be expected due to pre-existing antibodies.

There is no drug interaction studies for entolimod and this should be reflected on the SmPC. The applicant argues that those studies are not needed given that entolimod is likely to be used without supportive care. However, this statement is not supported. If entolimod is finally authorised it could be used in ARS settings in which supportive care will be given and also considering that the exposed

population will be under other medical treatments. Therefore, drug-drug interaction studies with products protocolised as medical care in such situations (e.g., antibiotics or filgrastim) or with the most common drugs administered in the most prevalent diseases in the population should have been performed. Given the absence of data, drug interactions should be considered as missing information in the RMP.

The applicant has included evaluation of ibuprofen concomitant medication in 17 healthy subjects. The applicant is requested to provide the full results of this investigation and to discuss the conclusions that can be drawn from the findings; particularly with respect to the claimed results that increase of cytokines were observed while clinical symptoms were ameliorated.

The pharmacokinetic profile seems not be affected by pretreatment with up to 400 mg of oral ibuprofen but this pretreatment appears to modify pharmacodynamic profile by increasing serum levels and AUC for the following cytokines: IL-6, IL-8, IL-10, TNF-α, and G-CSF. However, these findings have not been properly discussed by the applicant. If entolimod is finally authorised it could be used in ARS settings in which supportive care will be given and also considering that the exposed population will be under other medical treatments. Therefore, drug-drug interaction studies with products protocolised as medical care in such situations (e.g., antibiotics or filgrastim, potassium iodide, trisodium calcium diethylenetriaminepentaacetate (Ca-DTPA), Trisodium zinc diethylene triamine pentaacetate (Zn-DTPA), and Prussian Blue (ferric hexacyanoferrate9, anti-emetic agents,) or with the most common drugs administered in the most prevalent diseases in the population should have been performed.

No postmarketing experience exists since entolimod has not been marketed yet.

Assessment of paediatric data on clinical safety

The pharmacodynamics, pharmacokinetics, and safety of Entolimod have been evaluated in adults only. Safety in the paediatric population was not tested.

Nevertheless, the applicant claims extrapolation of the effects of Entolimod to paediatric patients in all age categories of human children (from birth to 11 years), adolescents (12 to 17 years), and adults (≥18 years). The product's safety was not investigated in human or juvenile animals, but the applicant claims that clinical safety was established in safety studies in nonhuman primates and human adults. This approach is not acceptable with respect to the major objections and open concerns currently not resolved.

3.3.10. Conclusions on clinical safety

No clinical safety data on the use of entolimod in the ARS setting is available since clinical trials in irradiated humans are neither ethical not feasible. Therefore, safety data on entolimod come from two studies in 150 healthy volunteers exposed to a single-dose intramuscular administration with a different formulation of entolimod to that proposed for marketing and a few oncological patients. In addition, safety data in primates from studies Rs-001 (conversion study) and Rs-23 (the pivotal trial in NPH) are available.

Most subjects reported AEs after entolimod administration and there seems to be a dose-dependent relationship for most of them. Several serious AEs have been identified in the studies in humans like tachycardia, hypotension, QT prolongation, increase of transaminases that need further clarification and that should be included in the RMP as important potential risks. Hypophosphatemia and hiperglucemia were observed in 74% y 86.3% of healthy volunteers, respectively. The mechanism of production of hypophosphatemia, that was grade 3 in 60.8% of subjects, is unknown. To what extent hyperglucemia can affect control of diabetic patients is also of concern. No drug interaction studies for

entolimod have been performed. This is relevant considering that entolimod would be expected to be used with best supportive care that includes among others antibiotics and, potentially, filgrastim.

Neutralising antibodies have been identified in healthy volunteers previously exposed to entolimod. Subjects not previously exposed to entolimod also have antibodies to prior bacterial infections what may limit the efficacy of entolimod in practice.

Unfortunately nonclinical toxicology studies were not fully predictive of the adverse events reported in humans. However, preclinical efficacy studies showed that the abnormal findings seem to replicate those seen in humans.

In general, the safety profile of entolimod cannot be considered well characterized.

No TLR5 agonist is currently approved; insofar the safety risks for this type of product are unknown. Evaluable safety population exposed with the proposed posology is restricted on 34 healthy volunteer only. Although significantly lower doses than those claimed efficacious in the pivotal trial Rs-23 in NHPs were used in healthy volunteers, the tolerability in the relevant dose range remains low in comparison to NHPS and mice and indicate a narrow therapeutic range.

Although most of the TEAEs can interpreted as trivial flu-like adverse events, significant cardiovascular adverse events (decreases in systolic and diastolic blood pressure, tachycardia, need for dopamine in one healthy volunteer) were also reported. In the intended human target population after a radiation disaster, cardiovascular adverse event due to entolimod are likely to have an impact on the overall outcome and the benefit risk balance at the end.

Considering the spectrum of the adverse events observed, entolimod may be seen at risk for inducing a CRS/"cytokine storm". Since high levels of IL-6 and several other cytokines are claimed to be responsible for the product's pharmacology and high IL-6 levels are described to be the key characteristic for drugs associated with CRS, this assumption seem plausible.

Furthermore, similar large differences in tolerability between animals (excellent tolerability) and humans (very low tolerability) were also observed during the development of TGN1412 which leads to a "cytokine storm" disaster in healthy volunteers in 2006.

Moreover, Il-6 induced activation of proinflammatory pathways may be another important risk as perhaps indicated by the fact that clinical relevant inflammation occurred in the limited safety population (cholecystitis, Grade 4 pneumonia).

The other main issue in this application remains the lack of representative and reliable safety information. A database of 34 relevantly exposed subjects is deemed insufficient even in an application under exceptional circumstances. These exceptional circumstances are triggered from the impossibility to generate efficacy data in humans; however, this does not mean that the level of safety information can be lowered or completely waived. On the other side, a potential risk for inducing a CRS/ "cytokine storm" is reasonably presumed, which however would preclude further evaluation of the product in healthy volunteers even from an ethical perspective.

From the applied indication for administration in the case of a radiation disaster only, it seems unlikely that after approval any new safety (or efficacy) data will become available for entolimod. Due to the lack of any post-marketing safety data standard approaches of pharmacovigilance will fail to provide any confirmation of benefit-risk assessment after approval.

In conclusion, safety is currently not adequately evaluated; preliminary data available are deemed to indicate significant risks associated with entolimod in the proposed posology.

Although one might argue that in the scenario of ARS a robust overall survival benefit may nearly counteract every risk, this view neglects the fact that potential risks should at least be clearly assessable in order to conclude on benefit-risk. Uncertainties are increased as evaluation of efficacy completely depends on animal data. Therefore, it is indispensable for a meaningful benefit risk assessment that safety for the applied product is adequately characterised.

In conclusion, it might seem reasonable to request the applicant to provide a justification that this safety database is sufficient to characterise the adverse effect profile of a single injection of entolimod at the proposed dose, including the impact of the known safety concerns (e.g. hypotension) in patients vulnerable to such effects

From a safety perspective the lack of adequate safety data remains critical, because patients, who had been exposed to potentially lethal irradiation as result of a radiation disaster, are highly likely to be polytraumatised (and burned). Additional toxicities added are likely to have a relevant impact on the overall benefit risk balance in the target population which remains not adequately assessable.

As said, entolimod infusion causes a profound reduction in blood pressure, with mean maximal changes in diastolic pressure in the range of more than 20 mmHg. Furthermore, the duration of hypotension is dose dependent. The applicant is proposing the use of entolimod in a disaster situation, based on a pivotal animal study mimicking a situation where supportive care, including the use of i.v. fluids was not administered. It is presumed that the proposed indication includes situation where supportive medical care is not available. The applicant should justify that entolimod is suitable for such use, notwithstanding the hemodynamic effects of the drug.

Moreover, the impact of prophylactic concomitant ibuprofen on safety and efficacy needs to be reliably clarified. And last but not least new safety data is also needed to resolve the significant remaining issues regarding the insufficient conversion of animal data into adequate dose for use in humans.

Neither entolimod's safety in special populations nor its impact on other drugs (DDI) is currently characterised. With respect to special populations, the applicant presents only some not sufficiently justified hypotheses regarding dosing according age, in renal and hepatic impairment as well as for the treatment of patients with increased cardiac risks; however, no data is available to evaluate any correctness of the conclusions and proposal provided.

3.4. Risk management plan

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table XX: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	• Potential for ischemic events during transient entolimod-mediated hypotension and tachycardia in patients with pre-existing vascular disease or low cardiac output • Potential for increased hepatotoxicity in patients with pre-existing liver disease

Summary of safety concerns	
Missing information • Use in the paediatric population • Use during pregnancy • Exposure while breastfeeding	• Data are not available regarding use in children • Data are not available regarding use during pregnancy • Data are not available regarding potential risks to breastfed children resulting from exposure to entolimod in breast milk

3.4.1. Discussion on safety specification

Having considered the data in the safety specification the Committee considers that the following issues should be addressed:

Important potential risks

- Ischemic events: This important potential risk should be renamed and only the term "Ischemic events" should be included in the table. Hypotension and tachycardia were reported in healthy volunteers and oncological patients included in any clinical studies but the risk could be higher in patients with pre-existing cardiovascular diseases. The applicant should characterise the risk in the corresponding section commenting that tachycardia and hypotension may lead to ischemic events.

- Hepatotoxicity: This important potential risk should be renamed and only the term "Hepatotoxicity" should be included in the table. A relevant number of patients treated with entolimod in the different clinical trials reported elevations of transaminases. The risk of elevation of transaminases could be higher in patients with pre-existing hepatic illness or taking hepatotoxic drugs concomitantly.

- QT prolongation: Several cases of QT prolongation have been reported in the clinical trials, in one of them in two thirds of the patients treated (study I196111-US) and one discontinued treatment due to QT prolongation. The applicant should discuss if QT prolongation should be considered an important potential risk.

Missing information

- Children have not been exposed to entolimod in clinical trials. The main studies (Rs-01 and Rs-23) were carried out in primates with an equivalent to 8-24 years in humans but data in younger animals are not available. Therefore, the assessor agrees that the use of entolimod in children should be considered missing information.

- There are available data for only 25 subjects older than 65 years coming for the studies in oncological patients so the safety profile of entolimod in these patients is not well characterised. In addition, old patients are likely to be polimedicated and their safety profile can be different. The applicant should discuss if the elderly should be considered missing information.

- The assessor agrees that the lack of data in pregnant and lactating women should be considered as missing information. Nevertheless, it should be renamed and only the term "Pregnancy and breastfeading" should be included in the table.

- No DDI have been carried out. The applicant argues that DDI are not needed given that entolimod is likely to be used without supportive care. However, this statement is not supported by the assessor. If

entolimod were finally authorised it could be used in ASR settings in which supportive care were available. Therefore, DDI with products recommended as medical care in such situations (e.g., antibiotics or filgrastim) should have been performed. The applicant should discuss if the lack of DDI studies should be considered missing information.

3.4.2. Conclusions on the safety specification

Having considered the data in the safety specification, the Committee considers that the following issues should also be a safety concern:

- QT prolongation should also be considered an important potential risk.
- Elderly should also be considered missing information.
- The lack of DDI studies should also be considered missing information.

Pharmacovigilance Plan

The applicant proposed the following on-going and planned studies in the Post-authorisation Pharmacovigilance Development Plan

Study/activity Type, title and category	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
PASS	Collection of information on patient characteristics, entolimod administration, safety, and mortality following a radiation disaster	• Description of basic demographic subpopulations receiving entolimod • Conditions of administration • Frequency of safety signals, including ischemic adverse events and adverse hepatic sequelae • Characterization of in-use safety profile in human children • Characterization of safety during pregnancy • Characterization of potential risks to breastfed children	Planned	Within 6 months following completion of patient follow-up.
Targeted follow-up questionnaires	Collection of relevant information regarding potential ischemic and hepatic adverse events	• Detailed information regarding outcomes relating to the important potential risks	Planned	With periodic safety update reports (PSURs) and RMP updates

3.4.3. Discussion on the Pharmacovigilance Plan

Studies in the pharmacovigilance plan aim to identify and characterise risks, to collect further data where there are areas of missing information or to evaluate the effectiveness of additional risk minimisation activities. They should relate to the safety concerns identified in the safety specification. Therefore, the applicant should update the table above in order to include the safety concern addressed, according to the summary of safety concerns.

Additionally, the study/activity category is missing in the table; therefore, the applicant should include this information in the table.

Studies in humans can only be observational following the occurrence of a radiation disaster. The PASS will be a prospective, non-interventional, observational study and will evaluate patient characteristics, circumstances of Entolimod use and outcomes in people exposed to potentially lethal irradiation occurring as the result of a radiation disaster. All people who receive Entolimod because of suspected radiation exposure will be considered for inclusion in the study.

The table of on-going and planned studies in the Post-authorisation Pharmacovigilance Development Plan should also include the studies/activities milestones and due dates.

Of note, targeted follow-up questionnaires are considered routine pharmacovigilance activities, and therefore, when updating the RMP according to the Rev. 2 accompanying GVP Module V Rev.2, these follow-up questionnaires should be removed from the sections and tables related with additional pharmacovigilance activities.

3.4.4. Additional pharmacovigilance activities to assess the effectiveness of risk minimisation measures

There are no additional risk minimization measures planned. Given the special context of use of Entolimod (radiation disaster), that seems appropriate. Therefore, there are no additional pharmacovigilance activities planned for assess the effectiveness of risk minimisation measures.

3.4.5. Overall conclusions on the PhV Plan

Entolimod will be used in acute situations were populations will be subjected to non-homogeneous whole-body irradiation, from which ARS will result. It is impossible to predict the number of individuals affected and the severity of the disease in each individual. The affected populations will also be very heterogeneous concerning pre-existing diseases and conditions.

ARS is a severe condition that can lead to death, due to haematopoietic, gastrointestinal or cerebrovascular syndromes.

Till now there are no effective countermeasures approved in Europe for this situation.

The applicant proposed targeted follow-up questionnaires as a routine activity in the PhV Plan to obtain structured information regarding the important potential risks of entolimod, which is endorsed. However, when updating the RMP according to the Rev. 2, these follow-up questionnaires should be removed from the sections and tables related with additional pharmacovigilance activities.

The applicant has planned a PASS to seek the missing information, namely the use in paediatric populations, in pregnant and breast feeding women, that can only be performed after the occurrence of a radiation disaster.

In addition, with regards to the objective of the study, the MAA should discuss the relevance of reformulating the objectives of the PASS to include not only the collection of information from people exposed to potentially lethal irradiation result of a radiation disaster but also individuals who receive accidental radiation exposure in a small scale (for example workers of nuclear facilities), taking into account the proposed indication for Entolimod, the efficacy and safety data already available and also the CHMP's comments on the proposed indication.

Of note, the applicant mentioned that relevant studies are planned as part of a formal PIP (EMEA-002020-PIP01-16) to address the issue of dosing recommendations in paediatric population and also the development of a lower strength solution for injection. Nevertheless, according to the GVP Module V (rev 2), the module SVI of Part II of the RMP should only include information about the potential for misuse for illegal purposes. Therefore, this data regarding the PIP should be removed from section SVI of Part II of the RMP.

The PRAC, having considered the data submitted and the conditions of use of entolimod, is of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

However, there are other concerns that the applicant should take into account.

Risk minimisation measures

Proposal from applicant for risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Potential for ischemic events during transient entolimod-mediated hypotension and tachycardia in patients with pre-existing vascular disease or low cardiac output	• Guidance in SmPC • Routine pharmacovigilance • Administration only under the supervision of a healthcare professional • Use restricted to designated centres	Not applicable
Potential for increased hepatotoxicity in patients with pre-existing liver disease	• Guidance in SmPC • Routine pharmacovigilance • Administration only under the supervision of a healthcare professional • Use restricted to designated centres	Not applicable
Use in the paediatric population	• Guidance in SmPC • Routine pharmacovigilance • Administration only under the supervision of a healthcare professional • Use restricted to designated centres	Not applicable
Use during pregnancy	• Guidance in SmPC • Routine pharmacovigilance • Administration only under the supervision of a healthcare	Not applicable

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	professional • Use restricted to designated centres	
Exposure while breastfeeding	• Guidance in SmPC • Routine pharmacovigilance • Administration only under the supervision of a healthcare professional • Use restricted to designated centres	Not applicable

Due to the special proposed indication for entolimod, the planned routine risk minimisation measures are acceptable. However, stating only "Guidance in the SmPC" or quoting the full text of the SmPC is not considered acceptable in the routine risk minimisation measures. In the Summary table of pharmacovigilance activities and risk minimisation activities by safety concern, the reference to the SmPC/PL section related with the safety concerns should be provided.

3.4.6. Additional risk minimisation measures

The applicant did not propose any additional risk minimisation measures.

The PRAC considers that no additional risk minimisation measures are necessary at this stage.

3.4.7. Overall conclusions on risk minimisation measures

The PRAC having considered the data submitted was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication. However, some updates are needed in the tables of section V of the RMP (see part 6 "other concerns)

3.5. Pharmacovigilance system

The Pharmacovigilance system as described by the applicant has the following deficiencies:

- The statement should be signed and dated by both, the QPPV and a representative person on behalf of the applicant.

Provided that the deficiencies are rectified prior to the applicant placing the medicinal product on the market, the CHMP may consider that the Pharmacovigilance system will fulfil the requirements. The applicant must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market.

4. Orphan medicinal products

Orphan designation

Entolimod was designated as an orphan medicinal product EU/3/15/1607 on 10th December 2015 in the following condition: Treatment of acute radiation syndrome.

Similarity

The application did not contain a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products.

5. Benefit risk assessment

5.1. Therapeutic Context

5.1.1. Disease or condition

Entolimod is being developed as a medical radiation countermeasure to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster in adults and paediatric population aged ≥2 years old.

Radioactive contamination and radiation exposure could occur if radioactive materials are released to the environment as the result of a nuclear/radiological event. Excessive radiation dose can cause short-term health effects including death, and long-term health risks especially cancer. Short-term health effects of radiation exposure are ARS and cutaneous radiation injury (CDCa). ARS is an acute illness caused by exposure of the entire body (or most of the body) to an external high dose of penetrating radiation in a very short period of time (usually a matter of minutes) (CDCb).

The time course and severity of clinical signs and symptoms of ARS are a function of different factors, mainly the absorbed radiation dose (Macià et al. 2011). The minimum absorbed dose for which signs or symptoms of ARS are described is 1 Gy (IAEA paper). At such dose hematopoietic ARS emerges, while gastrointestinal ARS occurs at higher doses, and the cerebrovascular ARS at doses higher than 20 Gy (Macià et al. 2011). In human beings, probability of death by ARS is 0% for absorbed doses of 1-2 Gy, 50-100% for doses in the range of 6-8 Gy, and 100% for doses >8 Gy (IAEA paper). The lethal dose required to kill 50% of the human population within 60 days (LD50/60) without medical treatment is about 3.5 Gy increasing to about 5–6 Gy with adequate supportive treatment (IAEA paper).

The hematopoietic ARS involves pancytopenia, with predisposition to complications such as infection (due to leukopenia) and bleeding (due to thrombocytopenia) that, if sufficiently severe, can result in death (Macià et al. 2011). The gastrointestinal ARS is due to denudation of intestinal mucosa which produces watery diarrhoea, dehydration and electrolyte loss, gastrointestinal bleeding and perforation (which facilitates the entry of bacterias into the bloodstream); death can occur due to sepsis, bleeding, dehydration and multisystem organ failure. Cerebrovascular ARS occurs presumably with endothelial cells damage and vascular leak with edema and consequently an increase in intracranial pressure, and death almost invariably occurs quickly (e.g. generally within 24–48 h). The radiation-induced multi-organ failure is thought to be due to damaged endothelial cells leading to a radiation-induced systemic inflammatory response syndrome mediated by the release of inflammatory cytokines.

Diagnosis of ARS is based on clinical and laboratory data (IAEA paper). The extent of radiation-induced injury to critical organs plays a vital role in the diagnosis, treatment and the prognosis of ARS (Singh et al. 2017). The International Atomic Energy Agency (IAEA) has published good summary of signs and symptoms of ARS and their correlation with absorbed doses (IAEA paper).

5.1.2. Available therapies and unmet medical need

The European approach for managing radiation casualties known as METREPOL bases on medical signs and symptoms and laboratory data, not on dose per se (Fliedner et al. 2001). Patients are asigned a response category, which require specific therapeutic strategies, based on the assessment of the damage to the critical organ systems as a function of time after radiation exposure using indicators of effect, i.e. observable clinical signs and symptoms. Specific therapeutic strategies are supportive medical care (antiemetics, analgesics, brain oedema therapy, adapted nutrition, antibiotics, skin treatment, and other medical/surgical approaches), blood component therapy, growth factor therapy, stem cell transplantation or surgery are other treatments.

Despite this protocolised approach for managing radiation casualties, no medicine is formally approved in the EU for use as a medical radiation countermeasure. Two granulocyte-colony stimulating factors (G-CSF) products, filgrastim (Neupogen®) and pegfilgrastim (Neulasta®), are approved medical radiation countermeasures by FDA for adult and paediatric human use. They are indicated to mitigate hematopoietic ARS, administering the first dose as soon as possible after suspected or confirmed exposure to radiation levels greater than 2 Gy, since they can effectively reduce the duration and severity of neutropenia and increase survival.

Efficacy data of both G-CSF products were derived from studies in nonhuman primates (NHP) conducted in accordance with United States governmental regulations as promulgated under the FDA Animal Rule. The Animal Rule states that for drugs developed to ameliorate or prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic substances, when human efficacy studies are not ethical and field trials are not feasible, FDA may grant marketing approval based on adequate and well-controlled animal efficacy studies when the results of those studies establish that the drug is reasonably likely to produce clinical benefit in humans. While there is no formal 'animal rule' framework in the EU, this should not be interpreted as precluding the possibility that animal models data in principle could play a critical role in the assessment of applications for European Union (EU) marketing authorisations for human medicines[1].

Entolimod is proposed to address an unmet medical need in Europe with the argument that filgastrim and pegfilgastrim are not adequate for use in a mass-causality radiation catastrophe since intensive supportive care may be limited. When used together with supportive care, both G-CSF demonstrated to reduce the risk of radiation-induced neutropenia in ARS (FDA paper). However, as stated in its SmPC by FDA, they had minimal direct in vivo or in vitro effects on the production or activity of hematopoietic cell types other than the neutrophil lineage, and therefore they are not expected to materially reduce the risks of radiation-induced thrombocytopenia or haemorrhagic complications). Neither are to act on radiation-induced gastrointestinal injury. Contrarily, entolimod is proposed as having effect on all three hematopoietic cell lineages (neutrophils, platelet, and erythrocytes) and on mitigating the erosive and haemorrhagic gastrointestinal toxicities of ARS.

Finally, the quality of the product is unclear because data provided are very poor and incomplete and do not ensure a controlled manufacturing process. Several deficiencies have been identified as Major Objections related to not sufficiently established manufacturing process to produce a consistent product, uncertaintines in the proposed control strategy, lack of process validation for drug substance (DS) and drug product (DP), comparability at DS and DP level not demonstrated (in particular between the clinical and the commercial process), insufficient characterization of DS, insufficient control of DS and DP (particularly in relation to control of impurities) and inconclusive stability data. . This additionally hampers confirming a positive benefit-risk relation and is deemed to preclude a marketing authorisation at present.

5.1.3. Main clinical studies

Human efficacy and safety studies cannot be conducted in situations such as a radiological/nuclear accident because they would not be ethical or feasible.

Entolimod efficacy data have been derived from a single pivotal study in lethally irradiated NHPs of ages estimated to correspond to human age categories of 8-24 years (study Rs-23). The identification of an effective human dose was done by a dose conversion study (Rs-001) in NHP. The dose conversion was based on the assumption that a given dose to human and animal producing the same effect on biomarkers in both (non-irradiated) species will have similar effect in survival in the two (irradiated) species.

- **Study Rs-23** was a GLP, randomized, blinded, placebo-controlled, parallel-group, dose-ranging study Rs-23 in 179 healthy, research-naïve irradiated NHP (rhesus macaques) evaluated the dose-dependent effect of entolimod on survival (primary), efficacy biomarkers, and haematology parameters. Single IM doses of placebo or 0.3, 1, 3, 6.6, 10, 40, or 120 µg/kg of entolimod, followed by 60-day observation period, were administered in NHP at 25 hours after TBI with 7.2-Gy dose. Ten animals per sex and group were allocated to each dose administered while in the placebo group 20/20 animals per sex were included. The age of the animals was not provided.
- **Study Rs-001** was a randomized, open-label, placebo-controlled dose-ranging study Rs-001 in 160 healthy, research-naïve, nonirradiated NHP performed to evaluate the PD responses to a single IM injection of entolimod. Single IM doses were administered including groups of placebo or 0.3, 1, 3, 6.6, 10, 40, or 120 µg/kg of entolimod. Each group included between 9-10 males and 10-11 females.The age of the animals at the start of dosing ranged between 3 to 7 years.

Safety data was presented after single-dose IM administration of entolimod in healthy humans (studies HU-7014 and HU-9001), in oncological patients (studies I196111-US, I196111-Russia and BL612-CBLB502) and in both preclinical efficacy studies (studies Rs-23 and Rs-001).

- Study HU-7014 was a phase 1 trial, single-centre, open-label, non randomized, single-dose, dose-escalation study that evaluated the PK and PD of entolimod. A single IM dose of entolimod of 2 µg (N=6), 6 µg (N=6), 12 µg (N=6), 24 µg (N=6), 30 µg (N=12), 35 µg (N=6), 40 µg (N=5) and 50 µg (N=3) was administered on a dose-per-subject basis in 50 healthy volunteers. Subjects were observed for ~36 hours following entolimod administration and then discharged. Subjects were required to return to the clinic on the morning of Day 5, on Day 12, and on Day 28 for follow-up and blood collection for analysis of anti-entolimod antibodies.
- Study HU-9001 was a Phase 2 trial, multicenter (2 centers) open-label, randomized, parallel-group study that evaluated the PK and PD of entolimod. They used one out of 4 different dosing regimens of entolimod in 100 healthy subjects: a single IM injection of 25 µg, 2 IM injections of 30 µg of entolimod administered 72 hours apart, a single IM injection of 35 µg, and a single IM injection of 35 µg preceded by 400 mg of orally administered ibuprofen (IBP) to mitigate the flu-like syndrome that can be associated with entolimod administration. Following entolimod administration, subjects were observed for ~8 hours and then discharged. All subjects except for those in the group receiving 2 entolimod injections were required to return to the clinic for follow-up on the morning of Days 2 and 6 (final visit). Subjects in the group receiving 2 entolimod injections were required to return to the clinic for follow-up on the morning of Day 2 and again on Day 4, when they received their 2nd entolimod injection. These subjects were subsequently observed for 8 hours and then discharged. Follow-up after the 2nd injection was on Days 5 and 9 (final visit).

In the combined HU 7014/HU 9001 healthy subject population, subjects ranged in age from 18 to 55 years.

- Study I196111-US was a single-centre, open-label, non-randomized, sequential dose-escalation study conducted in the US that evaluated the safety and tolerability of entolimod. A total of 26 subjects were enrolled and treated with absolute doses of 5 µg/dose (N=4), 10 µg/dose (N=3), 15 µg/dose (N=3), 20 µg/dose (N=4), 30 µg/dose (N=5), or 40 µg/dose (N=7). They presented with histologically or cytologically confirmed, locally advanced, inoperable or metastatic solid tumor for which no effective therapy existed. Patients aged from 42 to 82 years. Patients were administered repeated injections of entolimod: subcutaneous injections of entolimod on 5 consecutive Days (5, 10, 15, and 20 µg/dose absolute dose groups) or an intramuscular injection of entolimod on Day 1 and a subcutaneous injection of entolimod on Days 4, 8, and 11 (30 and 40 µg/dose absolute dose groups). Subjects were observed for 9 hours after the first entolimod injection. Subsequent injections were given on an outpatient basis, with further follow-up on the days of drug administration (for 8 hours after dosing), as well as on Days 15, 22, and 43. Subjects were evaluated every 3 weeks after Day 43 until disease progression or clinical, radiologic, or another discontinuation criterion was met. The study included a 30 day safety follow-up period after the last image that showed progression of disease or until resolution of any drug-related toxicity.

    - Study I196111-Russia was a 2-centre, open-label, nonrandomized, sequential dose-ranging study that evaluated the safety and tolerability of entolimod. Seven subjects were enrolled and treated with 4 doses of 30 µg of entolimod IM on Day 1 and SC, once daily, on Days 4, 8, and 11. Patients presented with histologically or cytologically confirmed, locally advanced, inoperable or metastatic solid tumor for which no effective therapy existed. Patients aged from 27 to 65 years. Subjects were to be observed for at least 36 hours after each entolimod injection (on Days 1, 4, 8, and 11 or Days 1 and 4), as well as at follow-up visits on Days 15, 22, and 43. Subjects were to be evaluated every 3 weeks after Day 43 until disease progression or another discontinuation criterion was met. The study included a 30-day safety follow-up period after the last image that showed progression of disease or until resolution of any drug-related toxicity.

    - Study BL612-CBLB502 was a 3-centre, randomized, placebo-controlled, single-blind study conducted in the Russian Federation to assess the safety, PD, and preliminary efficacy of entolimod. A total of 40 patients aged 33-84 years with colorectal cancer who had never received antitumor treatment and who were eligible for scheduled surgical resection of the primary tumour were included. Subjects received subcutaneous injections of the study drug, comprising entolimod (at either a dose of 0.35 µg/kg or 0.45 µg/kg) or placebo, administered as either 1 injection or as 2 injections separated by 3 (± 1) days. The last injection was to be administered 4 (± 2) days before surgical resection of the primary tumor. The study was conducted in 5 sequential treatment cohorts, each enrolling 8 subjects (with a 3:1 randomization such that 6 subjects received entolimod and 2 subjects received placebo). The study included a 7-day postsurgery follow-up and a long term follow-up up to 1 year.

The proposed indication in children is the same as that proposed in adults. No paediatric clinical study is planned as entolimod cannot be studied in healthy children.

5.2. Favourable effects

Entolimod efficacy data have been derived from a single pivotal study in lethally irradiated NHPs (study Rs-23). The pathophysiological mechanism of injury after acute total body irradiation at high dose rate

in humans is well-understood. The NHP model is considered acceptable since it most closely reproduces the clinical, histopathological, and pathophysiological aspects of radiation injury in humans.

In the study Rs-23, 72.5% of the animals in the placebo group have died at day 60 versus only 25% in the 10-µg/kg group (p=0.0021), resulting in 47.5% absolute increase in survival and a survival odds ratio of 7.9 in favour of entolimod administered intramuscularly at 25 hours after TBI with a gamma radiation dose of 7.2 Gy (intended to be an LD70/60 radiation dose). In the 40-µg/kg entolimod group the survival was 70% (p<0.0001), resulting in 42.5% absolute increase.

The probability of survival at Day 60 increased compared to placebo starting at a dose of 6.6 µg/kg with maximal effect at a dose of 10 µg/kg. The survival benefit compared to placebo plateaued across entolimod doses of 10 µg/kg (n=15/20; adjusted p=0.0021), 40 µg/kg (n=14/20; adjusted p<0.0001) and 120 µg/kg (n=14/20; nominal unadjusted p<0.0001) compared to survival of n=11/40 in the placebo arm.

In particular, the stable effect as shown in the plateau for survival for the three highest dose groups is a clear strength of this application. This seems to reduce probability for chance findings as reason for the survival benefit observed in NHPs and may indicate a really promising benefit for the target population, provided it can be translated into human population.

In the small populations investigated results of the primary analysis of survival in NHPs were claimed to be supported by 2 sensitivity analyses assessing the potential effects of euthanasia on the survival outcomes and by consistent results showing an entolimod survival benefit across all covariate subgroups analyzed.

Similarly, results of the primary analysis of survival in NHPs can be seen as describing consistent results across the covariate subgroups of body weight, body width, sex, study phase, entolimod-reactive antibody titer, and radiation dose, as none of the covariates analyzed demonstrated either an independent or an interactive effect on the study primary outcome of survival and the treatment effect seen in the primary analysis was maintained.

TBI produced expected hematological toxicity, resulting in neutropenia, thrombocytopenia, anemia, and lymphopenia in all treatment groups. In NHPs entolimod dose levels ≥10 µg/kg are claimed to be improved neutrophil, platelet count, and hemoglobin nadirs, and were associated with a lower proportion of days with cytopenias and anemia.

5.3. Uncertainties and limitations about favourable effects

The survival benefit was only observed in small cohorts of animals (10-20/group). Even in the pivotal trial Rs-23 the group size per dose was limited to 20 animals. The robustness and reliability of the outcome can easily be challenged, considering that the difference classifying a treatment group as statistical highly significant or non-significant depends at the end from the outcome in very few animals (2-3) per group only. Clinical studies in the intended target population marketing authorisations based on one rather than two pivotal trials are the exception, not the rule.

The claimed TRL5 associated mode of action is not sufficiently characterised with respect to primary and secondary pharmacology to allow concluding that increased overall survival is caused by entolimod. There remains a gap of plausibility between the mode of action as well as the PD claimed and the observed overall survival. Only short time increase of G-CSF (and in consequence ANC) and IL-6 are demonstrated for entolimod. However, these effects are restricted to the first 24h after administration due to the short half-life times of these cytokines. It seems implausible to conclude that the PD short time effects can be reasonably linked to an increased overall survival after lethally irradiation. From the claimed PD marker only G-CSF is characterised sufficiently with respect to

haematopoiesis. However, from comparable models with irradiated NHPs it was clearly demonstrated that only administration over longer times (until the nadir is passed) survival is increased. Insofar, it seems implausible that the 24 h effects of entolimod on G-CSF really explain the increased survival and reduction of neutropenia during the nadir weeks later. Moreover, the impact of short time IL-6 increases on haematopoiesis is also not plausible to explain the results and several other additional mechanisms and effects are mentioned, but not sufficiently proven. Insofar, a significant lack of plausibility remains between the characterised pharmacodynamics effects and the increases in overall survival shown.

As the pharmacological principle is new and insufficiently characterised, no other medicinal product has proven clinical relevant efficacy or safety of TLR5 activation confirmation of the survival benefit in a second pivotal trial might be necessary for this application. This is of particular relevance as it seems unlikely that after approval any new efficacy data will become available. Regular post-marketing data assessment will fail to provide any confirmation of benefit-risk assessment after approval. Moreover, the significantly lower tolerability of entolimod in humans compared with that observed in NHPs may indicate also a difference in efficacy between both species. The applicant is asked to justify approval based on a single pivotal trial, or to consider how additional evidence for efficacy in the indication applied for could be provided.Entolimod is highly immunogenic and therefore intended for single use only. However, naturally occurring antibodies neutralising the drug's efficacy are described in about 15 % of the US population, while the frequency in the European population is still unknown. Thus, trial Rs-23 was performed in a selected population of antibody negative NHPs. Moreover, a dominant TLR5 stop codon mutation occurs with a frequency of 10% in the human population. Subjects with this mutation have no or a reduced benefit from entolimod due to signalling defects. In consequence it seems that in a worst case scenario up to 25% of the patients may be exposed to the product although no benefit could be reasonably expected. However, these patients were not excluded in the applicant's calculation for the claimed survival benefit in the human target population.

As mentioned in the many major objections raised on quality issues, the product applied does not fulfil the quality standards necessary to discuss approval at present. Currently no marketing formulation was developed and the quality of the applied product is not acceptable. As entolimod batches used in previous non-clinical and human safety studies are not proven to be representative of the batches most recently manufactured and proposed for emergency-use there remain significant uncertainties whether the data shown were generated with the product now applied. Biocomparability needs to be shown.

The application strongly depends on the results of RS-23. The (formal) GLP status of the study is unclear. The applicant needs to verify in detail if or to what extent study Rs-23 was conducted in compliance and following the principles of GLP.

The applicant selected 10 µg/kg from irradiated primates (Study Rs-23) to estimate the dose in humans as the optimal dose in NHPs. However, a clear advantage of this dose cannot be concluded from the data on survival and haematological parameters that have been submitted. Additional data from study Rs-23 are needed to help clarify whether the 10 ug/kg entolimod dose is the optimal dose.

As the survival benefit was currently demonstrated only in NHPs it is essential to estimate a human dose that will provide the same efficacy (survival) in humans as was observed in pivotal animal efficacy studies. This is not possible at present, because the proposal for dose conversions based on G-CSF and ANC is neither methodological nor clinical adequately justified.

Study Rs-was 23 compared entolimod versus placebo alone (without BSC) as, according to the applicant, such supportive care would be restricted in the setting of mass casualty nuclear/radioactive accidents. However, there is protocolised standard care in ARS and even in case of mass casualty accident some usual standard care will be probably available. The proposed use of entolimod includes both situations where supportive care is fully available, and where this is limited. With regard to the former situation, the applicant should justify how the conditions of the pivotal NHP study (supportive care limited) has external validity with respect to a magnitude of benefit in case of full supportive care, including hematological growth factors. Conversely, the applicant should describe what conditions of use might be supported by the particular conduct of the pivotal NHP study, and justify that such conditions are foreseen to be realistic.

5.4. Unfavourable effects

No TLR5 agonist is currently approved; insofar the safety risks for this type of product are unknown.

In both studies in healthy volunteers (n=150) nearly 100% subjects presented with TEAEs, which seems to allow concluding that entolimod's toxicity in humans is not trivial. This assumption is confirmed by the fact that TEAEs categorized by the CTCAE as being severe (Grade 3) or potentially life-threatening (Grade 4) occurred in 28/120 (18.7%) subjects. This seems to confirm that entolimod's impact on safety in the human target population is significantly higher than that observed in NHPs toxicology studies. It should be noted that the manufacturing process has changed considerably during the product development and the comparability among lots employed in non clinical and clinical trials is lacking. Adverse findings reported in animal and clinical data are inconsistent.

At the proposed entolimod dose range of 0.4-0.6 ug/kg received by 51 healthy volunteers, the most frequent adverse events were chills (78.4%), headache (76.5%), nausea (49.0%), vomiting (47.1%), tachycardia (41.2%), pyrexia (39.2%), hypotension (31.4%) and increase of transaminases (27.4%). Most TEAEs were mild to moderate, 27.5% of them were considered Grade 3 or 4 (severe or life-threatening TEAEs). A dose-response relationship was observed for the occurrence of AEs after entolimod administration that is apparent for pyrexia, vomiting, SNC and gastrointestinal symptoms, laboratory parameters and hypotension. It is agreed that all these flu-like symptoms may be seen as caused by the cytokine release. However, most important about one third of the patient had significant decreases in blood pressure (31.4%) associated with tachycardia.

High levels of IL-6 insofar are likely initiating the proinflammatory IL-6-mediated signaling cascade, therefore occurrence of CRS cannot be excluded. Moreover, as all nearly all clinical symptoms of CRS were reported as "cytokine related adverse events" for entolimod and similarity with respect to very low toxicity in animals and significantly higher toxicity in man exists to other products known to induce a CRS, a risk for CRS is also seen for entolimod.

Moreover, Il-6 induced activation of proinflammatory pathways may add risks for patients with smouldering inflammatory disease as observed perhaps already in the limited safety population (acute cholecystitis in a healthy volunteer assessed as related and Grade 4 pneumonia in a patient).

Entolimod at the proposed dose was associated also with rapid increase of serum AST and ALT levels. At the proposed dose of 0.40-0.60 µg/kg, 50% of subjects presented with values higher than the upper limit of normality, and about 20% of healthy subjects with grade ≥3 severity. In most cases values returned to normal range by 120 h postdose (in 96% of subjects for ALT and 100% of subjects for AST).

Hyperglycaemia and hypophosphatemia were observed in most healthy subjects (86.3% and 74.5%, respectively), peaking at 8 hours and recovered within 24 hours in all them. Hypophosphatemia was grade ≥3 in 60.8% of the healthy subjects at the proposed dose.

Hypotension (diastolic and systolic) occurred at the proposed dose with nadir values below the lower limit of normality in 31.4% and 25.5% of subjects, for less than 1.5 hours, and fully recovered. The mean maximum decrease from baseline was -28.8 mm Hg for diastolic blood pressure and -22.8 mm Hg for systolic blood pressure. Also tachycardia and fever was present, in 65.7% and 58.8% of subjects with values above the upper normal limit, respectively, for few hours and fully recovered. Hypotension and tachycardia may lead to ischemic events. QTcB prolongation was observed after administration of entolimod with no clear dose-response relationship.

Regarding SAEs, one patient presented Grade 2 acute cholecystitis, one Grade 4 AST increase, one Grade 1 glucose intolerance and one Grade 2 hypotension. An additional one had serum transaminase elevations considered not related to entolimod.

Eleven subjects (8%) discontinued treatment prematurely due to adverse reaction, 7 due to increase in transaminases, 2 due to flu-like syndrome and 1 to asthenia. The three patients receiving entolimod at 0.40-0.60 μg/kg dose discontinued due to hypertransaminasemia.

Oncological patients presented with anemia, lymphopenia, increases in serum ALT or AST levels, serum alkaline phosphatase, GGT, hyperglycemia and hypophosphatemia, hypotension, tachycardia and fever amonst other adverse events. Grade 3 and 4 TEAEs as hypotension (n=2), serum AST elevation (n=3), atrial fibrillation (n=1), lymphopenia (n=2), serum lipase elevation (n=2), hypophosphatemia (n=1), extrasystoles and acute myocardial infarction in a subject with preexisting cardiac ischemia (n=2) were reported. Extrasystoles and acute myocardial infarction were considered as related to treatment by the investigator.

In oncological studies, the reason for discontinuation were severe hypotension, fever and QT prolongation, a Grade 3 increase in AST, bigeminal ventricular extrasystoles and acute myocardial infarction.

The necessity for an accurate dosing according body-weight as well as the restricted data available indicates a narrow therapeutic range of entolimod in humans. This means though also a high risk for overdosing in the intended target population, particularly in the case of an emergency setting as the highlighted radiation disaster.

5.5. Uncertainties and limitations about unfavourable effects

The absence of safety data in the target population, although understood, is an important shortcoming of this dossier since the safety profile of entolimod in the subjects of interest cannot be characterised.

The applicant has presented short-term safety data after the administration of entolimod mainly in healthy volunteers. Such data are considered relevant in the absence of safety information in the target population. However, the limitations of this information is obvious considering the complexity and severity of the clinical picture of the patients who are exposed to high doses of total body irradiation and that a number of concomitant medicines are expected to be concomitantly used as part of the recommended supportive care. In addition, the number of patients exposed is considered very limited taking into account that this medicinal product belongs to a novel therapeutic class (first TLR5).

The applicant should provide a justification that this safety database is sufficient to characterise the adverse effect profile of a single injection of entolimod at the proposed dose, including the impact of the known safety concerns (e.g. hypotension) in patients vulnerable to such effects.

If the same or other AEs would be observed in irradiated patients receiving (or not) full supportive care is unknown.

AEs in healthy volunteers were collected up to 28 days of entolimod administration. Although only one dose of entolimod is proposed the applicant claims that durable efficacy on clinical parameters is expected. So durable impact of entolimod on safety should not be ruled out. For the time being the safety profile of entolimod in healthy humans beyond 28 days is unknown.

Although it is acknowledged that the applicant may have tried to reduce imbalances between the dose groups, it is obvious from the trial design that the very small dose groups do not allow any adequate safety assessment in any subgroups, particularly, as age of inclusion was restricted from 18 to 47/55 years. Insofar, safety of patient above 55 years remains not assessable for this application.

Preclinical pivotal toxicology studies were not fully predictive of the adverse events reported in humans since they presented serious drawbacks and a mayor objection has been raised to the applicant for clarification (see non-clinical report).

The currently proposed posology is not adequately justified. Safety evaluation in healthy volunteers has shown a significantly clinical relevant lower tolerability of entolimod than that observed in NHPs. A difference regarding the safety outcome is likely to indicate also a difference in efficacy. In any case it justifies concluding that the currently presented dose conversion approach from a safety perspective was not successful. Moreover, the demonstration of higher safety risks in human means also that the benefit –risk balance in humans cannot be converted from the NHP trial Rs-23.

The TRL5 associated mode of action is not sufficiently characterised with respect to safety. Comprehensive data on the safety of entolimod on the major physiological systems (at least on the cardiovascular, respiratory and central nervous system) in humans in order allow assessment of the evident risks added by entolimod treatment is still missing.

A risk for exacerbation of pre-existing inflammation diseases derived from IL-6 induced activation of proinflammatory pathways in patients with chronic inflammatory comorbidities (COPD, RA, Crohns disease and many others) can be reasonably presumed, but is not further assessable from the limited data available.

The mechanism of action behind hyperglucemia, hypophosphatemia and increase in transaminases is currently unknown. Increases in serum ALT and AST following recommended entolimod doses may be indicative that patients with pre-existing hepatic disease could be at increased risk of liver injury. Considering the complete lack of knowledge regarding entolimod's metabolism, elimination pathways and potential for interactions, further data in these areas is considered a prerequisite to be able to assess dosing recommendations for special populations from a PK perspective.

A database of 34 relevantly exposed subjects to the proposed entolimod dose of 0.4-0.6 ug/kg withouth ibuprofen pretreatment is deemed insufficient even in an application under exceptional circumstances. These exceptional circumstances are triggered from the impossibility to generate efficacy data in humans; however, this does not mean that the level of safety information can be lowered or completely waived. Uncertainties are increased as evaluation of efficacy completely depends on animal data. Therefore, it is indispensable for a meaningful benefit risk assessment that safety for the applied product is adequately characterised.

The applicant is proposing the use of entolimod in a disaster situation, based on a pivotal animal study mimicking a situation where supportive care, including the use of i.v. fluids was not administered. It is presumed that the proposed indication includes situation where supportive medical care is not available. The applicant should justify that entolimod is suitable for such use, notwithstanding the hemodynamic effects of the drug.

From the applied indication for administration in the case of a radiation disaster and, although all radiological/nuclear accidents are considered a disaster, it seems unlikely that after approval any new safety data will become available for entolimod. Due to the lack of any post-marketing safety data standard approaches of pharmacovigilance will fail to provide any confirmation of benefit-risk assessment after approval.

Characterization of PK of entolimod in human subjects is incomplete and the comparability between the presented analyses is hampered since details on subject inclusion are not always clear. Moreover, there are no dedicated studies on bioavailability, elimination, metabolism, potential for drug interactions, and the influence of extrinsic and intrinsic factors on PK of entolimod. Neither are data regarding PK in renal impairment, hepatic impairment, elderly nor children.

5.6. Effects Table

Table X. Effects Table for entolimod to reduce the risk of death following exposure to potentially lethal irradiation occurring as the result of a radiation disaster

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Survival	NHP surviving at day 60 after TBI	%	• 75 (10-µg/kg entolimod) • 70 (40-µg/kg entolimod)	27.5	P=0.0021 (10-µg/kg entolimod) (n=20) P<0.0001 (40-µg/kg entolimod) (n=19)	Study Rs-23 (irradiated NHP)
Severe neutropenia	Days that animals were both alive and free of severe neutropenia (ANC<500 cells/µL)	%	62 (10-µg/kg entolimod)	29	Not very informative	
Clinically relevant neutropenia	Days that animals were both alive and free of clinically relevant neutropenia (ANC<1,000 cells/µL)	%	58 (10-µg/kg entolimod)	26	Not very informative	
Severe thrombocitopenia	Days that animals were both alive and free of severe thrombocytopenia (<20,000 platelets/µL of blood)	%	78 (10-µg/kg entolimod)	39	Not very informative	
Clinically relevant thrombocytopenia	Days that animals were both alive and free of clinically relevant thrombocytopenia (<50,000 cell/µL)	%	73 (10-µg/kg entolimod)	36	Not very informative	
Unfavourable Effects						
AEs of special interest	Patients with increase in transaminase levels upper limit of normality	%	50 (0.40-0.60 µg/kg entolimod)	-----	Additional clarification is required	Studies HU-7014 and HU-9001 (HV)
	>grade 3	%	20 (0.40-0.60 µg/kg entolimod)	-----		
	Patients with hyperglucemia	%	86.3 (0.40-0.60 µg/kg entolimod)	-----	Additional clarification is required	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Patients with Hypophosphatemia	%	74.5 (0.40-0.60 µg/kg entolimod)	-----	Additional clarification is required	
	>grade 3	%	60.8 (0.40-0.60 µg/kg entolimod)	-----		
	Patients with diastolic hypotension below the lower limit of normality	%	31.4 (0.40-0.60 µg/kg entolimod)	-----		
	Mean maximum decrease from baseline	mm Hg	-28.8 (0.40-0.60 µg/kg entolimod)	-----		
	Patients with systolic hypotension below the lower limit of normality	%	25 (0.40-0.60 µg/kg entolimod)	-----		
	Mean maximum decrease from baseline	mm Hg	-22.8 (0.40-0.60 µg/kg entolimod)	-----		
	Patients with fever above the upper normal limit	%	58.8 (0.40-0.60 µg/kg entolimod)	-----		
	QT-ptrolongation					

Abbreviations: NHP (nonhuman primates), TBI (total body irradiation), HV (healthy volunteers)

5.7. Benefit-risk assessment and discussion

5.7.1. Importance of favourable and unfavourable effects

The availability of a medical radiation countermeasure which would allow reducing morbidity and mortality after total body irradiation at high dose rate after a radiological/nuclear accident is clinically highly relevant and a strong benefit.

In the pivotal study the survival increase of 47.5% for the entolimod doses of 10 µg/kg of entolimod (which is the dose administered in NHP in which the proposed human dose is based on), and of 42.5% for the 40- µg/kg dose in comparison to placebo, has clearly clinical relevance. However, the the robustness and reliability of the outcome can be challenged as the group size was limited to 20 animals per group.

As the pharmacological principle is new and insufficiently characterised, no other medicinal product has proven clinical relevant efficacy or safety of TLR5 activation and a gap of plausibility remains with

respect to the claimed mode of action and the reported increase in overall survival, the applicant is asked to justify approval based on a single pivotal trial or to consider how additional evidence for efficacy in the indication applied for could be provided. Moreover, the (formal) GLP status of the pivotal study Rs-23 is unclear. The applicant selected 10 µg/kg from irradiated primates (Study Rs-23) as the optimal dose in NHPs to estimate the dose in humans. However, a clear advantage of this dose cannot be concluded from the data on survival and haematological parameters submitted and additional information is needed. This is a crucial issue as such dose in NHPs has been used to predict the dose to be given in human that would be used in clinical practice without any confirmation in the target population.

The selected secondary efficacy variables related to neutrophil and platelet counts would only allow to partially assessing the efficacy of entolimod in the haematological subsyndrome of ARS as they are not very informative and other clinically relevant endpoints related to the recovery of neutrophil cells and platelets have not been assessed.

The proposed use of entolimod includes both situations where supportive care is fully available, and where this is limited. With regard to the former situation, the applicant should justify how the conditions of the pivotal NHP study (supportive care limited) has external validity with respect to a magnitude of benefit in case of full supportive care, including hematological growth factors. Conversely, the applicant should describe what conditions of use might be supported by the particular conduct of the pivotal NHP study, and justify that such conditions are foreseen to be realistic.

A reliable dose conversion approach is crucial but the proposal for dose conversions is neither methodological nor clinical adequately justified. Moreover, the safety profile observed with the proposed converted dose indicates a significantly lower tolerability and higher level of toxicities than reported from the NHPs, although the human doses selected were significantly lower than those shown to be associated with an increase in overall survival in the NHPs.

In relation to the safety profile of entolimod, the absence of data in the target population is relevant given the complexity and severity of the clinical picture of the patients who are exposed to high doses of total body irradiation and that a number of concomitant medicines are expected to be concomitantly used as part of the recommended supportive care.

The applicant has only presented short-term (28 days) safety data after the administration of entolimod in healthy volunteers what is considered relevant in the absence of data in the target population. Only 150 healthy volunteers have been exposed to entolimod. Therefore, the safety profile in humans is not adequately characterised, especially considering that this medicinal product belongs to a novel therapeutic class (first TLR5 agonist).

In addition to common flu-like symptoms, increases in serum AST and ALT levels, hyperglycaemia, hypophosphatemia, hypotension, tachycardia, QTcB prolongation have been observed. The mechanism behind these observations is not clarified at present. Many of the reported symptoms are probably caused by the increased cytokine releasewhat may suggest that entolimod is at risk for inducing a CRS/"cytokine storm" in a relevant proportion of those exposed with the applied posology.

Entolimod infusion causes a profound reduction in blood pressure, with mean maximal changes in diastolic pressure in the range of more than 20 mmHg. Furthermore, the duration of hypotension is dose dependent. The applicant is proposing the use of entolimod in a disaster situation, based on a pivotal animal study mimicking a situation where supportive care, including the use of i.v. fluids was not administered. It is presumed that the proposed indication includes situation where supportive medical care is not available. The applicant should justify that entolimod is suitable for such use, notwithstanding the hemodynamic effects of the drug.

Preclinical pivotal toxicology studies were not fully predictive of the adverse events reported in humans since they presented serious drawbacks and a major objection has been raised to the applicant for clarification.

As also with respect to safety risk it is unlikely that after approval any new safety data will become available from post-marketing sources and standard approaches of pharmacovigilance will fail to provide more information, it is indispensable to characterise entolimod's safety profile before approval adequately. This is the only possibility to reduce the degree of remaining uncertainties for the applied target population and a prerequisite for an adequate risk assessment of entolimod.

5.7.2. Balance of benefits and risks

Clinical and nonclinical data submitted do not allow to conclude that entolimod is reasonably likely to produce clinical benefit in humans exposed to acute total body irradiation. Entolimod has shown in phase II trial Rs-23 several promising results indicating the possibility of a clinical relevant increase in overall survival in $LD_{70/60}$ gamma-irradiated Rhesus macaques. Even if an important and clinically relevant benefit was probably shown in the NHPs, the benefit cannot be extrapolated to the human target population due to the lack of a reliable dose conversion approach. Consequently it seems currently not possible to identify a dose for humans at which a similar efficacy can be reasonable presumed in the human target population.

Considering that efficacy is based in a single non-clinical pivotal study and the failure in dose converting, together with the lack of the characterisation of the mechanism of action, the lack of assessment of entolimod effect on relevant clinical parameters, the lack of comparative data versus best supportive care it remains difficult to define a clear benefit for the applied human target population at present. In addition, the toxicological profile of entolimod is not characterised and potential target organs of toxicity have not been identified. Furthermore, reproduction toxicity data provided was of dubious value for the assessment of the product.

For the evaluation of entolimod safety profile in humans in the applied dose range for adults only data from 51 subjects is available (and only 34 without ibuprofen pretreatment). This is clearly not sufficient for any reliable safety assessment. Even in an application under exceptional circumstances the level of safety information cannot be lowered to this degree; in particular, as the exceptional circumstances are triggered from the impossibility to generate efficacy data in humans mainly and no additional safety data can be expected from post-marketing sources or other pharmacovigilance instruments. As already the sparse data evaluable indicate that safety risks are not trivial and adverse events observed - considering the mode of action- may indicate a risk for a cytokine-release syndrome/ "cytokine storm", adequate safety characterisation is essential to provide a valid risk assessment for the applied product.

Although one might argue that in the scenario of ARS a robust overall survival benefit –provided it can be robustly confirmed and translated in the human population by a reliable dose conversion approach- may nearly counteract every risk, this view neglects the fact that potential risks needs at least to be clearly assessable in order to conclude on their impact on the benefit-risk balance.

Finally, the quality of the product is unclear because data provided are very poor and incomplete and do not ensure a controlled manufacturing process. Another issue is that the formulation used in the development is not the same as the one to be marketed and demonstration of similar bioactivity is missing. This is of paramount relevance as the inconsistency in the non clinical and clinical data provided could be a result in the lack of comparability among lots. The many major objections with respect to quality demonstrate that entolimod is not available in an adequately controlled and

standardised marketing formulation at present. This additionally hampers confirming a positive benefit-risk relation and is deemed to preclude a marketing authorisation at present.

5.7.3. Additional considerations on the benefit-risk balance

Given that all efficacy data is necessarily extrapolated from a non-human animal model, albeit the most appropriate one, the data cannot be regarded as comprehensive, therefore the applicant requested a MAA under exceptional circumstances. As such, in case of approval, the applicant should consider what measures they would plan to put in place so that relevant human safety and efficacy data could be collected in the event of product use.

5.8. Conclusions

The overall B/R of entolimod is negative.

6. References

- Barreiro LB, Marioni JC, Blekhman R, Stephens M, Gilad Y. Functional comparison of innate immune signaling pathways in primates. PLoS Genet. 2010;6(12):e1001249.

Buchmann K. Evolution of innate immunity: Clues from invertebrates via fish to mammals. Front Immunol. 2014 Sep 23;5:459.

- CDCa. Possible health effects of radiation exposure and contamination. Centers for Disease Control and Prevention. (online). Available from: https://emergency.cdc.gov/radiation/healtheffects.asp (accessed 30 November 2017).
- CDCb. Acute radiation syndrome. Centers for Disease Control and Prevention. (online). Available from: https://emergency.cdc.gov/radiation/arsphysicianfactsheet.asp (accessed 30 November 2017).
- Daniel W. Lee, Rebecca Gardner, David L. Porter, Chrystal U. Louis, Nabil Ahmed, Michael Jensen, Stephan A. Grupp and Crystal L. Mackall: Current concepts in the diagnosis and management of cytokine release syndrome ; Blood 2014 124:188-195; doi: https://doi.org/10.1182/blood-2014-05-552729
- Dickerson 2013. The Use of Leukocyte Growth Factors (LGFs) for Treatment of Radiation Injury from Radiological/ Nuclear Accidents, 1986-2012. U.S. Food and Drug Administration. FDA advisory committee briefing document: a joint meeting of the medical imaging drugs advisory committee and the oncologic drugs advisory committee. Available from: http://www. fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/MedicalImagingDrugsAdvisoryCommittee/UCM350151.pdf (accessed 30 November 2017).
- European Medicines Agency. EMA decision (P/0104/2017) on the agreement of a paediatric investigation plan and on the granting of a deferral for entolimod (EMEA 002020-PIP01-16). 2014 Apr 11. (online). Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/pips/P/0104/2017/pip_001598.jsp&mid=WC0b01ac058001d129 (accessed 30 November 2017)
- FDA paper. FDA approves radiation medical countermeasure. (online) Available from: https://www.fda.gov/emergencypreparedness/counterterrorism/medicalcountermeasures/aboutmcmi/ucm443245.htm (accessed 19 january 2018)
- FEMA paper. National Security Staff Interagency Policy Coordination Subcommittee for Preparedness & Response to Radiological and Nuclear Threats in 2010 in a Planning Guidance for Response to a Nuclear Detonation. (online). Available from: https://www.fema.gov/media-library/assets/documents/24879. (accessed 30 November 2017).
- Fliedner TM, Friesecke I, Beyrer K. Medical Management of Radiation Accidents: Manual on the Acute Radiation Syndrome. London: The British Institute of Radiology, 2001. (online). Available at https://oparu.uni-ulm.de/xmlui/bitstream/handle/123456789/1650/vts_6891_9573.pdf?sequence=1&isAllowed=y (accessed 18 January 2018).
- Gardner MB, Luciw PA. Macaque models of human infectious disease. ILAR J. 2008;49(2):220-55.

- Hawn, T.R., et al., A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. J Exp Med, 2003. 198(10): 1563-72.
- Hawn, T.R., et al., A stop codon polymorphism of Toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. Proc Natl Acad Sci U S A, 2005. 102(30): 10593-7.
- IAEA paper. Diagnosis and treatment of radiation injuries/ jointly sponsored by the International Atomic Energy Agency and the World Health Organization. —Vienna : The Agency, 1998. Safety reports series, ISSN 1020–6450 ; no. 2
- Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014 Sep 25;5:461.
- Leikin JB, McFee RB, Walter FG. A Primer for Nuclear Terrorism. Dis Mon 2003;49:479-516.
- Macià M, Lucas A, Casanovas E. Radiobiology of the acute radiation syndrome. Reports of practical oncology and radiotherapy 2011;16:123–30.
- Mahmood J1, Zaveri SR1, Murti SC1, Alexander AA1, Connors CQ1, Shukla HD2, Vujaskovic Z1. Caveolin-1: a novel prognostic biomarker of radioresistance in cancer. Int J Radiat Biol. 2016 Dec;92(12):747-753. Epub 2016 Sep 13.
- O'Neill et al., The history of Toll-like receptors - redefining innate immunity. Nature Reviews Immunology, 2013. 13: 453–460.
- Product Development under the animal rule. Guidance for industry. U.S. Department of Health and Human Services. Federal Drug Administration. October 2015. (online). Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatory Information/Guidances/UCM399217.pdf.
- REMMa. At risk/Special need populations. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available at https://www.remm.nlm.gov/specialpops.htm (accessed 30 November 2017).
- REMMb. Radiation + Trauma (combined injury). Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/radtrauma.htm (accessed 30 November 2017).
- REMMc. Index. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/index.html (accessed 30 November 2017).
- REMMd. Choose Appropriate Algorithm: Evaluate for Contamination and/or Exposure. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/newptinteract.htm#skip (accessed 30 November 2017).
- REMMe. Contamination: Diagnose/Manage. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/contamonly.htm#skip (accessed 30 November 2017).
- REMMf. Exposure: Diagnose/Manage Acute Radiation Syndrome. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online).

Available from: https://www.remm.nlm.gov/exposureonly.htm#skip (accessed 30 November 2017).

- REMMg. Exposure + Contamination. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/exposurecontam.htm#skip (accessed 30 November 2017).
- REMMh. Mass casualty. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/masscasualty.htm (accessed 30 November 2017).
- REMMi. Triage guidelines. Guidance on Diagnosis and Treatment for Healthcare Providers. Radiation Emergency Medical Management. (online). Available from: https://www.remm.nlm.gov/radtriage.htm#overview (accessed 30 November 2017).
- Seok J, Warren HS, Guenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2013;110(9):3507-12.
- Singh VK, Seed TM. A review of radiation countermeasures focusing on injury-specific medicinals and regulatory approval status: part I. Radiation sub-syndromes, animal models and FDA-approved countermeasures. International Journal of Radiation Biology 2017;93(9):851-869. DOI: 10.1080/09553002.2017.1332438.
- Singh VK, Newman VL, Seed TM. Colony-stimulating factors for the treatment of the hematopoietic component of the acute radiation syndrome (H-ARS): A review. Cytokine 2015;71:22–37.
- Vallender EJ, Miller GM. Nonhuman primate models in the genomic era: a paradigm shift. ILAR J. 2013;54(2):154-65.

7. Recommended conditions for marketing authorisation and product information

7.1. Conditions for the marketing authorisation

7.2. Summary of product characteristics (SmPC)

7.3. Labelling

7.4. Package leaflet (PL)

User consultation

The applicant will submit the results on the readability test at Day 121. This proposal is considered acceptable.