Amsterdam, 16 July 2021
EMA/352570/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Sitoiganap (WD)

International non-proprietary name: autologous glioma tumor cells, inactivated / autologous glioma tumor cell lysates, inactivated / allogeneic glioma tumor cells, inactivated / allogeneic glioma tumor cell lysates, inactivated

Note

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

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Joint Rapporteurs Recommendations

Based on the review of the data on quality, safety, efficacy, the application for Sitoiganap, an orphan medicinal product in the treatment of recurrent/progressive high grade glioma, is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions (see section VI).

In addition, satisfactory answers must be given to the "other concerns" as detailed in the List of Questions.

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

The quality dossier of Sitoiganap shows major deficiencies with regard to critical aspects such as control of starting material, product characterisation, the description and control of the manufacturing process, the manufacturing process characterisation and validation, and the drug product release and stability testing. It gives the impression that the manufacturing process is not sufficiently understood and controlled and its consistent performance not monitored. This raises concerns with regard to product quality, and consequently its safety and efficacy.

There is a lack of preclinical and clinical proof of principle able to support the claimed mechanism of action and the activity of Sitoiganap and of the co-administered drugs (GM-CSF, cyclophosphamide, bevacizumab), none of which is registered in the EU for the target indication. It is unclear which components (if any) of the proposed combination are needed for activity and how the claimed immune response induction is triggered and maintained overtime. The paucity of the data presented and the serious deficiencies and inconsistencies identified in the submitted dossier raise serious concerns over the conduct of the study and therefore the reliability of the results presented. All these issues, together with the very limited number of patients included in the non-preplanned preliminary analyses presented, do not allow for an evaluation of the efficacy and safety of the treatment. As a consequence a B/R assessment cannot be performed, thus remains undetermined.

Questions to be posed to additional experts

N/A

Inspection issues

GMP inspection

A MIA that covers all the manufacturing activities for human medicinal products is needed for ERC-NL (ERC The Netherlands - Epitopoietic Research Corporation (E.R.C.), Nistelrooise Baan 3, Schaijk, Netherlands). In addition, certificates of compliance with cGMP for Radboud Translational Medecine B.V. (Geert Grooteplein Noord 21, r142, Nijmegen 6525 EZ, Netherlands) and for Eurofins Bactimm B.V. (Middenkampweg 19, 6545 CH Nijmegen, The Netherlands) are needed (MO).

GCP inspection

Requesting a GCP inspection is considered justified.

New active substance status

Based on the review of the data the active substance contained in the medicinal product Sitoiganap is considered to be qualified as a new active substance in itself. However, the new active substance claim should be revised, as the current description of the active substances provided by the applicant is not accurate.

Additional data exclusivity /Marketing protection
NA

1. Executive summary

1.1. Problem statement

1.1.1. Disease or condition

High-grade gliomas are malignant and often rapidly progressive brain tumours divided into anaplastic gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma) and glioblastoma (WHO grade IV glioma's) based upon their histopathologic and molecular features (e.g., based on IDH-status (mutant vs wildtype), WHO classification 2016). The presenting symptoms of high-grade gliomas are dependent upon the location and size of the lesion and are similar to those produced by other primary and metastatic brain tumours. Patients typically present with progressive neurologic symptoms (like headache, seizures, focal neurologic symptoms) that evolve over the course of days to weeks.

Management

Newly diagnosed high-grade glioma patients

In patients with a newly diagnosed high-grade glioma, maximal surgical resection with preservation of neurologic function, followed by adjuvant radiotherapy and temozolomide (the latter for 6 months) are usually administered. Despite the survival benefit associated with adjuvant radiation and chemotherapy with temozolomide, the great majority of patients relapse following initial therapy. Progressive disease can be difficult to distinguish from radiation necrosis or other radiation-induced imaging changes occurring within 3 months after chemoradiation (pseudo-progression). Features such as the absence of symptoms, a methylated O6-methylguanine-DNA methyltransferase (MGMT) tumour status, the presence of a lactate peak and decreased cerebral blood volume on specialised imaging have been associated with pseudo-progression. Surgery may be required to distinguish between treatment-induced tumour necrosis and progressive disease. However, interpretation of biopsies in this setting can be challenging.

Recurrent high-grade glioma patients

Recurrent high-grade gliomas are associated with a poor prognosis. Median overall survival is less than one year, with significant tumour-related symptoms and morbidity. Treatment decisions for patients with recurrent and/or progressive disease are usually individualised and made via a multidisciplinary tumour board, since therapy is not curative and always associated with toxicity. In effect, therapy rarely restores neurologic function that has already been lost. Evaluation of prognostic parameters is also important in order to select patients for further treatment. Relevant parameters demonstrated to be predictive for benefit of second-line therapy include performance status, extent of disease, histologic grade (both at initial therapy and at recurrence), the relapse-free interval after the first line therapy, and recurrence pattern (ie, local versus diffuse).The management of patients with recurrent or progressive high-grade glioma, includes surgery, radiation therapy, and systemic therapy.

Patients with a localised recurrence, particularly after a prolonged period of stability, are better candidates for interventions such as reoperation or re-irradiation than those with primary refractory disease or diffuse, multifocal tumours. Surgical resection does not achieve durable tumour control and is typically followed by further systemic therapy, unless carmustine wafers are placed at the time of surgery. Re-irradiation of high-grade gliomas may be of benefit in selected patients, especially those

with a history of a low-grade tumour that has recurred as a high-grade tumour after an extended interval and/or in patients with localised tumours with a contraindication to systemic therapy.

Systemic options for patients with recurrent high-grade gliomas include second-line chemotherapy (eg, nitrosoureas (in particular lomustine as monotherapy or in combination), temozolomide re-challenge), TRK inhibitors in patients with NTRK-fusion-positive tumours (1 to 2% in adult glioblastoma) and experimental therapies in clinical trials. In the U.S. and Other Countries (but not in the EU) bevacizumab is also registered for this indication. All treatment options available have a palliative intent, and none has been directly compared with best supportive care to determine whether survival is improved by active therapy in this setting. Across trials of various systemic therapies, the median survival of patients with recurrent glioblastoma is approximately eight to nine months.

Temozolomide re-challenge has been studied in several phase II studies of patients with recurrent high-grade glioma with mixed results. In general, patients who have relapsed some months after completion of adjuvant temozolomide and whose tumours have a methylated MGMT promotor are the best candidates for re-challenge with temozolomide. For patients with glioblastoma treated with daily temozolomide the 6-month PFS ranged from 15% to 29%.

Nitrosoureas (eg, lomustine, carmustine, fotemustine) either as single agents or in combination regimens such as procarbazine, lomustine (CCNU), and vincristine (PCV) represent alternative treatment options as they have shown activity in phase II studies. In prospective studies in patients with recurrent glioblastoma, single-agent lomustine has been associated with a response rate of 9 to 14% and a median progression-free survival of 1.5 to 2.7 months. The PCV combination compared with temozolomide yields no statistically significant difference in PFS and OS (3.6 versus 4.7 months, and 6.7 versus 7.2 months, respectively). PCV represents a reasonable option in particular for tumours with 1p/19q-codeletion.

Bevacizumab has been registered in U.S. (but not in E.U.) for treatment of recurrent high-grade glioma Objective response rates with bevacizumab alone or in combination with irinotecan or lomustine may yield relatively high response rates (between 28 and 41.5%). However, bevacizumab has not been demonstrated to improve survival in patients with newly diagnosed or recurrent glioblastoma (in a trial of lomustine plus bevacizumab or lomustine alone no difference in overall survival was observed (9.1 versus 8.6 months; hazard ratio [HR] 0.95, 95% CI 0.74-1.21)).

Experience with NRTK inhibitors is very limited: only 7 patients with primary CNS tumour were reported to be treated with entrectinib at the time of registration in EU, of which one patient achieving tumour response. A total of 24 patients with primary CNS tumours were treated with larotrectinib at the time of approval, with confirmed ORR of 21% (5 patients, CR: 2 patients (8%), PR: 3 patients (12.5%)), with 2 additional patients (8%) with not yet confirmed PR and 15 patients (63%) with stable disease.

Importantly, challenges have been traditionally faced for the evaluation of tumour response and disease progression in the treatment of malignant glioma. For this purpose usually the Macdonald criteria have been applied, which rely upon measurement of areas of contrast enhancement). Moreover, the revised criteria by the Response Assessment in Neuro-Oncology (RANO) working group are usually implemented to address problems in assessing patients with pseudoprogression or in assessing progressive disease in patients with non-enhancing lesions.

Unmet medical need

In view of the poor prognosis of patients with recurrent and progressive high grade glioma despite the available treatment options, there is the need for novel treatments able to improve survival and quality of life.

1.2. About the product

Sitoiganap (also called Gliovac or ERC1671) consists of a haptenised irradiated glioma cell suspension and a lysate of haptenised irradiated glioma cells manufactured from a single tumour.

The proposed indication is

Treatment of adult patients with recurrent glioma.

Sitoiganap is claimed to elicit an immune reaction against the patients tumour cells. Sitoiganap is presented as a combination pack, containing 2 vials with autologous haptenised irradiated glioma cells, 2 vials with lysate of autologous haptenised irradiated glioma cells, 3 vials with allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour, and 3 vials with lysate of allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour.

Within the proposed indication Sitoiganap is administered intradermally with GM-CSF, cyclophosphamide and bevacizumab according to the following schema (as proposed in the provided SmPC):

Day 1-3: Cyclophosphamide 50 mg/day orally

Day 6: Sitoiganap A: vial 1 (0.1-1 x 10^6 allogeneic n°1 tumor cells + GM-CSF), vial 2 (0.1-1 x 10^6 allogeneic n°1 tumor cell lysates + GM-CSF),

Day 9: Sitoiganap D: vial 3 (0.1-1 x 10^6 autologus tumor cells + GM-CSF), vial 4 (0.1-1 x 10^6 autologus tumor cell lysates + GM-CSF),

Day 13: Sitoiganap B: vial 5 (0.1-1 x 10^6 allogeneic n°2 tumor cells + GM-CSF), vial 6 (0.1-1 x 10^6 allogeneic n°2 tumor cell lysates + GM-CSF),

Day 16: Sitoiganap C: vial 7 (0.1-1 x 10^6 allogeneic n°3 tumor cells + GM-CSF), vial 8 (0.1-1 x 10^6 allogeneic n°3 tumor cell lysates + GM-CSF),

Day 20: Sitoiganap D: vial 9 (0.1-1 x 10^6 autologus tumor cells + GM-CSF), vial 10 (0.1-1 x 10^6 autologus tumor cell lysates + GM-CSF).

A rest period of a minimum of 14 days and a maximum of 21 days is recommended in the SmPC. However, in the pivotal study cycles were administered every 28 days.

Moreover, it is noted that in the pivotal study treatment was administered with bevacizumab 10 mg/kg every 14 days.

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

Scientific advice has been sought by the CHMP twice, in 2014 (EMA/CHMP/SAWP/34863/2014) and in 2018 (EMA/CHMP/SAWP/485805/2018), respectively. In both occasions, CHMP outlined the exploratory and very preliminary data presented. The applicant was recommended to obtain pre-clinical and clinical proof of concept of the claimed mechanism of action of the compound as clear evidence was not presented at that time, and to provide adequate justification for the applied dosing regimen as well as for the proposed combination with cyclophosphamide, GM-CSF, and bevacizumab. It was underlined that none of the compounds administered in the combination was registered in the EU for the treatment of malignant glioma. Regarding the development programme, CHMP considered the data intended for a CMA (consisting of the results of 10 patients enrolled in the compassionate

use/exemption programme and 8 patients enrolled in the pivotal Phase II study) too preliminary for a benefit / risk evaluation. Concerns were also highlighted by the CHMP over the acceptability of bevacizumab as comparator of the Phase II trial (not registered in EU for the intended indication) as well as over the proposed primary endpoint (6-months PFS as considered of highly debatable clinical relevance) and the conduct and integrity of the trial, as several not-pre-planned interim analyses of the data were performed.

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

Despite the applicant statement that clinical studies were performed according to GCP and local legislation, several deficiencies have been identified in the submitted clinical package raising serious concerns on the conduct of the study. The integrity of the trial is therefore questioned. A GCP inspection is considered justified and could be triggered at a later stage depending on the responses provided by the applicant to the major objections raised that currently preclude any benefit/risk assessment.

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

Legal basis

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above-mentioned Regulation, based on the following criteria:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data.
- Unmet medical needs will be addressed,
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

The applicant has applied for a conditional marketing authorisation (CMA). However, the above mentioned requirements are not considered fulfilled.

New active substance status

Please refer to the comments reported above regarding the new active substance status.

Orphan designation

Orphan drug designation for the indication glioma has been granted by EMA (EU/3/13/1211) and by US FDA on 01/12/2011.

Information on paediatric requirements

Pursuant to Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0223/2020, P/0224/2020, P/0225/2020 and P/0226/2020 on the agreement of a paediatric investigation plan (PIP), the granting of a (product-specific) waiver and on the granting of a deferral. At the time of submission of the application, the PIP is not yet completed as some measures were deferred.

A waiver was granted to the paediatric population from birth to less than 3 years of age, on the grounds that the specific medicinal product is likely to be ineffective.

A clinical study was agreed to evaluate safety, activity and immunogenicity in children from 3 years to less than 18 years of age with relapsing malignant glioma. A deferral was granted for the date of initiation of this study by September 2021. The date of completion (last patient, last visit) is by September 2023 and is also deferred.

2. Scientific overview and discussion

2.1. Quality aspects

2.1.1. Introduction

Each batch of Sitoiganap (also called Gliovac or ERC1671) consists of a haptenised irradiated glioma cell suspension and a lysate of haptenised irradiated glioma cells manufactured from a single tumour.

The product is intended as immunotherapy for patients with recurrent glioma. It is, presented as a combination pack, containing 2 vials with autologous haptenised irradiated glioma cells, 2 vials with lysate of autologous haptenised irradiated glioma cells, 3 vials with allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour, and 3 vials with lysate of allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour. A full treatment is composed of Sitoiganap (ERC1671) administered with GM-CSF (Leukine) as adjuvant following a short three-day treatment period of a low-dose of Cyclophosphamide (Endoxan).

The irradiated haptenised glioma cells are presented as cryopreserved cell suspensions containing 1.25 – 12.5 x 10^5 cells in 250 µl Earle's Balanced Salt Solution (EBSS) containing 9.8% human serum albumin (HSA) and 77.7 mg/ml saccharose

The lysates of the irradiated glioma cells are presented as cryopreserved lysate of 1.25 – 12.5 x 10^5 irradiated haptenised glioma cells in 250 µl Water for Injections (WFI).

The drug product was classified as a somatic cell therapy medicinal product and has been designated as an orphan medicinal product for the indication: Treatment of glioma (EU/3/13/1211).

Active substance

The different drug substances are currently described in a single Module 3.2.S. This can be accepted as allogeneic and autologous tumour material is processed identically, and the manufacturing processes of the cell component and the lysate component are identical up to the last step.

General information

Four names are proposed for the active substance: autologous haptenised tumour cells, autologous haptenised tumour lysates, allogeneic haptenised tumour cells, and allogeneic haptenised tumour lysates. These names are considered not sufficiently specific as irradiation and the type of tumour are not indicated.

Sitoiganap is intended to elicit an immune reaction against the patient's tumour cells by providing a broad set of antigens derived from glioma tumour cells. The exact active ingredients of Sitoiganap are no defined at the cellular or molecular level. The rationale for the use allogenic material is to break the tolerance of the tumour by the presence of allogenic antigens. In addition, the patient is treated with cyclophosphamide (Endoxan) which is expected to abrogate immunosuppressive T reg function and with Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF, Leukine) for its immune-stimulating effect.

The properties attributed to the product are not supported by characterisation data or controlled by the product specifications. In addition, the current description of the products mechanism of action does not address the difference in properties of the different components of the product.

Details on the structure of the active substance are not provided.

Manufacture, process controls and characterisation

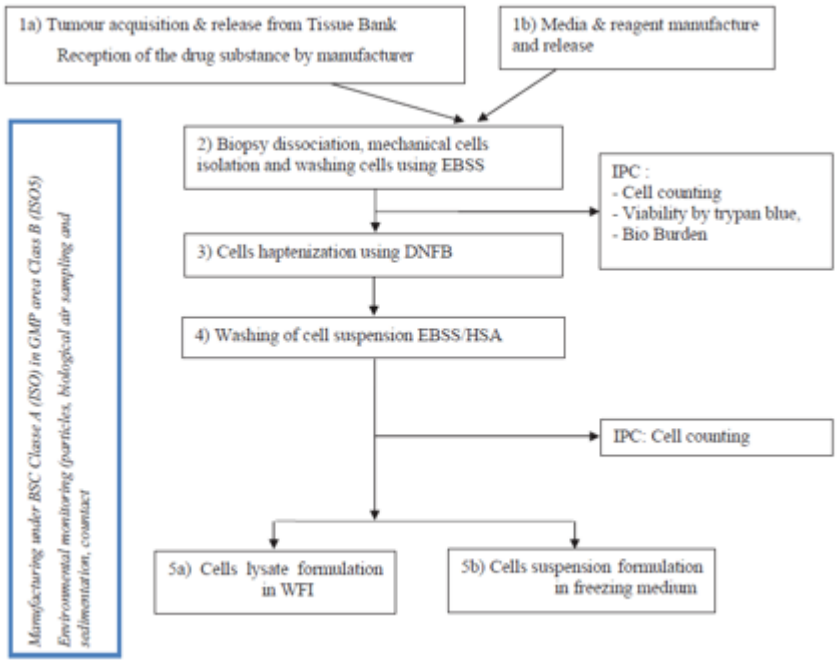
Manufacturer

Sitoiganap drug substance is manufactured by ERC, The Netherlands B.V. (ERC-NL). A valid MIA that covers all the manufacturing activities for human medicinal products is not provided. The provided QP declaration is not satisfactory. These issues are raised as **Major Objection**.

Manufacturing process and control

Briefly, the manufacturing process consists of collection of the tumour tissue, preparation of the cell suspension by cutting the tumour into pieces and transfer through a filter, washing of the cells, haptenisation with DNFB (1-Fluoro-2,4-dinitrobenzene), washing of cells, dividing the cell suspension in two components, and formulation of the two components in freezing medium (cell suspension) or WFI (lysate). After formulation, the vials are filled, irradiated and frozen (see drug product section).

Figure 1 - Flow diagram of the manufacturing process



The drug substance section describes how a single tumour is processed into a haptenised glioma cell suspension and a lysate of haptenised glioma cells, that will be filled, irradiated, and frozen to obtain the Sitoiganap drug product.

The active substances in Sitoiganap are the irradiated haptenised glioma cells and the lysates of the irradiated haptenised glioma cells. However, as the manufacturing process is continuous up to the final product and irradiation is performed at the level of the final container, it is understood that a somewhat arbitrary distinction is made between the drug substance manufacturing process (ends with cell lysis for the lysate component and with resuspension of cells in freezing medium for the cellular component) and the drug product manufacturing process (starts with filling of vials). There will be little gain in restructuring the CTD in line with the correct definition of the active substance, also because no drug substance specifications are defined due to the continuous nature of the manufacturing process. However, currently the starting material, drug substance, and drug product are not clearly defined and these terms are used inconsistently throughout the dossier.

The description of the manufacturing process lacks adequate detail on process parameters, operational ranges, and controls. In addition, criticality of the process steps and process parameters has not been defined and process evaluation/development and validation studies to justify the operational ranges are missing. The absence of an adequate manufacturing process description and justified control is considered a **Major Objection**.

Control of material

The tumour tissue used as starting material is obtained from an approved tissue bank (TTB-NL). The tissue is collected from a hospital by a subcontracted surgeon (hired by the tissue bank). Samples can be obtained from any hospital with the authority to perform the surgery.

A brief description is provided on the procedure used for collection of tumour tissue. This is accepted. However, the quality control and procedure for selection of allogenic tumour are not sufficiently described and justified. Compliance with relevant directives and regulatory guidance has been confirmed. Conditions for storage and transport are not adequately defined and justified. In addition, it is not clear if tumour tissue for allogeneic use is immediately manufactured into drug product or stored at TTB-NL or other sites. Together, these issues are raised as a **Major Objection**.

A description and justification of the quality control procedure and specifications for the starting material DNFB (1-Fluoro-2,4-dinitrobenzene), which is used for haptenisation, is not provided.

The provided overview of raw materials used in the manufacturing process seems incomplete when compared to the information provided in S.2.2 and information on the quality and control of the raw materials is insufficient.

Control of critical steps and intermediates

The information on the control of critical steps is limited to an overview of the IPCs that are performed. This is not sufficient. An overview, supported by process characterisation studies, of the process steps that are considered critical, including process parameters and operational ranges and information on process parameter criticality is missing.

In process controls are in place for sterility, endotoxin, cell count, and viability, but these are not clearly linked to the process steps in which they are performed and acceptance criteria are not defined. No controls are in place to control cell dissociation and haptenisation.

Overall, the manufacturing process control is insufficient. Important controls and supportive process characterisation studies to 1) define operational ranges, identify critical process parameters, and implement appropriate in process controls, and 2) demonstrate that the defined process conditions and

controls ensure that each process step performs as intended, are lacking. This is considered a **Major Objection**.

No intermediates are defined in the drug substance manufacturing process.

Process validation and evaluation

With the exception of media fill studies (see drug product section) no process development, process evaluation, or process performance qualification studies have been performed. The absence of process validation data is considered a **Major Objection**.

Manufacturing process development

The applicant indicates that product development has been performed in two steps, the first involve the changes carried out to adapt the manufacturing process from rodents to humans. This first manufacturing process was performed in BioElpida facility. The second step is performed to adapt the manufacturing process to GMP conditions at ERC-NL. The information provided on manufacturing process development is very concise. The 'animal method' and 'human method' are described, and it is indicated that GMP development of the product was performed in 2009-2010 and several improvements were made. It is unlikely that the product manufactured according to the 'animal method' is comparable to the product manufactured according to the 'human method'. The applicant that this product has been used in product characterisation or non-clinical studies. A justification that the results of these studies are representative for the commercial product is not provided. In addition, it is not clear whether the batches used for clinical trials were manufactured in the GMP facility and if any changes were introduced in the manufacturing process since the start of the clinical studies.

As indicated previously, no process development or process evaluation studies have been performed to support the design of the manufacturing process and its control (**Major Objection**).

Characterisation

The applicant did not perform any studies to elucidate the characteristics of the active substance. This is not acceptable and is considered a **Major Objection**. Simply defining the active ingredients as a broad number of antigens does not allow determining which quality attributes should be controlled. In addition, it ignores the effect of the haptenisation and irradiation steps. Even if full characterisation of the product is not feasible, at minimum data on cellular composition, protein content of the lysate, glioma antigens, the extent and variability of haptenisation, and batch to batch variability are needed to demonstrate that consistency of product quality, efficacy and safety is ensured by the manufacturing process and controls.

The applicant did not perform any studies with regard to impurities, but states that all cell types and molecules are considered as product related substances. This is not acceptable. As adequate impurity control is not demonstrated, this is considered a **Major Objection**.

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

Not applicable. The manufacturing process is continuous from raw material until drug product. The drug substance is not stored in a container and no testing is performed at the drug substance stage.

Stability

Not applicable. The drug substance is not stored.

2.1.2. Finished Medicinal Product

Description of the product and pharmaceutical development

Description of the product

The composition of Sitoiganap is presented in Table 1.

Table 1 - Composition of drug product

Component	Cell suspension (prepared in medium A)	Lysate suspension (prepared in Medium B)
Total tumor cells per unit of 250 µl	Between $1.25x10^5$ and $1.25x10^6$ cells	Between $1.25x10^5$ and $1.25x10^6$ cells equivalent
Total tumor cells per dose of 200 µL	Between $1x10^5$ and $1x10^6$ cells	Between $1x10^5$ and $1x10^6$ cells equivalent
	Medium A	Medium B
Saccharose, NF	77.7 mg	
Albumin human 20%, Eur Ph	0.49 mL	
Earle's Balanced Salt Solution EBSS	0.44 mL	
WFI		1 mL
Total Volume	1 mL	1 mL

Each batch of Sitoiganap consists of a haptenised irradiated glioma cell suspension in medium A (saccharose, human albumin 20%, Earle's Balanced Salt Solution and WFI) and a lysate of haptenised irradiated glioma cells in medium B (WFI) manufactured from a single tumour. The dosage form and composition of the drug product is not clearly defined in P.1 and inconsistent information is provided in Module 3 and the SmPC on the final dose preparation As the provided information does not allow to determine whether the dose provided by the clinical batches was consistent, this issue is considered a **Major Objection**.

The product is presented as a combination pack, containing 2 vials with autologous haptenised irradiated glioma cells, 2 vials with lysate of autologous haptenised irradiated glioma cells, 3 vials with allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour, and 3 vials with lysate of allogeneic haptenised irradiated glioma cells, each manufactured from a different allogeneic tumour.

Pharmaceutical development

Drug substance

The drug substance is defined as the haptenised glioma cells and the lysate haptenised glioma cells. Some inconsistencies in the description of the drug substance are noted. The drug substance is manufactured into drug product immediately and irradiation only occurs at the drug product stage.

Excipients

The excipients are Earle's balanced Salt Solution, saccharose, human serum albumin and WFI. Information on the selection of excipients is provided, but a justification for the quantity of the excipients is missing. In addition, the suitability of WFI for infusion of lysate suspension has not been discussed.

The information provided on formulation development is very limited. Information on the selection of the target cell concentration / lysate equivalent and a brief description of the development of the drug product is missing.

Overage
No overage is applied.

Physicochemical and biological properties
The information provided on the physicochemical and biological properties is largely identical to the information provided in S.1.3 and the same comments apply.

Manufacturing process development
The applicant states that no modifications were made to the manufacturing process from animal development except the GMP environment. This is not in line with the information provided in S.2.6. All changes that were introduced in the drug product manufacturing during development should be indicated and, if applicable, comparability data provided.

Information on the development studies conducted to establish that the dosage form, the formulation, the manufacturing process, and container closure system are appropriate for the purpose specified in the application is not provided. Additionally, the formulation and process attributes (critical parameters) that can influence batch reproducibility, product performance and drug product quality have not been identified and described.

Container closure system
The provided information does not allow assessment of the suitability of the container closure system. This is considered a **Major Objection**. Inconsistencies are noted in the provided information, and suitability of the container for storage at -80° C is not demonstrated. Limited product stability data are available, and information on the container closure systems used for the stability study or the storage conditions is missing.

Microbiological attributes
Products are tested for sterility. Non-sterile batches are rejected.

Compatibility
The applicant considers this section not applicable. However, according to the SmPC both the lysate and the cells are mixed 1:1 with 250 microgram GM-CSF (e.g. diluted in 250 microliter WFI) prior to administration. This information should be included and compatibility demonstrated. GM-CSF is not registered as a medicinal product in the EU. It has not been demonstrated that it is ensured that users have access to GM-CSF of suitable quality and that regulations for medicinal products are complied with (**Clinical Major Objection**)

Manufacture of the product and process controls

Manufacturer

Sitoiganap drug product is manufactured, quality controlled, primary packaged, stored and batch released by ERC, The Netherlands B.V. (ERC-NL). In addition, Eurofins Bactimm B.V. (quality control) and Radboud Translational Medicine B.V. ('processing of sterile medicinal product'). The responsibilities of each manufacturer included in this section is not clear enough.

As mentioned, a a MIA that covers all the manufacturing activities for human medicinal products is missing for ERC-NL and the QP declaration provided is not satisfactory. These issues are raised as a **Major Objection**.

Certificates of compliance with cGMP have been provided for Radboud Translational Medecine B.V. (Geert Grooteplein Noord 21, r142, Nijmegen 6525 EZ, Netherlands) and for Eurofins Bactimm B.V. (Middenkampweg 19, 6545 CH Nijmegen, The Netherlands).

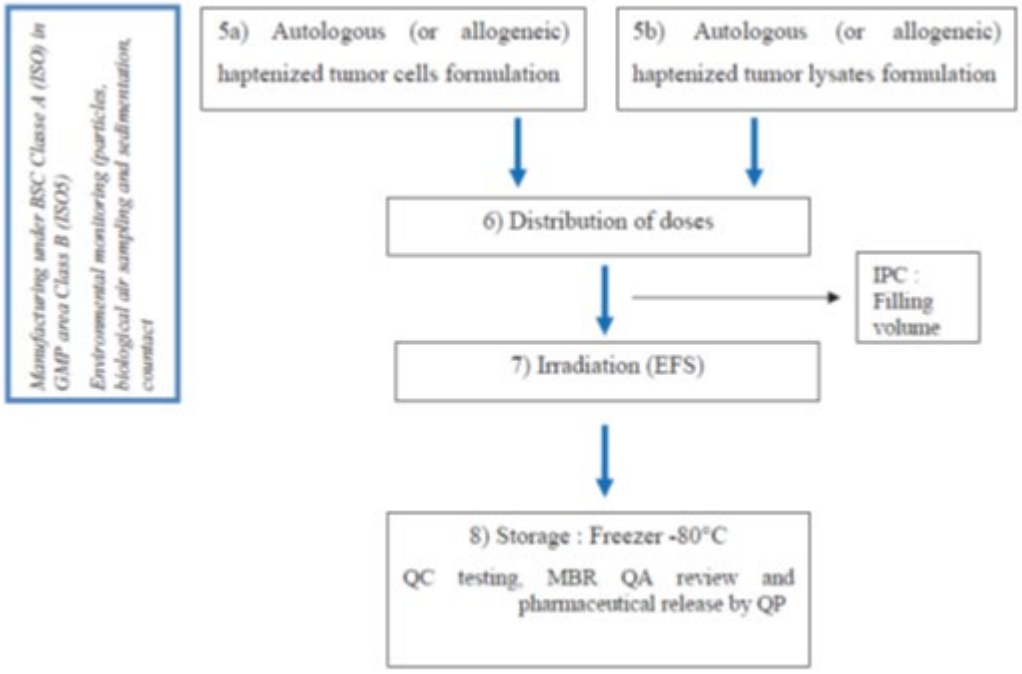
Batch formula

See Table 1 and remarks in the section on drug product composition. One batch is composed of cells and cells lysates derived from one tumour. Each tumour is treated separately and specifically as a single batch and a unique number is assigned and used all along the manufacturing and QC process to insure the full traceability. The average number of vials manufactured from one tumour is approximately 49 (range 37-134) with 24 vials of autologous haptenised and irradiated glioma tumor cells manufactured from one tumour and 24 vials of autologous haptenised and irradiated glioma tumour lysates manufactured from one tumour.

Manufacturing process and control

The manufacturing process is continuous from tumour tissue collection up to the final product. The drug product manufacturing process briefly consists of filling, irradiation and freezing.

Figure 2 - Flow diagram of the products manufacturing steps in a continuous process from active substances manufacturing steps



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As for the drug substance, the description of the drug product manufacturing process is too concise and detail on process parameters, operational ranges, and controls is missing. In addition, criticality of the process steps and process parameters has not been defined and process evaluation/development and validation studies to justify the operational ranges have not been performed. The absence of an adequate manufacturing process description and justified process control is considered a **Major Objection**.

Controls of critical steps and intermediates

The information on the control of critical steps is limited to a brief description of the controls that are performed during manufacturing. An overview of the process steps that are considered critical,

including process parameters and operational ranges and information on process parameter criticality is missing. The selection of critical steps should be justified by the process evaluation studies.

In process controls are in place for extractable volume (weight check) and irradiation (indicator of irradiation). Acceptance criteria are missing.

Overall, the manufacturing process control is insufficient. Important controls and supportive process characterisation studies are lacking. This is considered a **Major Objection**.

The product is frozen prior to irradiation and cells/lysate prior to irradiation are considered intermediate. The product is stored at -80°C (on dry ice) during the transport and irradiation process. The duration (transport and irradiation) must be less than 3 hours but varies based upon the intensity of the radiation source.

Process validation and/or evaluation

Four media fill runs were performed by processing a piece of TSB analogous to the processing of tumour tissue in the actual process. All acceptance criteria were met, suggesting adequate microbiological control of the process. However, it has not been confirmed that the process was challenged at worst-case conditions and the batch size supported by the media fill studies was not stated.

With the exception of media fill studies no process development, process evaluation, or process performance qualification studies have been performed. Especially for critical process steps that are not controlled by IPCs (e.g. the haptenisation and irradiation procedures), characterisation studies, including and evaluation of critical process parameters and effects on CQAs, are expected to demonstrate consistent performance. The effectiveness of the irradiation step in preventing cell division should be demonstrated. The absence of process characterisation and validation data is considered a **Major Objection**.

Control of excipients

The applicant considers this section not applicable as no excipient is added to the active substances formulation. This is agreed for the lysate component of the product, but not for the cellular component. For the latter, saccharose, human albumin and EBSS are considered excipients and appropriate information should be provided.

Product specification, analytical procedures, batch analysis

The specifications for Sitoiganap are presented in Table 2. Sitoiganap will be released only after final product has been tested and found to meet all acceptance criteria included in these specifications. Results are included in the master batch record.

Table 2 - Specification for Sitoiganap

Characteristics	Specifications	
Specifications	**Cells components**	**Lysates components**
Appearance	Vial Intact – cap closed	Vial Intact – cap closed
Identity	Label conform	Label conform
Quantity of Active substance	Between 1. 10^5 and 1. 10^6 Cells / dose	
Extractible volume	≥ 200 µL	≥ 200 µL
Proliferation	No proliferation	No proliferation
Potency	In consideration	In consideration
Impurity	In consideration	In consideration
Sterility (aerobic and anaerobic)	Comply with "Sterility (EP 2.6.27)"	Comply with "Sterility (EP 2.6.27)"
Endotoxin	< 200 EU/mL Comply with Bacterial Endotoxine (EP 2.6.14).	< 200 EU/mL Comply with Bacterial Endotoxine (EP 2.6.14).

Limited information is provided on the analytical methods for drug product release and a number of methods are still in consideration. For those methods that are in place, the provided method descriptions are in general too concise. Validation of the release test has not been demonstrated. This is considered a **Major Objection**.

No batch release data have been provided. This is considered a **Major Objection** as it cannot be determined whether the characteristics of the clinical batches have been consistent and support the proposed commercial specifications.

The drug product specifications are not in line with expectations. The absence of release tests for potency, haptenisation, proliferation, and impurities are considered **Major Objections**.

Once the applicant has adequately defined a set of specifications for cell components and lysate components, the general relevance of the release specification should be discussed. In addition, all control methods, regardless of whether they are applicable to control at release or to the shelf life should be validated.

A qualified in house reference standard is expected to be included in the potency assay. The parameters for characterisation of the reference standard should be submitted.

Container closure

The final product is stated to be packaged in NUNC CryoTube polypropylene vials with polypropylene screw caps made by Corning International and provided with a certificate of quality. The provided

information does, however, not allow assessment of the suitability of the container closure system as contradictory information is provided throughout the dossier. This issue is considered a **Major Objection**.

Stability of the product

A shelf life claim is not provided in P.8. The information in the SmPC suggests the applicant intends to claim a shelf life of 5 years when stored at -80°C. Data to support this shelf life are not provided. The provided stability data are limited to one batch that was tested after 6 and 12 months of storage for appearance, sterility, extractable volume, and (for the cellular component only) % cell haptenisation and cell count. The storage conditions and container closure system used in this study is not indicated. The study is not performed in line with ICH Q5C.

The absence of data supporting the shelf life of the product is considered a **Major Objection**. To justify the shelf life the product a stability study in line with ICH Q5C requirements should be performed.

In-use stability has not been demonstrated. The SmPC claim that the product may be administered up to 6 hours after thawing can therefore not be accepted.

Post approval change management protocol(s)

Not applicable

Adventitious agents

No adequate adventitious agents safety evaluation has been provided and this is considered a **Major Objection**.

It is agreed that no in process controls are in place for adventitious viruses, as the manufacturing process is short and does not contain any cell culture steps. No viral clearance steps are in place, which is acceptable for the type of product.

GMO

Not applicable

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

The Module 3 provided for Sitoiganap shows major deficiencies with regard to critical aspects such as control of starting material, product characterisation, the description and control of the manufacturing process, the manufacturing process characterisation and validation, the drug product release and stability testing, and the adventitious agents safety evaluation. These deficiencies have not been adequately addressed in the response of the applicant to the questions raised and no revised dossier has been provided. Based on the available information it cannot be assessed if the manufacturing and control of Sitoiganap can ensure consistent quality, efficacy and safety of the product.

Considering the poor quality of the Quality dossier, and the large number of unresolved deficiencies, the marketing authorisation application for Sitoiganap is not acceptable.

2.2. Non clinical aspects

2.2.1. Pharmacology

Mechanism of action

The applicant is developing a therapy to treat glioma. The therapy is based on the hypothesis that the immune system can be trained/activated to recognise and eliminate the tumour. For this purpose, the applicant intends to treat patients by intradermal injections with autologous and allogeneic tumour (glioblastoma) antigens (cells and cell lysates) in combination with an immune activation compound (GM-CSF)). A treatment cycle consists of two series of injections with four differently composed components, all combined with GM-CSF. Each of the series is preceded by oral use of cyclophosphamide, an immune- suppressive chemotherapy which is administered to deplete immunosuppressive T-regulatory cells. The hypothesis that immune surveillance and immune activation can have significant impact on tumour formation or tumour outgrowth is generally accepted. However, it remains unexplained how or in which way the here proposed treatment would results in anti-tumour immune activation for the current indication (or in general). For example, it was not discussed nor studied which immune cells will be activated upon the intradermal injection of the cells and of the cell lysates. In addition, the possibility of immune tolerance instead of activation was not considered but may occur with intradermal administration route. In an advice preceding the marketing application, the applicant was asked to further explain the mechanism of action: *'To elaborate on the composition and haptenization of the product and the anticipated role and added value of each of the separate components and how these in the very specific proposed dosing schedule would trigger an immune response to intracerebrally located tumours, as claimed.'* The product is very heterogeneous consisting of lysed tumour cells from the patient and allogenic tumour cell lysates, nonetheless no information whatsoever was submitted regarding the detailed contents and the role of each in the mechanism of action of the product. The effect of haptenisation on the product and on its intended function is not clear. The applicant notes that the intended immune reactions needed to evoke the anti-tumour effect are not easily mimicked by *in vitro* assays. However, it has not been sufficiently justified why MoA is not better explained or why in vitro studies supporting the MoA are missing to the dossier. The applicant should explain this deficit or submit (immune cell related) in vitro studies supporting (several aspects) MoA (**MO**).

Proof of principle studies

For each of the three pharmacodynamic presented studies below, several issues are noted that make it difficult to draw clear conclusions regarding the relationship between efficacy with product characteristics and/or specifics. Three general issues were noted for each of the studies: 1) description of the product and comparison to the clinical product was not clear (with regard to irradiation, haptenisation, cryopreserved or fresh formulated) 2) the design of the studies (lacking of controls and/or too few animals per condition) limits the conclusion on the contribution of Sitoiganap as part of the treatment to the anti-tumour effect, and 3) raw data of the tumour sizes at start of, during and at the end of the treatment are not included and amongst others claimed statistical significance cannot be checked. Below, the three pharmacology studies are briefly summarised, and study specific issues are noted.

Study 1

For the first *in vivo* pharmacology study, two strains of rats are used: Fisher 344 (F344) and Sprague Dawley (SD). Also, two types of tumour cells are used, glioma cell lines 9L and C6, for the purpose of tumour implantation as well as serving as 'ingredients' for the immunomodulatory product. Using these models, two types of experiments were conducted; an evaluation of the anti-tumour efficacy of

the prophylactic or of a therapeutic treatment. The prophylactic treatment is not regarded of relevance for the development of Sitoiganap and will not be further discussed here. Regarding the therapeutic treatment, two experiments (SD rats and F344 rats, n=1 per condition) were conducted that gave at most an indication that treatment with allogeneic or xenogeneic tumour (lysate) cells can have some therapeutic anti-tumour effect. The missing table 4.2 with the experimental design of animal studies in Fisher rats should be provided (OC). Overall, no conclusion can be drawn on the relation of the observed therapeutic effect with the composition and dose of the product (the so called ''vaccine''), the exact treatment regimen or the length of the therapy. Therefore, study 1 is considered of limited relevance to provide a treatment rationale with the clinical product and treatment regimen.

Study 2

In the second study, 4 different experiments are reported. All experiments are conducted with male Lewis rats of approximately 8-12 weeks of age that are implanted with CNS-1 tumour cells ($2x10^5$ cells/200 µl) subcutaneously. Although in this study more animals are used per tested condition, the tested conditions often differ to each other in more than one component which makes it difficult to compare conditions to each other. Furthermore, comparable treatments give different outcomes between different experiments within one study or between studies. Because of these limitations no conclusion can be drawn, making the study of limited relevance to provide a treatment rationale with the clinical product and treatment regimen.

Study 4

In study four, CNS-1 cells were implanted subcutaneously (SC) into the right flank of 8 -12 week old male Lewis rats to form tumours to evaluate the anti-tumour effect of the proposed treatment regimen. The 'vaccine' antigen preparation was composed of 'a mixture' of haptenised CNS-1 cells and RG2 cells ($1x10^6$ CNS-1 syngeneic and $1x10^6$ RG2 allogeneic glioma cells), together with lysates produced from $3x10^6$ CNS-1 syngeneic cells and $3x10^6$ allogeneic RG2 glioma cells, all or not combined with cyclophosphamide (30 mg/kg) and/or GM-CSF (30 µg/kg). It is regarded that study 4 is the best designed pharmacology study submitted with this dossier. Number of animals in the treatment groups are regarded enough for the purpose of determining differences between the various treatments. However, in this experiments, some control situations are missing. For instance, anti-tumour efficacy has not been evaluated for the treatment with GM-CSF and ''vaccine'' only. In addition, the evaluation of anti-tumour T-cell memory is conducted after only one cycle of treatments and relatively early after the end of the treatment and therefore this study does not mimic the clinic situation where at least 6 cycles are proposed and it is considered that anti-tumour T-cell memory should be evaluated also later than 10 days after finishing the treatment. Furthermore, in this experiment, it seems that in mice, not challenged a second time, the original tumours are growing out again. This would suggest that the anti-tumour effect only last for a limited period. This study supports the idea of using autologous and allogeneic antigens as immunomodulatory product to evoke an anti-tumour effect. However, it is not considered a rationale for clinical treatment with the proposed clinical product (components), the clinical proposed treatment regimen and the proposed co-medication.

Clinical relevance of the non-clinical studies

Used non-clinical disease model

For the purpose of providing the proof of principle for the proposed treatment schedule with Sitoiganap, the applicant studied the product and the treatment in a homologous animal model. While it is generally supported that an immunomodulatory product is tested in a homologous model, an explanation for/ justification of the selected animal model with regard to the relevance for the human situation is needed and the differences in the immune system of rat and human and the ability to fully investigate and cover the proposed clinical MoA in the animal model should be discussed. In addition,

the relevance of a tumour model of subcutaneous injected tumour cells for a tumour that originally locates to the brains, needs to be explained and justified (**MO**).

Applied route of administration

The animals were treated with the product via subcutaneous injection while the clinical RoA for the Sitoiganap components is intradermal (ID) or intramuscular (IM) injection. It is not explained to what extent subcutaneous injection that will hit the fat layer underneath the skin (used in the animal model) is comparable to intradermal injection that hits the dermis layer just beneath the epidermis (used in the clinic), with regard to the activation of the crucial immune cells. This issue awaits elaboration (**MO**).

Tested product and product regimen

The information on the product used in the NC studies is very limited and it is not clear how the composition of the product components used in non-clinical studies relates to the product components used in the clinic (e.g. in terms of number and concentration of cells, antigen composition, haptenisation, etc). Furthermore, the clinical product consists of thawed cryopreserved (lysate) cells, whereas the non-clinical product are freshly used (lysate) cells. The effect of these two types of formulations have not been evaluated in an animal model. Overall, the product as tested in the non-clinical studies is different from the clinical used product. Also the rationale for and clinical relevance of the chosen dose is unclear. The applicant should elaborate on these differences and explain their potential effect on the outcome of the studies substantiated with data and con (**MO**). In addition, it is not clear whether the cellular component and cell lysate after haptenisation and irradiation are capable to elicit the intended immune response against the unmodified epitopes of the patients tumour leading to elimination of the tumour. The effect of the variability in the product haptenisation in the immunogenic properties of the product should be determined. **(OC)**

Tested co-medication

Sitoiganap is not given as a sole treatment. Two types of co-medication that are proposed for the clinic are also evaluated in the non-clinical studies. From this evaluation and the evaluation of several other types of co-medication, it is not immediately clear why is chosen for co-treatment with GM-CSF and Cyclophosphamide above other types of co-medication (amongst other resiquimod (TLR7/8, B 7.1, etc) as the choice is not justified by data nor a discussion. In an advice preceding the MA, the applicant was asked to elaborate on the role and timing of GM-CSF administration as well as thoroughly justify the role of CY in the intended indication, including the possibility that CY will interfere with the aimed mechanism of action, which requires the immune system. This is not sufficiently addressed and therefore, the applicant is asked to provide a rationale for the proposed co-medication and provide supporting data (**MO**).

Quality of the data

The applicant provided their data in graphs only. Raw data e.g. the tumour sizes per day before, during and after treatment for each individual animals as well as safety data for each individual animal is not included. Consequently, claimed statistically significant results cannot be confirmed. The request for raw data is part of the combined NC/C pharmacology major objection (**MO**).

Secondary pharmacology, safety pharmacology and pharmacodynamic interaction studies.

The absence of studies addressing the secondary pharmacology is agreed.

For advanced therapy medicinal products, it is not required to address safety pharmacology in a separate study. However, the applicant should support the safety for CNS, respiratory and cardiovascular systems with data from the toxicology studies. This is not considered sufficiently

covered in the toxicology studies. The applicant is asked to provide an overview of the design of the study, the method of analysis and the accompanying results of the evaluation of safety aspects regarding CNS, cardiovascular and respiratory system in the toxicity study and discusses the GLP compliance of this study (**OC**).

The applicant notes that pharmacodynamic drug interactions with the proposed co-medication cyclophosphamide and GM-CSF is studied in the toxicology study. However, the toxicology study has been conducted in NOD-SCID mice that are unable to mount an adaptive immune response and are thus not capable of showing the pharmacodynamic action of the product, neither the effect of the proposed immune modulatory co medication. Thus, the toxicology study is not considered appropriate for evaluation of pharmacodynamic interactions. The applicant is asked to justify absence of dedicated PD interaction studies and discuss the potential PD interaction of cyclophosphamide, GM-CSF and bevacuzimab and substantiate this with data. Clinical data could be used as supportive justification for the clarification of this issue (**OC**).

2.2.2. Pharmacokinetics

Standard PK evaluations are not applicable for ATMPs such as Sitoiganap. It is therefore endorsed that dedicated absorption, metabolism and excretion studies have not been conducted.

The applicant has conducted 1) an *in vivo* biodistribution study (in combination with a toxicology study, see section 4) aimed to determine duration of degradation of the drug product following administration, and 2) an *in vitro* stability study to evaluate the viability of the product between thawing and patient administration (leading to a post-thawing shelf-life). The study designs, results and corresponding conclusions of the applicant are discussed here.

In vivo biodistribution study

The applicant has conducted a combined biodistribution and toxicity study (called: tumourigenicity study) in female NOD SCID mice. Mice were once IP injected with 100 mg/kg cyclophosphamide on day 0, 4 times ID injected with 3.1 µg/mouse GM-CSF and 4 times with a non-specified dose of Sitoiganap (group 2) or unknown type of placebo (group 1) on day 3, 10, 17 and 24. One additional mouse was left untreated (group 3). On day 30, animals were sacrificed for evaluation of the presence of human DNA in 7 tissues.

No human DNA was found in liver, kidney and lung for group 2. In contrast, a few animals from group 1 showed positiveness of at least 1 out of 3 replicates (low amount of DNA) in these tissues, while these animals had not been treated with Sitoiganap. Low levels of human DNA were observed in spleen, brain and heart in several animals from both group 2 and group 1. Human DNA in skin samples was found in group 2 animals, although only 2 animals were considered positive (but with higher DNA levels compared to the other tissues analysed). No positive tissue samples were found in group 3 (untreated mouse).

The applicant has designed a single dose PK study in NOD SCID mice. However, the possibility to interpret the study data is very limited. Considering substantial limitations of the study and absence of proper discussion of the design and results, a PK major objection was raised (**MO**).

In vitro stability study

The applicant has conducted an *in vitro* stability study (i.e. determination of viability after thawing) on the finished product intended for human use. Metabolic activity directly after thawing and after 24h, 48h and 72h (at room temperature) was compared, using different U87 human glioma cell line and Sitoiganap concentrations and components. Based on the results, the applicant considers that the *in vitro* stability (shelf-life after thawing) should be fixed at a maximum of 6h.

The design of the study, including materials and method, is poorly described. There are several shortcomings: it is not clear whether the real human product (haptenised, irradiated cells and lysate) have been used, whether control cells have been treated the same way as Sitoiganap cells (i.e. irradiation procedure and freezing formulation) and thus whether U87 cells are reliable as control, and whether the MTT method is suitable for viability testing of irradiated cells, as no validation data on this method and the proliferation status of the cells have been provided. Furthermore, information in module 2.6.5 on cell concentrations used does not seems to be in line with the original study report. Taken together, considering these shortcomings and the absence of an analysis point prior to T24, the claim of 6h in-use stability is not supported. This is further addressed in the Quality AR.

The applicant did not further mention why this study was submitted in the non-clinical part of the dossier and not in the quality part. Since the study is specifically related to *in vitro* quality characterisation of the product, this study is not considered relevant for supporting clinical use from a non-clinical perspective. Therefore, no questions will be raised to the applicant.

2.2.3. Toxicology

Based on the PD studies, the applicant has concluded that "*repeated cycles of treatment are preferred compared to a single treatment cycle*". Considering that the clinical treatment regimen can take up to 12 treatment cycles (according to SmPC section 4.2), the absence of a single dose toxicity study can be endorsed.

Toxicity study

The applicant has conducted a combined biodistribution and toxicology/tumorigenicity study (aimed to determine potential safety effects of the drug product following administration (study has been described above). As noted above, this study has been performed in NOD-SCID mice, but this is not considered a relevant model, as the clinical (pharmacological) effect and (potential) adverse events cannot be mimicked in these immunodeficient animals. Furthermore, in this study 4 administrations of Sitoiganap have been given, with each administration containing another active ingredient (cells and lysates from different batches). In contrast, the clinical regimen consists of 5 administrations (with in total 4 different Sitoiganap components) per cycle, and the patient is to be treated repeatedly with a 14 to 21-day or longer resting period between the cycles (according to section 4.2 of the SmPC). Therefore, the repeated use (i.e. more treatment cycles) in the clinic has not been mimicked in the NC toxicity study.

Of note, although the study is termed 'tumorigenicity study' the design is lacking endpoints (e.g. proliferation of the cells *in vitro* and *in vivo*) to assess tumourigenicity, a longer follow-up time would be needed for this (to evaluate potential formation of neoplasms) and the use of autologous product would be preferred. as autologous cells may contribute the most to the risk for tumorigenicity in humans (i.e. such cells will not be removed via an allogeneic immune response).

Assessment of study design
The study design to determine the safety profile of the product and treatment regimen has several shortcomings, which limits interpretation of the study data. Considering substantial limitations of the study, absence of proper discussion of the design and results, and considering that this study has not provided any safety support for the clinic, a toxicology major objection has been raised (**MO**).

Assessment of study results
According to the applicant, there was no difference in toxicity between group 1 and group 2. The only observation made in the tissues analysed was the mononuclear/macrophage infiltration in a few mice from group 2, but without evidence of human DNA in these samples. Nevertheless, the detection of human DNA by PCR Alu is debatable (see section 3). Furthermore, and in agreement with the conclusion of the second reading of the histopathological slides, it is likely that this observation in the skin is a remnant of the intradermal injections.

It was noted that administration of cyclophosphamide resulted in a body weight decrease in all group 2 mice (between day 0 and 2), but only in one group 1 mouse. Moreover, brain weight in group 2 was lower compared to group 1. The applicant did not further elaborate on the potential cause(s) for these observed differences.

One animal from group 2 was diagnosed with liver inflammation, and the applicant states that this could be due to a toxic response. It is not clear whether the applicant thereby refers to a reaction on treatment with cyclophosphamide, GM-CSF or Sitoiganap. In addition, the applicant did not discuss the presence of mild haemorrhage(s) in the brain of 2 mice from group 2 (one of which also had immune cell infiltrates in the skin), while these haemorrhages were not observed in group 1 mice. Of note, these observations were not made in the second reading of the histopathology (study 8), indicating the absence of concordance between both readings.

Proliferative capacity study

The applicant assessed the amount of DNA in the Gliovac cells and cell lysates and compared this to the amount of DNA present in a positive control (U87 cells and cell lysates) both at t=0 and t=96hr. The description of the study method is very limited. For example, it is not clear 1) whether or not a negative control (cells that cannot proliferate) was used, 2) whether clinical grade product was used or whether this were research batches, 3) how the products were treated, 4) why only one dose of Gliovac cells was tested, while this is not the highest anticipated clinical dose, 5) why only one batch of Gliovac cells appear to be used, and 6) whether the DNA level method was properly validated and suitable to test for the absence of proliferation.

The assay presented by the applicant to assess the proliferative capacity is regarded a quite poor assay to ascertain the absence of any proliferative cell in the drug product. This is considered to be crucial information for the safety of the product, and it is regarded that this should have been conducted under GLP or GMP. The absence of a negative control in the assay as well as the lack of information on the batch characteristics and quality and the validity of the DNA assay complicates conclusiveness on the proliferative capacity of the Gliovac cells. The absence of proliferative cells in the drug product needs to be confirmed to ensure the quality of the clinical product which should be in place in the Quality section of the dossier (see Quality AR).

Other studies

The absence of a dedicated genotoxicity, carcinogenicity, reproduction, antigenicity, dependence, metabolite and phototoxicity studies can be endorsed, considering the type of product (cells) and

indication, in line with ICH S9. Remarks and questions related to the SmPC (section 4.3, 4.6 and 5.3) are provided in the separate PI document.

DNFB is used to haptenize the tumour cells and may be present in the final DP. Whether or not this impurity remains below an acceptable level in the Sitoiganap vials is assessed in the Quality AR.

2.2.4. Ecotoxicity/environmental risk assessment

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, Sitoiganap is not expected to pose a risk to the environment. However, responses on quality questions related to DNFB may have an influence on the ERA requirements for Sitoiganap.

2.2.5. Discussion on non-clinical aspects

Pharmacology

Conclusion and discussion

The many deficiencies and unclarities regarding the pharmacodynamic activity of the product could have been addressed in OCs when the clinical PD data would be overwhelming and clear. However, also the clinical PD data do not support the claimed activity of Sitoiganap. It is thus not clear which components (if any) of the proposed combination are needed for activity and how the claimed immune response induction against the malignant glioma is triggered and maintained overtime and whether this results indeed in (immune-mediated) control or even eradication of the tumour. This lack of non-clinical or clinical support for the dose, the need for allogenic and autologous tumour antigens, and for intact (irradiated) (haptenised) cells and cell lysates, for the co-administered drugs (GM-CSF, cyclophosphamide, Bevacizumab) and for the proposed treatment schedule in order to achieve an effective anti-tumour response is considered to be a major deficiency to this application. The raised major objection on non-clinical and clinical PD is raised was not sufficiently addressed and the issue is not solved. It is concluded that the pharmacological activity of sitoiganap is insufficiently studied. There is a lack of preclinical and clinical data supporting the claimed mechanism of action, the activity of Sitoiganap and the need for the co-administered drugs (GM-CSF, cyclophosphamide, Bevacizumab) in the targeted indication. As a consequence the impact of product composition (including haptenisation), dose and treatment regimen on the desired anti-tumor activity and the contribution of the co-administered drugs is not known.[MO] (**see LoQ**).

Several other concerns have been posed. One relating to absence of data and an adequate discussion on the products safety related to CNS, respiratory and cardiovascular. Another on the absence of dedicated PD interaction studies including a discussion. One on the effect of haptenisation on the product and its function. And a fourth issue is related to the text on the product's mechanism of action in section 5.1 of the SmPC (**see LoQ**).

Pharmacokinetics

The applicant has designed a single dose PK study in NOD SCID mice. However, the possibility to interpret the study data is very limited because the study and its design have several significant limitations and uncertainties related to the animal model, treatment regimen (in relation to the clinic), amount/choice of tissues evaluated and time point of analysis, which have not been appropriately discussed by the applicant. In addition, the suitability of the PCR Alu assay has not yet been

established, as no validation data were provided. This limits the interpretation and the translatability of the results to patients. A conclusion on distribution and persistence of Sitoiganap (to target tissues) and relationship with its mechanism of action is therefore not possible and no conclusions on the aspects that would be relevant to evaluate in (non-clinical) safety studies (i.e. duration, follow-up, target organs, see the *Guideline on human cell-based medicinal products*, EMEA/CHMP/410869/2006) could be drawn. Considering that no PK data have been obtained clinically either, the distribution and persistence of Sitoiganap is insufficiently addressed and the conclusions of the applicant cannot be endorsed. The MO raised on the lack of understanding regarding the distribution and persistence of Sitoiganap was not sufficiently addressed and the issue is not solved. It is concluded that the distribution and persistence as well as the safety of Sitoiganap has insufficiently been studied. The performed study is insufficient because of the flawed design, interpretation is hampered and ,results may not be reliable. Consequently the safety profile is cannot be determined and the impact of any potential risk minimisation strategies cannot be estimated. [MO] **(see LoQ).**

The applicant noted that product is distributed in the blood circulation and destroyed with blood cells in the spleen. This is not fully understood and not supported by the data provided by the applicant. In addition, in the dossier it is stated that "the product is generally absorbed between last injection (D24) and sacrifice day (D30), i.e within 6 days." Regarding pharmacokinetic assessment, the term "absorbed" is not fully understood. Therefore, additional discussion regarding the non-clinical pharmacokinetics of the product and the fate of the product in the spleen is necessary. Clinical data could be used as supportive. In its response, the applicant notes that the product is no longer found at the injection site at D30 (6 days after the last injection at D24) and notes that it is 'destroyed' by phagocytes. The applicant 'believes' that in this manner the product is moved via the interstitial space to the spleen and the lymphatic system for removal. In human apparently, it does not remain at the site of injection either. However, no supportive data is provided. The hypothetic nature of the answer is regarded unsatisfactorily, but the question will not be further pursued**.** In some tissues human DNA was detected in more control animals than in treated animals (lungs, kidney, liver). The applicant 'believes' that the animals in the control groups did not receive the human material (sitoiganap alike) and notes that this was probably an error in the processing of the study. More specifically in the procedure of cutting organs in two parts, one for histology and one for detection of human DNA. Therefore the quality of the study cannot be guaranteed and makes the request for justification for the absence of GLP compliance even more urgent. The applicant should reflect on the quality of the study, clarify and discuss all processing errors together with the CRO and justify the absence of GLP **(see LoQ).**

Toxicology

The only safety study has several limitations and uncertainties related to the animal model, treatment regimen (in relation to the clinic) and potential PD interactions, amount/choice of parameters (and tissues) evaluated and time point(s) of analysis, which have not been appropriately discussed by the applicant, while this was requested in the Scientific Advice from 2018 (EMA/CHMP/SAWP/485805/2018). The study is of limited relevance for the clinic due to the improper design and the differences of the test-product with the clinical product. Information has not been provided on : on and/ or rationale for 1) the different parts of the treatment regimen (e.g. components A1, A2, B1, B2 and dose of Sitoiganap, type and dose of placebo, used dose/frequency of administration/route of administration of cyclophosphamide and GM-CSF and evaluation of their potential PD interaction, full treatment frequency per cycle and number of cycles compared to clinical treatment), 2) the choice for the NOD SCID model (instead of immune competent and/or homologous model) and omission of toxicology evaluation in male animals, 3) the absence of clinical observations (other than body weight) and laboratory measurements (including haematology, clinical chemistry and

urinalysis) which should be provided when available, 4) selected tissues (including 'brain' as a whole instead of several individual parts and absence of e.g. draining lymph nodes and gonads) and extraction procedure of the samples for histopathology analysis, and 5) selected follow-up time and time point for analysis after final administration; In addition, pivotal toxicity studies should be GLP-compliant, although some considerations specifically for ATMPs are available (Good laboratory practice (GLP) principles in relation to ATMPs, EMA, 26 January 2017). However, absence thereof has not been justified. All these issues limit the interpretation and the translatability of the results to patients. A conclusion on safe dose(s) for humans, target organs for toxicity to be monitored in the clinic and duration of follow-up periods to detect adverse events in patients (see also requirements in the *Guideline on human cell-based medicinal products*, EMEA/CHMP/410869/2006) is therefore not possible, while the applicant was requested to discuss safety aspects related to clinical use of the product and the need for clinical intervention methods in the Scientific Advice. The toxicity of Sitoiganap was insufficiently addressed and non-clinical safety support for the clinic was not available. Therefore, the MO was not considered sufficiently addressed and the issue is not solved. It is concluded that the distribution and persistence as well as the safety of Sitoiganap has insufficiently been studied. The performed study is insufficient because of the flawed design, interpretation is hampered and ,results may not be reliable. Consequently the safety profile cannot be determined and the impact of any potential risk minimisation strategies cannot be estimated. [MO] **(see LoQ).**

It appears that cyclophosphamide and GM-CSF are essential parts of the treatment, next to Sitoiganap. This would require that the complete treatment should be compared to no treatment at all. Since only one mouse was available for this 'no treatment', this is not considered a relevant control group. This limitation to the study design, only one animal in a control group, was not explained properly **(see LoQ).** Because of the choice of study groups, no conclusions can be drawn on the overall toxicity of the complete treatment regimen (although this is considered necessary for the benefit/risk determination). Nevertheless, there is already quite some clinical experience with cyclophosphamide and GM-CSF. Since the other control group contained only one animal, it can be accepted that the isolated effect of Sitoiganap (by comparing group 1 and group 2) was evaluated in the current non-clinical study.

Regarding the study results, several unclarities have been noted that deserve further discussion or explanation. This concerns 1) observed differences in body weight and brain weight between group 1 and group 2 mice, 2) the histopathology data on the brain was lacking due to issues in the trimming of the samples, with most brain samples damaged, whereas Sitoiganap seem to lower brain weight in the animals 3) the cause of, relation to treatment and clinical relevance of observed liver inflammation and haemorrhages in group 2 mice and the (absence of) concordance between both histopathological readings (i.e. study 7 versus study 8) for these observations 4) the absence of a sensitisation study or proper evaluation of local tolerance 5) the absence of a discussion on the risk of Sitoiganap for reproductive and developmental toxicity (**see LoQ**). The applicant was also requested to discuss the effect of Sitoiganap (and co-medications) on specific immune cell types, including a relevant discussion on the potential immunotoxicity by Sitoiganap and by the co-medications. The applicant referred to the answer provided to question 77 a for further information regarding CY and GM-CSF. The discussion is regarded very limited. It would have been appreciated when own data or information from literature on the effect of CY and GM-CSF on changes in levels of the various immune cells and the expected effects would have been incorporated in the discussion on the potential for immunotoxicity. This was not provided. Although the question is not fully addressed, the issue will not be further pursued, as it this is not regarded to be an issue that needs to be addressed by means of collecting more non-clinical data at this stage.

A few, possibly more administrative issues, have been noted. Therefore, an updated module 2.6.5 and 2.6.7 with more detailed and correct information on both study designs and outcomes is needed and

the correct GM-CSF dose (in µg/kg) given to the mice should be provided. Also the full report of study 110211 with all the missing information and all the data and results in the same study report should be provided. Furthermore, several other concerns related to the deficiencies in the RMP related to the non-clinical studies and related to the SmPC sections 4.3, 4.6 and 5.3 are raised, but are not (sufficiently) addressed. The specific comments related to the SmPC are now addressed in the specific assessment of the PI in a separate document. The non-clinical sections in the RMP need to be rewritten and some non-clinical findings should be added. More specific comments for updating the RMP and SmPC are provided below:

Effects on the brains (e.g. haemorrhage) should be added as an important potential risk identified in the non-clinical studies in a specific table on non-clinical identified risks in Part II: Module SVIII of RMP. Furthermore, the occurrence of injection site reactions as and AE should be reflected in the RMP and the SmPC.

The applicant should rewrite section Part II - SII and provide a table in section Part II: Module SVIII on the potential and identified risks and missing information. The applicant should submit and updated RMP. In addition, the applicant should provide and updated SmPC and addresses all the comments and questions in there.

2.2.6. Conclusion on non-clinical aspects

Based on the provided dossier and response to questions the intended effect and potential safety issues of Sitoiganap therapy are insufficiently understood. The non-clinical dossier still shows major deficiencies, the quality of the *in vivo* studies is of concern, the claimed pharmacodynamic activity is insufficiently supported, the interpretability of the biodistribution study is limited and the safety profile has not been adequately studied. The applicant did not addressed on these issues adequately. Some minor issues will not be further pursued, but most concerns, including the majors one's remain. For the full assessment of the applicants response be referred to the D150 non-clinical JAR. The conclusion that the product cannot be granted market approval from non-clinical perspective remains as the two major objections and several other concerns formulated based on the non-clinical information submitted by the applicant which are not yet adequately addressed.

2.3. Clinical aspects

2.3.1. Pharmacokinetics

No formal PK assessment has been performed for Sitoiganap nor for the concomitant medications. No information at all on clinical pharmacokinetics were provided.

2.3.2. Pharmacodynamics

In support of the PD and PK/PD of Sitoiganap, the applicant has presented the results of pre-clinical in vivo experiments as published by Stathopoulos et al.,(described in the non-clinical section) as well as the published case report of a patient with glioblastoma multiforme treated with Sitoiganap where biopsies after treatment and post-mortem were performed. In addition preliminary results of

CD3+/CD4+ lymphocyte counts of patients enrolled in the ongoing Phase II study submitted as pivotal for the application for MAA have been provided (described in the efficacy section).

According to the applicant, the tumour biopsies of the case report show infiltration of tumour samples by CD68+ macrophages after treatment and post mortem.

2.3.3. Discussion on clinical pharmacology

Pharmacokinetics
No information was provided regarding the clinical pharmacology development of the product. Although the absence of a conventional clinical pharmacokinetic program for a human cell-based medicinal product like Sitoiganap (ERC1671) is acceptable, information related to the human pharmacokinetic of Sitoiganap, study requirements, possible methodologies and their feasibility should be discussed and included in the dossier in line with the EMA guidelines [Guideline On Human Cell-Based Medicinal Products (EMEA/CHMP/410869/2006) and Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018)]. (**OC**)

Given the nature of the product (lysed or irriadiated autologous and allogenic tumour cells) and the intradermal injection the lack of clinical PK data on the product can be accepted provided that the non-clinical studies can provide sufficient understanding of the distribution and persistence of the product. However it appears that this is not the case, and that additional discussion on this issue is needed (see non-clinical **MO**).

Information/data on the systemic exposure of the concomitant medications GM-CSF (as immunostimulant) and cyclophosphamide (as immunomodulator) and the methods used to determine the exposure levels are lacking and should be provided. (**OC**)

Pharmacology
The proof of concept to potentially support the claimed mechanism of action of Sitoiganap is essentially based on pre-clinical in vivo experiments reported by Stathopoulos et al. It is acknowledged that several articles published in the literature in the recent years could support, on the basis of pre-clinical in vivo models, a potential rationale for activity of autologous and allogenic tumour cell lysates administered intra-dermally in patients with glioblastoma (Belmans et al., 2017, Maes et al., 2011, Oh et al., 2009). On the other side, it is also underlined that in the recent past several immune-modulating agents / vaccines with very promising antitumour activity in preclinical models, failed to show (significant) antitumor response in patients with malignant gliomas, as demonstrated by the fact that actually no immune-modulating product / vaccine is registered in EU for the treatment of glioblastoma.

The results of the case study showing macrophage infiltration are considered interesting in suggesting the presence of inflammation as basis of an immune response induced by Sitoiganap. However, information available is very limited and it is unclear whether, and eventually how, the findings could be related to Sitoiganap treatment.

No proof of concept in the clinic has been presented for the contribution of the different components of the proposed combination (i.e., both Sitoiganap autologous and allogenic components, cyclophosphamide, GM-CSF) in evoking the claimed immune response necessary to achieve anti-tumour activity. It is also unclear in which way the claimed immune response is evoked and expected to be maintained overtime. The activity of Sitoiganap is not self-evident from the information

presented by the applicant. A pre-clinical and clinical rationale for the addition of bevacizumab (at the proposed dose) in the ongoing pivotal Phase II study is also lacking.

No dose finding study was performed with Sitoiganap. Therefore, dose-response relationship cannot be assessed. In the guideline on cell-based products (EMEA/CHMP/410869/2006) it is stated that selection of the dose should be based on the findings obtained in the quality and the non-clinical development of the product and should be linked with the potency of product. It is expected that the dose is confirmed during the clinical studies. However, no formal justification has been provided by the applicant for the applied dose-regimen, combination and timing of administration of Sitoiganap autologous and allogenic components as well as concomitant medications. It is therefore unclear whether the proposed dose of $1x\ 10^5 – 10^6$ for cell vials is necessary to achieve the claimed anti-tumour activity and whether lower doses could be sufficient too. This is considered relevant in particular for patients where the $1x\ 10^5 – 10^6$ dose is not attained at the end of the manufacturing process.

According to the applicant the immune-response induced by Sitoiganap is further supported by the preliminary results of the CD3+/CD4+ helper T-lymphocyte counts monitored in peripheral blood at baseline and every 2 weeks in patients enrolled in the ongoing Phase II trial presented as pivotal evidence for the intended indication. As stated by the applicant, both the maximum count and the end-of treatment count highly correlated with the OS in patients treated with Sitoiganap and bevacizumab, but not in the placebo and bevacizumab group. Moreover, data on pre-treatment CD4+ T lymphocyte count in the peripheral blood of 15 patients enrolled in the study and with available information correlate with OS more strongly in the Sitoiganap group (vaccine and cross-over) than in the placebo, non-cross-over group. However, it is underlined that the relevance of CD4+ helper T lymphocyte counts in peripheral blood is not well established in glioblastoma patients /clinical trials with cell-based vaccines / immune modulating agents. Information on other relevant immune-related cells/components is missing. Moreover, only results of the correlation with OS have been presented, results of correlation analyses with other efficacy parameters are lacking. Furthermore, the credibility of the results is seriously challenged as patients that crossed over to Sitoiganap after progression in the control arm were included and counted in the experimental arm.

No assessment on secondary pharmacology is possible due to the lack of information presented by the applicant.

As a final comment, in the briefing document the applicant refers to Sitoiganap as a vaccine product. However, according to the EU nomenclature it is classified as an immune-modulating agent.

2.3.4. Conclusions on clinical pharmacology

Overall, it is considered that the pharmacology of Sitoiganap has been insufficiently addressed. The claimed mechanism of action is mainly based on general immunological principles and theoretical assumptions and some potentially interesting findings but lack a clear and unambiguous experimental support. Also the contribution of the different components of the proposed combination (i.e., both Sitoiganap autologous and allogenic components, cyclophosphamide, GM-CSF) in evoking the claimed immune response necessary to achieve anti-tumour activity has insufficiently been demonstrated.

The clinical pharmacology data is not considered adequate to support the application.

2.3.5. Clinical efficacy

The clinical development plan to support the proposed CMA of Sitoiganap-GM-CSF, cyclophosphamide and bevacizumab in patients with recurrent/progressive glioblastoma and gliosarcoma (WHO grade IV malignant gliomas, GBM) is based on interim results of a Phase II study (ERC1671-H02, NCT01903330). In addition, the results of 10 patients with recurrent/progressive high grade malignant gliomas treated with the Sitoiganap under a compassionate use/hospital exemption protocol have been presented as supportive evidence. Moreover, 8 patients received Sitoiganap (in several cases in combination with nivolumab / pembrolizumab) in the context of the FDA "Right to try" act.

Dose-response studies and main clinical studies

No dose finding study has been performed to support the proposed Sitoiganap dosing regimen nor to support the combination with GM-CSF, cyclophosphamide and bevacizumab proposed for CMA. Some argumentations based on general immunological concepts/assumptions and few pre-clinical experiments have been presented to support the claimed mechanism of action.

The proposed dose for Sitoiganap is 10^5-10^6 tumour cell components in each vial. The dose was chosen based on preliminary preclinical, manufacturing and clinical data, and is similar with the dose used with another cellular ATMP (Mvax - cryopreserved, autologous, hapten-modified melanoma vaccine) used in oncology. There was no dose escalation assay in clinical trials.

Rationale for Proposed Combination of Sitoiganap plus Bevacizumab

In the clinical trial submitted within this application, bevacizumab was added as standard treatment upon FDA request. Malignant gliomas express high levels of VEGF, a key regulator of angiogenesis. Levels of VEGF expression correlate with both tumour grade and microvessel density; these findings suggest that therapeutic strategies targeting VEGF may provide an effective approach to suppress malignant glioma growth.

The applicant states that Sitoiganap and bevacizumab work through complementary pathways, and both have activity in glial tumours, with no overlapping toxicities. In addition, bevacizumab might modulate a more functional and robust immune response to the antigens present in the Sitoiganap vaccine.

The applicant's hypothesis is that a combination of these two mechanisms of action could enhance the ability to affect the two main driving forces of tumour proliferation: the glioma cells by augmenting their immune recognition by activated lymphocytes and the vascular compartment by reducing the local concentration of VEGF resulting in diminished tumour angiogenesis. Bevacizumab is administered at 10 mg/kg.

Rationale for GM-CSF dose and schedule

Sargramostim is a recombinant human granulocyte macrophage colony stimulating factor (rhu GM-CSF). GM-CSF is a hematopoietic growth factor which stimulates proliferation and differentiation of hematopoietic progenitor cells and activation of dendritic cells.

According to the applicant, the addition of GM-CSF acts as an immunostimulant, by specifically increasing the proliferation of myeloid cells as well as the proliferation and asymmetric division of bone marrow stem cells into platelets, neutrophils, monocytes and macrophages, eosinophils and basophils. In addition, GM-CSF attracts T-lymphocytes and stimulates antigen presentation by dendritic cells.

Rationale for cyclophosphamide

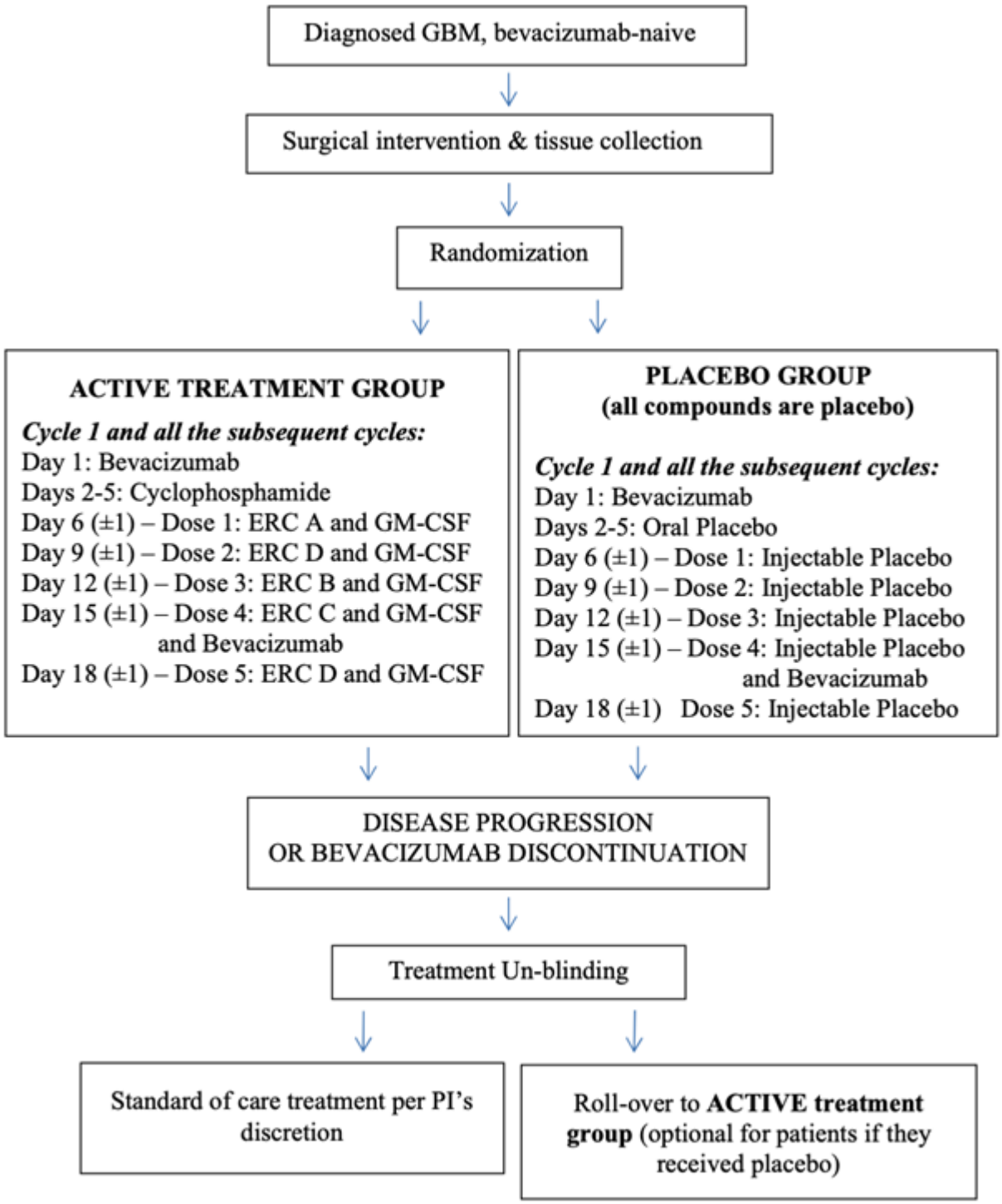
Cyclophosphamide (CY), an alkylating agent, has a bimodal effect on the immune system depending on the dose and schedule of administration. A low dose causes a shift in the T cell population resulting in the change from immunosuppression to immunopotentiation, by reducing the tolerant Treg population. High-dose CY reduces the total number of T lymphocytes thereby causing immunosuppression. In the treatment regimen, 50 mg/m2 CY is considered low dose, and is used as part of the treatment.

2.3.6. Main study - Study ERC1671-H02 (NCT01903330)

Title: A randomized, double-blinded, placebo-controlled, Phase 2 study of Sitoiganap (Gliovac, ERC1671), GM-CSF, cyclophosphamide plus bevacizumab vs placebo plus bevacizumab in recurrent/progressive, bevacizumab-naïve glioblastoma multiforme and gliosarcoma patients (WHO grade IV malignant gliomas, GBM).

Study ERC1671-H02 is an ongoing, investigator-initiated, randomised (1:1), double-blind phase II trial comparing Sitoiganap - GM-CSF, - cyclophosphamide – bevacizumab vs placebo – bevacizumab in recurrent/progressive, bevacizumab-naïve glioblastoma multiforme and gliosarcoma patients. A total of 84 patients are planned to be randomised (1:1). The study was designed as an exploratory trial.

Figure 3 - ERC1671-H02 - study design



* according to the inclusion criteria, patients were required to be progressive after surgery, temozolomide and radiotherapy.

The clinical study report document provided by the applicant related to trial ERC1671-H02 only consists of a short synopsis of the study protocol with a brief summary of few patient characteristics and results. Results are presented in terms of few summary tables/graphs. In the response document a Clinical Study Protocol (and several amended versions) has been submitted by the applicant.

Study Participants

The ERC1671-H02 study population included adult patients (≥18 years) with histologic diagnosis of glioblastoma or gliosarcoma (WHO Grade IV), Karnofsky performance status ≥70%, life expectancy of at least 12 weeks, who had received prior therapy including surgery (intended as biopsy, partial or full resection), conventional radiation therapy and temozolomide, were relapsed or refractory, had progressive and pre-surgical bi-dimensionally measurable disease (as per iRANO criteria). Adequate bone marrow, renal and hepatic functions were required for inclusion too. Patients with systemic corticosteroid therapy were allowed if they were taking a dose of ≤ 4 mg dexamethasone or equivalent per day during the week prior to Day 1. Patients with prior treatment with bevacizumab or other VEGF-inhibitors and/or with known contraindications to bevacizumab treatment (i.e., uncontrolled hypertension, proteinuria, cardiovascular disease, etc) were excluded.

The study is being conducted at 3 study centers in the US (University of California, University of Southern California, Dana Faber Institute).

Treatments

Treatment administration was performed according to the following schema:

Day 1: Bevacizumab
Days 2-5: Cyclophosphamide (or placebo)
Day 6 (±1) – Dose 1: ERC A/Placebo and GM-CSF/Placebo
Day 9 (±1) – Dose 2: ERC D/Placebo and GM-CSF/Placebo
Day 12 (±1) – Dose 3: ERC B/Placebo and GM-CSF/Placebo
Day 15 (±1) – Dose 4: ERC C/Placebo and GM-CSF/Placebo and Bevacizumab Day 18 (±1) – Dose 5: ERC D/Placebo and GM-CSF/Placebo

One cycle is defined as 28 days. Treatment is repeated every 28 days until progression of disease or intolerance.

RC1671/Placebo and GM-CSF/Placebo are administered intradermally, while cyclophosphamide or placebo is administered orally. GM-CSF/Placebo dose is 500 mcg and cyclophosphamide or placebo dose is 50 mg/day. Bevacizumab is administered as standard of care at 10 mg/kg dose every 14 days.

Every treatment cycle is composed of administration of the 'vaccine'product components A, B, C and D. Each component (A, B, C and D) is composed of two vials- one containing irradiated tumour cells and the other containing the cell lysate. The number of cells per vial is of $1x10^5$-$1X10^6$ cells for each of the separate components (ERC A, B, C and D) suspended in 0.25 mL of volume. For the lysate vials, the same number of cells ($1x10^5$-$1X10^6$) are lysed in a total volume of 0.2 mL. The volume of each vial needs to be 0.2 mL. ERC A, ERC B and ERC C are generated from allogeneic GBM tumours from the ERC tumour bank, while ERC D is generated from the autologous tumour, collected from the study patient during surgery.

It is unclear whether the use of dexamethasone > 4 mg/day was allowed during the study and whether data on its use were collected.

Bevacizumab was chosen as comparator/backbone treatment even though this product is not registered for this indication in the EU.

Treatment was to be continued until either evidence of progressive disease, unacceptable toxicity (if bevacizumab has to be discontinued due to severe, persistent toxicity, the patients will continue on ERC1671/Placebo and GM-CSF/Placebo as a single agent), non-compliance with study follow-up, or withdrawal of consent.

Cross-Over to Active Treatment Group

At the time of proven disease progression or if the patient is discontinued from bevacizumab due to toxicity, the group assignment was unblinded. The patients enrolled in the Active Treatment Group were offered standard or palliative care. The patients enrolled in the Placebo Group were offered the opportunity to cross-over to the Active Treatment Group. Patients who did not participate in the cross-over option of the study returned for an End of Therapy visit where alternate treatment and/or care options were discussed. All patients continued to be followed for survival. All cross-over patients who received Sitoiganap no longer followed the study calendar and were followed per local standard practice. The treating physician had the option to add additional standard therapy e.g. radiation therapy, Optune, etc., to their treatment plan.

Objectives

According to the original study protocol submitted the primary objective consisted of demonstration of superiority of the experimental arm versus the control arm in terms of 6-month progression free survival (6-month PFS). Secondary objectives included evaluation of radiographic response, progression free survival (PFS), overall survival (OS), safety and immune response.

In the response document (answer to Q123) the applicant states that the original primary endpoint (6-month PFS) was not amended during the study. However, in answer to Q124 and according to the last amended version of the study protocol provided by the applicant 12-month OS probability is presented as the primary study outcome with radiographic response, progression free survival, safety and immune response being mentioned as secondary endpoints. It seems that, despite the applicant's statement, the protocol was amended to include 12-month OS as primary outcome/endpoint. In such a case it remains uncertain whether the modification was data driven.

Outcomes/endpoints

In the original study protocol the primary endpoint was 6-month PFS probability. Secondary endpoints included OS, PFS and radiographic response.

However, when looking at the protocol characteristics as presented in clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT01903330), at answer to Q124 in the response document and at the most recent version of the study protocol submitted, OS rate at 12 months is presented as primary endpoint, whereas PFS at 12 months, immune response etc. are presented as secondary endpoints. Despite the applicant's statement that the primary endpoint has never been amended it seems that the protocol has been amended after the study had commenced; it is unclear whether protocol integrity has been protected.

Criteria for definition of PFS/radiographic response or progression do not appear to be unequivocal, as for instance, regarding progression free survival, it is unclear whether only radiographic progression was considered an event or whether, and eventually how, events of clinical deterioration due to progression and / or death in absence of disease progression were counted. Similarly, radiographic response was categorised per iRANO or MacDonald Criteria, suggesting lack of homogeneity in the criteria implemented for assessment of response in all patients treated. Moreover, it is unclear how response and progression was assessed in patients with no measurable disease after surgery. No information has been provided on who would assess PFS, but it is assumed that this was done by the investigators in view of the exploratory design. It is therefore unclear whether revision by a blinded IRC was implemented. The frequency of radiologic assessment seems also to have been amended during conduct of the trial, as observed in the different submitted versions of the study protocol.

Randomisation and blinding (masking)

Participants had an equal chance to be assigned to the ERC1671 arm or the Standard of Care arm (1:1). When the University of California at Irvine (UCI) was the only participating site, randomisation was determined following informed consent by the sponsor using software manufactured by Randomize.net. The software purported to use a block-size of four. This process held for the first 14 enrolees. Based on literature, and not study results to date, the study team decided to stratify randomisation on methylation status of the MGMT gene, the switch was beyond the ability of the original randomisation software. Consequently, the study statistician (at UCI) developed a SAS program to produce random assignments, one-to-one, with a block size of four. The program produces a randomisation schedule of arbitrary length (for multiples of four participants) by randomly permuting all six possible ways in which blocks of four consecutive participants can be assigned, two to each arm. The only constraint is that, across blocks, four consecutive assignments to the same arm are not allowed. The results are exported from SAS into an excel spreadsheet. Separate runs were made for MGMT positive and MGMT negative/unknown. These sheets were sent to the sponsor, with instructions to make study-arm assignments strictly following the order of assignments on the spreadsheet, each slot to be used only once. The sponsor provided copies of the sheets to the statistician at UCI for monitoring purposes. Neither the sponsor's agents nor the UCI statistician has any contact with potential nor actual participants, and are completely isolated from recruitment, examination, follow-up, nor initial data entry role in any contact with potential or engaged participants, their recruitment, examinations, nor follow-up.

It remains unclear why a rule that disallowed more than three consecutive assignments was included in addition to block randomisation. Such a rule does not fit within a valid randomisation procedure. If the SAS program included a random number seed then the randomisation process can be replicated, however it remains unclear whether the full randomisation procedure could be replicated given the first part was performed using Randomize.net and the second, stratified randomisation was generated using SAS.

Reasons for unblinding included provision for roll-over of placebo patients to active-treatment arm (see Treatment Plan), serious toxicity which may be related to Sitoiganap, death (in case patient is lost to follow-up) and data analysis. In the event of serious adverse events, the study statistician will communicate accrual numbers, stratified by study center, MGMT status, and study arm as may be required by the DSMB. It remains unclear whether the Investigator and patient were sufficiently blinded to treatment allocation.

Statistical methods

According to the original version of the study protocol submitted in the response document the sample size was determined assuming a 6-month PFS rate of 40% in the target population treated with bevacizumab monotherapy (as reported by Friedman et al 2009). With a total sample size of 35 patients per arm, two sample chi-square test, and one-sided significance level of 20%, the study has 80% power to detect a difference between the null hypothesis proportion of 40% vs the alternative proportion of 60%. However, in the last version of the study protocol submitted in the response document, "a likelihood-ratio chi-square test will compare the proportions alive at 12 months between the two study arms. Because this is a pilot study, the risk of type-one error is set at 20 percent for this analysis. When OS12 for placebo and treatment groups is in the anticipated range, a sample-size of 42 per group (84 total) yields satisfactory power for the likelihood-ratio chi-square test with an alpha of 20%. As shown in the table below, power is 79% or more if the placebo OS12 is 15% or less and the

treatment OS12 is 30% or more. Power is at least 73% if OS12 in the placebo group is 25% or less and OS12 in the treatment arm is 40% or more."

Looking at the results presented at the time of CHMP scientific advice as well as the several updates of the study results published in the literature, it seems that the applicant and study personnel is looking at the study results during the study without any reference to pre-planned time-points.

The clinical trial database has been managed by the University of California at Irvine. Independent quality assurance personnel have reviewed the database and no deviations have been reported. Discussions are planned with the United States FDA to transfer the data into an ERC database. That work is almost complete and is expected to be independently monitored once the data has been transferred.

Results

The results of the study have been presented according to an unplanned analysis performed with the cut-off date of 01 December 2017 after inclusion of 9 patients (n=5 in the Sitoiganap arm and n=4 in the control arm). Study Participant flow was not provided and not clear from the documentation submitted. One patient enrolled in the experimental arm was considered not evaluable due to discontinuation prior to completion of the first cycle. Reason for the discontinuation is not mentioned.

The results of an updated analysis with cut-off date 20 June 2020, after enrolment of 22 patients, are presented too.

All the results are provided in the form of abstracts published or presented at congresses. No detailed information with additional data/analysis/single patient data has been provided. No detailed Clinical Study Report has been provided by the applicant. In the response document the applicant states that not all the efficacy endpoints have been evaluated "in order to preserve the integrity of the trial".

No detailed information related to conduct of the study has not been provided. The study protocol seems to have been amended 17 times. Within the response document the applicant has provided all the 11 versions of the study protocol implemented during the conduct of trial up to date. The implemented changes in the different versions of the protocol are not readily visible and no summary has been presented regarding the kind (i.e., major or minor), nature (i.e., content) and reasons for the amendments performed by each version of the protocol hampering any assessment on this issue. A list of protocol deviations has been presented in the response document. However, a summary listing with number (%) of minor and major protocol deviations observed, respectively, has not been presented.

Baseline data

Of the 9 patients enrolled at the time of the cut-off (01 December 2017), median age was 57 years (49-65), and 2 out 9 patients were female. The average KPS was 80 (70-100). No other information has been presented over other relevant demographic and baseline disease characteristics of the patients enrolled in the study, as well as over distribution of patient parameters considered predictive for benefit of second-line therapy in high grade glioma (i.e., extent of disease, histologic grade (both at initial therapy and at recurrence), relapse-free interval, and recurrence pattern time). Data on comorbidities, co-medications and post study therapy have not been presented too. In particular, it is unclear whether dexamethasone doses > 4mg/day were allowed during the study and eventually how the use of dexamethasone was handled.

Table 3 - Patient characteristics – study ERC1671-H02

Characteristics	Active Treatment Group + Bevacizumab (n=5)	Placebo Control + Bevacizumab (n=4)
Age (average [range])	59 (49 – 65)	57 (48 – 74)
Male (n [%])	4 (80%)	3 (75%)
KPS (average [range])	80 (70-100)	90 (70-100)
Relapses (n [%])		
1	5 (100%)	3 (75%)
IDH1/2 status (Wild-type [%]	5 (100%)	4 (100%)
MGMT promoter	Unmethylated 4 (80%) Undetermined 1 (10%)	Unmethylated 3 (75%) Undetermined 1 (25%)

Numbers analysed

The presented results concern the first 9 patients included in the study, corresponding to 10.7% (9/84) of patients planned to be enrolled in the trial.

Outcomes and estimation

According to the original study protocol submitted by the applicant the primary study endpoint was 6-month PFS probability, which seems have been amended later on as in the latest version of the study protocol presented 12-month OS is presented as primary endpoint. Only results on response rate, median OS and median PFS have been provided.

Response Rate

The response rate claimed by the applicant for the Sitoiganap arm was 75% (3/4) vs 25% (1/4) for the control arm. According to the applicant patients in the Sitoiganap arm experienced durable responses, with one patient with a partial response after cycle 1 maintained for more than 7 months and alive over 2 years. However, the analysis does not follow a ITT principle as 5 patients (and not 4) were enrolled in the experimental arm. No information has been reported on the number (%) of patients in both arms achieving CR, PR, SD and PD, respectively. Individual and median time on treatment / duration of response / duration of treatment have not been provided.

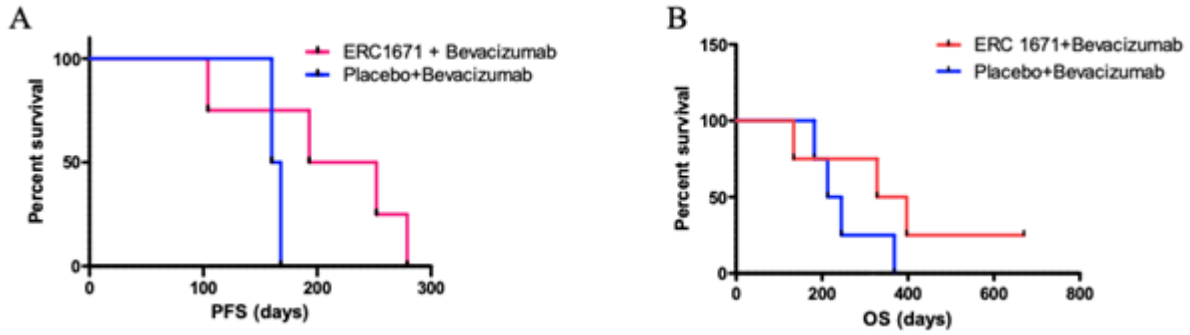
It is unclear which method was used for evaluation of response (i.e., all with iRANO or all with McDonalds), whether assessment was performed by investigators or by a blinded independent review committee, and whether the reported responses where "confirmed" (i.e., confirmation after at least 4 weeks was considered necessary).

Overall Survival (OS) and Progression Free Survival (PFS)

Median OS of patients treated in the Sitoiganap arm was 12.1 months (363 days), with one patient surviving more than 2 years. Median OS in the control arm was 7.6 months (229 days), with all patients having died within 1 year.

Median PFS was 7.3 months (223 days) in the Sitoiganap arm compared with 5.4 months (164 days) in the control arm.

Figure 4 - PFS and OS ERC1617-H02 study (cut off 01 December 2017)



Only median values are presented without any information on 95% confidence interval values as well as HR and p-values.

Immune correlations

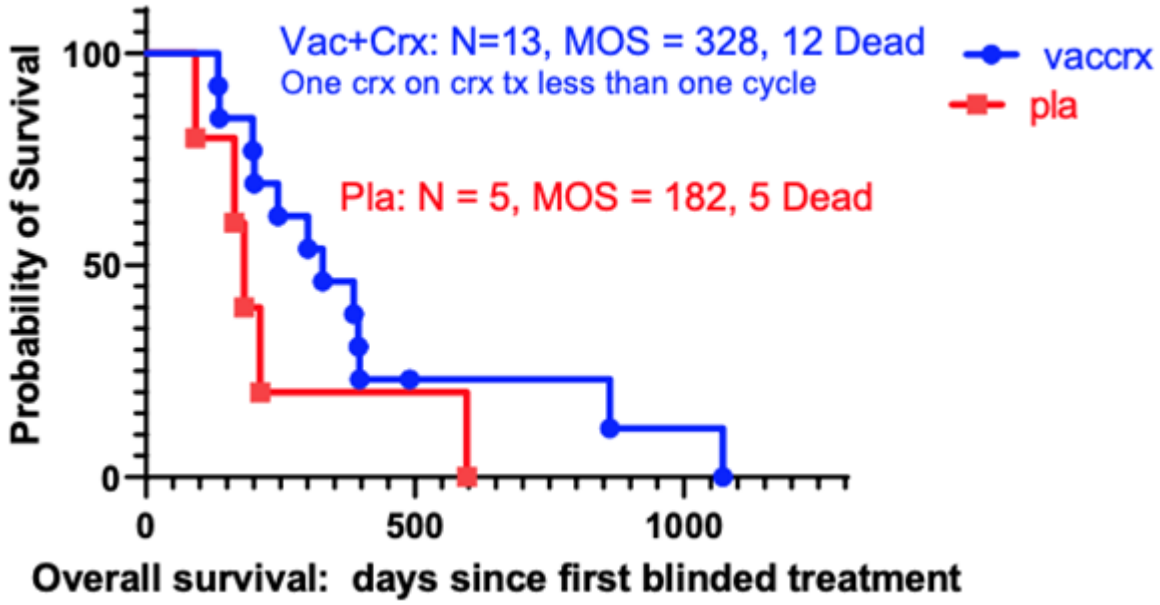
An analysis to evaluate a potential correlation between CD3+/CD4+ helper T-lymphocytes counts and OS was presented. However, information is lacking regarding the actual number of patients and samples per treatment arm included in the analysis, the number of missing samples, the handling of missing data as well as data on standard deviation and standard error.

Updated analysis (cut off 20 June 2020) (submitted for presentation for the Society of Neuro-Oncology Meeting, November 2020).

At the 20 June 2020 cut-off date, 22 recurrent bevacizumab-naïve glioblastoma patients have been randomised to Sitoiganap /GM-CSF/cyclophosphamide + bevacizumab or placebo + bevacizumab. Median age is 56.5 years (33-74), 7 patients (32%) are female, and average KPS is 82.3 (70-100). Of the 22, two discontinued before completing one cycle of therapy and two remain on blinded treatment. Currently 18 patients are unblinded due to further progression: 8 were on Sitoiganap and 10 on placebo. Five of those on placebo crossed to Sitoiganap at progression. All but one of the 18 are now deceased.

Median overall survival of unblinded patients randomised to Sitoiganap + bevacizumab (n = 8) is 264.5 days (8.7 months) from the start of study treatment, compared to 182 days (6 months) for those randomised to placebo + bevacizumab who did not cross over (n = 5). Median overall survival of unblinded patients on 'vaccine' Sitoiganap treatment at randomisation or crossover is 328 days (11 months) after first study treatment (n = 13).

Figure 5 - OS: Sitoiganap 'vaccine' at randomisation or crossover vs placebo no crossover: evaluable and unblinded (N=18), Logrank p<0.18



Of note, in the updated analysis only results on median OS and immune correlations have been presented, whereas results of 6-month PFS probability (primary study endpoint), median PFS and ORR (the last two presented at the previous cut off analysis) have not been provided. Moreover, it is unclear whether an ITT principle has been followed. Furthermore, it is noted that no statistically significant difference in OS is observed between study arms (logrank p<0.18) and that median OS appears significantly reduced in the experimental arm at the updated analysis (8.7 months) compared with the previous analysis (12.1 months). Finally, the analysis presented includes patients that crossed over to Sitoiganap after progression, implicating the introduction of a selection bias.

Reasons for study discontinuation in the 2 patients discontinuing treatment before completing the first cycle of treatment have not been provided by the applicant but could raise concerns over potential treatment related toxicity.

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 ERC1671-H02

Title: A randomized, double-blinded, placebo-controlled, Phase 2 study of Sitoiganap (Gliovac, ERC1671), GM-CSF, cyclophosphamide plus bevacizumab vs placebo plus bevacizumab in recurrent/progressive, bevacizumab-naïve glioblastoma multiforme and gliosarcoma patients (WHO grade IV malignant gliomas, GBM).			
Study identifier	NCT01903330		
Design	Investigator-initiated, double blind, placebo controlled, study. Crossover from the control to the experimental arm allowed at time of disease progression.		
	Duration of main phase:	Not available	
Hypothesis	Superiority, Type 1 error relaxed (20%) Planned to be enrolled 84 patients. Patients actually enrolled at the time of the cut off analysis (1 December 2017) presented: 9 patients		
Treatments groups	Sitoiganap-GM-CSF-cyclophosphamide-bevacizumab	N=5	
	Placebo-bevacizumab	N=4	
Endpoints and definitions	Primary endpoint	6-month PFS probability in the original clinical study protocol - 12-month OS in the latest version	
	Secondary endpoint	RR	Definition and criteria for assessment not unequivocal
	Secondary endpoint	OS	Definition: death due to any cause
Database lock	01 December 2017		
Results and Analysis			

Analysis description	**Primary Analysis**
Analysis population and time point description	Information not available. ITT principle does not seem to have been followed

Descriptive statistics and estimate variability	Treatment group	*Sitoiganap-GM-CSF-cyclophosphamide-bevacizumab*	*Placebo-bevacizumab*	
	Number of subject	N=5	N=4	
	OS	12.1 months	7.6 months	
	variability statistic	Not available	Not available	
	RR	75% (3/4)	25% (1/4)	ITT principle not
	variability statistic	Not available	Not available	
	PFS variability statistic	7.3 months Not available	5.4 months Not available	ITT principle not followed
Notes	No detailed Clinical Study Report available. No detailed information available on methods implemented, statistical analysis plan and conduct of the study. Major deficiencies and inconsistencies identified in the dossier.			

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

The applicant provided an inter-study comparison comparing survival after resection of recurrent glioblastoma, claiming an improvement in survival with Sitoiganap (10.6 months) vs surgery alone (5.29 months, $p<0.0002$). According to the applicant's claim, patients receiving Sitoiganap are more likely to survive one year following start of treatment than patients receiving currently available treatments for malignant glioma. More patients are reported to be alive at one year following treatment with Sitoiganap (38%) when compared to temozolomide (33%) and CCNU (34%). Also, the Sitoiganap treated patients (which almost all have an unmethylated MGMT promoter) had statistically superior OS as compared with the standard treatment (CCNU, temozolomide/TMZ or PCV).

Table 5 - Inter-study comparison comparing survival after resection of recurrent glioblastoma

			One-Sided 95%CI	One-Sided 80%CI
Cohort		MOS	lower	Lower
ERC Vac*		***10.58***	***8.05***	***9.89***
ERC placebo		5.98	p < 0.05	p < 0.2
Barker		5.25	p < 0.05	p < 0.2
Wick, 2017	CCNU (all)	8.6		p < 0.2
	CCNU unmethylated MGMT	7.15	p < 0.05	p < 0.2
	CCNU+bev (all)	9.1		p < 0.2
	CCNU+bev unmethylated MGMT	8.02	p < 0.05	p < 0.2
Batchelor, 2013	CCNU (all)	9.8		
Weller, 2015	TMZ 7/14 (all)	9.8		
	TMZ 21/28 (all)	10.6		
	TMZ unmethylated MGMT	7.9	p < 0.05	p < 0.2
Brada, 2010	TMZ	7.2	p < 0.05	p < 0.2
	PCV	6.7	p < 0.05	p < 0.2
Kappelle, 2001	PCV	7.6	p < 0.05	p < 0.2
Schmidt, 2006	PCV	7.9	p < 0.05	p < 0.2

Furthermore, the applicant claims that OS with Sitoiganap is superior to all previously published studies including the studies which had similar surgical requirements (see meta-analysis of all NABTC studies, J. Clarke et al, 2011, Neuro-Oncology).

The information provided by the applicant regarding the above presented analysis are very scarce. In effect, it is unclear how many patients were included in the analyses presented, whether the analyses include all patients treated with Sitoiganap to date (i.e., Phase II trial, compassionate use/exemption protocol, single-patient protocol, Phase 0 trial) independently of co-administration with bevacizumab (or whether data on only patients receiving also bevacizumab are included) and whether there were patients excluded from the analyses presented, and if so how many. In the last figure reported above N=31 patients treated with Sitoiganap are mentioned, but it is unclear from which database they come from. Moreover, looking at the studies presented for comparison as historical controls (Baker et al, Clarke et al, etc) it is clear that they were referring to different specific situations than the one presented to date.

Clinical studies in special populations

Only adult patients (>18 years old) have been treated with Sitoiganap- GM-CSF-cyclophosphamide-bevacizumab to date.

No information has been provided regarding the number of patients >65 years included in the studies performed to date with Sitoiganap - GM-CSF-cyclophosphamide-bevacizumab.

Patients with hepatic and renal impairment were excluded from the studies performed with Sitoiganap to date.

In vitro biomarker test for patient selection for efficacy

No information has been provided by the applicant.

Glioma patients are characterised for their tumour mutation profile (mainly MGMT status and also by the presence of e.g. IDH 1/2 mutations). Patients included in the study are expected to have been

characterised in this regard (though only data from the first 9 patients randomised seem to be available, see comments above). However no information regarding the tests used has been provided.

Supportive study

Case reports: Hospital exemption / compassionate program

Ten (10) recurrent glioblastoma patients, after standard of care treatment, including surgery, radio- and chemotherapy with temozolomide, and for US patients, also bevacizumab, were treated under a compassionate use/hospital exemption protocol (9 patients described in Schijns VE et al, 'vaccine' 2015) and under a single-patient protocol approved by the US FDA (1 patient described in Bota DA et al. Perm. J. 2015). Sitoiganap was given intradermally, together with human GM-CSF and preceded by low dose cyclophosphamide. Six-months OS for the 10 Sitoiganap patients was 100%, vs 33% reported in historical controls represented by Barker et al 1998.

Median age was 53 years (26-62), with 6/10 being females. The average KPS was 80 (60-100). Out of these 10 patients, all in terminal stage of disease, 6 had not received bevacizumab before, during or after Sitoiganap treatment. Out of the 4 patients that had received bevacizumab, 2 had bevacizumab treatment until disease progression, and stopped bevacizumab prior to surgery and start of Sitoiganap. One patient received bevacizumab until disease progression followed by surgery and 4 cycles of Sitoiganap and was continued on bevacizumab afterwards. One patient received bevacizumab until disease progression and continued on both Sitoiganap and bevacizumab for an additional 2 months.

The applicant claims that according to the dataset of these recurrent glioblastoma patients treated with Sitoiganap, 6-month OS is 100%, 12-month OS is 40%, and median OS is 46 weeks (10.5 months) and compares these data to istorical controls (Barker 1998) reported to have a 6-month OS of 33% and median OS of 23 weeks. This comparison indicates significantly increase (log rank test $p< 0.0001$) in OS of patients treated with Sitoiganap according to the calculations by the applicant. A more recent review of single-arm bevacizumab studies performed (Taal et al 2014, Field et al 2015, Heiland et al 2015) indicates a 6-month OS between 18% and 62% and a 12-month OS between 10% and 26%, as compared with the 6-month OS of 100% and 12-month OS of 40% for Sitoiganap.

It is emphasised that results were presented in the form of published abstracts, and several relevant inconsistencies are identified in the data presented. For instance, the applicant reports an average KPS PS of 80 with a range between 60 and 100. The applicant seems also to have picked up several patients out of different databases with KPS ≥80 to be included in the analysis without an adequate justification. No matching of patients disease and baseline characteristics between the databases has been done.

"Right to try" act

Patients with glioma have sought access to Sitoiganap in the United States via a Compassionate use pathway called Right-to-Try. Since March of 2019, eight (8) GBM patients were treated with Sitoiganap, GM-CSF, cyclophosphamide, bevacizumab and either nivolumab or pembrolizumab (Table 6). All eight patients had failed first-line therapy with radiation and temozolomide, and two of them had also failed second-line therapy.

Table 6- Patient characteristics treated with Sitoiganap via Right to Try compassionate use

Patient	Gender	Age	KPS	MGMT	IDH1
1	m	44	80	unmeth	wt
2	m	54	70	meth	wt
3	m	37	90	meth	wt
4	m	40	70	meth	mut
5	m	46	70	unmeth	wt
6	m	70	80	unmeth	wt
7	f	73	70	meth	wt
8	m	59	70	unmeth	wt

According to the applicant, early results in this population of patients are promising, with a median overall survival of 307 days (10 months). No additional information in this population was provided.

2.3.7. Discussion on clinical efficacy

Sitoiganap is a cell-based advanced therapy medicinal product. The clinical development plan submitted in support of the proposed CMA of Sitoiganap (Gliovac, ERC1671) in combination with GM-CSF, cyclophosphamide and bevacizumab in adult patients with recurrent/progressive glioblastoma and gliosarcoma patients (WHO grade IV malignant gliomas, GBM) is based on the preliminary results of a Phase II study (ERC1671-H02, NCT01903330) in n=9 patients (n=22 in updated analyses cut off 20 June 2020). In addition, the results of 10 patients with recurrent/progressive glioblastoma treated with Sitoiganap (without bevacizumab) under a compassionate use/hospital exemption protocol and n=8 patients who have received the proposed treatment combination (in addition to either nivolumab or pembrolizumab) in the context of the FDA 'Right-to-try' act have been presented as supportive evidence.

The proposed combination Sitoiganap, GM-CSF, cyclophosphamide and bevacizumab regimen as well as the claimed mechanism of action is not justified by a sound preclinical and clinical proof of concept/evidence and is based solely on general immunological concepts/assumptions. The non-clinical information lack good quality (raw) data, and is hampered by significant limitations in the design of the in vivo studies and in understanding of the relevance of the animal model. Furthermore, the product explored in pre-clinical studies does not seem to correspond to the product explored in the clinical setting.

In addition, no clinical dose finding study has been conducted, not only for Sitoiganap but also for the other components of the combination.

In absence of any pre-clinical or clinical support of a proof of concept, the argumentations to support the claimed mechanism of action and deliver PoC remain highly hypothetical. It is therefore unclear which components of the proposed combination (if any) are needed for activity and how the claimed immune response induction is triggered and maintained overtime. Thus, the justification for the proposed dose and dosing schema is lacking, not only for Sitoiganap but also for the other components of the combination.

It is underlined that none of the three additional medicinal products proposed as part of the treatment strategy are currently approved in the EU for the proposed indication and that GM-CSF is not available at the EU market.

Design and conduct of clinical studies

No formal dose ranging studies have been conducted as part of the clinical development program of Sitoiganap. It is stated that the dose has been selected based on preliminary preclinical and manufacturing data (described above), but further description of the dose selection has not been provided. No adequate justification has been presented for the selected dose, not only for Sitoiganap but also for all the other products intended to be given in combination.

Study ERC1671-H0 is an investigator-initiated, currently ongoing, randomised (1:1), double-blinded phase II trial comparing Sitoiganap - GM-CSF, - cyclophosphamide – bevacizumab vs placebo – bevacizumab in recurrent/progressive, bevacizumab-naïve glioblastoma multiforme and gliosarcoma patients. As stated by the applicant, the study was designed as an exploratory Phase II trial, and not to support regulatory decision making. Importantly, the current design according to which Sitoiganap is added to GM-CSF, cyclophosphamide and bevacizumab and compared to bevacizumab in addition to placebo, does not allow to establish the contribution of the different monocomponents to the proposed treatment strategy, i.e. the effect of Sitoiganap cannot be isolated.

As initial relevant issue, while a clinical study report document has been provided by the applicant related to trial ERC1671-H02 , it only consists of a short synopsis of the study protocol with a brief summary of few patient characteristics and results. Upon request a clinical study protocol (and several amended versions) has been presented by the applicant in the response document. The protocol seems to have been amended several (17) times, but detailed information on the amendments is lacking. An overview with time, nature and reasons for amendments is lacking, hampering any assessment of whether a data-driven approach was followed. A list of protocol deviations by patient has been presented, but no summarizing tables have been provided. Results are presented in the form of a few summary tables/graphs seriously hampering any critical assessment. These are considered major deficiencies of the dossier precluding any assessment. Moreover, relevant inconsistencies have been identified within the dossier raising serious concerns on the appropriate conduct of the study and the reliability of the data that would justify a GCP inspection. Furthermore, because of the major deficiencies in reporting, it is not possible to ensure that all the deficiencies, knowledge gaps or concerns related to evaluation of the effect of treatment have been identified.

While the general design of the study, i.e. randomised, double blind, controlled design is endorsed, multiple choices were made regarding the design of the study which hamper the assessment of the B/R based on this study. The primary objective of the trial as claimed by the applicant (6-months PFS probability, see assessment of answer to Q123 in the Clinical AR) is not considered able to support benefit in the target population. In view of the short life expectancy of these patients (far below 12 months), the lack of available subsequent treatment options, and the well-known difficulties in assessing (radiological) response and progression in the proposed target population, OS would represent the preferred endpoint to support clinical benefit. However, the allowance of cross-over at the time of progression complicates evaluation of OS and is thus very unfortunate. Moreover, despite the applicant's statement in answer to Q123, the protocol seems to have been amended to change the primary endpoint from 6-month PFS to 12-month OS, as suggested by the latest version of the study protocol submitted, the applicant's answer to Q124 and the clinicaltrials.gov website. As information on timing and reason for the change are missing, it is unclear whether protocol integrity was protected.

Further, the use of bevacizumab as control is highly questionable. It is emphasised that bevacizumab is not registered for the proposed indication in the EU, where, since introduction of temozolomide as first line, comparative studies in recurrent glioblastoma have typically used lomustine as comparator. Therefore, as already pointed out by the CHMP at the time of scientific advice, a comparator consisting of best investigator's choice including among others bevacizumab and lomustine would have been more appropriate to accommodate different practices in different regions. Similar concerns apply to

cyclophosphamide and GM-CSF, used in the experimental arm only, which are also not authorised in the EU for the proposed indication. In fact GM-CSF is not even marketed in the EU.

Furthermore, the choice for the relaxed alpha control (choice for one sided significance level of 20% (i.e. Type I error of 20%) is not acceptable for confirmatory evidences and also the described approach for data analyses (i.e., "secondary analyses will examine the data in many ways in order to inform future efforts in this area, without regard to study-wide type- 1 error") exemplify that this study should can only be seen as hypothesis generating and not appropriate to provide confirmatory evidence needed for a B/R assessment.

All-in-all it is clear that the study was performed as an exploratory exercise and not with the intention to support regulatory decision making, as has been acknowledged by the applicant.

The enrolment criteria define an adult high grade glioma population with recurrent/progressive glioblastoma and gliosarcoma (WHO grade IV malignant gliomas) after surgery/RT/temozolomide, who are bevacizumab naïve and lack of significant renal, hepatic and cardiovascular co-morbidities. In the response document the applicant has agreed to align the treated population with the one proposed in the wording of indication. However, the intended use of Sitoiganap, i.e., the fact that Sitoiganap is proposed to be administered as combination therapy with bevacizumab, GM-CSF and cyclophosphamide, should also be addressed in the wording of indication. Therefore the proposed indication would need to be further amended.

A clinical study protocol (and several subsequent amended versions) has been provided by the applicant clearly indicating the exploratory nature of the trial. The provided definition of endpoints is not considered unequivocal. For instance, regarding progression free survival, it is unclear whether only radiographic progression was considered an event or whether, and eventually how, events of clinical deterioration due to progression and / or death in absence of disease progression were counted. Similarly, radiographic response was categorised per iRANO ***or*** MacDonald Criteria, suggesting lack of homogeneity in the criteria implemented for assessment of response in all patients treated. Furthermore, it is unclear how response and progression was assessed in patients with no measurable disease after surgery. The frequency of radiologic assessment seems also to have been amended during the conduct of the trial, as observed in the different submitted versions of the study protocol. The impact of such amendments on the evaluation of study results has not been discussed by the applicant.

Regarding the statistical methods applied, no sound and appropriately pre-specified methodological and analytical approach was applied. The randomisation methods are not understood. It remains unclear why a rule that disallowed more than four consecutive assignments was included in addition to block randomisation. Such a rule does not fit within a valid randomisation procedure. If the SAS program included a random number seed then the randomisation process can be replicated, however it remains unclear whether the full randomisation procedure could be replicated given the first part was performed using Randomize.net and the second, stratified randomisation was generated using SAS. It is also unclear whether blinding could be properly maintained during conduct of the study.

Importantly, looking at the results presented at the time of request of CHMP scientific advice as well as the various updates of the study results published in the literature, it is clear that several unplanned interim analyses have been performed. It is also unclear whether access to the clinical trial database was managed according to guidelines. All these observations raise major concerns on the integrity of the trial and therefore on the reliability of the results presented. A GCP inspection is warranted.

The preliminary results of the study are essentially based on the analysis of the first 9 randomised patients (cut-off date 01 December 2017) representing 10.7% (9/84) of the planned patients. A subsequent update after enrolment of 22 patients has been provided (cut-off date 20 June 2020), but

it is presented in the form of abstract submitted for congress presentation. These patient numbers are considered too limited to allow any meaningful conclusion, also in the context of a CMA.

The patient flow is unclear and regarding the patients enrolled, very limited information has been presented on demographic and baseline characteristics, over distribution of patient parameters considered predictive for benefit of second-line therapy in high grade glioma (i.e., extent of disease, histologic grade (both at initial therapy and at recurrence), relapse-free interval, and recurrence pattern time) as well as on co-medications (especially dexamethasone), comorbidities and post-study therapies. This hampers the identification of imbalances able to potentially affect study results and thus the interpretability of the data. Recruitment dates should also be clarified.

Efficacy data and additional analyses

Results provided, as per 1 December 2017, include 9 patients, with a KPS $\geqslant$70, randomised and treated; one of which was non-evaluable, due to discontinuation prior to completion of the first cycle. Despite 6-months PFS is mentioned as primary study endpoint in the protocol submitted by the applicant, only results regarding response rate, median OS and median PFS have been presented. A cherry-picking data driven approach cannot be excluded.

ORR was 75% (3/4) in the experimental arm vs 25% (1/4) in the control arm. The duration of responses was not provided.

Median OS was 12.1 months (363 days) in the Sitoiganap arm (with one patient surviving more than 2 years) vs 7.6 months (229 days) in the control arm (with all patients having died within 1 year).

Median PFS was 7.3 months (223 days) in the Sitoiganap arm compared with 5.4 months (164 days) in the control arm. The relevance of this data as claimed by the applicant is not straightforward and results should be discussed.

The analyses presented were not performed according to an ITT principle, as the data of one patient enrolled in the experimental arm and discontinuing the study before completion of the first cycle where not included in the analyses. The data presented are also considered incomplete as only median values are presented without any information on 95% confidence interval values as well as HR and p-values.

The updated analyses presented after enrolment of 22 patients (cut off 20 June 2020) present the same deficiencies as outlined for the primary analysis. Furthermore, it is noted that no statistically significant difference in OS is observed between study arms (logrank p<0.18) and that median OS appears significantly reduced in the experimental arm at the updated analysis (8.7 months) compared with the previous analysis (12.1 months). This analysis presented includes patients that crossed over to Sitoiganap after progression, implicating the introduction of a selection bias. Information regarding patients who crossed over to the 'active group' is not available. In this regard, the numbers analysed within this trial should also be clarified, in particular the ITT population as well as per protocol population have not been provided.

Major methodological deficiencies have been identified in the inter-study comparisons presented by the applicant with historical controls analyses presented. For example the study published by Barker was performed in another setting and with a different approach (it essentially consisted of a retrospective evaluation of patients enrolled in two trials who underwent secondary surgical resections) hampering a fair comparison, and was published in 1998 and it is therefore unclear whether the standard of care applied at that time could be considered representative for the one currently applied in EU for this condition. Also regarding the other mentioned studies involving bevacizumab no matching of patient disease and baseline characteristics has been done. In addition, the database of Sitoiganap treated patients used for these comparisons is not clear, and patient selection cannot be excluded. Overall a data driven approach for these inter-study comparisons cannot be excluded and reliability of the

claimed treatment effect is uncertain. Thus the conclusions claimed by the applicant over superiority of Sitoiganap on historical controls are not supported and results can be considered exploratory at best.

The analyses aimed to capture a correlation of CD3+/CD4+ lymphocyte counts with OS in patients treated with Sitoiganap-GM-CSF-cyclophosphamide-bevacizumab are considered highly exploratory at this stage and the results not conclusive. Special populations, e.g. elderly as well as patients with renal and hepatic impairment should in principle be studied and if no data are available the potential differences in these populations should be discussed and justified.

Methods used to characterize patients according to MGMT status (a stratification factor) and the presence of IDH mutations have not been described albeit patients included in the study were expected to be characterised in that respect (of note only data from the first 9 patients randomised seem to be available). Moreover, the implications of the tumour characteristics in relation to the treatment with Sitoiganap, or the concomitant treatments proposed to be administered together with Sitoiganap (i.e. bevacizumab, GM-CSF and cyclophosphamide) have not been discussed.

2.3.8. Conclusions on clinical efficacy

The dossier submitted by the applicant is insufficient for a B/R assessment. A proof of concept for the claimed activity of the drug/combination proposed is lacking. Major deficiencies and inconsistencies have been identified in the dossier raising serious concerns on the conduct of the study and the reliability of the results presented, where a cherry picking data driven approach cannot be excluded. Further to the above, the current design does not allow to establish the contribution of the different monocomponents to the proposed treatment strategy, i.e. the effect of Sitoiganap cannot be isolated in the intended treatment setting. All these issues, together with the very limited number of patients included in the analyses presented, do not allow a B/R assessment and would justify a GCP inspection. However, a GCP is not triggered at this time considering that it will not be able to solve all other major deficiencies of the dossier identified.

2.3.9. Clinical safety

The pivotal study for the proposed indication for treatment of adult patients with recurrent glioblastoma is pivotal study NCT01903330: a randomised, double-blinded, placebo-controlled Phase 2 study of Sitoiganap in combination with bevacizumab. Patients in the sitioganap arm in this study were treated Q28 days with Sitoiganap (4 different components ERC-A through D; whole tumour cells [1 x 10^5 - 1 x 10^6 cells]), GM-CSF 500µgr (both on D6, D9, D12, D15, D18); cyclophosphamide 2x 25mg (D2-5) and bevacizumab 10mg/kg (D1, D15). Control arm patients were to be treated with bevacizumab 10mg/kg (D1, D15) plus placebo (oral placebo D2-5, injectable placebo D6, D9, D12, D15 and D18).

Safety evaluation

Patients enrolled in this study were evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements [D1,D6,D12,D18 of every cycle until EOT]. AEs are graded for severity using NCI Common Terminology Criteria for Adverse Events v.4.0. Patients were evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study. Patients discontinued from the treatment phase of the study for any reason were evaluated ~30 days (28–42 days) after the decision to discontinue treatment.

Bevacizumab-Specific:

Hypertension was monitored through routine evaluation of blood pressure prior to each bevacizumab treatment. Optimal control of blood pressure according to standard health. In patients with bleeding, hemostasis evaluation, was to be performed as clinically indicated. Proteinuria will be monitored through urinary protein/creatinine ratio or urine dipstick for protein performed on spot urine at least every 6 weeks. Patients who have an ongoing bevacizumab -related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed. If patients on treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure.

Patient exposure

The total extent of exposure to sitoiganap has not been reported.

Adverse events

Data reported as of December 1st, 2017 - eight patients enrolled – which completed the safety lead-in analysis of the ongoing, Phase II, double-blinded, placebo-controlled study. As per treatment protocol, nine study participants were unblin[d]ed. Unblinding revealed that four patients had received Sitoiganap, four had received placebo. One patient was marked as non-evaluable due to discontinuation prior to completion of the first cycle. Median age was 57, with 2/8 female patients. The average KPS was 80 (70-100).

Clinical results in the pivotal study for toxicity show an equal distribution of AEs between the active treatment and placebo groups, with no grade 4 or 5 toxicities (Table). The most common adverse events were "Heacache'[Headache] and Injection site reaction.

Among documented grade 3 toxicities, headaches were the most common. Among all toxicities, injection site reactions (induration, erythema and ulceration) were most frequently noted. Although these skin reactions were mild, they indicated the development of immune responses. However, they were not consistently noted in all patients. Similarly, other observed mild systemic reactions, including self-limiting fever and chills, represent expected outcomes related to the intended immune stimulation.

Table 7 - **Toxicities (only the grade 3 toxicities or the toxicities reported at least as 5 separate events are highlighted as separate rows).**

Adverse Events (ERC1671 plus Bevacizumab)	Grade 3	Total
Injection site reaction	0	67
Arthralgia	0	70
Gait disturbance/Fall	1	4
Back Pain	1	6
Headache	2	9
Anxiety	0	6
Total Events of any grade	**4**	**162**

Adverse Events (Placebo plus Bevacizumab)	Grade 3	Total
Gait disturbance/Fall	1	38
Muscle weakness	2	4
Hydrocephalus	2	7
Delirium	1	1
Urinary Incontinence	1	4
Thromboembolic event	1	4
Total Events of any grade	**8**	**58**

Updated results (June 20, 2020)

To date 22 recurrent bevacizumab-naïve GBM patients have been randomised to Sitoiganap/GM-CSF/Cyclophosphamide + Bevacizumab or Placebo + Bevacizumab. Of the 22, two discontinued before completing one cycle of therapy and two remain on blinded treatment. Currently 18 patients are unblinded due to further progression: 8 were on vaccine and 10 on placebo. Five of those on placebo crossed to vaccine at progression. All but one of the 18 are now deceased.

Clinical results for toxicity show no difference in the distribution of AEs between the Vaccine and Placebo groups, with no Gr4/Gr5 AEs in either group. The only Grade 3 AE were attributed to Bevacizumab (HTN, wound dehiscence, HA). No SAE were noticed immediately following the vaccination (report to November 2019).

Table 8 - **Toxicities and grades**

ERC1671 (active group and cross-over

Attribution Grade	Unrelated Or Unlikely	Possible	Probable	Definite	Total
1	75 (27.3%)	5 (1.8%)	25 (9.1%)	83 (30.2%)	188 (68.4%)
2	47 (17.1%)	5 (1.8%)	13 (4.7%)	5 (1.8%)	70 (25.5%)
3	10 (3.6%)	3 (1.1%)	3 (1.1%)	1 (0.4%)	17 (6.2%)
4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	132 (48.0%)	13 (4.7%)	41 (14.9%)	89 (32.4%)	275 (100%)

Placebo Group (no cross-over)

Attribution Grade	Unrelated Or Unlikely	Possible	Probable	Definite	Total
1	28 (37.3%)	3 (4.0%)	9 (12.0%)	9 (12.0%)	49 (65.3%)
2	15 (20.0%)	0 (0.0%)	2 (2.7%)	0 (0.0%)	17 (22.7%)
3	9 (12.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (12.0%)
4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	52 (69.3%)	3 (4.0%)	11 (14.7%)	9 (12.0%)	75 (100%)

Updated results- 1st response to the RSI

This summary is based on all subjects receiving at least one dose of blinded study treatment from 27 March 2014 through 4 May 2020. The number of such subjects is 22. As of 30 June 2020, 19 (86.4%) of the 22 participants are deceased. Half of the 22 participants were randomised to active treatment (N=11) and a half to placebo (N=11). One patient assigned to active therapy was lost to follow up after only six days of treatment. Maximum days on blinded treatment was 357 (median = 128.5; total patient days = 3319). Five of the 11 patients randomised to placebo elected, upon disease progression, to cross over to active therapy. One of these was on active treatment for only eight days.

Maximum days on active therapy as cross-over was 176 (median = 43; total patient days = 390).

Table 9 - Number of patients and patient-days on treatment

	Treatment Type				
	Active Vaccine			Placebo	
Source	N	Patient Days		N	Patient Days
Randomization	11	1394		11	1925
Cross Over	5*	390		0	0
TOTAL = 3709	xx	1784		xx	1925

Table 10 - **Number and rate of all recorded AE by treatment type (with approximate 95% confidence intervals on the rate of AE per patient-treatment days**

	Treatment Type		
	Active Vaccine	Placebo	Pool
Number of AE	259	195	454
Patient Days on Treatment	1784	1925	3709
Rate (AE/Pat Days Tx)	0.145	0.101	0.122
95% CI Lower Bount	0.128	0.087	0.111
95% CI Upper Bound	0.164	0.117	0.135

These approximate 95% CI suggests that active vaccine may confer a greater risk of AE of all types than placebo, even though the number of patient days on active therapy is somewhat smaller than the placebo condition. What follows are stratifications aimed at illuminating the nature of those AE more prevalent under active treatment.

Table 6 - Grade of observed AEs by treatment type

	Treatment Type					
	Active		Placebo		Pool	
Grade	N of AE	Col %	N of AE	Col %	N of AE	Col %
Grade 1	192	74.13	124	63.59	316	69.60
Grade 2	52	20.08	51	26.15	103	22.69
Grade 3	15	5.79	20	10.26	35	7.71
Total	259	100	195	100	454	100

Note there is no AE of grade four or above. For each treatment type, the majority of AE is grade one. The distribution of grades seems similar between the two treatment types, except for grade 1 AEs, which are seen more often in the active treatment group (explaining the Table 2 data).

Table 12 - Grade of observed AEs by attribution and treatment type

Assessed Relation to Study Treatment	Treatment Type											
	Active Treatment						Placebo					
	Grade						Grade					
	1		2		3		1		2		3	
	N AE	Col %	N AE	Col %	N AE	Col %	N AE	Col %	N AE	Col %	N AE	Col %
Unrelated	53	27.60	30	57.69	10	66.67	70	56.45	35	68.63	16	80.00
Unlikely	4	2.08	7	13.46			3	2.42	1	1.96		
Possibly	18	9.38	3	5.77	2	13.33	8	6.45	5	9.80	2	10.00
Probably	41	21.35	8	15.38	2	13.33	23	18.55	7	13.73	2	10.00
Definitely	76	39.58	4	7.69	1	6.67	20	16.13	3	5.88		
Total	192	100	52	100	15	100	124	100	51	100	20	100

Under placebo conditions, the majority of AE were thought unrelated to study treatment for each grade. Under the active treatment, the majority of AEs were considered unrelated to the study treatment for grades 2 and 3, but not for grade 1.

ERC is reporting data from the report all-causality adverse events by System Organ Classification (SOC) and preferred term (PT) in accordance with ICH-E3. The period covered is from the date the first dose of blinded study treatment was administered to the first participant (27 March 2014) through the end of June 2020. A summary of reported adverse events is as follows:

Table 7 - List of AE descriptions thought probably or definitely related to study treatment by treatment type: grade 1

Abbreviated Description	Treatment Type					
	Active Treatment			Placebo		
	N AE	Col %	N pat	N AE	Col %	N pat
Injection-site reactions[1]	109	93.2	6	2	4.7	2
Headache[2]	3[2]	2.6	3	11	25.6	4
Fatigue	2	1.7	2	8	18.6	4
Irritation	2	1.7	1			
Flu-like symptoms	1	0.9	1			
Hypertension[3]				18[3]	41.9	3
Epistaxis[4]				3[4]	7.0	2
Intracranial Hemorrhage[5]				1[5]	2.3	1
Total	117	100	xx	43	100	xx

[1] includes cases where injection-site redness, itch, swelling, bruising are mentioned
[2] Headache in the active-treatment condition: 1/3 mentions bevacizumab
[3] Hypertension in the placebo condition: 13 of 18 instances mention bevacizumab: 8/8 in one subject and 5/8 in another
[4] Epistaxis in the placebo condition: 3/3 mention bevacizumab
[5] Intracranial Hemorrhage in the placebo condition mentions bevacizumab

Table 8 - List of AE descriptions thought probably or definitely related to study treatment by treatment type: Grade 2

Abbreviated Description	Treatment Type					
	Active Treatment			Placebo		
	N AE	Col %	N pat	N AE	Col %	N pat
Hypertention[1]	5	41.7	2	5	50.0	2
Thrombosis[2]	3	25.0	2	2	20.0	2
Redness	2	16.7	1			
Hemorrhage[3,4]	1[3]	8.3	1	1[4]	10.0	1
Fatigue[5]	1	8.3	1			
Vasogenic Edema[6]				1	10.0	1
Headache				1	10.0	1
Total	12	100	xx	10	100	xx

[1] In the active-treatment condition, 5/5 instances of hypertension mention bevacizumab. In the placebo condition 3/5 mention bevacizumab. The same cross-over patient accounts for 4/5 instances under active treatment and 3/5 under placebo (024).
[2] In the active-treatment condition, 2/3 instances of thrombosis mention bevacizumab. A cross-over patient is responsible for 2/3 under active treatment and 1/2 under placebo (008).
[3] The single case of hemorrhage under active treatment is hemorrhoidal, and bevacizumab is mentioned. The single patient is a cross-over (024)
[4] The single case of hemorrhage under placebo is intracranial and mentions bevacizumab. The patient is a cross-over (010)
[5] Under active therapy, bevacizumab is mentioned in the complaint of fatigue, which is from a cross-over patient (024)
[6] Under the placebo, the case of vasogenic edema is from the same subject as the intracranial hemorrhage (010)

Table 95 - List of AE descriptions thought probably or definitely related to study treatment by treatment type: Grade 3

Active Treatment		
Description	N AE	N Patients
Wound Dehiscence (bevacizumab)	1	1
Headache (bevacizumab)	1	1
Rash (Pruritic)	1	1
Total	3	2[1]
Placebo		
Description	N AE	N Patients
Headache (bevacizumab)	1	1
Hypertensive Urgency (bevacizumab)	1	1
Total	2	2

[1] In the active treatment group, the wound dehiscence and headache occurred in the same patient.

Patient listings were provided which specify the type of AEs recorded per patient, the onset and end date of AEs, the grade and whether the AE was considered serious. It was also registered whether medication was administered (yes/no) and any procedure was performed (yes/no) and whether the AE was considered related to a treatment component (bevacizumab, GM-CSF, cyclophosphamide, sitoiganap). It was also indicated whether treatment components were interrupted or discontinued. In addition, patient listings of laboratory abnormalities and injection site reactions were provided.

Safety reporting in compassionate use patients

In compassionate use patients (PD study report 1) it is reported that no significant side effects potentially attributable to the combination were witnessed. In the phase 0/ compassionate use treated patients (Study report 2) it is reported that no significant side-effects potentially attributable to the

combination were witnessed. For all 8 patients, only local erythema (4 patients) and headaches (2 patients) were reported as AE. Both the HA's and local erythema were rated as grade 2 or less.

Serious adverse events and deaths

Serious adverse events

No high grade toxicity has been observed at the interim results of the clinical trial.

Deaths

In the case glioma, it should be notice that patient death is the outcome observed inevitably. There was no death reported as provoked by Sitoiganap administration.

Laboratory findings

Not available.

Safety in special populations

Not reported.

Immunological events

Not reported.

Safety related to drug-drug interactions and other interactions

Not reported.

Discontinuation due to AES

Not reported.

Post marketing experience

Not reported.

2.3.10. Discussion on clinical safety

It appears that the safety profile for Sitoiganap is intended to be established based on the safety data from phase 2 study NCT01903330: a randomised, double-blinded, placebo-controlled study of Sitoiganap in combination with bevacizumab versus bevacizumab+placebo in patients with recurrent/progressive glioblastoma and gliosarcoma - WHO classification grade IV malignant gliomas. Patients in the Sitoiganap arm were also treated with GM-CSF and cyclophosphamide. Thus, note that AEs are reported for Sitoiganap combination therapy and that the contribution of Sitoiganap to the observed safety profile is unclear due to the concurrent therapies given.

In general, the clinical study report (CSR) related to trial ERC1671-H02/NCT01903330 only consists of a short synopsis of the study protocol with a brief summary of few patient characteristics and some efficacy and safety results. There are large deficiencies in the reporting of safety data and relevant details to explain what data are presented have not been provided. In the response to the RSI some listings of reported AEs, injections site reactions and laboratory abnormalities per patient were provided however overall the safety data collected lacks most data requested, the data is not detailed and complete enough.Thus, the safety reporting is not considered to be in accordance with ICH E3 (CPMP/ICH/137/95). Furthermore, multiple inconsistencies in the reported data can be noted throughout the CSR. As a consequence the assessment of the safety findings is severely hampered and the interpretability is limited. It is therefore also impossible to ensure that all deficiencies, uncertainties or knowledge gaps in the safety profile of Sitoiganap have been identified during this assessment.

Furthermore, the quality dossier (Module 3) of Sitoiganap shows major deficiencies with regard to critical aspects such as product characterisation, the description and control of the manufacturing process, the manufacturing process characterisation and validation, and the drug product release and stability testing. This raises concerns with regard to product quality, and consequently its safety (and efficacy).

Data on the extent of exposure have not been provided. Throughout the CSR Sitoiganap treatment is mentioned in the pivotal study, in "PD studies" or via hospital exemption and in a Phase 0 study, however reports on the patient numbers are inconsistent and therefore the total number of patients exposed to Sitoiganap is unknown. Moreover, the number of patients exposed to specified dose levels and the numbers exposed for a given duration are unknown. Further, relationships between safety and dose were not established.

The population for the primary safety analysis is not defined and the time frame for safety reporting is not clear. It appears that the safety population is intended to consist of patients in the pivotal study which completed the safety lead-in analysis and that have been unblinded and assigned to treatment with Sitoiganap. This would imply that the evaluation of the safety profile is based on either 4 of 5 patients in the Sitoiganap arm, and 4 patients in the control arm (data cut-off December 2017). From the updated analyses (data cut-off June 2020) it seems that 8 patients have been treated with Sitoiganap. Ten patients were randomised to the control arm (placebo plus bevacizumab) of whom 5 seem were crossed-over to receive the experimental arm at progression. In the response to the RSI, the applicant indicated that the start of the study is 27 March 2014 and that updated safety data for N=22 patients was collected through the earlier of death or onset date of the most recent AE prior to 30 June 2020. This is not understood as it appears that AEs were monitored until 30 June 2020 unless patients died, while in the CSR it was stated that "Patients discontinued from the treatment phase of the study for any reason were evaluated ~30 days (28–42 days) after the decision to discontinue treatment." It is also not understood how an onset date of an AE can mark the end of AE reporting.

Even when there would be no deficiencies in the reporting, this number of patients is considered far too small for evaluation of the safety profile of sitoiganap, also when considering the rarity of the disease (EMA/CHMP/205/95 Rev.5).

There are very large deficiencies in the reporting of AEs. Information (e.g. numbers, severity, listings) on all-causality and drug-related AEs occurring on treatment by the System Organ Classification (SOC) and preferred term (PT) were not provided. These deficiencies do not allow to determine frequently occurring AEs, to compare the toxicity between the two study arms and to determine safety concerns/specifications.

The following AEs have been described to occur: headache, injection site reactions (induration, erythema and ulceration), arthralgia, gait disturbance/fall, back pain, anxiety, self-limiting fever and chills. Headache is described to be the most frequently occurring Grade 3 AE and no Grade 4 AEs were

reported in the sitoiganap arm. The number of patients in which these AEs occur is not reported. Note that these AEs are observed in patients receiving Sitoiganap in combination therapy and it is unclear if they can be attributed to Sitoiganap or to the other components of the treatment paradigm. In the control arm AEs gait disturbance/fall, muscle weakness, hydrocephalus, delirium, urinary incontinence and thromboembolic event have been described to occur. There were also Grade 3 AEs observed attributed to bevacizumab (wound dehiscence, HTN and HA [abbreviations not explained]), however it is unclear at which frequencies and in which arm these were observed. In the response to the RSI updated safety data was provided. The applicant did not present a summary of all-causality AEs but a summary of Grade 1-3 AEs "thought probably or definitely related to study treatment" was provided. These AEs were not reported using PTs and SOCs. Overall, because of the above mentioned limitations in safety reporting it is not possible to interpret these data.

Of note, also from the non-clinical package no clinically meaningful and clinically predictive safety data could be obtained due to several limitations and uncertainties related to the animal model, treatment regimen (in relation to the clinic), type/choice of parameters (and tissues) evaluated and time point(s) of analysis (refer to NC report).

It is reported that no SAE were noticed immediately following the vaccination and that there was no death reported as provoked by Sitoiganap administration. However, these statement are very general and do not exclude the possibility of SAEs and treatment related deaths during the study. Also more detailed data (including listings) on SAEs and deaths during the study have not been provided. In the response to the RSI, it was indicated that as of 30 June 2020, 19 (86.4%) of the 22 participants are deceased and the deaths were all believed to be from progression of disease. However, more detailed data to support these statements were not provided. Further, data on AEs leading to discontinuation or treatment modifications have not been provided. Therefore SAEs, and other significant adverse events cannot be identified and it is unknown whether AEs may be lethal. It is also unknown whether certain events require monitoring. Further, the reversibility, tolerability and management of the toxicity of sitoiganap cannot be determined.

A list of laboratory abnormalities was provided, however no summarizing tables were submitted and thus frequently occurring laboratory test changes for Sitoiganap cannot be established.

No safety data in special populations was provided, though the small sample size of the study prohibits meaningful subgroup analyses. As a consequence patient groups at increased risk for more toxicity could not be determined.

Formal DDI studies as outlined by the Guideline on the Investigation of Drug Interactions have not been performed and this is acceptable given the nature of the product. No data on overdoses or on immunological events has been provided. Note that only sparse data have been provided on immunological response in the efficacy section and therefore it is not known what type of immunological response can be observed after Sitoiganap, GM-CSF, cyclophosphamide and bevacizumab administration. It is also unclear whether Sitoiganap may elicit auto-immunity.

In the phase 0/compassionate use treated patients it is reported that no significant side-effects potentially attributable to the combination were witnessed. However, also here important details on the design/methods of these studies and on the methods for collecting safety data have not been reported and the available data are of poor quality, which hampers the interpretability of these data. Therefore, the contribution of these data to the assessment of the safety of Sitoiganap is limited.

The above described deficiencies and limitations to the safety data do not allow to interpret the provided safety data. As a consequence, it is not possible to determine the safety profile for Sitoiganap, the safety concerns/specifications cannot be established and the safety information in the SmPC cannot be assessed. The applicant should resolve the deficiencies and report safety data in

accordance with ICH guidelines. Further, the large deficiencies in the safety data and the poor quality of the available data questions whether the collection of the safety data was conducted in accordance with GCP (ICH E6; EMA/CHMP/ICH/135/199) and ICH E2A (Clinical Safety Data Management; CPMP/ICH/377/95). A GCP inspection is considered appropriate.

2.3.11. Conclusions on clinical safety

Overall, large deficiencies have been noted in the safety reporting. For instance, the extent of exposure is unknown, frequently occurring AEs cannot be established and it is not possible to identify SAEs, other significant adverse events, AEs resulting in death and safety concerns/specifications. Also management and tolerability of Sitoiganap toxicity cannot be evaluated. As a consequence it is not possible to determine the safety profile for Sitoiganap, nor to determine which information regarding the safety profile should be provided to the health care professional in the (safety related) sections in the SmPC .

Moreover, the safety data which have been provided are of poor quality, and inconsistencies are noted throughout the CSR. As a consequence the provided data are not interpretable. Further, the small sample size, and the administration of concomitant medication (bevacizumab, cyclophosphamide and GM-CSF) also hinder the evaluation of the safety profile of Sitoiganap.

Because of all the deficiencies and the poor quality of the safety reporting a GCP inspection is considered justified and could be triggered at a later stage depending on the responses provided by the applicant to determine whether collection of the safety data has been performed in according with ICH guidelines and to verify the reliability of the safety reporting.

Of note, new issues may arise if the requested data is provided by the applicant.

2.4. Risk management plan

2.4.1. Safety Specification

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table 106 - SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Non-specific systemic reaction (Fatigue, fever, arthralgia, myalgia, back pain and headache) Gait disturbance/fall Injection site reaction Anxiety
Important potential risks	Anaphylaxis Immune-related liver disorder Immune-related kidney disorder Gastrointestinal disorder Endocrinopahty Embryofetal toxicity
Missing information	Safety profile in patient with comorbid disease Safety profile in immunodeficient patient

2.4.2. Discussion on safety specification

The proposal for safety specifications by the applicant cannot be assessed. The most important reason for this is that the safety profile for Sitoiganap could not be established due to the poor quality of the presented data, the small sample size of the pivotal study and large deficiencies in the safety reporting (refer to safety discussion). There are also many deficiencies in the non-clinical, pharmacology and quality reporting, which also do not allow to assess the data provided by the applicant.

Further, there are several comments to be made on the data that has been provided in the RMP. In general, the structure and content of the risk management plan do not fulfil the requirements of GVP module 5,rev.2, nor does the applicant follow the respective Guidelines on risk minimisation. The data provided in the RMP is of poor quality. It appears that the applicant has listed most of the AEs which have been reported to occur on Sitoiganap treatment as safety specifications. However, it should be noted that important identified risks should be undesirable clinical outcomes for which there is sufficient scientific evidence that they are caused by the medicinal product. Potential risks should include only the risks that are undesirable clinical outcomes and for which there is scientific evidence to suspect the possibility of a causal relationship with the medicinal product, but where there is currently insufficient evidence to conclude that this association is causal. Both should warrant further evaluation as part of the pharmacovigilance plan and risk minimisation activities (EMA/838713/2011 Rev 2). Further, several of the risks are described using very broad terms (e.g. systemic reaction, Immune-related liver disorder, immune-related kidney disorder, gastrointestinal disorder, endocrinopathy, immunodeficient etc) and require re-formulation.

In the RMP section "Details of important identified risks, important potential risks, and missing information" (SVII.3) the backgrounds for selecting the safety specifications are listed. However, the information by the applicant is not always understood, often not supported by (non) clinical data of Sitoiganap, not adequately motivated, arguments for preventability are not understood and not described with sufficient details and without supportive scientific evidence. Moreover, the text does not appear to follow guidelines (EMA/838713/2011 Rev 2) and guidance. In the response to the RSI the applicant indicated that there was no data in women who are pregnant or lactating. It is not understood why these patients were not listed under missing information in the RMP.

An updated RMP was submitted, but the applicant did not address the major deficiencies in the RMP, as described in the AR and the LOQ Other Concerns.

Thus, the issues related to the establishment of the safety profile for Sitoiganap as well as the issues related to the data provided in the RMP should be resolved before conclusions on the safety specifications can be made.

2.4.3. Conclusions on the safety specification

Having considered the data in the safety specification, it is considered that the following issues should be addressed:

- The structure and content of the risk management plan do not fulfil the requirements of GVP module 5, rev. 2 nor does the applicant follow the respective Guidelines on risk minimisation on SAFETY AND EFFICACY FOLLOW-UP - RISK MANAGEMENT OF ADVANCED THERAPY MEDICINAL PRODUCTS EMEA/149995/2008. The quality of data presented in the RMP precludes a decision at this stage on the pharmacovigilance and risk minimisation activities needed for the safe and effective use of the product. The applicant is asked to address the major deficiencies in the RMP, as described in the AR and the LOQ Other Concerns and provide an updated RMP of sufficient quality to support a decision regarding the required pharmacovigilance and risk minimisation activities.

- The safety profile for Sitoiganap could not be established due to the poor quality of the presented data, the small sample size of the pivotal study and large deficiencies in the CSR. There are also many deficiencies in the non-clinical, pharmacology and quality reporting. These should be resolved before a conclusion can be made on the adequacy of the important identified/potential risk and missing information. Further, the applicant should justify why the important identified risks and the important potential risk influence the B/R balance, warrant further evaluation as part of the pharmacovigilance plan and risk minimisation activities in accordance with the GVP guideline (EMA/838713/2011 Rev 2). Also note that in the RMP sections on the safety specifications, particularly in the section "Details of important identified risks, important potential risks, and missing information" rewording and reformulation is needed, in accordance with the GVP guideline and guidance.

2.4.4. Pharmacovigilance plan

Routine pharmacovigilance activities

Routine pharmacovigilance practices involve the following activities:

- Describing, establishing and maintaining a pharmacovigilance system for listed drug product.
- SOPs for Individual case safety reports (ICSR) handling, reporting, quality management, signal detection, database set-up, privacy protection.
- Maintenance of the central data processing system, data entry.
- Communication of safety concern and variation
- Handling of spontaneous and clinical trial case reports.
- Adverse reaction reports collection.
- Adverse reaction evaluation.
- Signal detection and signal management.
- Follow-up on missing information.
- Detection of duplicates, duplicate check.
- Preparation of Periodic safety update report (PSURs), and Risk management plan (RMPs).
- Reporting to authorities if necessary.
- Risk management plan development and fulfillment.

- Introduction of changes related to safety of Sitoiganap or pharmacovigilance system in registration materials.

Other forms of routine pharmacovigilance activities for safety concerns:

The following activities will be performed to:

Objective	**Milestone**
Control of product distribution	Request a "Treatment access form" for each patient
Enhanced passive surveillance	Request the "Follow up form" at least 1x/cycle (month)

Analysis of risk-benefit, review of AEs	Periodic safety report (1x/year)
Communication of safety concern	DHPC writing
Implementation of variation	EMA submission

Additional pharmacovigilance activities	
Non-Clinical	NA
Clinical - Interventional	SITOIGANAP-H02 - Ongoing : Safety and efficacy in relapsing high grade glioma PASS – To plan : Post-authorisation safety study
Clinical – Non interventional	

PRAC Assessment comment

Above tables do not fulfil requirements of the GVP module V, re.2 in the respective section and need to be revised. In addition, content, respectively planned pharmacovigilance activities need to mentioned explicitely per risk. The MAA is requested to use the templates provided by EMA for the tables and sections.

2.4.4.1. Summary of planned additional PhV activities from RMP

Table 17- On-going and planned additional pharmacovigilance activities

Study Status Category	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
ERC1671-H02 Phase 2 Study of ERC1671 in Recurrent Glioblastoma Multiforme On-going	To evaluate safety and tolerability of ERC1671 plus bevacizumab among patients with recurrent glioma. To determine the 6-PFS in relapsing patient treated with ERC1671 vs placebo To evaluate radiographic response, PFS and OS of patients with recurrent glioma treated with ERC1671 plus bevacizumab To characterise of the immune response to ERC1671 treatment in adult patients with relapsed glioma.	General safety and tolerability of ERC1671 plus bevacizumab among patients with recurrent glioma.	Start Date Study completion	March 2014 March 2019
ERC1671-H04 Long term safety study **PASS** Planned	Primary • To further evaluate the longterm safety profile of ERC1671 in the treatment of patients with glioma when used under conditions of routine clinical care	- Long term safety - Adverse events under condition of routine clinical care	Protocol submission Regular updates	July 2018 Data will be reviewed on an on-

	• To evaluate the incidence of allcause mortality and adverse events of special interest in patients with glioma. Secondary • To further evaluate the longterm effectiveness of ERC1671 in the treatment of patients with glioma when used under conditions of routine clinical care • To quantify discontinuation of treatment due to adverse events or due to lack of or loss of therapeutic response. • • To further elucidate the risk of abnormal liver function tests • To further elucidate the risk of abnormal renal function tests			going basis as a part of signal detection and reported within PSURs, when available.

To define with EMA

PRAC assessor's comment

Though the MAA provided a structurally revised RMP, which formally complies with the GVP module V format rev.2 in its major requirements the content still does not fulfil the requirements (e.g.missing information on clinical trial details, scientific background, PHVplan adjustments). Particularly, the parts including data on which the PHV-plan is based (safety concerns-justifications for inclusion e.g.) have not been updated or further information added for a more comprehensive view. It is still not possible to assess the eligible safety concerns from the data presented and consequently it is rather not possible to assess whether the routine measures proposed are able to minimise the named risks sufficiently.

The PHV-plan as presented above is still not acceptable. The data the PHV plan is based on, are far too limited to conclude on any risks arising from treatment.

A draft version of the PASS protocol was not provided with responses to LOQD120 and is mandatory for further assessment.

Conclusion

The MAA should further substantiate the risk minimisation measures proposed in relation to the safety concerns. Importantly scientific background of each safety concern and (when applicable) context of its occurrence in the respective clinical trial needs to be presented more in detail.

The risk minimisation measures and the PHV plan as provided above are not acceptable respectively insufficient. A PASS protocol has not been drafted and provided yet. The MAA has again added a remark "to be defined with EMA" which is not foreseen in the application procedure. It is the MAA's obligation to present appropriate pharmacovigilance measures, which will then be evaluated by the PRAC whether they are sufficiently monitoring (and minimising) the identified risks.

The MAA is requested to present a Pharmacovigilance plan which addresses all risks identified sufficiently and further to this in case of a PASS proposed to monitor a draft of the PASS protocol must be provided with responses to LOQ.

2.4.4.2. Overall conclusions on the PhV Plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that the proposed post-authorisation PhV development plan is not sufficient to identify and characterise the risks of the product and the applicant should propose PhV studies.

The applicant should propose a post-authorisation PhV development plan sufficiently monitoring the risks.

2.4.5. Plans for post-authorisation efficacy studies

Summary of Post authorisation efficacy development plan

Table 18- Planned and on-going post-authorisation efficacy studies that are conditions of the marketing authorisation or that are specific obligations.

Study **Status**	**Summary of objectives**	**Efficacy uncertainties addressed**	**Milestones**	**Due Date**
Efficacy studies which are conditions of the marketing authorisation				
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				

PRAC assessment comment *In view of the sparse and incomprehensible data set of the clinical development studies a post authorisation efficacy study with integrated safety aspects would be very much endorsed.*

2.4.6. Risk minimisation measures

Routine Risk Minimisation Measures

Summary of additional risk minimisation measures

Table 19- Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Please see below the table from the updated RMP v0.4 from response to LOQD120

Safety concern	Routine risk minimisation activities	Additional risk minimization measures
Non-specific systemic reaction	EU SmPC, Section 4.8 EU SmPC Section 4.4	None
Gait disturbance/Fall	EU SmPC, Section 4.8	None
Injection site reaction	EU SmPC, Section 4.8	None
Anxiety	EU SmPC, Section 4.8	None
Anaphylaxis	EU SmPC, Section 4.8	None
Immune-related liver disorder	EU SmPC, Section 4.8	None
Immune-related kidney disorder	EU SmPC, Section 4.8	None
Gastrointestinal disorder	EU SmPC, Section 4.8	None
Endocrinopathy	EU SmPC, Section 4.8	None
Embryofetal toxicity	EU SmPC, Section 4.6 EU SmPC, Section 4.8	None
Safety profile in patient with comorbid disease	EU SmPC, Section 4.8	None
Safety profile in immunodeficient patient	EU SmPC, Section 4.3 EU SmPC, Section 4.5 EU SmPC, Section 4.8	Contraindications
Legal status	EU SmPC, Section 4.2	

Table 20- Description of routine risk minimisation measures by safety concern

Safety concern	Routine risk minimisation activities	Additional risk minimisation measures
Non-specific systemic reaction	EU SmPC, Section 4.8 : Patients treated with SITOIGANAP have an increased risk of non-specific systemic reaction, e.g, skin reaction, back pain, arthralgia and headache. Systemic reactions are common reactions following immunisation and can be treated according to corresponding guidelines. They are expected according the mechanism of action of SITOIGANAP. Headache is the second most frequently noted side effect upon SITOIGANAP administration. It may also indicate the development of an immune response efficient in the brain. However, it is not consistently noted in all patients and no clear correlation between efficacy and headache or other systemic reaction can be concluded. Monitoring of the reaction should be performed. If severity implies use of corticosteroids, SITOIGANAP should be temporarily stopped. EU SmPC Section 4.4 : Immunosuppressive drugs and corticosteroïds may not be used concomitantly with SITOIGANAP. SITOIGANAP can be temporarily stopped if necessary.	None
Gait disturbance / Fall	EU SmPC, Section 4.8 : Patients treated with SITOIGANAP have an increased risk gait disturbance and fall. Gait disturbance may arise from the cancer itself, its location in the brain, the compression it exerts on brain structures or as a consequence of edema, e.g., immunologically-induced edema. It may originate in several other situations such as physical member damage (joints, muscles, vessels or soft tissues) or injury or infection. It may be temporary or constitute a long-term (chronic) problem. It may be barely noticeable or severe as to cause an inability to perform tasks of daily living. Treatments of unsteady gait depend on the underlying cause. There is not contraindication to the continuation of SITOIGANAP administration.	None
Injection site reaction	EU SmPC, Section 4.8 : Patients treated with SITOIGANAP have an increased risk of injection site reaction. Low-grade skin reaction is the most frequently noted side effect upon SITOIGANAP administration. It may indicate the development of an efficient immune response. However, it is not consistently noted in all patients and no clear correlation between efficacy and local skin reaction can be concluded. Monitoring of the reaction should be performed. If severity implies use of corticosteroids, SITOIGANAP should be temporarily stopped.	None
Anxiety	EU SmPC, Section 4.8 : Anxiety may be a complication of cancer, cancer treatment, including immunotherapy, and change in behaviour with safety concern should lead to referral of the patient to neuropsychology or neuropsychiatry services.	None

Anaphylaxis	EU SmPC, Section 4.8 : Anaphylaxis is defined as a reaction "with skin or mucosal involvement, airway compromise and/or reduced blood pressure with or without associated symptoms, and a temporal relationship to allergen exposure". Immediate systemic allergic reactions after immunological therapy such as commonly used vaccines are extremely rare. Rare, serious adverse events are an unfortunate risk of many effective medications across all medical specialties. In the risk:benefit analysis, the very small risk of experiencing a serious adverse event needs to be weighed against the potential significant benefits of employing that therapy in the treatment of this life-threatening disease, i.e., glioma. Anaphylaxis to the treatment is a contraindication to receipt of further doses.	None
Immune-related liver disorder	EU SmPC, Section 4.8 : Signs and symptoms of hepatitis may include eye or skin yellowing (jaundice), pain on the right side of the stomach area and tiredness. Liver function should be monitored in case of symptoms. In the event of abnormal liver tests, physicians may consider stopping SITOIGANAP administration temporarily or permanently.	None
Immune-related kidney disorder	EU SmPC, Section 4.8 : Signs and symptoms of kidney disorder may include dysuria, hypertension, fatigue, fever, vomiting, appetite loss, cramp, back aches, blood in urea. Renal function should be monitored in case of symptoms. In the event of abnormal renal tests, physicians may consider stopping SITOIGANAP administration temporarily or permanently.	None
Gastrointestinal disorder	EU SmPC, Section 4.8 : Signs and symptoms of gastrointestinal disorder may include diarrhea, colitis, pain, hematochezia, weight loss, fever and vomiting, dysphagia, epigastric pain, endoscopic lesions. In the event of gastrointestinal toxicity, physicians may consider stopping SITOIGANAP administration temporarily or permanently.	None
Endocrinopathy	EU SmPC, Section 4.8 : Signs and symptoms of endocrine problems may include headache, tiredness and weight changes. Endocrine functions should be monitored in case of symptoms. In the event of endocrinopathies, physicians may consider stopping SITOIGANAP administration temporarily or permanently. Prompt recognition of signs and symptoms and implementation of the recommended management guidelines may prevent serious complications such as adrenal crisis.	None

Embryofetal toxicity	EU SmPC, Section 4.6: No study has been conducted to establish the effect of this "Treatment" on fertility or on embryos and fetuses (notably the risk of malformations). The risks and consequences of pregnant women are therefore unknown. This is why pregnant women are not allowed to be treated and why the use of an effective contraceptive method is compulsory in women of childbearing age and for men and their female partners 30 days prior to the start of the treatment, during the treatment and for 3 months after the end of the treatment. You can discuss suitable contraceptive methods and how long you will need to use them for with your physician. If you think you might be pregnant, please inform the physician immediately. Women who breastfeed will not be treated to avoid any unknown possible toxicity of the treatment on the infant. EU SmPC, Section 4.8 : Based on its mechanism of inducing a immune reaction, SITOIGANAP may cause fetal harm when administered to a pregnant woman. SITOIGANAP should not be administered to pregnant women, nor in women of childbearing potential not using effective contraception. Effective contraception should be used during treatment and for at least 3 months following the last dose of SITOIGANAP.	None
Safety profile in patient with comorbid disease	EU SmPC, Section 4.8 : The impact of comorbid disease and cotreatment on SITOIGANAP efficiency and safety has to be studied, in particular regarding renal disorder, but also brain hemorrhage, significant vascular disease, recent peripheral arterial thrombosis (less than 6 months), coagulopathy, ulcer, uncontrolled hypertension, heart failure, diabetes.	None
Safety profile in immunodeficient patient	EU SmPC, Section 4.3: Contraindications : Known immunosuppressive disease, active systemic autoimmune diseases such as lupus, receipt of systemic immunosuppressive therapy, human immunodeficiency, virus (HIV) infection, Hepatitis B or Hepatitis C EU SmPC, Section 4.5: SITOIGANAP should not be administered with immunosuppressive drugs	None

Overall conclusions on risk minimisation measures

The PRAC Rapporteur having considered the data submitted was of the opinion that the proposed risk minimisation measures are not sufficient to minimise the risks of the product in the proposed indication(s).

Moreover, the formal requirements of this section are still not fulfilled: the table which should have been copied here is found under part V.1 of the RMP.

2.4.7. Summary of the risk management plan

The public summary of the RMP requires revision.

2.4.8. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.4 is not acceptable.
Pharmacovigilance system

Having considered the data submitted in the application, it was not appropriate to conclude on pharmacovigilance system at this time.

Having considered the data submitted in the application, a pre-authorisation pharmacovigilance inspection is required.

3. Benefit risk assessment

3.1. Therapeutic Context

3.1.1. Disease or condition

High-grade gliomas are malignant and often rapidly progressive brain tumours divided into anaplastic gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma) and glioblastoma based upon their histopathologic and molecular features (e.g., based on IDH-status (mutant vs wildtype), WHO classification 2016). The amended target indication applied for by the applicant is treatment of "adult patients with recurrent/progressive glioblastoma and gliosarcoma - WHO classification grade IV malignant gliomas". However, in the pivotal study Sitoiganap was administered in combination with bevacizumab, GM-CSF and cyclophosphamide, which are not included in the currently proposed indication.

The aim of new treatments in patients with recurrence/progressive high-grade gliomas is to prolong overall-survival, or to improve symptoms, whilst minimising toxicity.

3.1.2. Available therapies and unmet medical need

Recurrent/progressive high-grade gliomas after first line therapy are associated with a poor prognosis, with median overall survival less than one year and significant tumour-related morbidity. Treatment decisions are usually individualised, since therapy is not curative and always associated with toxicity. Treatment options include surgery, radiation therapy, and systemic therapy. Involvement of a multidisciplinary tumour board is usually implemented for the decision of the best therapeutic approach to be followed. Systemic second-line treatment options include chemotherapy (e.g., lomustine as monotherapy or in combination, temozolamide rechallenge) and experimental therapy in a clinical trial. In the U.S. and Other Countries (but not in EU) bevacizumab is registered for this indication. Moreover, two TRK inhibitors (i.e., larotrectinib and entrectinib) have been recently approved in the EU for the treatment of patients with NTRK-fusion-positive tumours.

3.1.3. Main clinical studies

The main evidence to support efficacy consists of the preliminary results of the ERC1671-H02 (NCT01903330) study, an ongoing, investigator-initiated, randomised (1:1), double-blind phase II trial comparing Sitoiganap - GM-CSF, - cyclophosphamide – bevacizumab vs placebo – bevacizumab in

adult patients with recurrent/progressive, bevacizumab-naïve glioblastoma multiforme and gliosarcoma (WHO classification grade IV malignant gliomas).

In addition, the results of 10 patients with recurrent/progressive glioblastoma (grade IV gliomas) treated with Sitoiganap-cyclophosphamide-GM-CSF under a compassionate use/hospital exemption protocol have been presented as supportive evidence.

3.2. Favourable effects

The results of the ERC1671 study have been presented based to an interim analysis performed after inclusion of 9 patients (n=5 in the Sitoiganap arm and n=4 in the control arm, cut-off 01 December 2017), all enrolled at the University of California Irvine. The results as claimed by the applicant are as follows:

"The overall response rate was 75% (3/4) in the Sitoiganap arm vs 25% (1/4) in the control arm. Patients in the Sitoiganap arm experienced durable responses, with one patient with a partial response after cycle 1 that was maintained for more than 7 months and who was alive over 2 years after treatment.

Median OS of patients treated in the Sitoiganap arm was 12.1 months (363 days). Median OS in the control arm was 7.6 months (229 days), with all patients having died within 1 year.

Median PFS was 7.3 months (223 days) in the Sitoiganap arm compared with 5.4 months (164 days) in the control arm."

The results of an updated analysis with cut-off date 20 June 2020, after enrolment of 22 patients, were presented too. *"Median overall survival of unblinded patients randomized to Sitoiganap + bevacizumab (n = 8) was 264.5 days (8.7 months) from the start of study treatment, compared to 182 days (6 months) for those randomized to placebo + bevacizumab who did not cross over (n = 5). Median overall survival of unblinded patients at the time of cut-off randomized for Sitoiganap at the beginning of the study or taking Sitoiganap after crossover was 328 days (11 months) after first study treatment (n = 13)."*

3.3. Uncertainties and limitations about favourable effects

The quality dossier of Sitoiganap shows major deficiencies with regard to critical aspects such as product characterisation, the description and control of the manufacturing process, the manufacturing process characterisation and validation, and the drug product release and stability testing. It gives the impression that the manufacturing process is not sufficiently understood and controlled and its consistent performance not monitored. This raises concerns with regard to product quality, and consequently its efficacy and safety.

Also major deficiencies in the non-clinical and clinical dossier are noted. The clinical study report document provided by the applicant related to trial ERC1671-H02 only consists of a short synopsis of the study with a brief summary of few patient characteristics and results. Relevant details on patient flow, including patients recruited in each group, discontinuations, patients currently treated and the follow-up of those patients unblinded, baseline characteristics, methods to be applied are missing. The clinical study protocol seems to have been amended 17 times, but adequate information on the kind (i.e., major/minor), nature (i.e., content) as well as timing and justification for the changes is lacking, hampering evaluation on whether trial integrity was preserved. Results are provided in the form of a few summary tables/graphs. These major deficiencies seriously hamper any (critical) assessment.

Moreover, relevant inconsistencies have been identified within the dossier raising serious concerns on the appropriate conduct of the study and the reliability of the data.

The claimed mechanism of action (MoA) relies on general immunological principles, however there is no pre-clinical or clinical proof of concept data to support this MoA of the drug and the combination with GM-CSF, cyclophosphamide and bevacizumab. It is unclear which components (if any) of the proposed combination are needed for activity and how the claimed immune response induction is triggered and maintained overtime. Justification for the proposed dosing scheme is lacking not only for Sitoiganap but also for the other components of the combination. It is underlined that none of the three additional medicinal products proposed as part of the treatment strategy are currently approved in the EU for the proposed indication. Importantly, GM-CSF is currently not available in the EU questioning the feasibility of Sitoiganap treatment in EU in clinical practice.

Study ERC1671-H02 was designed as exploratory study and not with the intention to support regulatory decision making, as demonstrated by the relaxed type I error applied (one-sided 20%) and the approach regarding data analysis. Multiple factors in the study design significantly hamper the evaluation of a treatment effect of Sitoiganap and thus establishing a benefit of treatment. Along with the discrepancies and the uncertainties identified in the selection of the primary endpoint, that, in contrast with the applicant's statements, seems to have been amended underway from 6-month PFS to 12-months OS rate, also the choice for bevacizumab as comparator/backbone is considered problematic as this drug is not registered for this indication in EU. Furthermore, as Sitoiganap is co-administered with cyclophosphamide and GM-CSF and no pre-clinical support is available, it is impossible to disentangle the contribution of Sitoiganap (if any) to the claimed activity of the combination. The allowance of cross-over further complicate assessment of the results.

Regarding study conduct, several relevant deficiencies in the methods applied/conduct of the study have been identified which raise major concerns on the integrity of the trial and therefore on the reliability of the results presented. These include apparent changes of the primary endpoint after study was commenced, the conduct of not-pre-planned interim analyses, and the reported management of the clinical database. No reassurance that blinding of the applicant and study personnel was preserved has been presented.

Regarding the randomisation strategy, it remains unclear why a rule that disallowed more than four consecutive assignments was included in addition to block randomisation. Such a rule does not fit within a valid randomisation procedure. If the SAS program included a random number seed then the randomisation process can be replicated, however it remains unclear whether the full randomisation procedure could be replicated given the first part was performed using Randomize.net and the second, stratified randomisation was generated using SAS.

Furthermore, the results of the ERC1671-H02 study provided to date consist of only 10.7% (9/84) of the planned patients which is considered too preliminary to allow any meaningful conclusion. Data from the updated analyses are too scarce to allow any conclusion. The reported results derive from not-preplanned unblinded review of the data in the context of an ongoing study which is considered unacceptable in particular when such data are submitted for regulatory review with the intention to support an approval. The presented results are likely to be biased, treatment effect overestimated and a reliable inference is no longer possible.

As information on several prognostic and other relevant parameters of patients included in the analyses (i.e., extent of disease, histologic grade (both at initial therapy and at recurrence), relapse-free interval after first line) as well on the use of co-medications (like dexamethasone) is lacking it is not possible to determine the effect thereof on the study results.

Moreover, a cherry picking/data driven approach cannot be excluded as, only results of ORR, median PFS and median OS are presented. Furthermore, the analyses presented have not been conducted according to an ITT principle, as the data related to one out of the five patients enrolled in the Sitoiganap arm discontinuing study treatment before completing the first cycle of therapy have been excluded from the analysis, leading to inflation of the claimed effect.

The applicant has presented overall response rate results. No reassurance has been presented over homogenous implementation of criteria for evaluation of response (as both MacDonald ***or*** iRANO criteria were allowed) and it is unclear whether, and eventually how, the identified amendments related to schedule of radiological evaluation could have affected the assessment of response. Also information on the duration of the responses has not been provided.

The updated analyses presented after inclusion of 22 patients presents the same deficiencies as the ones identified at the preliminary analyses, challenging reliability of the results. Moreover, it is noted that at the updated analysis only results of median OS (and not 6-month PFS, median PFS and ORR) have been provided. The analysis on median OS including patients crossing over to Sitoiganap after progression is challenged by selection bias.

The interstudy comparisons presented are challenged by selection bias and by the lack of matching in relevant parameters (study population, treatment setting, etc) and as well as by the use of non-contemporary literature (1998) and therefore are not considered supportive for the claimed treatment effect.

3.4. Unfavourable effects

The following AEs have been described to occur in the Sitoiganap arm: headache, injection site reactions (induration, erythema and ulceration), arthralgia, gait disturbance/fall, back pain, anxiety, self-limiting fever and chills.

Headache is described to be the most common documented Grade 3 AE. Wound dehiscence, HTN and HA [abbreviations not explained] were Grade 3 AEs considered attributed to bevacizumab. No Grade 4 were reported in the sitioganap arm.

It is reported that no SAE were noticed immediately following the administration and that there was no death reported as provoked by Sitoiganap administration.

In the phase 0/compassionate use treated patients it is reported that no significant side effects potentially attributable to the combination were witnessed.

3.5. Uncertainties and limitations about unfavourable effects

The safety data has not been reported in accordance with ICH E3 (CPMP/ICH/137/95), i.e. there are very large deficiencies in the reported safety data. These deficiencies include:

- definitions of the primary safety population
- data on the extent of exposure
- data on all-causality and drug-related AEs by the System Organ Classification (SOC) and preferred term (PT)
- summarising tables on laboratory abnormalities
- detailed data on SAEs, deaths and other significant adverse events

- data on AEs leading to discontinuation or treatment modifications
- safety data in special populations, safety related to drug-drug interactions, other interactions, overdoses and immunological events
- listings of individual patient data and narrative statements of events of particular interest.

In addition, the quality dossier, does not allow to determine whether the manufacturing process and control ensure product safety (refer to uncertainties and limitations about favourable effects).

While data on the phase 0/compassionate use studies have been provided in support of the safety profile, important details on the design/methods of treatment and on the methods for collecting safety data have not been reported. Therefore, it is highly uncertain whether these data are reliable and are thus not able to support claims on the safety profile. Further, the provided NC data does not provide relevant information for determining the clinical safety profile.

Because of all these deficiencies it is not possible to assess the safety profile of Sitoiganap, to determine safety concerns/specifications, to evaluate the management and tolerability of Sitoiganap toxicity and to determine which information should be provided in the SmPC and thus to assess the content of the safety related sections in the SmPC.

Moreover, the following issues are identified, which hinder assessment of the safety profile even if the deficiencies were to be resolved:

- In the CSR very few safety data has been presented and the available safety data is of poor quality; the AEs are presented in a non-systematic manner, without the use of standard terms. Also the safety reporting is inconsistent, lacks details and does not define/explain what data are shown. As a consequence these data are very difficult to interpreted and the reliability of the data is questioned.
- the sample size of the pivotal study is considered too small for establishing the safety profile of sitioganap, even when considering the rarity of the disease.
- the administration of concomitant medication (GM-CSF, bevacizumab and cyclophosphamide) does not allow to evaluate the safety profile for Sitoiganap monotherapy

It cannot be excluded that new safety issues may arise if the requested data is provided by the applicant. Of note, due the large deficiencies in the safety reporting, it is uncertain whether the collecting of safety was conducted in accordance with GCP.

3.6. Effects Table

Table 21- Effects table for Sitoiganap-GM-CSF-cyclophosphamide-bevacizumab

Effect	Short Description	Unit	SGCB	BP	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	SGCB	BP	Uncertainties/ Strength of evidence	References
Median OS	Definition NA	months	12.1	7.6	No ITT principle applied, only 10.7% (9/84) of pts included, no pre-planned IA, no pre-specified primary study endpoint. Statistical significance unclear	
ORR	Criteria for assessment NA	%	75	25	No ITT principle applied with overestimation of effect in the experimental arm, criteria for response applied unclear, no preplanned IA.	
PFS	Definition NA	months	7.3	5.4	No ITT principle applied, frequency of assessment unclear, no preplanned IA.	
Unfavourable Effects						
Headache	AE of headache	N (%)	NR	NR	-Large deficiencies in safety reporting - Available data of poor quality, inconsistent and non-interpretable - unclear if safety was collected in accordance with GCP -small sample size -administration of concomitant medication	
Injection site reactions	Injection site reactions (induration, erythema and ulceration)	N (%)	NR	NR		
SAEs	Serious adverse events	N (%)	-	NR		

Abbreviations: NA: not available; SGCB: Sitoiganap, GM-CSF, cyclophosphamide, bevacizumab; BP: bevacizumab, placebo; OS: overall survival; ORR: overall response rate; PFS: progression free survival; IA: interim analysis.

Notes: Data on the primary study endpoint defined in the study protocol (6- month PFS) have not been provided.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Considering the poor prognosis of recurrent/progressive glioblastoma with life expectancy far below one year and the substantial disease-related morbidity, a novel treatment option able to improve

survival associated with an acceptable safety profile would be welcomed/considered of clinical benefit. However, in the case of Sitoiganap, the major deficiencies of the dossier identified hamper an adequate assessment of the quality of the product and of the efficacy and safety of treatment. The quality dossier gives the impression that the manufacturing process of Sitoiganap is not sufficiently understood and controlled and its consistent performance not monitored. This raises concerns with regard to product quality, and consequently its safety and efficacy.

There is a lack of preclinical and clinical data on proof of principle able to support the claimed mechanism of action and the activity of Sitoiganap and of the co-administered drugs (GM-CSF, cyclophosphamide, bevacizumab), none of which is registered in the EU for the target indication. It is unclear which components (if any) of the proposed combination are needed for activity and how the claimed immune response induction is triggered and maintained overtime. The paucity of the data presented on the clinical study and the serious deficiencies and inconsistencies identified in the submitted dossier raise serious concerns over the conduct of the study and therefore the reliability of the results presented. A cherry-picking data driven approach cannot be excluded/ seems to have been followed, with reported results deriving from unblinded review of the data in the context of unplanned interim analysis/es.

All these issues, together with the very limited number of patients included in the non-preplanned preliminary analyses presented, do not allow for an evaluation of the efficacy and safety of the treatment. As a consequence a B/R assessment cannot be performed, thus the B/R remains undetermined.

In view of the major concerns identified on the conduct of the study, a GCP inspection is considered justified.

3.7.2. Balance of benefits and risks

In view of the major deficiencies identified the B/R of Sitoiganap for the proposed indication is considered not assessable at this time and thus negative.

3.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

A conditional marketing authorisation (CMA) has been requested by the applicant in the initial submission. However the arguments provided by the applicant on the fulfilment of the requirements for a CMA according to current EU regulation are not agreed upon.

It is considered that the product could fall within the scope of Regulation (EC) No 507/2006 concerning conditional marketing authorisations, as it aims for the treatment of a life-threatening disease and is designated as an orphan medicinal product. However, the product is **not** recommended for a conditional marketing authorisation as the dossier does not fulfil the requirements for a CMA, i.e. the benefit-risk balance is negative (as discussed), the applicant is unlikely to be able to provide comprehensive data after authorisation, it has not been demonstrated that the product will address an unmet medical need, and the benefits to public health of the immediate availability do not outweigh the risks inherent in the fact that additional data are still required.

As the requirements are not fulfilled, a conditional marketing authorisation is NOT considered appropriate.

GCP inspection

An inspection should be considered.

3.8. Conclusions

The overall B/R of Sitoiganap in combination with GM-CSF, cyclophosphamide and bevacizumab is considered **NOT assessable** and therefore **negative**.

4. Recommended conditions for marketing authorisation and product information in case of a positive benefit risk assessment

4.1. Conditions for the marketing authorisation

In view of the major objections it is premature to recommend any conditions for marketing authorisation.

4.2. Summary of product characteristics (SmPC)

Please refer to the separate SmPC AR for comments regarding the SmPC

Pursuant to Article 23(1) of Regulation No (EU) 726/2004 (REG), Sitoiganap is included in the additional monitoring list for the following reasons: new active substances.

4.3. Labelling

4.4. Package leaflet (PL)

User consultation

The applicant considers the user consultation 'not applicable' as the product will be distributed and administered in hospital only by healthcare professionals.

Conclusion from the checklist for the review of user consultation

The provided justification is not considered valid. The applicant is requested to provide a full user consultation test or to provide a valid justification for not submitting a user consultation test.

5. Appendices (as appropriate)

Not applicable.

6. QRD checklist for the review of user testing results

PRODUCT INFORMATION

Name of the medicinal product:	Sitoirganap
Name and address of the applicant:	Epitopoietic Research Corporation-Belgium (E.R.C.) Rue Jean Sonet, 10 5032 Isnes , BELGIUM
Name of company which has performed the user testing:	Not applicable
Type of Marketing Authorisation Application:	CMA
Active substance:	autologous glioma tumor cells, inactivated autologous glioma tumor cell lysates, inactivated allogeneic glioma tumor cells, inactivated allogeneic glioma tumor cell lysates, inactivated
Pharmaco-therapeutic group **(ATC Code):**	Not yet assigned
Therapeutic indication(s):	Treatment of recurrent glioma in adult patients
Orphan designation	☒ **yes** ☐ **no**
Rapporteur/CoRapporteur	**Netherlands / Spain**

- Full user testing report provided	☐ yes	☒no
- Focus test report provided	☐ yes	☒no
- Bridging form provided[1]	☐ yes	☒no

- In case bridging form[1] has been provided, please perform the assessment in the bridging form and state the overall conclusion/recommendations below:

_____
_____
_____
_____
_____
_____
_____

- Is the justification for bridging acceptable?	☐ yes	☐ no
- Is the justification for not submitting a report acceptable?	☐ yes	☒ no

Reasons

The provided justification for not performing a user testing report is: "Administration in a hospital setting only". This is not considered a valid justification. The applicant is requested to provide a user consultation or a valid justification for not performing a user test.

[1] QRD form for submission and assessment of user testing bridging proposals [EMA/355722/2014]