21 April 2017
EMA/353057/2017
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal Assessment report

Zafiride

International non-proprietary name: NGR-hTNF

Procedure No. EMEA/H/C/004455/0000

Note

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

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

Table of contents

List of abbreviations

AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
$AUC_{0-\infty}$	Area under the serum or plasma concentration *versus* time curve extrapolated up to infinite time
$AUC_{0-t(last)}$	Area under the serum or plasma concentration vs. time curve up to the last detectable concentration
BIC	Best Investigator's Choice
BSC	Best Supportive Care
C_{max}	Maximal serum or plasma concentration
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DB	Double blind
DCR	Disease Control Rate
DSBM	Data Safety Monitoring Board
DLT	Dose limiting toxicity
DS	Drug substance
DP	Drug product
EKG	Electrocardiogram
ECOG PS	Eastern Cooperative Oncology Group Performance Status
EORTC	European Organization for Research and Treatment of Cancer
ESF	Eligibility Screening Form
γ GT	Gamma-Glutamil transpeptidase
GMP	Good manufacturing practice
HAS	Human Serum Albumin
hTNF	human TNF
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
ILP	Isolated Limb Perfusion
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
LCSS	Lung Cancer Symptom Scale
LDH	Lactate dehydrogenase
LVEF	Left ventricular ejection fraction
MCD	Maximal clinically tolerated dose
MCU	Medical Care Utilization
MPM	Malignant Pleural Mesothelioma
NCI	National Cancer Institute
NGR	Asparagine-Glycine-Arginine
NLR	Neutrophil-to-lymphocyte ratio
NP	Not provided
NT	Not tolerated
NYHA	New York Heart Association
PD	Progressive Disease
PFS	Progression free survival
PK	Pharmacokinetic
PP	Per-protocol Population
PPS	Post-progression survival
PR	Partial Response
PT	Prothrombin Time
QoL	Quality of Life
OS	Overall Survival
R	Randomised
RECIST	Response Evaluation Criteria in Solid Tumours

ROI	Region of Interest
SA	Single arm
SL	Single line
SAE	Serious Adverse Event
SD	Stable Disease or Standard deviation of the mean
SP	Safety Population
$t_{1/2}$	Apparent terminal half-life
TFI	Treatment-free interval
t_{max}	Time to peak serum or plasma concentration
TNF	Tumour necrosis factor
ULN	Upper limits of normal
VEGF	Vascular endothelial growth factor

1. Recommendation

Based on the CHMP review of the data on quality, safety and efficacy, the CHMP considers that the application for Zafiride, an orphan medicinal product in the treatment of *adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy,* **is not approvable** since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time.

In this dossier multiple major deficiencies have been identified regarding the quality, pre-clinical, clinical pharmacology and clinical efficacy and safety data. These undermine the validity of the obtained preclinical and clinical data and prohibit interpretation of the results. These have to be resolved as the current dossier cannot be considered of sufficient quality to support the application.

The details of these major objections are provided in the list of questions (Section VI).

Proposal for questions to be posed to additional experts

Not Applicable.

Proposal for inspection

GMP inspection(s)

The CHMP has been assured that acceptable standards of good manufacturing practice (GMP) are in place at the sites responsible for the manufacture and assembly of this product, with the exception of the site responsible for batch certification. EudraGMP certificate reference numbers of the manufacturing authorisation of each of the involved manufacturing sites have been provided. However, considering the noted deficiencies of the quality data in this MAA, inspection prior to authorisation could be considered, depending on the answers to the LoQ.

Furthermore, AIFA has confirmed that the site responsible for batch release (MolMed SpA Via Olgettina 58 20132 Milano Italy) is not authorised for the batch certification of recombinant proteins. The site shall request an extension of the current manufacturing authorisation and the applicant shall provide a valid manufacturing authorisation for the batch certification of this type of products. That may require an inspection (the need for inspection is to be confirmed by AIFA).

GLP inspection

Issues regarding GLP have been identified and are discussed in section 2.4 of this overview. If this important issue is not clarified, then a triggered GLP inspection would be appropriate.

GCP inspection(s)

For routine GCP inspections

A routine GCP inspection is not planned.

For triggered GCP inspections

Not applicable at this stage in the procedure. However, issues regarding GCP have been identified and are discussed in section 2.4 of this overview. Most importantly, it is currently not clear whether the

phase I-II studies have been approved by the local medical ethics committee(s)If this important issue is not clarified, then a triggered GCP inspection would be appropriate.

New active substance status

Based on the review of the data, the CHMP considers that the active substance NGR-hTNF contained in the medicinal product Zafiride is to be qualified as a new active substance in itself.

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

The acclaimed indication is:

"*Zafiride is indicated for the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy."*

The proposed posology is 0.8 micrograms/m^2 every week as long as clinical benefit is observed or until unacceptable toxicity occurs.

2.1.2. Epidemiology

Malignant pleural mesothelioma (MPM) is a rare disease and cure is only seldom accomplished. In Europe, the incidence is about 1.6 per 100,000 inhabitants corresponding to 8,000 new diagnoses annually. Based on a 5-year survival of 5%, the complete prevalence is 1.9 per 100,000 corresponding to 10,000 prevalent cases in the EU [Gatta, 2001]; thereby fulfilling the criteria for an orphan disease. MPM occurs predominantly in men (ratio of men to women 5:1) [Larsson, 2007]. The median age of diagnosis is 68 years.

Most patients (80%) are diagnosed in stage III/IV and are in need of systemic therapy (following surgery). However, poor performance score and low chemo- and radio-sensitivity of the tumour result in poor prognosis. The median overall survival is approximately one year.

2.1.3. Biologic features, aetiology and pathogenesis

The most frequent risk factor for malignant mesothelioma is exposure to asbestos with occupational exposure being documented in 70-80% of those affected. Other potential factors are radiation, semian virus 40 (SV40) and exposure to erionite. The mean latency period of MPM after exposure to asbestosis is around 40 years (range 15-67 years).

There are three main histologic subtypes of mesothelioma: epithelioid, biphasic and sarcomatous. Epithelial tumours are the most common (about 50% of cases) and patients have a mean overall survival of 19 months. The least common is the sarcomatous type (10%), and this tumour has the worst median OS, about 13 months. The mean OS for the biphasic tumour type is intermediate to that of the epithelial and sarcomatous tumour.

MPM is often regarded as a locally invasive cancer. However, a large post mortal examination in 318 patients showed extra-pleural localisation in 90% of cases and lymph node involvement in 53% of cases [Finn, 2012].

The mechanism of MPM carcinogenesis by asbestosis is not entirely understood. Several molecular pathways are involved in malignant pleural mesothelioma; these include disturbed cell cycle regulation, apoptosis, growth factor pathways, and angiogenesis.

The presence of angiogenesis in the tumour, as assessed by microvessel count (MVC), is an independent poor prognostic factor in MPM [Edwards, 2003]. In this respect, VEGF expression correlates with microvessel density and VEGF (vascular endothelial growth factor) overexpression in tumour or pleural fluid predicts low survival in MPM patients. High levels of VEGF have been observed in the serum of patients with MPM, not in normal patients exposed to asbestosis. However, no correlations were found between tumour vessel density and serum or pleural effusion VEGF levels [Strizzi, 2001]. Several anti-angiogenetic drugs have been tested in mesothelioma [Remon J, 2013]. Recently, it was shown that the addition of the anti-VGFR bevacuzimab could prolong the OS when added to pemetrexed and cisplatin in the first line setting [Zalcman, 2016].

The MPM is not sensitive to radiotherapy. Radiotherapy is only used with palliative intend to provide symptom relieve.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

MPM typically originates from the lower parietal pleural and the costradiapharmatic sinus. The most common symptoms (≥ 60% of patients) include dyspnoea and chest pain. Pleural effusion is often present. Other frequently reported symptoms are tiredness, cough while seated and constipation.

The chest X-ray usually states unilateral pleural effusion and thickening. The typical signs on the chest CT are a rind-like tumour along the pleural cavity together with diffuse or nodular thickening. These signs may be suggestive of the diagnosis.

Mesothelioma is a heterogeneous cancer, and besides this, the pleura is also a common site for metastatic disease. The histo-pathological analysis of pleural tissue is mandatory for final MPM diagnosis [ERS/ATS Task Force 2010]. To this end, thoracoscopy is recommended to obtain adequate histology, determine stage of the disease optimally and allow pleural fluid evacuation. During thoracoscopy, multiple deep and large biopsies from both the normal and seemingly abnormal pleura should be obtained to provide sufficient and adequate samples for diagnosis. The diagnosis of MPM should be made on light microscopy combined with appropriate immunohistochemistry allowing subtyping according to histology [ESMO 2015]. When thoracoscopy is not feasible or contra-indicated, ultra-sound guided true cut biopsies can be used. Importantly, an independent expert panel should be involved to confirm the diagnosis, particularly in a clinical trial or in any case where there is doubt about the diagnosis [ERS/ATS Task Force, 2010].

Staging describes the anatomic spread of a tumour. At least five different staging systems for MPM are available. In the absence of a uniform, robust and validated staging system, experts advocate the use of the most recent TNM-based IMIG/UICC classification [ESMO 2015, ATS/ERS Task Force 2010]. The limitation of this system is its inaccuracy in describing tumour spread and node involvement. Therefore, modified versions are used to describe the staging of the pleural mesothelioma.

As stated above, malignant pleural mesothelioma is a heterogeneous disease in terms of histology and stage at diagnosis. Several prognostic factors have been described in large multicentre series, adding to the heterogeneity of the patient population. In this respect, two prognostic scoring systems have been devised by the European Organisation for Research and Treatment of Cancer (EORTC) and the Cancer and Leukaemia group B (CALGB). However, performance status and histopathological subtype are currently the only prognostic factors of clinical importance that are routinely assessed in the management of patients with malignant mesothelioma [ATS/ERS Task Force, 2010].

2.1.5. Management

The treatment options for patient with MPM are surgery, radiotherapy and chemotherapy.

There is limited evidence for the efficacy of surgery in patients. Due to the intricate location and relation with other organs, it is virtually impossible to obtain free resection margins. Surgery is limited to patients with stage I-III disease. In operable patients, the decision whether to perform a pleurectomy/decortication (P/D) or an extra pleuro-pneumectomy (EPP) may not be made until surgical exploration. The success rate of surgery is variable (ERS ATS Task Force 2010, EMSO 2015].

As stated above, MPM is hard to treat because most patients (80-95%) present with advanced disease. For patients with advanced or recurrent malignant mesothelioma and fit for chemotherapy, a combination doublet platinum chemotherapy with either pemetrexed or raltitrexed (antifolates) should be initiated [Vogelzang 2003; Van Meerbeek 2005]. The pemetrexed/cisplatin combination shows response rate of 45.5%, and a median OS survival of 13.3 months (95% CI 11.4-14.9), with a median PFS of 6.1 months (95% CI 5.3-7.0) [SmPC pemetrexed].

After failure of first line therapy, there is currently no second line standard of care. No randomised study has shown the benefit of second line chemotherapy on survival of quality of life after failure of primary chemotherapy. From non-randomised studies it is known that the median OS for patients fit for chemotherapy after failure of first line platinum/pemetrexed is 6-7 months [Krug 2015; Kindler 2016] and this might be prolonged to 8.4 months if treated with second line chemotherapy [Jassem 2008].

Guidelines recommend the use of second line chemotherapy. Single agent vinorelbine has shown useful activity in a phase II trial [ESMO 2015]; another therapeutic option might be gemcitabine [ESMO 2105]. A retreatment with pemetrexed can also be considered in case of a prolonged (≥ 6-12 months) symptomatic and objective response after first line therapy (ERS/ATS). Retrospective analyses showed a prolonged OS on second line chemotherapy treatment in MPM in those patients with a prolonged PFS or TFI in response to first line therapy [Zucali 2014, Manegold 2005].

Vinorelbine can be administered as intravenous or oral application. Most clinical studies have been conducted as intravenous therapy. Vinorelbine can be administered at weekly intervals at 30 mg/m^2 [Stebbing, 2009] or 25 mg^2 on day 1 and 8 in a 3-week cycle. The use of vinorelbine as oral therapy is limited to one study only. These treatments reported median OS of 6.2-9.6 months, with the longest OS observed with the once weekly dose [Ceresoli, 2015].

Gemcitabine as monotherapy has been administered i.v. at a dosage of 1250 mg/m^2 on day 1 and 8 of a 3 week cycle. In this small study (n=27 patients), gemcitabine showed a response rate of 7% (95% CI1-24%) with a median overall survival of 8 months [van Meerbeek, 1999].

Neither the ERS/ATS nor the ESMO guidelines recommend doxorubicin as second line therapy.

2.2. About the product

It concerns an application for Marketing Authorisation for Zafiride (NGR-human Tumour Necrosis Factor alpha, NGR-hTNF) produced in Escherichia Coli cells by recombinant DNA technology. The medicinal product falls within the mandatory scope of the Centralised Procedure according to Regulation (EC) No 726/2004, Article 3(1) - Annex (4) - Orphan designated medicinal product. NGR-hTNF has been granted orphan drug status in 2008 for the treatment of malignant mesothelioma by EC on June 03, 2008 (EU/3/08/549).

Marketing authorisation is requested for Zafiride in the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy.

The proposed dose regimen of Zafiride is 0.8 micrograms/m^2 every week as long as clinical benefit is observed or until unacceptable toxicity occurs.

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

The applicant received scientific advice from the CHMP on October 2008. The scientific advice pertained quality aspects and the clinical development.

Originally, at the time of the SA in 2008, the applicant had planned the pivotal study with an open-label design, comparing NGR-hTNF and best supportive care (BSC) with BSC alone. However, with the intention of improving overall recruitment and avoid systemically bias once randomisation results were known, the SAWP/CHMP advised the company to perform a double-blind, placebo-controlled, randomized study, comparing NGR-hTNF plus best investigator's option (BIO) (and not BSC) versus placebo plus BIO. The applicant has complied with this by letting the investigators chose a single-agent chemotherapy to be given in combination with NGR-hTNF/placebo one hour after the end of the NGR-hTNF/placebo administration.

The CHMP/SAWP suggested not to exclude any anticancer treatment and let all potential options (although not life prolonging) be available to investigators and patients. However, in the protocol surgery, immunotherapy, anticancer hormonal therapy and radiotherapy (except palliative) was excluded from BSC. The applicant was advised to standardise the concomitant therapy (e.g. antibiotics, nutritional support and optimal symptom control as well as pain management), however this does not seem to have been followed up.

It was noted that infusion of NGR-hTNF usually was associated with onset of rigors. To minimize the potential of transient constitutional symptoms, which might un-blind the trial, the CHMP/SAWP suggested considering a premedication. The applicant has chosen paracetamol 1000 mg p.o./i.v. as prophylaxis 30 to 60 minutes prior to the start of each infusion of NGR-hTNF/placebo.

The CHMP/SAWP recommended rewording the first inclusion criterion somewhat more strictly, so that the only preceding treatment was a pemetrexed-cisplatin combination treatment, which failed. The applicant amended the inclusion criterion as follows: "Prior treatment with no more than one systemic pemetrexed-based chemotherapy regimen administered for advanced or metastatic disease. Prior use of a biological agent in combination with a pemetrexed-based regimen and prior administration of intra-pleural cytotoxic agents were allowed, while a prior exposure to anthracyclines precluded the use of doxorubicin." It is therefore noted that the advice from CHMP/SAWP regarding precision of the preceding treatment was not followed. In addition, the criterion does not specifically requires that the patients had to fail/progress in order to stop the pemetrexed treatment. This is considered problematic considered the applied indication.

In their original proposal for the pivotal study design, the applicant suggested to test two primary endpoints (OS and PFS), however, the SAWP/CHMP strongly advised to choose OS as the primary endpoint and PFS as a key secondary endpoint. The company has complied with this.

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

The CHMP has been assured that acceptable standards of good manufacturing practice (GMP) are in place at the sites responsible for the manufacture and assembly of this product, with the exception of the site responsible for batch certification. EudraGMP certificate reference numbers of the manufacturing authorisation of each of the involved manufacturing sites have been provided. However, considering the noted deficiencies of the quality data in this MAA, inspection prior to authorisation could be considered, depending on the answers to the LoQ.

Furthermore, AIFA has confirmed that the site responsible for batch release (MolMed SpA Via Olgettina 58 20132 Milano Italy) is not authorised for the batch certification of recombinant proteins. The site shall request an extension of the current manufacturing authorisation and the applicant shall provide a valid manufacturing authorisation for the batch certification of this type of products. That may require an inspection (the need for inspection is to be confirmed by AIFA).

The applicant stated that all the pivotal preclinical safety studies were conducted in compliance with GLP. However, several GLP issues have been noted: Studies 0415-2011-R and 0416-2011-R report that a histopathological peer review has been performed, the raw data were archived, but the outcome was not reported. A possible GLP deviation can be derived by the apparent discrepancy between the codes of the studies and the study duration, which suggests that they began in 2011 with a date of the final report in 2016, which does not seem to justify the long period. Furthermore, study 0416-2011-R is missing a completed GLP and QA compliance statement, as key personnel signatures and a QA statement are lacking. The Applicant is requested to provide the histopathological peer review report, a justification for the discrepant GLP and QA statements and the specified timelines between initiation and the final report of the pivotal toxicology studies.

GCP concerns regarding the conduct of the clinical studies were raised. These pertained to:

- In the application no evidence has been provided that the uncontrolled phase I/II studies were monitored/approved by an independent review board/independent ethics committee (IRB/IEB).
- Additional clarifications are needed if the IRB/IEC has (have) been notified regarding the increase in sample size in two studies (i.e. NGR-004 and NGR015), and the lack of installation of the planned Independent Data Safety Monitoring Board in the pivotal study NGR-015.

In the pivotal study NGR015 the following important GCP-related issues were identified:

- Not fulfilling the inclusion and exclusion criteria was most frequently classified as a minor protocol deviation, while these should be considered as a protocol violation (as the safety of the patients and the integrity of the data might be jeopardized).
- The inclusion of n=21 patients that were rechallenged with a pemetrexed treatment is a violation of the inclusion criterion: *Prior treatment with no more than one systemic pemetrexed-based chemotherapy regimen administered for advanced or metastatic disease."* However, these patients were not labelled as having violated/deviated from the protocol.

- A total of n=77 (19%) patients did not have a post-tumour assessment. This raised the following concerns:
  - These patients should have been classified as preliminary withdrawn and the reasons for not having post-tumour assessment should have been provided in the dossier.
  - The PFS was a key secondary outcome. The high number of patients not having a tumour assessment raised concerns regarding the adherence to the protocol
  - The number of patients not having a post tumour assessment (n=77) is inconsistent with the number of patients for whom no tumour assessment was possible (n=55) and not further explained.
- Concerns if the blinding of the study data is maintained because of the inclusion of a substudy within this trial and the deblinding of 7 patients in one site because of the concerns regarding the administered doses.
- Inconsistencies are observed in the published data and the final study protocol and in the data presentation in the clinical overview version 0.0 and version 2.0. These changes seem to indicate that after the analyses the database has been adjusted.
- The applied definitions of protocol violations and protocol deviations differ from commonly used definitions. Non-adherence to the in- and exclusion criteria is usually considered as a protocol violation, but another definition was used in the study. Also, only the adverse events with a clear relationship with the study medication were included in the overall safety analyses. These two unusual interpretations of common GCP concepts do raise serious concerns whether the studies were conducted according to GCP (see also section 1).
- the applicant should clarify whether the pivotal study NGR015 has been inspected by any EU or non-EU inspectorates (FDA, Health Canada) during the study period or within the last three years since finalization. Inspection reports should be submitted if available

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

- Legal basis

  This application has been submitted as a full application through a centralized procedure for a new active substance, NGR-hTNF, in accordance with Directive 2001/83/EC, Article 8(3).

- Accelerated procedure

  Not applicable.

- Conditional approval

  The applicant applies for a conditional approval.

  In order to obtain a conditional approval, the following criteria have to be met:
  - An unmet medical needs will be fulfilled
  - The benefit/risk is positive
  - It is likely that the applicant will be able to provide comprehensive data
  - The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required

It is agreed with the applicant that malignant pleural mesothelioma (MPM) is a serious debilitating and life threatening disease and has an orphan indication [EU/3/08/549). Therefore, *if* the benefit/risk would be assigned as positive, the first criterion would be met.

The applicant considered that benefit/risk is positive *in the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy.* In this respect, the improvement in OS survival is considered remarkable because of the limited life expectancy in this patient population with a median overall survival of OS 6 months with no approved treatment available.

CHMP comment

The current dossier is deficient regarding quality, PK, and clinical, and this precludes a benefit/risk assessment (see Benefit/risk section in this report). Since the benefit/risk cannot be assigned as positive, it cannot be determined whether the unmet medical need for the applied application can be fulfilled upon the treatment with NGR-hTNF. Therefore, the main requirement for a conditional approval has not been met.

The applicant proposes three additional confirmatory studies to provide comprehensive data. In the first study, patients who progress after the first line treatment of mesothelioma will be included and in the second study patients who are fit for switch maintenance therapy will be studied. (N.B. Patients who are fit for switch maintenance show no progression after first line chemotherapy.) The last proposed study, option C would be a formal phase III study in the second line setting, limiting the inclusion to patients with a short treatment free interval showing progression after first line treatment.

CHMP comment

The first two of these studies include a different study population than was studied in the pivotal study aiming to support the current application. Clarification is needed for several aspects, these pertain to e.g. whether the same dose and dose frequency will be applied as in the current pivotal study and to what extent results are can be extrapolated from one type patient population to the other. Otherwise, these studies are unlikely to be suited to support the conditional approval.

The study that would best fit to support the conditional approval of the current application would be study C, **if** the recruitment of this study is complete at the time of opinion on the current dossier. This requirement has to be met as enrolment for this proposed study will be difficult in case NGR-hTNF would receive conditional approval. Also, the ethical feasibility of this trial can be questioned in case Zafiride has been granted a conditional approval.

In conclusion: at this time it cannot be determined if NGR-hTNF could fulfil the unmet medical need in the second line treatment of malignant mesothelioma. Also, it is not clear whether the proposed studies will provide the confirmatory data as needed. Therefore, the requirements for fulfilling a conditional approval are currently not met.

- Exceptional circumstances

  Not applicable

- Biosimilar application

  Not applicable

- 1 year data exclusivity

  Not applicable

- Significance of paediatric studies

  Not applicable. Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision EMA/288360/2016 on the granting of a class waiver, CW/1/2011.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

Zafiride (NGR-hTNF) is a direct-acting, first-in-class peptide-targeted agent (PTA) prepared by conjugating the human Tumour Necrosis Factor-α (TNF-α) with the CNGRCG peptide. NGR-hTNF is a recombinant protein consisting of hTNF coupled (via N-terminus) to the C-terminus of the peptide CNGRC(G). NGR-hTNF drug product is a liquid manufactured from a bulk drug substance consisting of 0.2 mg/ml of protein in citrate (50mM sodium citrate, 3.5% mannitol, 1.0% sucrose, pH 6.2) buffer. NGR-hTNF is a sterile concentrate for solution for infusion. Before infusion to patients, NGR-hTNF drug product is diluted to the appropriate concentration with 0.9% NaCl containing 1 mg/ml human serum albumin (HSA). The presence of HSA is necessary to avoid loss of NGR-hTNF, when present at very low concentrations, by absorption to tubing.

NGR-hTNF has been developed both as single agent and in combination with standard chemotherapy for the treatment of several solid tumours. The mechanism of action of NGR-hTNF is determined by specific effects due to the two components of this compound. The NGR peptide specifically binds to a CD13 isoform selectively expressed by endothelial cells of human tumour vessels during neo-angiogenesis, whereas it does not bind to various CD13+ epithelial and myeloid cells, thus sparing tumour-unrelated human tissues. As a result presence of the NGR peptide increases *in vivo* tumour vessel homing of NGR-hTNF compared to hTNF. In addition, the binding of NGR to CD13 increases the stability of the NGR-CD13/TNF-TNFR interaction. The combination of a highly selective homing to tumour blood vessels (due to selective CD13 binding) and increased apoptosis of angiogenic endothelial cells (due to stabilisation of the NGR-CD13/TNF-TNFR interaction) *in vivo*, significantly contributes to the control of tumour growth.

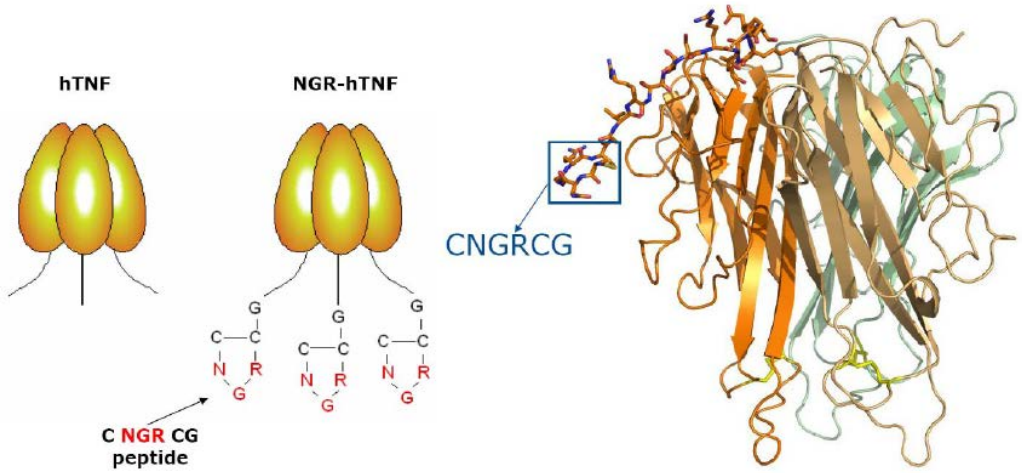
3.1.2. Active Substance

General Information

NGR-hTNF is a recombinant protein consisting of hTNF coupled (via N-terminus) to the C-terminus of the peptide CNGRC(G).

The active form of NGR-hTNF is a trimeric protein made up of three identical subunits (monomers) non-covantely linked, with an intra-chain disulphide in the CNGRC domain and another intra-chain disulphide in the TNF domain.

Figure 1: (A) Schematic representation of hTNF and NGR-hTNF; (B) three dimensional structure (resolution 2.8Å) of NGR-hTNF.



(A) (B)

NGR-hTNF is a non-covalent homo-trimeric protein of approximately 50 kDa that behaves like TNF in gel filtration chromatography. Each of the three subunits (Mr 17939,4) consists of 163 amino acid residues, including four cysteines (2 in the CNGRC domain and 2 in the TNF domain) that form intra-subunits disulfide bonds. The N-terminal methionine is removed after synthesis by *E. coli* cells.

The mechanism of action of Zafiride is characterized by the specific targeting of the molecule to the tumour vasculature, followed by multiple downstream effects mediated by the interaction of both of the two components of the molecule (see above).

The N-terminus of NGR-hTNF is heterogeneous and isoforms of the molecule are described.

No information is provided regarding integrity of CD13 targeting by the NGR moiety in these isoforms. In addition, deamidation of the asparagine in NGR occurs, *in vivo*. The control system (release specifications, stability protocol) does not adequately control deamidation **[MO]**. A potential consequence of inactivity of the NGR group is lack of CD13 targeting, eliminating this component of the pharmacological mechanism of action, but leaving the TNF related (non-targeted) cytotoxic effects intact. **[MO]**

Manufacture, characterisation and process controls

Manufacturers

NGR-hTNF Drug Substance is manufactured in accordance with cGMP at the following facilities; Fujifilm Diosynth Biotechnologies, UK, (manufacturing and quality control testing) and MolMed Spa, Italy (performing quality control testing).

The applicant mentions the involvement of contract manufacturing and testing sites. All contract manufacturing and testing sites should be included under Manufacturer(s). GMP status (for EU: manufacturing licence; for non-EU: QP declaration, and last inspection by EU competent authority) must be provided. **[MO]**

Manufacturing process

NGR-THFalpha is produced in host strain *E. coli* BL21, transformed with expression vector plasmid pAVEwayTM/NGR-TNFalpha. The vector pAVEway has the gene encoding the recombinant protein NGR/TNFalpha under control of a promoter and optimised lac operator sequence that enables complete

repression of in the absence of inducer. pAVEway strain BL-21. was selected for the preparation of the master cell bank (MCB) and the subsequent working cell bank (WCB).

The manufacturing process consists of an initial fermentation of *E. coli*, during which induction of protein expression with Isopropyl β-D-1-thiogalactopyranoside (IPTG) is performed. *E. coli* cells are harvested. Resuspended cells are lysed to release the product and the supernatant clarified. The soluble protein is then purified through chromatographic steps. A final 0.2 µm filtration is carried out before the drug substance is filled in containers for freezing and storage in 2L PETG bottles placed in double sealed bags at <-65°C. The NGR-hTNF drug substance is transported to the drug product manufacturer (NPM) in dry ice.

A *description of the manufacturing process,* including tables with operating ranges and/or set points of process parameters and tables of in process controls for each manufacturing step, is provided. However, there are discrepancies between the descriptions of the same steps provided in different parts of the dossier, as well as in the overviews of critical process parameters and attributes for the different steps. Reprocessing is not unequivocally described. The applicant should harmonise and correct the description of the process in different sections of the MAA. **[MO]**

Based on the description of the batch numbering system it seems that more batches have been manufactured than reported in the MAA. Explanation is asked. **(OC)**

Control of materials

A list of raw materials of non-biological origin used for the manufacturing of NGR-hTNF drug substance is provided. Most of the incoming material is controlled by Ph. Eur. grade materials or acceptance of the manufacturer's CoA. No products of human or animal origin were used in the manufacture of NGR-hTNF.

Fujifilm prepared a Master cell Bank and a Working Cell Bank.

Control of the manufacturing process

The applicant states that the proposed commercial manufacturing process for NGR-hTNF drug substance is controlled by ensuring process parameters, in-process controls and in-process specifications are within their expected ranges.

The proposed control strategy is unacceptable in its present form. Most tests are done "for information only", without defined acceptance criteria. The existing proposal of the applicant contains a too limited range of critical output parameters. Lack of validation data prohibit assessment (PPQ batches still have to be manufactured). **[MO]**

Manufacturing process development

Overall, three DS manufacturing processes were developed: subsequently processes A, B and C. Process A material was used for non-clinical studies and for Phase I and II clinical trials. Process B material was used for phase III clinical studies and in addition for non-clinical studies. Process C is the commercial process with which only two batches were manufactured, with small further change of process C between the first (non-GMP) and the second (GMP) batch. Between process A and B, major changes to the process have been made, e.g. a new cell line was constructed, the fermentation process was upscaled, the purification process was profoundly changed, and the formulation buffer was changed (PBS to citrate buffer, pH from 7.2 to 6.2, protein concentration increased). The range of characterisation tests used for the process A batch included for comparability assessment was reasonable for a process in development, however quantitative information regarding the composition regarding isoforms is lacking. For drug substance batches from process B and C more quantitative

information regarding isoform composition is available, however for process C batches too limited testing of primary and higher order structure was included in the comparability and characterisation studies. Process changes between processes B and C are smaller than between A and B.

Characterisation

Module 3.2.S.3 is highly incomplete and partially not consistent with other parts of the documentation (e.g. information included in manufacturing process development and comparability studies). This module should be entirely rewritten and supplemented with the data presented in manufacturing process development and comparability studies) as well as new data.

Characterisation studies were performed on two Process C batches manufactured using the commercial process (one GMP, one non-GMP, with small differences in manufacturing process, due to change of facility). According to the provided text, the following attributes were evaluated: appearance, identity, purity, potency. It is noted, that potency only includes cytotoxicity testing (not CD13 targeting). In addition, these batches were characterized for product related impurities (the applicant mentions: aggregates as dimers, trimers, hexamers, post-translational modifications of protein), process related impurities (host cell proteins, residual DNA), bioburden and endotoxins. Test methods to detect/characterise impurities were: SE-HPLC, SDS-PAGE (reducing and non-reducing), UPLC Peptide Mapping, cIEX-UPLC, ESI Mass Spectrometry, MRM MS, Free –SH, Q-PCR Residual DNA, Host Cell Protein ELISA, Bioburden, Endotoxin, Tetracycline residuals, IPTG residuals, Antifoam DF-204 residuals, PEI residuals, α√β3 binding assay (deamidation assay). Unlike for the process B and A batches, for process C batches, no amino acid analysis, N-terminal sequencing, circular dichroism or tryptophan intrinsic fluorescence was done for the Process C batches.

The available characterisation studies provide only superficial information on results of analyses. Since description and validation of analytical procedures was also extremely short and deficient, full critical assessment of the provided characterisation results (as part of the comparability studies) is not (yet) possible.

It is concluded that characterisation data are insufficient, due to small number of analysed batches, insufficient range of included characterisation tests, insufficient discussion of available relevant characterisation results in the section on characterisation. The company should include the PPQ batches in an additional comparability exercise and include parameters on primary and higher order structure as well as on deamidation (also during stability), and include testing for integrity of the NGR-moiety and its functionality (CD13 binding assay, as part of potency testing), and include an adequately justified proposal for CQA's for product related impurities, based on risk assessment and/or non-clinical c.q. clinical data, in accordance with ICH Q11.

More detailed presentation and discussion of results is required for assessment. In addition, the relevant data should be properly reviewed and discussed in Module 3.2.S.3. **[MO]**

Specification

The active substance specification includes control of identity, purity, potency and other general tests. Release specifications for the drug substance cannot be finally assessed, since insufficient manufacturing process validation data, batch analysis data and stability data are available. In addition, the choice of proposed release parameters is not based on appropriately justified CQAs (based on risk analysis and design of control system), and the limits are not in line with the few available batch

analysis data. A number of concerns has been reised regarding the active substance specifications, analytical method description and validation.

The specification set for TNF activity of NGR-hTNF is too wide. **[MO]** No clear description is provided which deamidated forms are measured. The definition of NGR-hTNF isoforms is not provided clearly anywhere in the MAA. **[MO]**

Reference standard

Due to insufficient package of release, characterisation and stability data, the qualification of the reference standard is not acceptable. Supplemental data should be provided in accordance with the final release specifications and requested additional characterisation and stability data. For instance, potency and deamidation should be included, acceptance limits should be specified. **[MO]**

Container Closure system

The NGR-hTNF DS is filled into 2L PETG gamma irradiated bottles with High Density PolyEthylene screw closure. The container and the closure are only qualified for sterility, cytotoxicity and pyrogenicity. The company should also provide information on Extractables and Leachables. Under process validation (Module 3.2.S.2.5) the company announces that studies are planned. **(OC)**

Stability

Insufficient stability data are available. **[MO]**

The PPQ batches from process C which are going to be manufactured will also be put on stability. The company should revise the stability protocol for these batches, and include additional stability indicating tests. **[MO]**

Comparability exercise for Active Substance

Comparability of release and characterisation data was studied of 1 process A batch to three (2 GMP) batches of process B drug substance and of two (1 GMP) process C batches to two (both GMP) process B drug substance batches.

No comparability data of in process parameters was provided. Considering the differences between the processes, this is only relevant for processes C vs B.

Since only comparability data from 1 process C GMP batch are available and only with a limited number of parameters and a limited storage period, further comparability data from the PPQ batches are asked, with an extended package of test methods. **[MO]**

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

NGR-hTNF drug product is a liquid, manufactured from thawed bulk drug substance, formulated at the target concentration of 0.2 mg/ml in 50 mM sodium citrate, 35 mg/mL mannitol, 10 mg/mL sucrose, pH 6.2. DP is filled in Type I glass 3 ml vials with a filling volume of 1.0 ml. The infusion product is prepared at the hospital pharmacy.

Before infusion to patients, NGR-hTNF drug product is diluted to the appropriate concentration with 0.9% NaCl containing 1 mg/ml human serum albumin (HSA). The procedure for the preparation of the infusion solution as provided to the hospital pharmacist should be part of the SmPC.The DP as manufactured according to the commercial process has not been produced yet. This is considered a **MO.**

Manufacture of the product and process controls

Manufacturers

NGR-hTNF Medicinal Product for commercial use is manufactured by NerPharMa starting from NGR-hTNF Drug Substance produced according to manufacturing process C.

Manufacturing process

The description of the manufacturing process is far too general for a MAA and should be provided in more detail. Each step in the process should have the appropriate process parameters identified, such as time, temperature, or pH and holding times of the intermediates.

Three PPQ lots were manufactured according to process B, to demonstrate that DP commercial manufacturing process, will consistently produce DP of reproducible quality meeting validation criteria and release specifications. Because Process B closely resembles commercial manufacturing, the process qualification to identify critical steps (see table below) can generally be accepted. An extensive comparison of the steps of process B with the commercial process steps has been provided with justifications for each of the differences. However, no commercial scale GMP DP batches have yet been manufactured, which is considered a major objection.

In addition to the PPQ activities, also compatibility studies with process materials and primary packaging materials were carried out, however no data have been provided.

Mixing speed and time and homogeneity during filling have been validated. Transport of DP and maintaining temperature during shipping has been validated.

None of the excipients used in the manufacturing of NGR-hTNF DP in liquid dose form are of human or animal origin.

Product specification

The medicinal product specification includes control of identity, purity, potency and other general tests**.**

The proposed set of specifications has to be re-evaluated based on when an appropriate characterisation of DS has been completed. In this respect, CQAs remain to be established and several of the product-specific Drug Product (most the same as for DS) release tests have not properly been validated. This is considered a **MO**. Several of the proposed specifications are too wide considering the results of the clinical batches (both from process A and process B).

Stability of the product

No stability data are available for commercial process batches. These should be generated.

Comparability exercise for Finished Medicinal Drug Product

In the absence of commercial process DP batches no comparability data are available for the batches from the commercial process with the clinical batches. **[MO]**

Adventitious agents

NGR-hTNF is produced in a bacterium (*E. coli*) production system and is purified from the E. coli supernatant. No virus or mycoplasma testing was performed as there are no known bacterium viruses that have been reported to be transmitted to humans or risk of mycoplasma growth.

No animal-derived materials are used for the production of NGR-hTNF. Both the master cell bank (MCB) and working cell bank (WCB) were prepared using animal component-free media. Therefore, the risk of NGR-hTNF being contaminated with TSE agents is negligible

Sufficient information has been provided regarding adventitious agents. Microbiological control is obtained by means of IPCs throughout the manufacturing process and was assessed as part of the manufacturing process and its control strategy.

GMO

N/A

3.1.4. Discussion on chemical, pharmaceutical and biological aspects

Drug substance

The provided dossier is severely deficient. The definition of the active substance is not understood, as forms with changes in the NGR part are considered as part of the active substance. However, these forms may have different activity and/or affinity/specificity for binding to CD13.

In addition, the description of the drug substance manufacturers and of the drug substance manufacturing process is incomplete and/or contain errors, the control system is not acceptable, the commercial manufacturing process is not yet validated. PPQ batches of the commercial process still have to be manufactured and there are only release and stability data of 1 non-GMP DS batch and 1 GMP batch, precluding assessment of process validity, consistency and of Drug Substance stability. The sections on characterisation and control of drug substance are not acceptable, containing incomplete information and/or justification. Additional attributes should be included in the release specifications and in the stability protocol. Methods are insufficiently described and analytical validation studies either are not provided or still have to be carried out. Further data is asked regarding the potency assay.Comparability data are incompletely reported and comparative data are available on only few batches used in clinical studies vs. the commercial process (not yet clinically tested) and include too little parameters. Data on the reference standard for the commercial process are incomplete and not acceptable in their current form.Taking into account all uncertainties and lack of substantial proof of the quality, control and consistency of the drug substance, quality of the drug substance is considered insufficiently proven.

Drug product

For the Drug Product, no GMP batches for the commercial process have been manufactured yet. This is considered a MO. Consequently, the process validation data are highly incomplete and no comparability with the clinical batches has been demonstrated. Both are considered MO. The control of drug product

is not acceptable. Firstly, because the CQAs have not been established as result of the insufficient characterisation of DS. Therefore the proposed release and stability specifications need to be re-evaluated. Secondly, because the non-compendial methods are not appropriately validated. Several of the proposed specifications are considered too wide based on the results of the clinical batches.

3.1.5. Conclusions on the chemical, pharmaceutical and biological aspects

The provided quality dossier is considered insufficient. In the quality assessment. A number of Major Objections have been raised which preclude a positive opinion on Zafiride.

3.2. Non clinical aspects

3.2.1. Pharmacology

Primary pharmacodynamics

NGR-hTNF is a recombinant human tumour necrosis factor (TNF) linked to a NGR peptide. TNF is able to bind TNFR1 and 2 both present on these cells, and has a cytotoxic effect. The peptide is able to bind CD13 present on endothelial cells of early tumour vasculature. The NGR peptide is added to TNF to increase the specificity by homing TNF to tumour vascular endothelial cells and stabilizes its binding to TNFR. The cytotoxic effect of TNF is thereby specifically targeted to the tumour associated vessels and aimed at making tumours more accessible for the immune cells and chemotherapeutics.

In vivo antitumour activity and localisation of NGR–mouse TNF

The antitumour activity of NGR-mouse TNF (NGR-mTNF) was studied *in vivo* in mouse carrying B16F1 melanomas or RMA-T lymphomas. NGR-mTNF co administered with melphalan showed a synergistic effect in reducing tumour growth, which appeared to be dependent on the interaction of the TNF moiety with TNF receptors since it was abolished in the presence of anti-TNF antibody. When the NGR peptide was removed from the molecule (by trypsin treatment) the *in vivo* antitumour activity, but not the toxicity, dropped to the level of mTNF although *in vitro* cytolytic activity of NGR-mTNF against LM cells was not changed. In presence of an excess of CNGRC, a peptide binding CD13, antitumour activity, but not the toxicity, of NGR-mTNF was reduced in vivo. Antitumour effects of NGR-mTNF were also inhibited after incubation with an anti-CD13 monoclonal antibody (mAb R3-63). NGR peptide coupled to quantum dots (Qd) showed localisation to CD31 and CD13 positive blood vessels associated to a murine colon carcinoma, but not to normal vessels and tissues, including intestine, central nervous system, kidney, lung, heart, muscle, and bone marrow, confirming the specificity of NGR in targeting tumour associated blood vessels. Binding can be abrogated by downregulation of CD13 with CD13-specific shRNA. Although binding of the NGR moiety of the product to CD13 has been studied by means of co-localisation and competition assays no information exists on kinetics of this interaction.

In vivo antitumour activity studies revealed an efficacy/toxicity ratio of NGR-mTNF (8,8/0,6) 14 times greater the RMA-T lymphoma model and 12–15 times in the B16F1 melanoma than that of mTNF despite similar toxicity. Testing a range of doses (from 0.01 to 10000 ng/mouse) revealed a U shaped dose-response curve where the antitumour effect of 10 ng/mouse was lower than that of 0.01-0.1 ng/mouse and 1000-10000 ng/mouse.

In an adjuvant setting (mouse model of breast tumour metastasis) NGR-TNF reduced both size and number of lung metastases 7 days after inoculation with 4T1 tumour cells, whereas when treatment was started 13 days post inoculation this only resulted in reduction of metastasis. This may be related to the increase of neo-angiogenesis, 7 days after tumour challenge, identified by the increase of CD13

expression on CD31+ tumour vasculature. Increased infiltration of CD3+ T cells in the lung of NGR-mTNF treated mice particularly localizing within the metastatic lesions, suggest an active role of the immune system in the anti-metastatic activity of NGR-mTNF. As NGR-mTNF does not affect wound healing, treatment could start immediately after surgery.

The role of the immune system was further studied in the spontaneous model of neuroendocrine pancreatic tumours in RIP1-Tag2 versus RIP1-Tag2 RAG1-/- mice. At an early stage, low doses of NGR-mTNF had a direct cytotoxic effect inducing endothelial cell apoptosis, reducing vessel density and eventually inhibiting the development of angiogenic islets both in immunodeficient and immunocompetent mice. When the expression of CD13 on endothelial cells was down-modulated, the antitumour effects of NGR-mTNF were mainly mediated by different mechanisms involving the immune system such as an increased infiltration of activated CD8+ T cells. The Applicant also claims that genes encoding proinflammatory cytokines and vascular stabilizing factors were upregulated, but does not present data confirming these findings.

Chemotherapeutic drugs at maximum tolerated doses or vascular disrupting agents (VDAs), which induce massive tumour hypoxia and necrosis, recruit Bone Marrow derived Dendritic Cells (BMDC). BMDC are potent immunosuppressants as well as proangiogenics and thereby contribute to the rapid regrowth of the tumour after the initial insult. However, NGR-mTNF decreased tumour blood vessel density (evaluated as CD31 expression) by induction of apoptosis of tumour and tumour endothelial cells measured by cleaved caspase 3 (ClCasp3), without causing tumour hypoxia & necrosis and the subsequent mobilisation or recruitment of proangiogenic BMDCs at the tumour site.

Localisation of NGR–humanTNF to and signalling of CD13 and TNFR 1 & 2 receptors

CD13 expression on normal and neoplastic renal tissue from human origin was evaluated using two different anti CD13 antibodies, 13C03 and WM15. The 13C03 epitope, but not the WM15 epitope, was expressed in the brush border of the renal proximal tubule epithelial cells. None of the epitopes was expressed on neoplastic cells, but both epitopes were expressed on stromal cells surrounding neoplastic lesions. Results indicate that at least two CD13 isoforms exist and that the WM15 epitope is specific for expression of tumour associated endothelial vessels. Sections of renal cell carcinoma, breast and prostate metastases incubated with NGR-humanTNF (NGR-hTNF) pre-complexed with TNF mAb78 (NGR-TNF/78) and 13C03 or WM15, show a NGR-hTNF localisation very similar to WM15 and distinct from those of 13C03 and could be totally competed by co-incubation with an NGR-mIFNγ conjugate. Biacore analysis confirmed that the presence of the CNGRCG sequence does not modify the interaction of the NGR-hTNF with TNF receptors (TNFR1 and TNFR2) as determined the kinetic parameters (K_{on}, K_{off} and K_D) and dissociation kinetics with faster K_{off} values for the latter of the two receptors. NGR-hTNF has an NGR-mediated higher affinity for TNFR than hTNF (hTNF IC50 ≈20 nM vs NGR-hTNF IC50 ≈5 nM), indicating that there is an increase in the affinity of NGR-hTNF for TNFRs on NGR-binding cells but not on NGR-nonbinding cells

MEK, Erk1/2 and Akt, out of 33 kinases tested were differentially activated by NGR-TNF over hTNF (Broad Phospho-site Screen assay, Kinexus). NGR-hTNF inhibits Ras, Mek, Erk1/2, Akt, and IκB-α more than hTNF. These signalling effects can also be obtained by incubation with NGR-mIFN (binding only CD13, since mouse IFN is not recognized by human cells) and hTNF separately. These results indicate that both NGR and TNF portions contribute to the activation of signalling pathways elicited by NGR-hTNF, through the engagement of both CD13 and TNFRs. In addition, NGR-hTNF exerts higher activation of caspases 3, 8 and 9 than hTNF thereby inducing more effectively apoptosis of tumour vessel endothelial cells in tumour-bearing mice. The applicant concludes that binding of NGR to CD13 determines not only the “mere homing” of NGR-TNF but also a) the stabilization of the NGR-CD13/TNF-TNFR interaction and b) a direct effect of CD13, leading to a highly selective homing on tumour blood

vessels and apoptosis of angiogenic endothelial cells *in vivo,* thus significantly contributing to the control of tumour growth.

NGR-mTNF or NGR-hTNF in combination with chemotherapeutic drugs in cancer therapy

A synergistic antitumour effect was observed in the B16F1 and RMA tumour models in mice both at high doses (3-9 µg) NGR-mTNF as well as low doses (0.01- 0.1 ng) of NGR-hTNF administered together with melphalan or other chemotherapeutic drugs (doxorubicin, cisplatin, gemcitabine and paclitaxel). Synergism was stronger with NGR-**m**TNF than 0.1 ng of NGR-**h**TNF, suggesting that TNFR2 (triggered only by NGR-mTNF in the mouse) contributes to the overall antitumour activity of NGR-TNF. NGR-mTNF increases the number of cells reached by doxorubicin, which is easy visible since it is a fluorescent drug, suggesting that NGR-mTNF results in more permeable tumour vasculature and increases accessibility of the tumour for immune cells and chemotherapeutics. Evidence was obtained to suggest that 2 h is the time necessary for NGR-mTNF to induce optimal biological effects to increase the efficiency of chemotherapeutics. The increase of efficacy was not accompanied by an increased toxicity.

Secondary pharmacodynamics

The combination of hTNF alpha, specifically targeting TNFR1 and 2 receptors, with the NGR peptide, binding to a certain CD13 isoform, makes the product specific for targeting TNFR1 &TNFR2 co-localised with CD13. Secondary pharmacodynamics are not foreseen and the absence of studies addressing potential secondary pharmacodynamics can be agreed.

Safety pharmacology

In accordance with ICH S6, safety pharmacology was assessed as part of repeated dose toxicity. However, the applicant also performed separate safety pharmacology studies in which NGR-hTNF is administered via a bolus injection. Since this is not the clinical route of administration, these studies are regarded supportive. Safety pharmacology of the product administered via clinical route of administration, intravenous infusion, is addressed in the repeated dose toxicology

Effects in the Irwin test and on body temperature in rats dosed with 1, 13.1 and 89.4 µg/kg NGR-hTNF resulted in mild features mainly associated with changes in autonomic function (piloerection and exophthalmos) in all animals (also vehicle) but with a more prolonged effect in animals dosed with 89.4 µg/kg NGR-hTNF. Mid and high dose also resulted in mild effects on body gait. In conscious male rats, upon intravenous administration of 1, 13.1 and 89.4 µg/kg NGR-hTNF resulted in a significant increase in respiration rate at 240 min but not at 15 min post-dose 89.4 µg/kg NGR-hTNF. Cardiovascular effects of conscious, telemetered Cynomolgus monkeys upon administration of 0.20, 6.40 and 5.49 µg/kg NGR-hTNF showed significant dose-independent increases in heart rate with both 6.40 and 5.49 µg/kg NGR-hTNF that were associated with the shortening of the RR, PR and QT intervals. Shortening of the QT interval was partially corrected using the QTcF interval and completely corrected using the QTcQ interval.

Thus, provided that hTNF is also active in rat and Cynomolgus monkey, above summarized studies support that up to doses of 1 µg/kg NGR-hTNF in the Rat and 0.20 µg/kg NGR-hTNF in Cynomolgus monkey, a cause for concern for adverse reactions on vital functions in humans is not present.

Pharmacodynamic drug interactions

The product was proposed to be synergistic with chemotherapeutic drugs by making the tumours more accessible for the chemotherapy. This effect is sufficiently addressed in the primary pharmacodynamic studies. The omission of further PD interaction studies is agreed.

3.2.2. Pharmacokinetics

The analysis methods were generally fit for purpose, in part due to the very high exposures that have been reached. Imprecision, inaccuracy, negative bias or failing incurred sample reanalysis was noted for several assays. Assays were not developed according to the standards established in the EMA guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009). Pharmacokinetics are characterized by slow clearance and relatively low volume of distribution, suggesting that the majority of NGR-hTNF remains in circulation. While no accumulation has been observed, exposure increases more than dose proportionally in rats and appears to plateau in the high dose group. In monkeys, high intra-animal variability confounds a male-female comparison. In males, exposure increases more than dose proportional between low and mid dose groups, and dose proportionally afterwards. Lower exposure is noted in rats after 85 days compared to day one estimates. Very high intra-animal variability is observed and very low exposures have been reached in individual animals and in dose groups after repeated dosing in rat and Cynomolgus monkey. High variability with low exposures in individual animals, particularly after repeated dosing is usually indicative of immune responses, or high variability can be the result of problems with the assay. High inter-animal variation and the lack of data on anti-drug antibodies in the 13 week monkey studies compromise a definitive conclusion on the level of exposure to NGR-hTNF in both rat and monkey.

A biodistribution study was conducted in mice, which received NGR-hTNF via an intraperitoneal infusion. In this publication NGR-hTNF appears to distribute to organs and blood relatively equally and does not accumulate in kidney. In contrast, toxicokinetic data from pivotal chronic IV studies in both Cynomolgus monkey and rat suggests that NGR-hTNF largely remains in serum and is considered to be the clinically relevant representation of distribution.

As a protein therapeutic, NGR-hTNF is not expected to be metabolised or excreted. Pharmacokinetic interaction with small molecules is not expected and there was virtually no influence of NGR-hTNF on the pharmacokinetics of doxorubicin or cisplatin, two cytostatics expected to be used in clinical treatment with NGR-hTNF. NGR-hTNF also does not influence CYP450 in Cynomolgus monkeys at doses as high as 12.5 µg/kg/day for 14 days. Statistically significant increases are not considered biologically relevant.

3.2.3. Toxicology

Single dose toxicity

Very high single doses of Zafiride in mice or rats were relatively well tolerated. At the highest doses tested, Zafiride induced acute inflammatory reactions. While the data are usable to establish a top dose in chronic studies, the relevance of the findings for humans is limited since such high doses will never be achieved under normal clinical conditions.

Repeat dose toxicity

It is unclear to what extent Cynomolgus monkey or rat can be considered relevant toxicological species. In GLP compliant chronic IV infusion studies with this species, no toxicity was observed at all, although liver was a target organ in the bolus IV studies with only mild or moderate severity. However, neither justification suggests that the models were selected based on being a pharmacologically active species.

Exposure in bolus IV studies in rats and Cynomolgus monkeys is considered sufficiently high to assess toxicity. Studies in rats using a bolus IV route of administration resulted in NGR-hTNF related high rates of mortality. The cause of death was primarily attributed to severe acute haemorrhagic

inflammatory reaction in the caecum. At the end of treatment there were major decreases in red cell parameters in both sexes and in all surviving NGR-hTNF-treated groups. This finding was associated with extramedullary haematopoiesis. These findings were not repeated in Cynomolgus monkeys. The relation to the Mode of Action of the product with regards to the cause of mortality and haematology findings, and the observed species differences was not sufficiently discussed by the Applicant. In Cynomolgus monkeys, bolus IV injection of NGR-hTNF was much better tolerated. Increased transaminases (ALT, AST) and histopathological changes in liver including multifocal portal liver inflammation suggest that liver would be a target organ, in line with the known toxicity of TNFα.

Marked mortality, which was previously observed in rats, no longer occurred after switching to a slow IV infusion of NGR-hTNF. No or only minor changes were observed in NGR-hTNF treated rats were observed. In a non-GLP 5 week IV infusion study, one female monkey given 400 µg/kg/week was found in moribund condition and sacrificed. At necropsy, the liver was found to be yellowish and friable. Subsequent groups of animals given 200 µg/kg/week showed increased liver transaminase activity suggesting that at high exposure, TNF-mediated liver toxicity occurred. In the pivotal chronic 13 week IV infusion study in monkeys, no relevant findings were observed on cardiovascular, respiratory or central nervous system, nor ECG on haematology, serum chemistry, urinalysis, gross necropsy or histopathology parameters at any dose tested.

Considering the very high exposures that have been reached at which adverse reactions were observed, these are generally not considered to be meaningful for humans. The absence of further adverse effects in monkey given very high doses of NGR-hTNF is not understood in the light of the toxicity of TNF alone in human. In addition, data on the pharmacological activity of the product in rat and monkey, for instance a tissue cross reactivity study or a cytotoxicity study on rat or monkey cells, is lacking.

Pharmacokinetic and toxicokinetic data should be interpreted with caution. Nevertheless, absolute exposure margins in animals were very high compared to clinical exposure. It can be accepted that individual animals in all dose groups were exposed throughout the IV infusion repeat dose study, which allow a toxicological assessment. Very large inter-animal variation is apparent in exposure. Anti-drug antibody analyses have been performed for the bolus IV studies in Cynomolgus monkeys. A low neutralising antibody titre was detected in 9 out of 32 animals, three of which were control group animals. 4 out of 10 animals in the high dose group showed slight elevations in antibody titres. These were below the LOQ and were not considered to be a strong response. No anti-drug-antibody titres have been determined in the pivotal 13 week toxicology studies in either rat or Cynomolgus monkey. Absence of anti-drug antibody data compromise the definitive interpretation of the toxicokinetic data.

Reproductive toxicity

Fertility endpoints were incorporated in pivotal repeat dose toxicology studies without notable changes that would suggest that fertility is impaired in males or females. NGR-hTNF is teratogenic in rats but only at high exposures. In a non-GLP rat embryofoetal development study, administration of NGR-hTNF resulted in decreased body weight in dams on the day of treatment and severely increased post-implantation loss (49.8%) in the high dose treated group (240 µg/kg). Decreased foetal weight was noted, with skeletal malformations observed in foetuses exposed on GD10 and GD16. In the pivotal study, there was a minimal decrease in maternal bodyweight and bodyweight gain in the highest dose group tested, 240 µg/kg/day. Uterus weight was decreased at final necropsy in high dose dams. Severe post implantation loss was noted in the high dose group, in particular dams treated on GD 6 and 12 (36.5%). Embryofoetal loss in high dose dams treated on GD 10 and 16 was 15.5%. In contrast, embryofoetal loss in dams given 85 µg/kg/day on GD 10 and 16 was 3.6% and comparable to control animals (3.5%). Mean foetal body weight was slightly decreased in offspring of high dose

dams treated on GD 10 and 16. External malformations were noted in 2.6% and 3.5% of foetuses exposed to high dose NGR-hTNF on GD 6 and 12 or 10 and 16, respectively and represented 13% and 14.3% of litters, respectively. Short fore and/or hindlimbs were prominent findings in 4 pups with malformations. Considering the high exposures achieved in the high dose group, these results are not considered to be clinically relevant.

There were no visceral malformations in any examined animals. Visceral variations were increased roughly 2-fold in foetuses exposed to high dose NGR-hTNF on GD 10 and 16 compared to controls (6,7% vs 3.7%), but not on GD 6 and 12 (3.8%). Foetuses exposed to 240 µg/kg also showed a marked increase of thoraco-lumbar changes, namely 27 pre-sacral vertebrae, the 14th supernumerary rib and retardation of ossification of thoraco-lumbar centra. In contrast, in dams given 30 µg/kg or 85 µg/kg NGR-hTNF the type and distribution of variations were comparable to the incidence in control animals. Given the extremely high exposure multiples, even at the lowest dose tested, these results are not likely to be clinically relevant.

Other toxicity studies

In line with what is expected for protein therapeutics and NGR-hTNF in particular, no specific studies were conducted to evaluate immunotoxicity, dependence or impurities. Local tolerance was assessed in the pivotal repeat dose toxicity studies without untoward effects. Antigenicity of NGR-hTNF in both rat and Cynomolgus monkey has not been assessed (see toxicokinetics). NGR-hTNF did not increase toxicity of two common cytostatics, doxorubicin and cisplatin, in a repeat dose toxicity study in Cynomolgus monkeys. The only observed toxicity was attributable solely to either doxorubicin or cisplatin.

3.2.4. 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, NGR-hTNF is not expected to pose a risk to the environment.

3.2.5. Discussion on non-clinical aspects

Pharmacology

Variants of NGR-hTNF

Three variants of the NGR-hTNF exist, that differ from each other in the NGR part. It is not clear whether all these variants bind CD13 with same kinetics and whether or not these variants have same antitumour activity. In addition, it is not known how much of each variants is present in each of the batches used in the preclinical studies. It is also not known which batches are used for the non-clinical PD studies. A multidisciplinary major objection is posed reflecting these shortcomings **(multidisciplinary MO)**.

Mode of Action – Signalling

Biacore data indicate that binding to TNFR and CD13 stabilizes the NGR-CD13/TNF-TNFR interaction and giving reason to assume increase NGR-hTNF affinity for the target cells. It remains unclear if CD13 and TNF receptors in the cell membrane are already in close proximity before binding to NGR-hTNF or whether binding to NGR-hTNF brings them together and whether this close proximity also influence downstream signalling. Furthermore, based on signalling data, the applicant concludes that binding of NGR to CD13 determines not only the “mere homing” of NGR-TNF but also a) the stabilization of the

NGR-CD13/TNF-TNFR interaction and b) a direct effect of CD13, leading to a highly selective homing on tumour blood vessels and apoptosis of angiogenic endothelial cells in vivo, contributing to the control of tumour growth. From the signalling data it is assumed that signalling directly via NGR binding to CD13 may contribute to the inhibition of the kinases that are inhibited by TNF and also contribute to the activation of Caspase 3, 8 and 9. How signalling via CD13 occurs exactly is not investigated. Binding kinetics of (different isoforms of) NGR interacting with CD13 (e.g. Biacore data) are lacking. It may be possible that binding of NGR to CD13 is dependent on the variant and that relative presence of the different isoforms in the mixture / product may influence the antitumour activity of the product. This issue is covered by the multidisciplinary major objection.

Proof of Concept - Effective dose

In the study to investigate the role of binding of NGR-mTNF to its receptors, mice were dosed 0.1 ng per dose per mouse. In contrast, in the study to investigate the role of the NGR peptide mice were dosed more than 10000 x higher (1, 3 or 9 µg mTNF or NGR-mTNF). The NGR peptide is added to the TNF moiety to increase its specificity in the picogram range. Furthermore, the dose-response curves of NGR-mTNF and mTNF (from 0.01 to 10000 ng/mouse) in the RMA-T lymphoma- models, showed that antitumour effect of 10 ng/mouse was lower than that of 0.01-0.1 ng/mouse and 1000-10000 ng/mouse. This suggests a a U-shaped dose–response curve. Thus, in vivo tests in the mouse have been conducted with doses at both sides of the U shaped dose-response curve, without further justification of the selected doses, or comments on the observed dose-response curve. In addition, extrapolation of effective dose in the mouse model to the human situation was not discussed.

Pharmacology - Summary

Binding of NGR-hTNF to human tissues and in vitro signalling of NGR-hTNF in human cells have been shown by the applicant, although clarification on binding and direct signalling via the NGR binding to CD13 is lacking. Proof of concept of use of low and high doses of the NGR-mTNF variant has been observed in the mouse bearing human tumours. However, in vivo data on NGR-hTNF is scarce and has the shortcoming that it only can bind TNFR1 in the mouse. In addition, it is not yet clear to which extent the mouse variant of the product can be compared with the clinical product, which may limit direct extrapolation of an effective dose and the concordant effect to the clinical situation.

Pharmacokinetics

The immunoassay with ECL detection was used to determine NGR-hTNF in the pivotal repeat dose toxicity studies performed in rat and monkey using the CMS/HSA formulation, which is comparable to the commercial product. Although formally validated, it is unclear whether this method is able to discriminate between NGR-hTNF and hTNF since the capture was done with a cytokine antibody. The Applicant is requested to clarify and discuss potential consequences for the interpretation of pharmacodynamic and toxicokinetic data.

Single dose and repeated dose absorption studies noted in the pharmacokinetics tabulated summary refer to toxicology studies in rat and Cynomolgus monkeys. Formal pharmacokinetic absorption studies have not been performed. It should be noted that in the bolus IV studies, the target dose has not been reached because of absorption of the drug product to the glass vials. Absorption occurred dose dependently, with more drug product being absorbed with increasing concentration. Furthermore, detectable levels of NGR-hTNF were measured in some of the plasma samples from control animals in the 13 week repeat dose toxicity study in monkeys and rats, and in the EFD study. While these levels are low and do not affect the conclusions of the studies, the Applicant suggests that interference with an endogenous compound is the cause for the positive findings in control animals. Rather than AUC^{-}_{inf} or AUC_{0-t}, the Applicant has used AUC_{last} to estimate exposures. This is accepted, although it is a

less precise estimate of the exposure. The methods used to measure plasma concentrations of NGR-hTNF in rats and Cynomolgus monkeys are acceptable. The analytical methods used to measure antibodies to NGR-hTNF in Cynomolgus monkey plasma is acceptable.

High variability with low exposures in individual animals, particularly after repeated dosing is usually indicative of immune responses, or high variability can be the result of problems with the assay. Pharmacokinetic data should be interpreted with caution. The Applicant is invited to discuss high intra-animal variability and the potential for anti-drug antibodies in the pivotal 13 week IV infusion studies and to provide an estimate on how many animals were exposed for the duration of the study. The Applicant is also requested to comment on the absence of anti-drug antibody analysis in the pivotal IV infusion studies, and to submit these data if available.

A biodistribution study was conducted in mice, which received NGR-hTNF via an intraperitoneal infusion. These data were submitted as a peer reviewed publication. For protein therapeutics distribution studies are not meaningful although tissue cross reactivity study can be useful. However, TCR data was not submitted. Plasma protein binding has not been investigated. Intraperitoneal injection is generally not an ideal route of administration to assess distribution of protein therapeutics. While intraperitoneal delivery is a parenteral route of administration, and IV injection in mice can be technically difficult, pharmacokinetic properties of an intraperitoneally administered product are more similar to oral administration because the primary route of absorption is via the mesenteric vessels, which drain into the portal vein and pass through the liver into circulation.

Toxicology

It is unclear to what extent Cynomolgus monkey or rat can be considered relevant toxicological species. The Applicant has justified use of the rat ‘as it has been extensively used in toxicology studies and a large amount of biological data is available for this species’. Liver-effects, seen sporadically and without a clear dose response, were observed at all dose levels in the 13-week rat study. They were described by the pathologist as bile duct proliferation, peribiliary mononuclear inflammation, peribiliary fibrosis, centrilobular and/or nodular multifocal hepatocellular fibrosis. The findings were not reversed after treatment free recovery period and could be interpreted as early indicators of cirrhosis. This hepatotoxicity is a concern due to the nature of the lesions (liver structure alterations, non-reversible) and the absence of NOAEL. The applicant should discuss the mechanism of these lesions and in particular the possibility of an off-target binding of NGR-TNF variants on CD13 isoforms on liver structures (biliary or other). There are few adverse findings in monkeys but the findings observed in the 14-day study (increased ALT, AST, reduced cholesterol, reduced albumin/globulin ratio in both sexes) indicated that the liver may be target organ for toxicity. The applicant should discuss the clinical relevance of the liver findings in animals and provide an overview of results from liver monitoring in patients. Futhermore in the 13-week monkey study, only control and high dose groups were examined for histopathology. However, examination of all animals at all doses groups is required for non-rodent species according to the guideline for repeat-dose study (CPMP/SWP/1042/99 Rev 1 Corr*). The applicant should provide these data. Cynomolgus monkey was selected ‘as NGR-hTNF is a biological product incorporating human Tumour Necrosis Factor, it was considered more appropriate to use the non-human primate, rather than the dog or minipig, as the second mammalian species for toxicology testing. (It has also been documented that the dog is extremely sensitive to TNF and rapid generation of an immune reaction to a human protein prevents repeated dosing in the dog)’. In GLP compliant chronic IV infusion studies with this species, no toxicity was observed at all although liver was a target organ in the bolus IV studies with only mild or moderate severity. However, neither justification suggests that the models were selected based on being a pharmacologically active species. Affinity binding assays for rat and Cynomolgus TNF-receptors have not been submitted. A tissue-cross-

reactivity assay is also missing from the non-clinical package. The apparent lack of toxicity at very high exposures is surprising given the known safety profile of TNF after IV infusion in humans, chiefly dose limiting hypotension and hepatotoxicity (Roberts et al. 2011). The Applicant should also demonstrate that NGR-hTNF is pharmacologically active in both rat and Cynomolgus monkey and to discuss potential differences in activity between the toxicology species and humans in terms of TNF-R binding. Affinity binding (Biacore) data of NGR-hTNF to rat and monkey TNF-R are expected at minimum (**MO**).It should be noted that at this stage, new studies in animals should not be considered. Furthermore, and in retrospect, long term studies in 2 species would not be required according to ICH S6 when the 1 month studies show comparable results. In this situation, a 13 week study in a single pharmacologically relevant species would have been sufficient.

As stated in the Assessor's comments on PK, animals in the bolus IV study did not receive the target dose because of absorption of NGR-hTNF to the glass vials. Nevertheless, exposure is considered sufficiently high to assess toxicity after a bolus injection in animals. Bolus IV studies do not use the intended clinical route and are considered supportive. However, the marked mortality in the rat studies, haemorrhage of caecum and haematological findings in surviving animals should be discussed in relation to the Mode of Action of the product. This should take into account the observed species differences and the potential for clinical adverse reactions with a similar mechanism of actionResults from bolus IV studies are difficult to interpret and the high mortality in rats is not considered to be clinically relevant because bolus IV not the intended clinical route, the formulation (PBS formulation) used in the bolus IV studies does not represent the clinical formulation and because such high exposures will not be reached in the clinic. Nevertheless, milder comparable adverse reactions should be considered. The citrate mannitol sucrose buffer diluted with HSA formulation used in chronic toxicology studies where NGT-hTNF was administered via slow IV infusion more closely matches the formulation and route of administration proposed for clinical use and are considered as the pivotal chronic toxicology studies. GLP compliant bolus IV studies where NGR-hTNF are supportive.

Pharmacokinetic and toxicokinetic data should be interpreted with caution. Nevertheless, absolute exposure margins in animals were very high compared to clinical exposure. It can be accepted that individual animals in all dose groups were exposed throughout the IV infusion repeat dose study, which allow a toxicological assessment. Very large inter-animal variation is apparent in exposure. For example, low dose female rats can be considered practically unexposed at day 85. Furthermore, exposure on D85 in animals was generally lower than on day 1. This seems to suggest that clearing anti-drug antibody could be formed in animals with low exposure. Anti-drug antibody analyses have been performed for the bolus IV studies in Cynomolgus monkeys. A low neutralising antibody titre was detected in 9 out of 32 animals, including control group animals. However, because this is neither the intended route of administration, nor the clinical formulation applied for, these cannot be considered as relevant to assess results from the IV infusion studies. However, no anti-drug-antibody titres have been determined in the pivotal 13 week toxicology studies in either rat or Cynomolgus monkey.

3.2.6. Conclusion on non-clinical aspects

There are other concerns that should be resolved and preclude a marketing authorisation at this time. The non-clinical assessment also has consequences for section 4.3, 4.6 and 5.3 of the SmPC.

3.3. Clinical aspects

The development programme

The applicant submitted five phase I, one phase II (study NGR010) and one pivotal phase III study (Study NGR015) to support the application. Another phase II trial is ongoing (NGR019). No specific trial was designed to study pharmacokinetics and pharmacodynamics of NGR-hTNF; these topics were investigated in the Phase I-II studies.

However, the clinical development plan lacks consistency, as the phase I-II studies are conducted with a tri-weekly dosing scheme, while the current applied application consists of a weekly dosing scheme. No pharmacodynamic data were obtained for this weekly dosing scheme, while bridging data for the tri-weekly dosing scheme to the weekly dosing scheme is missing. The clinical development programme did not yield biomarker data to support the mode of action.

This application is based on a single, pivotal, randomised, double blind, placebo-controlled trial (NGR015). After the study failed to meet the primary endpoint, the protocol-defined subgroup analyses for survival showed a significant interaction only for one patient subgroup: those with a median TFI of < 4.8 months. This subgroup is based on the treatment-free interval (TFI), i.e. time from the last treatment application to the start of the randomised treatment.

The applicant used the prespecified cutpoint (median value) to define the subgroup of interest, i.e. the subgroup with a short TFI defined by a TFI <4.8 months, and a specific post hoc sensitivity analysis to select a TFI <6 months. Importantly, PFS and TFI are not the same. Moreover, several types of clinical data were not provided, e.g. the main outcomes of the complementary subgroup with a TFI >6 months were not included in the dossier.

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

Table 1 Overview of the clinical studies.

Study ID	No. of studies / locations	Phase	Design	Posology	objectives	Subjects by arm Entered	Duration	Gender Male/female Median age	Diagnosis/inclusion criteria	Primary endpoint
EORTC 16041	2 (NL, DE)	I	SA, MTP	0.2-60 µg/m2	DLT, MTD, Ba PK	70	Depending on clinical outcome	NP	Solid tumours	DLT, MTD
IPR/07 NGR002	1 (IT)	I	SA, MTP	0.2-0.4-0.8-1.6 µg/m²/3w	MTD/DLT Vessel permeability	16	Depending on clinical outcome	6/10 60 yrs	Advanced solid tumours	Safety, DCR DCE-MRI
IPR/09, NGR003	4 (IT, NL)	I	CBT, SA	NGR-hTNF 0.2-0.4-0.8-1.6 µg/m²/3w Docetaxel 75mg/m²	Safety, dose recommendation	15	Docetaxel: max 6 cycles or 550 mg NGR-hTNF: Until PD, NT, or PDc	10/5 58 yrs	Advanced or metastatic solid tumours	DCR, safety
IPR/10 NGR004	2 (IT)	I	CBT, SA	NGR-hTNF 0.2-0.8 µg/m²/3w Cisplatin 80 mg/m² (max 6 cycles)	Safety, dose recommendation	22	Cisplatin: max 6 cycles NGR-hTNF: Until PD, NT, or PDc	14/8 60 yrs	Advanced or metastatic solid tumours	DCR
NGR013		I		NGR-hTNF 60-325 µg/m²/		48			Advanced or metastatic solid tumours	
IPR/16 NGR010	4 (IT)	II	SA	0.8 µg/m²/3w	Tumour activity	43	Depending on clinical outcome.	27/16 64 yrs	Radiologic confirmed MPM after 1st line pemetrexed chemotherapy	PFS
		II	SA	0.8µg/m²/w		14		8/6 67yrs		
IPR/22 EudraCT no. NGR015	41*	III	SA	0.8 µg/m²/w + BSC ± second line chemo		400/386	NGR-hTNF Until PD, NT, or PDc	301/99 66 yrs	confirmed MPM after 1st line pemetrexed based chemotherapy	OS
NGR019		II	R, DB PC, DB	0.8 µ/m²/3 week starting after 3-7 weeks post treatment	Improvement PFS/OS	100	Until PD, NT, or PDc		MPM without disease progression after 1st chemotherapy.	PFS, OS

MTP = multiple posologies. SA = single arm, MTP = monotherapy, CBT = combination therapy, R = randomised DB = double blind, PC = placebo controlled, PDc = patient's decision, NT = not tolerated, PD = progressive disease. MTD = maximal tolerated dose, DLT dose limiting toxicity, BA = biological activity, PK = pharmacokinetic NP = not provided, DCR = disease control rate (CR+PR+SD).

*Study NGR015 was conducted in 12 centres in the (IT, UK, IE, NL, PL. USA, Egypt, CA, SE, BE, ES, FR), DCE-MRI – dynamic contrast enhanced magnetic resonance imaging

**. Study NGR013 Study report not provided

Table made by assessor.

3.3.1. Pharmacokinetics

The proposed dosing regimen for NGR-hTNF is 0.8 µg/m^2 administered as 1 hour intravenous infusion once weekly. Pharmacokinetics (PK) and pharmacodynamics of NGR-hTNF were assessed in three single-agent phase I trials (EORTC16041, NGR002 and NGR013) and in two phase I combination trials with doxorubicin (NGR003) and cisplatin (NGR004). In all PK-yielding studies except NGR013, NGR-hTNF was administered in doses ranging from 0.2 to 1.6 µg/m^2, mostly as an 1 hour infusion. In all these studies, NGR-hTNF was administered every 3 weeks, which is different from the applied posology, advising once weekly administration. The PK of NGR-hTNF is only assessed in patients, NGR-hTNF has not been administered to healthy volunteers.

Distribution. The volume of distribution of approximately 30-50 l/m^2, when administered at a 0.8 µg/m^2 dose, indicates distribution to the extravascular tissues.

Excretion and metabolism. After iv infusion, NGR-hTNF has a relatively short $t_{1/2}$ of 1-2 hours. NGR-hTNF, being a protein, it is expected to be degraded by enzymatic proteolysis into small peptides and amino acids.

Dose-linearity and time-dependence of PK. Based on the PK data provided, there does not appear to be an indication for non-linear PK of NGR-hTNF. Also, because only one dose (0.8 µg/m^2) will be applied, no further data are considered necessary.

As expected in light of the short NGR-hTNF $t_{1/2}$ of 1-2 hours and the 3-weekly administration schedule, no accumulation is observed upon repeated administration of NGR-hTNF. Considering this short $t_{1/2}$ of NGR-hTNF, no accumulation is expected at the aimed 0.8 µg/m^2 once weekly posology either.

Interindividual variability. Inter-individual variability in the extent of systemic exposure to NGR-hTNF in patients in Study NGR001 was generally low to high, with geometric mean CVs ranging from 16% to 88% for AUC0-4h and 11% to 83% for C_{max}.

Special patient populations. Clearance of NGR-hTNF is believed to occur via enzymatic proteolysis. Therefore renal or hepatic impairment is considered unlikely to affect systemic exposure to NGR-hTNF and the lack of NGR-hTNF PK data related to renal or hepatic impairment is acceptable. No discussion on PK in relation to gender, race, weight and age was provided. As mesothelioma is not seen at young age, and NGR-hTNF has been granted with the waiver for a Paediatric Investigation Plan by the PDCO, the lack of NGR-hTNF PK data in children is acceptable.

Interactions. No in vitro drug-drug interactions studies were conducted for NGR-hTNF. According to the Applicant, studies NGR003 and NGR004, in which NGR-hTNF was combined with doxorubicin and cisplatin, respectively, indicate that no major PK interactions occur between NGR-hTNF and these co-medications given at the normal dose.

3.3.2. Pharmacodynamics

No specific clinical studies were submitted addressing the mechanism of action, but the support for the biological activity of NGR was evaluated in subpopulations of the phase I-II studies. An overview of the parameters to assess the biological activity of NGR-hTNF is provided in

Table 2 Measured parameters to support the pharmacodynamic activity in the phase I-II studies of NGR-hTNF-alfa

Studies	EORTC	NGR002	NGR003	NGR004	NGR010	NGR013[1]
Number included	70	16	15	22	43 14	48
DCE MRI (n)	31[2]	12			NP	37
Translational research						
Soluble receptors RI& RII (n)	NP[3]	15[4, 5]	NP[5]	[175]		46
Anti NGR-TNF antibodies		15	15	17		
Pharmacokinetics	x	X	X	X	X	X
Neoplastic response	70	15	15	22	57	NP

[1] study NGR013: no study report provided
[2] assessment limited to liver and head and neck tumours/metastatic disease
[3] included after amendment 6 dd. 21 may 2007, to assess the feasibility of a weekly administration because to the potential effect of sTNFR1 and sTNFR2 on the pharmacological and toxicological properties of NGR-hTNF. If sTNF-RI and sTNF-RII blood levels return to baseline value within a week from treatment start, a weekly administration could be envisaged.
[4] one patient is not included because of grade 3 toxicity on the first infusion
[5] only assessed over the first 3 cycles
DEC-MRI Dynamic Enhanced Contrast Magnetic Resonance Imaging
NP not provided
ND not done

The pharmacodynamic effects were investigated in patients receiving a tri-weekly dosing schedule of NGR-hTNF. The antitumor activity of TNF is hypothesised to depend on an indirect mechanism associated with selective obstruction and damage of tumour-associated blood vessels and on activation of immune mechanism rather than a direct toxic effect on tumour cells [Curnis 2000].

- DCE-MRI: effect on tumour vasculature

The effect of NGR-TNF on the tumour vasculature was measured using Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI). DCE-MRI is a non-invasive technique which can assess tissue perfusion and oxygenation within the tumour microenvironment.

The DCE-MRI measures the K_{trans}. The K_{trans} is the transfer constant from plasma to extravascular extracellular space of tumour. Depending on the balance between capillary permeability and blood flow in tumour tissues, a reduction of K_{trans} would mean either a reduction of the number of capillaries, a reduction of permeability or both situations.

For the assessment of the DCE-MRI, only the K_{trans} results of study NGR002 (low doses (0.2-0.4-.08 and 1.6 µg/m^2/3 weeks) and study NGR013 (60-325 µg/m^2/3 weeks) were taken into account. In study NGR002 the doses were administered in 1 hour, in study NGR013 the doses were administered within 2 hours.

In study NGR002, a total of n=12 provided data for the first dosing, with n=3 are exposed to the applied dose of 0.8 µg/m2/3w and presumably a total of n=9 patients provided data for the subsequent doses. The K_{trans} showed initially small non-significant increases (except for the 0.8 µg/m^2/3 weeks dose), while the next cycles showed a reduction. In contrast with to the other low doses, the 0.8 µg/m^2/3weeks showed a direct decrease in K_{trans} (table 2), with larger increases over time (Table 3).

The high doses administered in study NGR013 (60 to 325 µg/m^2/3 weeks) showed a rapid decrease in Ktrans following the first and subsequent cycles.

Table 3 Median change from baseline in Ktrans within the regions of interest

	Median change	**95% CI**	**P value**
Dose µg/m^2/3weeks			
0.2 (n=3, 10 ROI)	+4%	np	P=0.70
0.4 (n=3, 9 ROI)	+13 %	-7% to +52%	P=0.04
0.8 (n=4, 15 ROI)	-17%	-23 to +3%	P= 0.02
1.6 (n=2, 4 ROI)	+13%	np	P=0.10
Overall	4%	np	P=0.32

NP not provided: Please note that the number of included patients need to be clarified (see clinical AR).

- Soluble receptors

TNF-alfa can induce the shedding of soluble receptors sTNF-RI and sTNF-R2. These circulating receptors can compete for TNF-α with cell-surface receptors, thus blocking its bioavailability and activity and serving as physiological attenuators of the TNF- α activity.

The soluble receptors were measured in the dose finding studies NGR002, NGR003, NGR004, NGR013 and part of the EORTC during the first three cycles of treatment. For study NGR003 only the results of the first cycle were provided.

At the low doses, up till 0.8 µg/m^2/3 weeks, no soluble sTNF-R1 and STNF-R2 were induced, not with monotherapy (study NGR002 and EORTC 16041) and not in combination therapy with cisplatin (study NGR004). In combination with doxorubicin, a small increase of sTNF-R1 and s-TNF-R2 was observed at doses ≥ 0.8 µg/m^2/3 weeks dose, but at higher dose 1.6 µg m^2/3 weeks a significant increase was shown. All studies showed that the soluble TNF-R1 and TNF-R2 were induced at doses 1.6 µg/m^2/3 weeks and higher, with an apparent plateau at the 25 µg/m^2/3 weeks dose (EORTC 16041, NGE 013).

- Antibodies

No antibodies against NGR-TNF were shown in the 47 patients tested at the 0.2-1.6 µg/m^2/3 weeks doses base. For study NGR003 only the results after the first dose were provided.

- Chromagrafin A

As stated by the applicant, chromagraphin A may protect vessels from induced vascular leakage. According to the applicant, the difference of chromagrafin A levels at 1 h and 4 h was significant ($p<0.0047$; two-tailed t test, paired).

3.3.3. Discussion on clinical pharmacology

Pharmacokinetics

A major issue with the dossier at the current stage is the fact that the reliability and robustness of the provided pharmacokinetic data cannot be assessed, since information on the *applied analytical methods, and their validation,* used for analysis of NGR-hTNF in the serum or plasma samples from clinical studies is virtually absent in the dossier including the clinical study reports. The Applicant should, therefore, provide the relevant validation reports for all clinical pharmacology studies. Further,

these validation reports should be discussed, e.g. in relation to analogies and differences in the NGR-hTNF analytical methodology applied for the different clinical studies. Further, no (complete) *bioanalytical reports* were provided. Such reports, allowing assessment of the sample analysis, should be submitted.

With regard to the analysis of serum or plasma samples for NGR-hTNF, the Applicant states that individual concentration-time profiles for each patient were *adjusted for baseline levels* by subtracting the time-zero value to all other time-point values. However, no detailed information on this was found in any of the study reports, and the information provided for Study NGR001 and 003 seems to indicate that correction was not always done in the same way. The Applicant should provide the individual analysis data for all NGR-hTNF samples from all clinical studies with and without correction.

No information on *stability and storage conditions* of the samples for NGR-hTNF was provided for any of the clinical studies. This information should be provided and discussed for each study, clarifying if samples have been measured within an acceptable period of time with respect to stability. Further, in the scarce information from the bioanalytical report DXKP1025 for Study NGR003, it is indicated that the storage conditions of the samples was changed during the conduct of Study NGR003 (from -20°C to -80°C). This situation, however, was not further discussed. Information on storage of NGR-hTNF samples from other studies is lacking. A detailed description of stability tests, as well as a detailed description of the storage conditions of the NGR-hTNF samples for all clinical studies, both at the clinical facility and the analytical facility, should be provided. In this light the reason and consequences of the change in storage condition from -20°C to -80° should be extensively discussed.

No data was provided on *Incurred Sample Reanalysis*. Therefore the robustness of the bioanalytical method cannot be assessed and this should be demonstrated by the Applicant.

Further, the clinical study reports including pharmacokinetics seem to lack a high number of critical data, and neither protocol nor information on approval of the protocols and amendments by an Investigational Research Board (IRB) was provided for any of the clinical pharmacology studies.Copies of these approvals hould be provided. At this stage, however, it is proposed to ask the Applicant to provide this missing information. Based on the response and information provided, a decision can be taken whether a GCP inspection should be triggered.

Another major issue is the fact that all pharmacokinetic studies were conducted with NGR-hTNF process A formulation. Although the final formulation process C is markedly different from process A formulation, e.g., with respect to the relative amount of isoforms present in the formulation, no pharmacokinetic data have been obtained with the to be marketed process C formulation. Assuming the Applicant can resolve the above-mentioned issue related to the reliability and robustness of the NGR-hTNF analytical methods, the Applicant should discuss the relevance of the provided pharmacokinetic data, and reassure that these pharmacokinetic data provided are suitable for the to be marketed Process C product as well.

Inter-individual variability. Inter-individual variability in the extent of systemic exposure to NGR-hTNF in patients in Study NGR001 was generally low to high, with geometric mean CVs ranging from 16% to 88% for AUC_{0-4h} and 11% to 83% for C_{max}. Intra-individual variability was not calculated by the Applicant. Such analysis should be provided.

Special patient populations. Since clearance of NGR-hTNF is believed to occur via enzymatic proteolysis, renal or hepatic impairment is considered unlikely to affect systemic exposure to NGR-hTNF. The lack of NGR-hTNF pharmacokinetic data related to renal or hepatic impairment is, therefore, acceptable. However, confirmation of the absence of an effect of renal or hepatic impairment should be provided based on an overall analysis of all available PK data, e.g. by using a population

pharmacokinetic approach. Pharmacokinetic data have not been discussed in relation to gender, race, weight and age. Such discussion, e.g. by means of a population pharmacokinetic study, should be provided by the Applicant. Further, a table should be completed in order to provide an overview of the number of elderly patients included in the submitted pharmacokinetic studies. As mesothelioma is not seen at young age, and NGR-hTNF has been granted with the waiver for a Paediatric Investigation Plan by the PDCO, the lack of NGR-hTNF pharmacokinetic data in children is acceptable.

Interactions. No in vitro drug-drug interactions studies were conducted for NGR-hTNF. Proteins like NGR-hTNF are known to have a low potential for pharmacokinetic drug interactions as they are not metabolized by liver cytochrome P450 (CYP) or other drug metabolizing enzymes. However, a discussion should be provided on the likelihood of (indirect) drug-drug interactions related to inhibitions of enzymes or drug transporters by NGR-hTNF.

According to the Applicant, studies NGR003 and NGR004, in which NGR-hTNF was combined with doxorubicin and cisplatin, respectively, indicate that no major pharmacokinetic interactions occur between NGR-hTNF and these co-medications given at the normal dose. However, the NGR-hTNF C_{max} when given in combination with cisplatin appears to be higher than the C_{max} when NGR-hTNF is given at a 0.8 µg/ml dose as a single agent or in combination with doxorubicin (i.e., 9-18 pg/ml and approximately 34 pg/ml, respectively). This apparent difference should be explained by the Applicant. Further, the NGR-hTNF AUC's calculated by the Applicant in Study NGR004 appear to differ from the AUCs reported in the other studies NGR001, 002 and 003. The Applicant is invited to explain the apparent differences.

Pharmacodynamics

No specific pharmacodynamic study was conducted to support the anti-tumour effect of NGR-TNF. Pharmacodynamic measurements were included in dose finding studies with the main pharmacodynamic parameters to support the application being the DCE-MRI and the soluble TNF receptors (sTNF-RI and sTNF-R2).

Design and conduct of the studies

All phase I-II studies were conducted with a tri-weekly dosing scheme, except for a small cohort included in study NGR010. Only this small cohort (n=14) was exposed to the applied weekly dosing scheme, limiting the direct pharmacodynamic support for the weekly dose administration. The pharmacodynamic measurements were limited to the DCE-MRI, as the effect on the soluble sTNF-RI and STNF-R2 was not measured in this setting. It needs to be justified, how the results obtained by the tri-weekly dosing schedule, can be used to support to the weekly dosing schedule.

The main study to support the pharmacodynamic effects of NGR-hTNF was study NGR002. The study was preliminary stopped because of the optimal tolerability of the low dose range (0.2-1.6 µg/m^2/3 week), and the preliminary results of biomarkers, magnetic resonance and pharmacokinetics (page 44 of clinical study report NGR002). A specification of these outcome measures including a discussion supporting the preliminary stopping would have been appreciated.

NGR-hTNF's proposed mode action is a targeted anti-angiogenetic effect. The target is the CD13, but CD13 has not been evaluated as a possible biomarker. The proposed anti-angiogenetic effect is also not supported with biomarkers like VEGFR reduction, although the inclusion of such a biomarker might have improved the internal validity of the trial.

DCE-MRI measures the K_{trans}. Although K_{trans} is an incompletely validated endpoint, several early-phase clinical trials of VEGF signalling inhibitors have shown changes in Ktrans consistent with reductions in VEGF-dependent tumour perfusion, vascular permeability or both [Mross, 2009; Miller

2005]. However, the K_{trans} is not easily to be interpreted, because it depends on the balance between capillary permeability and blood flow in tumour tissues. A reduction of K_{trans} would mean a reduction of amount of capillaries, a reduction of vascular permeability or both.

Efficacy

DCE-MRI measurements were made in study EORTC16041, NGR002, NGR010 and NCG013. Only the results of study NGR002 and NGR013 are extensively discussed to support the proposed mode of action. The results of the other studies are not taken into account, despite the fact that only the small cohort (n=14) included in study NGR010 could provide support for the applied weekly posology.

The effect after the first dose administration for the low dose range (0.2-1.6 µg/m^2/3w) is provided by the limited number of n= 12, with n=3 are exposed to the applied dose of 0.8 µg/m^2/3w. A total of n=9 patients provided data for the subsequent doses. This data is considered rather limited.

The applicant inferred the preclinical experience directly to the observed changes in the K_{trans} for the low doses. The applicant argues that the preclinical low dose NGR-hTNF cause tumour vessel stabilization, and the later cycles vessel damage. The observed clinical reduction in K_{trans} would mimic these changes. However, we consider that no arguments have been provided why the reduction in K_{trans} would not be a result of reduced vessel permeability.

- Soluble receptors.

The provided data are probably obtained from the first three cycles. In man, TNF induced soluble receptor may block the biological activity of NGR-TNF. The provided data showed that soluble receptors were not induced at doses ≤ 1.6 µg/m^2/3w.

- Biomarkers.

During the study, the applicant investigated various biomarkers in study NGR002. It needs to be clarified if CD13 or VGEF biomarkers indeed have not been measured. NGR-hTNF may have an effect on the angiogenesis, but no data was provided showing a correlation between the VEGF, the CD13 ligand, Ktrans or tumour response. Therefore, apparently, no biomarker is identified that supports the proposed mode of targeted anti-angiogenetic efficacy of NGR-hTNF. Identification of a biomarker (or multiple) would have increased the internal validity of the trial and could have supported the acclaimed mode of action.

- Chromagranin A

Incomplete data for chromogranin A has been provided. Although the applicant stated what the clinical relevance of this protein is unknown, chromogranin A has been measures as it may counteract the effect of NGR-hTNF.

- Antibodies

No antibodies against NGR-TNF were shown in the 47 patients tested at the 0.2-1.6 mcg/m2 doses at a 3 weekly base. The number is small, and the dosage schedule is less intense than the currently applied application of 0.8 µg/m^2/3 week. Furthermore, neither validation reports of the immunogenic assessment methodology, nor bioanalytical reports with individual results of the analysed samples analysed for anti-drug antibodies were provided.

3.3.4. Conclusions on clinical pharmacology

Overall, due to the lack of key information on the analysis of the samples for NGR-hTNF in the various clinical studies, the reliability of the NGR-hTNF pharmacokinetic data reported in this assessment

report cannot be scrutinized. At this stage, the pharmacokinetic data cannot be used to support the current application. Further, the clinical study reports including pharmacokinetics seem to lack a high number of critical data, and neither protocol nor information on approval of the protocols and amendments by an Investigational Research Board (IRB) was provided for any of the clinical pharmacology studies.Copies of these approvals should be provided. Furthermore, no PK data were provided for the to be marketed formulation C, which appears markedly different from the process A formulation used in the pharmacokinetic studies.

Even in case the applicant would be able to provide reassurance regarding the reliability of the provided pharmacokinetic data and the applicability of these pharmacokinetic data for the to be marketed formulation, a number of concerns are raised and these are discussed below.

The overall pharmacodynamic data show important insufficiencies to support the proposed mode of action and the applied weekly posology.

Antibodies against NGR-TNF were measured in 47 patients tested at the 0.2-1.6 mcg/m^2 doses at a 3 weekly base. The number of patients tested is small, and the dosage schedule is less intense than the currently applied posology of 0.8 µg/m^2/3week. Further, no validation reports of the immunogenic assessment methodology nor bioanalytical reports with individual results of the samples analysed for anti-drug antibodies were provided.

The proposed mode of action is a targeted anti-angiogenetic effect. The target is the CD13, but CD13 has not been evaluated as a possible biomarker. The proposed anti-angiogenetic effect is also not supported with other potential biomarkers, like VEGFR reduction.

In addition, DCE-MRI were conducted to support the anti-angiogenetic effect. Only a limited and selected number of DCE-MRI's are taken into account to support the application. The interpretation of the observed changes in Ktrans also needs to be explained.

The phase I-II studies provide limited support for the proposed mode of action and for the applied posology of 0.8 µg/m^2/week. The provided pharmacodynamic data is obtained in clinical studies being conducted with a tri-weekly administration of NGR-hTNF. Therefore, justification is needed if the observed long term effects (i.e. effects on tumour vascularity, soluble TNF receptors) of the tri-weekly posology will be comparable to the weekly administration.

In short, the overall pharmacokinetic and pharmacodynamic data shows important insufficiencies to support the applied posology. The main support for the applied posology needs to be provided by the results of the pivotal study.

3.3.5. Clinical efficacy

Dose-response studies

The safety and efficacy of NGR-hTNF was investigated in the phase I-II studies (EORTC16041, NGR002, NGR003, NGR004 and NGR-010. Study EORTC16041 was a dose escalating study to establish the maximal tolerated dose and the dose limiting effects. Study NGR002 investigated the safety and efficacy of NGR-hTNF as monotherapy. The studies NGR003 and NGR004 investigated the safety and efficacy of NGR-hTNF in combination with doxorubicin (75mg/m^2/3w, study NGR003) and cisplatin (80 mg/m^2/3w, study NGR004) in doses of 0.2-0.4-0.8 and 1.6 µg/m^2/3w. During these trials, NGR-hTNF was administered as a tri-weekly infusion. The tumour response was evaluated in accordance to Response Evaluation Criteria in Solid Tumours (RECIST).

Safety

The maximal tolerated dose identified by study EORTC16041 was 45 µg/m^2 during 1 hour dose administration in a 3-weekly schedule. Dose limiting toxicities were dyspnoea and hypoxia. NGR-hTNF doses up till 1.6 µg/m^2/3w were also well tolerated if combined with doxorubicin 75 mg/m^2 /3w or cisplatin 0.8 mg/ m^2/3w and the MTD was not reached.

Tumour response

The tumour response was evaluated in accordance to the Response Evaluation Criteria in solid tumours for the studies NGR 002, NGR003 and NGR004, i.e. standard RECIST.

Study NGR101 and NGR015 used the modified Recist criteria. Overall, the best DCR and RR was observed for the 0.8 µg/m^2/3w dose, flowed by the 1.6 µg/m^2/3w dose (Table 4).

Table 4 The disease control rate and response rate for the different doses across the different studies EORTC 16041, NGR002, NGR003 and NGR004

	Disease control rate (CR+PR+SD) (n,%)				**Response rate (CR+PR) (n, %)**			
Posology µg/m^2/3w	**0.2**	**0.4**	**0.8**	**1.6**	**0.2**	**0.4**	**0.8**	**1.6**
EORTC 16041	1/3	ND	2/3 (66)	ND	0	ND	2/3 (66)	0
NGR002	1/3 (33)	1/4 (25)	4/4 (100)	1/4 (25)	0	0	1/4 (25)	0
NGR003	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	1/3 (33)	0/3	1/3 (33)	0/3
NGR004	0/3	0/3	6/12 (50)	2/3 (66)	0	0	1/12 (8%)	0
Overall response	**5/15 (33%)**	**4/10 (40%)**	**15/22 (68%)**	**6/12 (50%)**	**1/15 (7%)**	**0/10 (0%)**	**5/22 (23%)**	**0/12 (0%)**

Table made by assessor. Study EORTC 16041 and NGR002 are single dose administration, study NGR003 and NGR004 are combination with doxorubicin and cisplatin respectively. ND not done

Conclusion:

The maximal tolerated dose is 45 µg/m^2/3 week administered as an 1 hour infusion. The best tumour responses were observed with the 0.8 µg/m^2/3 week posology.

Main clinical study

Study NGR015

Title study: Randomised, double-blind phase III study of NGR-hTNF plus best investigator's choice (BIC) versus placebo plus BIC in previously treated patients with advanced malignant pleural mesothelioma (MPM)

Study NGR015 was the pivotal, global, multicentre, randomised, double-blind phase III study comparing the safety and efficacy of NGR-hTNF plus best investigator's choice care (BIC) versus placebo plus BIC in recurrent malignant pleural mesothelioma in previously pemetrexed treated patients.

Study participants

The pivotal phase III study NGR015 included adult patients with radiologically confirmed disease progression after first line treatment with a pemetrexed based chemotherapy for a histologically or cytologically confirmed advanced or metastatic malignant pleural mesothelioma.

Patient had to have an ECOG score 0-2 and adequate organ function. Patients with significant cardiac, hepatic or renal dysfunction or known cerebral metastases were excluded, and patients completing radiation therapy or chemotherapy within four weeks or having surgery within two weeks before treatment start were excluded as well.

Stratification factors used were:

- Candidate for chemotherapy (yes vs. no),
- Type of chemotherapy (doxorubicin, gemcitabine or vinorelbine),
- ECOG performance status (0 vs. 1-2).

Treatments

Patients were randomized (1:1) to receive either NGR-hTNF (administered i.v.) every week at a dose of 0.8 µg/m^2 or placebo plus Best Investigator's choice (BIC).

Best Investigator's Choice (BIC) included either best supportive care alone or combined with a single-agent chemotherapy (doxorubicin, gemcitabine, or vinorelbine).

The following posology was defined for the single agent chemotherapy treatment (on investigator's decision):

- Doxorubicin: 60-75 mg/m² every 3 weeks (maximum of 6 cycles)
- Gemcitabine: 1,000-1,250 mg/m² on days 1 and 8, every 3 weeks, for a maximum of 6 cycles, OR
- Vinorelbine: 25 mg/m² iv on days 1 and 8 every 3 weeks, for a maximum of 6 cycles (or weekly for 12 weeks) or, 60 mg/m² per os on days 1 and 8 every 3 weeks for a maximum of 6 cycles (or weekly for 12 weeks).

Objectives

The primary objective was to compare overall survival (OS) in patients randomized to NGR-hTNF plus BIC versus patients randomized to placebo plus BIC.

The key secondary outcome measures were the PFS and the Disease control rate (DCR). Another secondary outcome measurement was the time to symptomatic progression.

The primary endpoint was the OS defined by as the time from the date of randomization until the date of death due to any cause.

Outcomes/endpoints

The primary endpoint was the OS defined by as the time from the date of randomization until the date of death due to any cause.

The key secondary outcome measure was the PFS defined as the time from the date of randomization until disease progression or death due to any cause.

DCR was defined as the percentage of patients who have a best-response rating of complete response, partial response, or stable disease.

The response to treatment was standardized according to the modified RECIST criteria for Malignant Pleural Mesothelioma (Byrne, 2004). Malignant mesothelioma most commonly grows as a 'rind' around the pleural surface. Without defining the selection of measurement sites, the usual applied RECIST criteria could be applied differently among investigators.

Time to symptomatic progression is defined as time from randomization to the date of the Lung Cancer Symptom Scale (LCSS) assessment on which symptomatic progression is identified.
The LCSS sub score is the average symptom burden index computed as the mean score for all six major symptoms like appetite loss, fatigue, cough, dyspnoea, haemoptysis, and pain. However, haemoptysis was excluded from the adapted version used for MPM. Symptomatic progression will be defined as a worsening in the average symptom burden index by 25% in the LCSS sub score.

Sample size

Based on a hazard ratio of experimental patients relative to control patients being 0.726, a total of 195 in each study arm would be needed to reject the null hypothesis, i.e. the experimental and control survival curves are equal with probability (power) 0.80 and the type I error (alfa) of 0.05, using an unstratified log-rank test at a two sided significance level.

Randomisation

Patients were randomised to receive NGR-hTNF or placebo with a ratio of 1:1. Randomisation was stratified by:

- Candidate for chemotherapy (yes vs. no) and,
- Type of chemotherapy (doxorubicin, gemcitabine or vinorelbine),
- ECOG performance status (0 vs. 1-2).

Statistical methods

The primary population for the efficacy was the intend-to-treat (ITT) population that include all randomised patients. The per-protocol population (PP) included all patients included in the ITT population who received at least 6 weeks of study treatment and the patients who terminated treatment before 6 weeks because of progression or death and adhered to the protocol.

Patients included in the per-protocol population analysis should have at least one post-baseline tumour assessment and no more than one line of pemetrexed-based systemic therapy.

The safety population included all randomized patients who were exposed to study medication

- The primary and secondary efficacy variables

The primary hypothesis tested was that NGR-hTNF would have an improved overall survival compared with BIC. The log-rank test (unstratified) was used to compare median survival in the two treatment arms. A stratified log-rank test was performed with the stratification factors used for randomization. Kaplan-Meier curves were displayed, and median survival estimates with 95% confidence intervals were given. Cox regression analyses (unstratified and stratified) were performed and treatment-by-covariate interaction terms included to assess whether effects differed for a number of pre-specified subgroups. Covariates of interest were age (< or ≥ median value), gender, chemotherapy on study, ECOG PS (< or ≥ 1.27), histology, EORTC prognostic score, best response to prior therapy (CR/PR or SD/PD/unevaluable), interval from first-line completion to second-line initiation (TFI)(< or ≥ median value), and neutrophil-to lymphocyte ratio (NLR)(< or ≥ median value).

For the TFI, the median value was chosen. TFI was computed as the time between the end of first line therapy and start of second line therapy. The start date of TFI was the last chemotherapy cycle, while for 12 patients receiving maintenance therapy it was the end of maintenance treatment. The end date of TFI was the start of the second-line therapy, while for 21 patients being rechallenged with pemetrexed, it was the start of rechallenge.

- Missing data

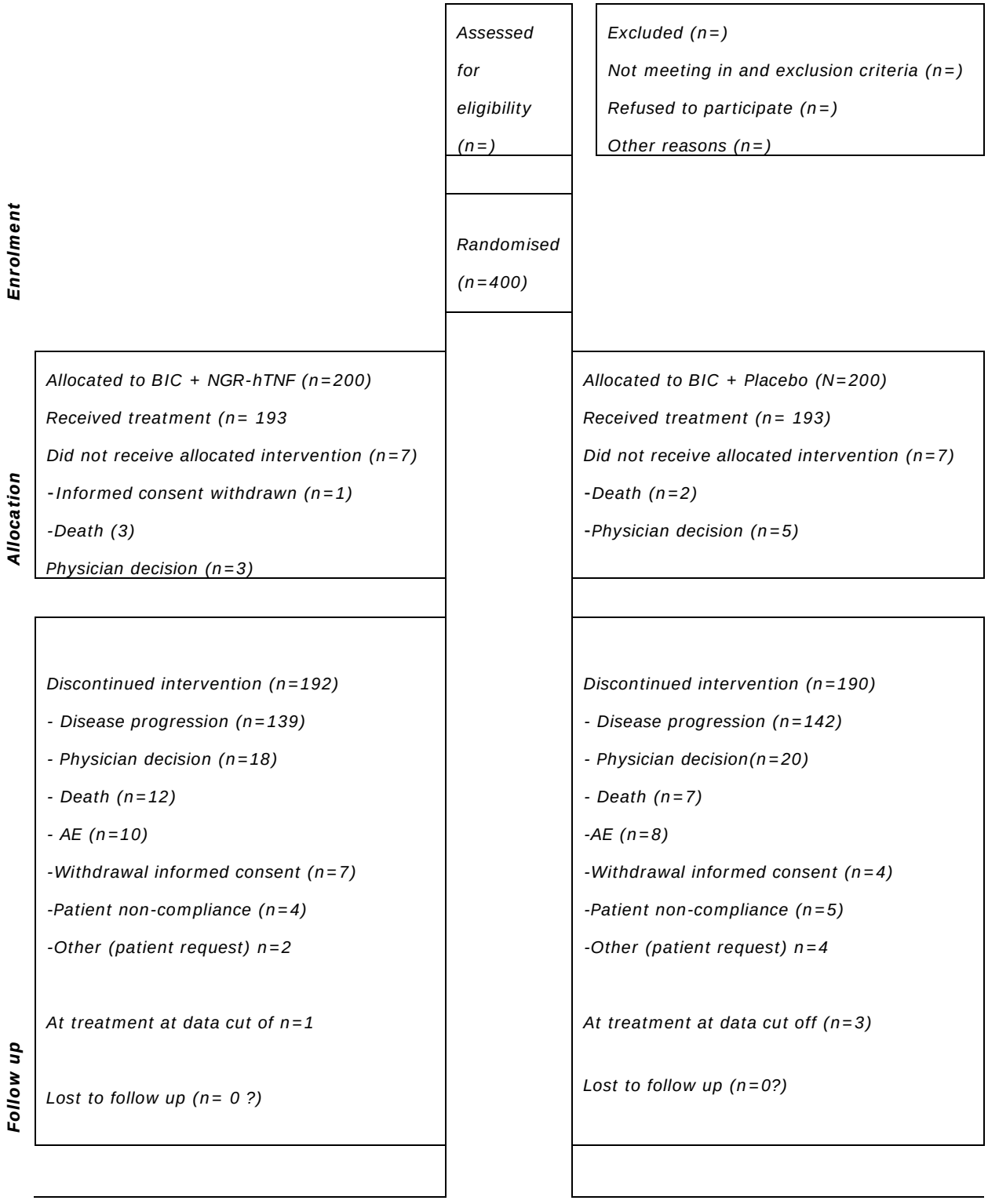
For handling missing data, a complete-case analysis was used by excluding patients with missing values from the data set. The following was defined for the primary and secondary outcomes:

- For the primary efficacy outcome (OS), patients who were randomized but not exposed to study drug and have no further follow up were be censored on the day of randomization.
- For the secondary outcome (PFS), the two main censoring rules were that (1) patients with no tumour assessments after baseline but who are still alive at the time of the clinical cut-off will be censored at day of randomization; (2) in case there was missing information, censoring of the data may be defined as the date of last visit at which progression status was adequately assessed. The recorded progression date is the date of the protocol-scheduled clinic visit immediately after all radiological assessments.

Results

- **Participant flow**

Figure 1 The participant flow of study NGR015



Analyses	*Analyses ITT n=200* *Analyses PP (n=156)* *- Excluded from PP (n=44) because* *- Lack of post baseline tumour assessment on measurable disease (n?)* *- Treatment discontinuation within 6 weeks (n?)* *Violation of defined major protocol deviation (n=1)* *Analyses Safety Population (SP) (n=193)* *Excluded from safety population (n=7)*	*Analyses ITT n=200* *- Analyses PP (n=163)* *Excluded from PP (n=37)because* *- Lack of post baseline tumour assessment on measurable diseases (n?)* *-Treatment discontinuation within 6 weeks (n?)* *-Violation of defined major protocol deviation (n=2)* *Analyses Safety population (n=193)* *Excluded from safety population (n=7)*

Not provided data is missing data.

Recruitment

Between April 2010 and January 2013, a total of 400 patients were recruited from 41 sites in 12 countries across Europe (n=339), Egypt (n=36), and USA & Canada (n=34). Patients were followed up for a median of 19.1 months for NGR-hTNF and 18.3 months for placebo. Data cut-off date was 29th April 2014.

Conduct of the study

The study was amended 4 times with an additional 3 local amendments. The first amendment, dated January 20th 2010, specified the inclusion of a vinorelbine substudy to evaluate the safety with NGR-hTNF. The third amendment, dated Sept 15 2011, included an additional stratification according to baseline chemotherapy (doxorubicin, gemcitabine or vinorelbine).

The safety of the study should be monitored by an independent data safety monitoring board that reviewed the safety data once the first 80 patients have completed six weeks of therapy. However, the safety monitoring board was not initiated.

The date of data base lock was not provided and no procedures were described how to adjust the data after data base lock.

At cut-off date, eight patients have been unblinded:
☐ 7 patients at one site were unblinded due to uncertainty related to the doses were administered (see section 14.3.3)
☐ 1 patient was unblinded after PD for site request, to allow the patient to enter a different trial.

Post hoc analyses for the identification of a subgroup of interest

Based on the significant treatment-by-TFI interaction test using a median cut-point of 4.8 months, this interaction was further investigated.

The treatment free interval (TFI) was defined by the end of the first line and the start of the second line therapy. The start date of TFI was the last chemotherapy cycle, while for 12 patients receiving maintenance therapy it was the end of maintenance treatment. The end date of TFI was the start of the second-line therapy, while for 21 patients being rechallenged with pemetrexed, it was the start of rechallenge.

The TFI was dichotomized in short or long according to the median for the trial sample (4.8 months, 95% CI 4.2-5.1). Thereafter, the effect on OS with different cut- points was further investigated and based on these results the cut-off value was set at <6 months, ≥6 months. The cut-off for the subgroup of interest was set at 6 months, because the observed improvement in mOS for NGR-hTNF was still maintained at this threshold, and the threshold of 6 months was considered by the applicant to be more practical and easy to use in calculations than the median (4.8 months).

Numbers analysed

A total of 400 patients were included in the ITT population; a total of n=200 patients were randomised to each treatment. A total of 319 patients were included in the PP population; n=156 patients were randomised to NGR-hTNF and n=163 patients were randomised to placebo. A total of 386 patients were included in the safety population, a total of n=193 patients were randomised to each treatment. The subgroup of interest, patients with a TFI <6 months, included n=236 patients; n= 117 patients randomised to NGR-hTNF and n= 119 patients randomised to placebo. The complementary subgroup, patients with a TFI> 6 months, included n= 81 patients randomised to NGR-hTNF and n= 81 patients randomised to placebo. Two NGR-hTNF treated patients were not included in the TFI analyses because of these patients the TFI was not assessable.

Baseline characteristics

- **ITT population**

The study included a total of 400 patients: 301 males and 99 females. Most patients were Caucasian (99%). The mean age at study entry was 66 years. The ECOG performance scale (PS) =0 was reported in n=119 (30%) patients, PS=1 in n=259 (65%) and PS=2 in n= 22 (6%) of patients. Most patients reported a beneficial EORTC prognostic score (<1.27) (n=282, 71%). Most patients were previously exposed to asbestosis (n=238, 60%).

The most frequently reported histology of MPM was of the epithelial type (n=333, 83%) followed by mixed type (n=34, 9%), sarcomatous type (n=19, 5%) or unknown (n=14, 4%). At the time of diagnosis, a total of n= 77 (19%) has stage I-III disease, a total of n= 163 (40%) patients had stage IV disease; in an additional n=160 (40%) patients the stage of disease was unknown. A total of n=100 (25%) responded to previous therapy (CR+PR), the baseline neutrophil-to lymphocyte ration (NLR) was less than the median (<4) in n=214 (54%) patients.

During the study, all patients received best supportive care. Except for n=19 (5%) of patients, all patients received second line single arm chemotherapy (n=181, 95%). Gemcitabine was most frequently prescribed (n=211, 52% %), followed by vinorelbine (n=159, 40%) and doxorubicin n=11 (3%).

- **Short-TFI population with TFI <6 months**

A total of 236 patients were included in the short TFI<6 months group. A total of n=127 (54%) were younger than 66 years. Most patients were male (n=178, 75%), with a ECOG PS 1 to 2 (n=171, 72%) and with a favourable EORTC prognostic score (ECOG <1.27) (n=157, 67%).

The most frequently included MPM histology was epithelial type (n=188, 80%). The data for the disease stages, including the number of patients with unknown disease stages at diagnosis, is not provided. A total of n=46 (19%) showed a previous response to prior therapy (CR or PR). The NLR <4 was observed in n=128 (54%) of patients.

During the study, all patients received best supportive care. Except for n=9 (4%) of patients, all patients received second line single arm chemotherapy (n=227, 96%). A total of n=126 (53%) received gemcitabine, a total of n= 94 (40%) received vinorelbine and a total of n=7 (3%) received doxorubicin.

The dossier does not contain a comparison of the baseline characteristics between NGR-hTNF and placebo for this subgroup.

Outcomes and estimation

- **ITT POPULATON**

The final analysis was performed when the total study population showed approximately 306 deaths (75%). A total of n=149 (75%) of patients died in each arm. The study did not demonstrate statistically significant improvement in the OS, PFS or DCR of NGR-hTNF over placebo (

Table **5**). Also, no significant differences were observed in the duration of disease control and improvement in the QoL assessed by the Lung Cancer Symptom Scale score either.

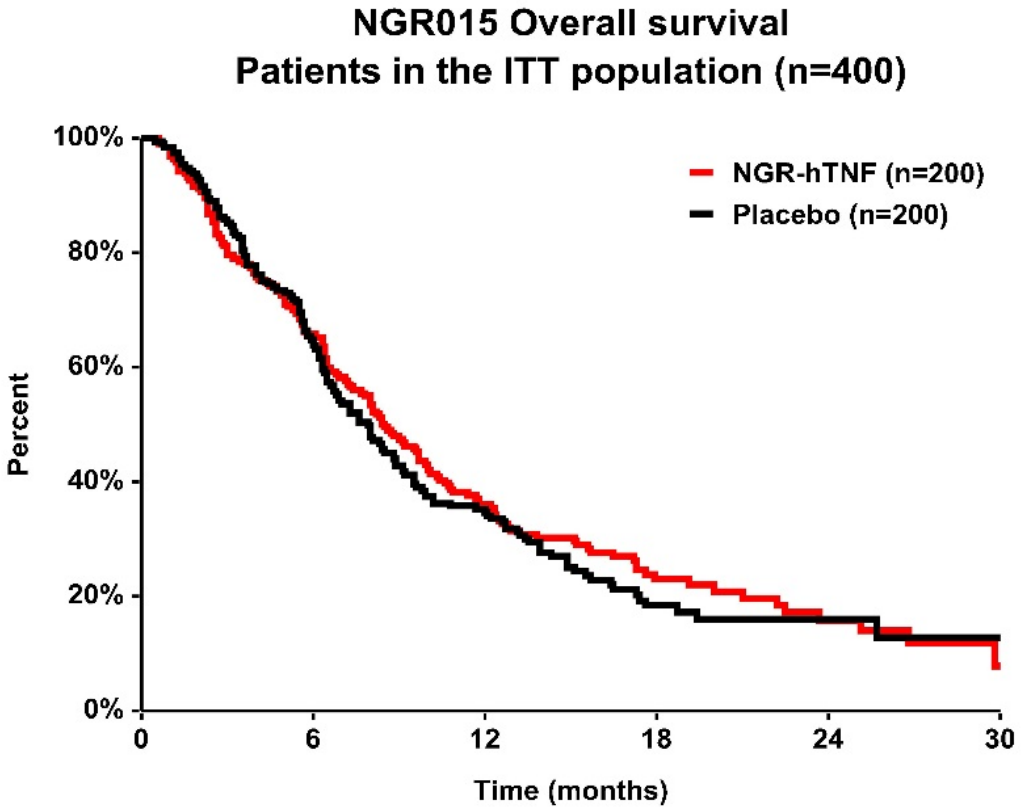
Table 5 Summary of the primary and key secondary outcome measures of study NGR015

	NGR-hTNF	Placebo	Outcome, p-value
Primary outcome measure			
Median OS (month, 95% CI)	8.5 (7.2-9.9)	8.0 (6.6-8.9)	Stratified HR 0.97 (0.77-1.22) p=0.79 Unstratified HR 0.94 (0.75 - 1.18) p=0.59
Secondary outcome measures			
Median PFS (month, 95% CI)	3.4 (2.7-4.1)	3.0 (2.3-3.7)	Stratified HR 0.96 (0.78-1.19) p=0.72 Unstratified HR 0.95 (0.78-1.17) p=0.65
Disease control rate (CR/PR/SD)	N=114 (57%)	N=109 (55%)	Odds ratio 1.13 (0.76-1.68), p=0.62
CR/PR (n/n)	N=3 (2%)	N=6 (3%)	

Source table 7, table 18 and table 23 of Clinical overview version 02

The PP analysis confirms the results of the ITT analyses, no statistically significant improvements for NGR-hTNF were observed for the OS, PFS and DCR.

Figure 2 Overall survival for the ITT population NGR015 (n=400)

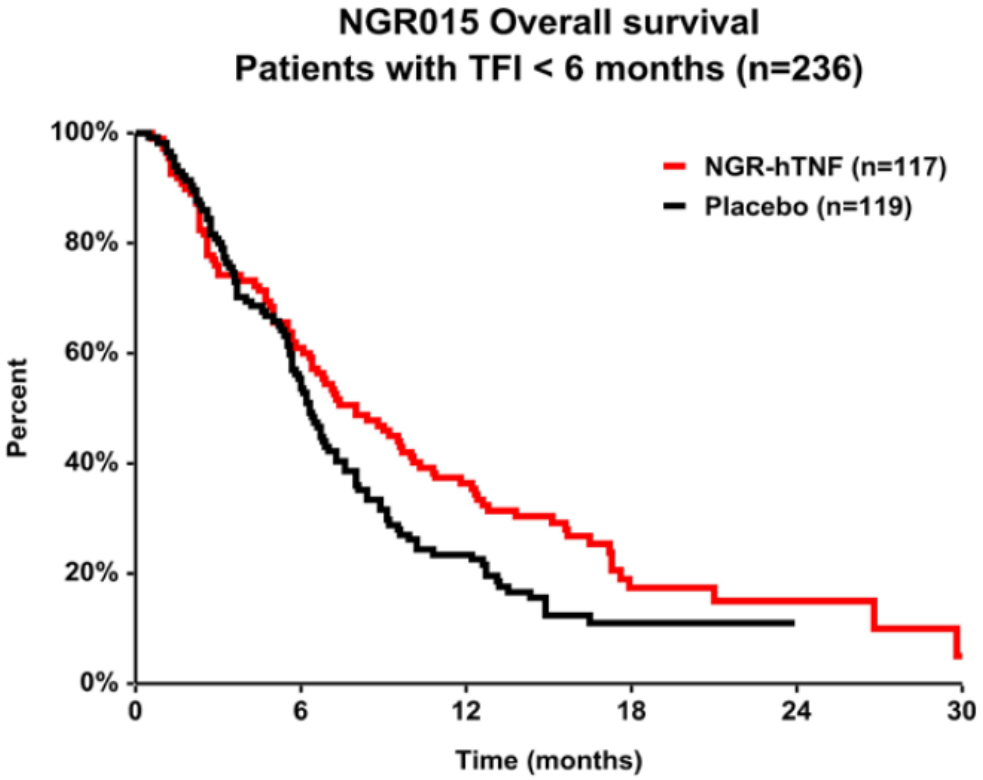


Source: figure 1 of the clinical overview version 2.0.

- **SHORT-TFI POPULATION WITH TFI <6 MONTHS**
- The study population with a short TFI <6 months consisted of n=236 patients. The final analysis was conducted when 185 (78%) events were observed.
- **Primary outcome mOS**

The mOS for NGR-hTNF was 8.0 months (95% CI 6.3-10.1), for placebo 6.4 months (95% CI 5.7-7.6). The stratified HR was 0.70 (0.52-0.95), p=0.02 (Figure 3).

Figure 3 Overall survival by treatment for the patient subgroup with TFI shorter than 6 months (n=236)



Source: figure 5 clinical overview version 2.0

- **Secondary outcome measures**
- **PFS**

The median PFS was 3.0 months (95% CI 2.3-4.1) for NGR-hTNF and 2.0 months (1.5-3.0) for placebo, resulting in a stratified HR of 0.75 (95% CI 0.57-0.99), p= 0.04. The KM curves for the PFS were not provided for the TFI <6 months.

- **Disease control rate**

The DCR for NGR-hTNF compared with placebo was n=62 (53%) vs. n=57 (48%). The response rate (CR+PR) was comparable between the two treatments e.g. 2% and 3% respectively.
More patients in the NGR-hTNF group had non assessable disease (21%) compared with the placebo group (Table 6)

Table 6 Best overall response for the subgroup with TFI < 6 months

Best overall response TFI shorter than 6 months	NGR-hTNF n=117	Placebo n=119
Best overall response at any time		
Disease control rate (DCR)	62 (53%)	57 (48%)
Complete or partial response (CR o PR)	2 (2%)	3 (3%)
Stable disease (SD)	60 (51%)	54 (45%)
Progressive disease (PD)	30 (26%)	50 (42%)
Nonassessable (NA)	25 (21%)	12 (10%)
Odds ratio (95% CI)	1.23 (0.74 - 2.04)	
Fisher exact test p-value	0.44	
Duration of disease control		
≥ 6 months	38%	27%
(95% CI)	(26 - 50)	(15 - 39)

Source table 26 of Clinical overview version 2

- **Per Protocol analyse**

No per protocol analyses was provided for this subgroup with TFI <6 months.

Subgroup analyses

- **ITT population**

A covariate-by-treatment interaction test was conducted with the pre-specified covariates. A statistical significant interaction was observed for the treatment free interval. This subgroup showed a statistically significant interaction between the TFI and OS (HR=0.53; 95% CI, 0.33 to 0.84; p=0.007). To check for the independence of interaction between treatment group and TFI, also the nonsignificant interactions of the other baseline factors (age, sex, PS, histology, EORTC score, NLR, best response to prior therapy and selected chemotherapy) were included in the regression model, with treatment-by-TFI interaction remaining statistically significant (HR=0.42; 95% CI, 0.27 to 0.65; p=0.0006).

- **Short-TFI population with TFI <6 months**

For the subgroup with a TFI <6 months, also the predefined subgroup analyses were conducted. An improvement in OS in favour of NGR-hTNF was observed in almost all defined subgroups, with the exception of non-epithelioid /unknown histology and the patients with a high baseline NLR (Table 7). In these subgroups the HR was near 1 (0.98 and 1.06 respectively).

Table 7 OS according to baseline covariates for the patients with TFI < 6 months (n=236)

Overall survival Short TFI subgroup	N	HR	95% CI	Median (months)		p-value
				NGR-hTNF	Placebo	
All patients	236	0.74	0.55 - 0.98	8.0	6.4	0.04
Age (median)						
< 66 years	127	0.68	0.44 - 1.02	8.0	6.7	0.07
≥ 66 years	109	0.81	0.53 - 1.22	7.4	6.3	0.32
Sex						
Female	58	0.51	0.25 - 1.00	16.5	8.4	0.05
Male	178	0.78	0.55 - 1.07	6.8	5.8	0.13
PS						
0	65	0.48	0.27 - 0.85	10.9	6.9	0.01
1 to 2	171	0.83	0.58 - 1.17	7.1	6.1	0.29
Tumor histology						
Epithelial	188	0.70	0.50 - 0.97	9.0	6.7	0.04
Nonepithelial/unknown	48	0.98	0.51 - 1.86	4.0	5.7	0.94
EORTC score						
Good (< 1.27)	157	0.64	0.44 - 0.91	10.1	7.3	0.02
Poor (≥ 1.27)	79	0.88	0.52 - 1.45	4.7	4.2	0.61
NLR (median)						
Low (< 4)	128	0.54	0.35 - 0.82	12.6	7.6	0.004
High (≥ 4)	107	1.06	0.69 - 1.61	4.9	6.0	0.78
Best response to prior therapy						
CR/PR	46	0.58	0.29 - 1.14	8.4	5.8	0.12
SD/PD/NE	190	0.77	0.55 - 1.06	7.3	6.7	0.11
Chemotherapy on study						
No	9	0.91	0.21 - 3. 87	6.3	7.3	0.90
Yes	227	0.74	0.54 - 0.99	8.0	6.3	0.05
Gemcitabine*	126	0.68	0.45 - 1.00	8.0	6.3	0.05
Doxorubicin	7	0.50	0.08 - 3.04	14.2	8.0	0.45
Vinorelbine*	94	0.78	0.53 - 1.42	7.3	6.1	0.58

*HR estimated by an unstratified Cox model. HR less than 1 denotes a favorable result for NGR-hTNF versus placebo. *Median follow-up significantly longer in the gemcitabine than in vinorelbine cohorts (24.0 vs 16.9 months, respectively; p=0.01)*

Source table 16 of the clinical overview version 2.0

This subgroup analyses was also conducted for the PFS in the patients with a TFI shorter than 6 months. NGR-hTNF included PFS improvements across all pre-specified patients subsets except for the non-epithelial/unknown subtype and the patients with a CR/PR on previous therapy.

Table 8 PFS according to baseline covariates for the patient subgroup with TFI shorter than 6 months (n=236)

Progression-free survival Short TFI subgroup	N	HR	95% CI	Median (months)		P-value
				NGR-hTNF	Placebo	
All patients	236	0.75	0.57 - 0.98	3.0	2.0	0.04
Age (median)						
< 66 years	127	0.62	0.42 - 0.91	2.8	2.6	0.01
≥ 66 years	109	0.89	0.59 - 1.33	3.4	1.8	0.59
Sex		0.81	0.46 - 1.39	4.8	2.5	0.44
Female	58	0.72	0.52 - 0.99	2.7	1.8	0.04
Male	178					
PS						
0	65	0.64	0.38 - 1.07	5.3	2.9	0.09
1 to 2	171	0.79	0.57 - 1.08	2.6	1.7	0.15
Tumor histology						
Epithelial	188	0.74	0.54 - 1.00	3.0	1.8	0.05
Nonepithelial/unknown	48	0.81	0.43 - 1.49	2.3	3.1	0.50
EORTC score						
Good (< 1.27)	157	0.77	0.55 - 1.06	3.5	2.2	0.12
Poor (≥ 1.27)	79	0.67	0.40 - 1.10	2.3	1.8	0.11
NLR (median)						
Low (< 4)	128	0.67	0.46 - 0.95	4.7	2.3	0.03
High (≥ 4)	107	0.89	0.59 - 1.33	2.2	1.7	0.57
Best response to prior chemotherapy						
CR/PR	46	0.73	0.39 - 1.34	1.9	2.1	0.31
SD/PD/NE	190	0.77	0.57 - 1.04	3.0	2.0	0.10
Chemotherapy on study						
No	9	1.00	0.23 - 4.26	2.6	3.2	1.00
Yes	227	0.76	0.57 - 0.99	3.0	1.8	0.05
Gemcitabine	126	0.72	0.49 - 1.04	3.2	2.0	0.08
Doxorubicin	7	0.40	0.06 - 2.43	8.0	4.8	0.32
Vinorelbine	94	0.82	0.52 - 1.25	2.7	1.7	0.35
HR estimated by an unstratified Cox model. HR < 1 denotes a favorable result for NGR-hTNF versus placebo.						

Source table 22 of the clinical overview version 2.0

Sensitivity analyses for the OS of the TFI <6 months

To further assess the robustness of survival outcomes in the short TFI subset, sensitivity analyses were undertaken to check whether the treatment effect on survival changed using alternative TFI thresholds, landmark analyses, or different censoring rules:

1. To assess the impact of TFI threshold the cut-off value of TFI 4.8 months was compared to TFI 6.0 months.
2. To account for guarantee-time bias, landmark analyses were set at 12 weeks after treatment initiation.
3. a. To appraise the impact of censoring, survival data of untreated patients (censored at randomization) were censored at last contact or counted as events at time of death. This included 9 (5%) patients in the TFI <4.8 months and 11 (5%) of patients in the TFI <6 months.

   b. Similarly survival data of patients who had received new cancer treatments were censored at the start of new anti-cancer therapy. This pertained n=41 (5%) of the patients with a TFI <4.8 months and 51 (22%) of the patients with a TFI <6 months.

These additional sensitivity analyses showed that the statistically significant improvement on the HR was generally maintained (Table 9).

Table 9 Sensitivity analyses of OS for the subgroup of patients with a short –TFI:

Sensitivity analyses for OS	HR	95% CI	P value
1. TFI thresholds			
TFI cutoff at 4.8 months (n=198), unstratified	0.68	0.49 - 0.95	0.02
TFI cutoff at 4.8 months (n=198), stratified	0.66	0.47 - 0.92	0.01
TFI cutoff at 6 months (n=236), unstratified	0.73	0.54 - 0.98	0.04
TFI cutoff at 6 months (n=236), stratified	0.70	0.52 - 0.95	0.02
2. Landmark analyses			
TFI cutoff at 4.8 months (n=177), landmark at 6 weeks, unstratified	0.68	0.48 - 0.95	0.02
TFI cutoff at 4.8 months (n=177), landmark at 6 weeks, stratified	0.65	0.46 - 0.92	0.02
TFI cutoff at 6 months (n=174), landmark at 12 weeks, unstratified	0.60	0.43 - 0.85	0.004
TFI cutoff at 6 months (n=174), landmark at 12 weeks, stratified	0.58	0.40 - 0.82	0.002
3. Censoring rules			
No censoring at randomization of untreated cases			
cutoff at 4.8 months (n=198), unstratified	0.72	0.52 - 0.99	0.04
cutoff at 4.8 months (n=198), stratified	0.69	0.50 - 0.96	0.03
cutoff at 6 months (n=236), unstratified	0.77	0.58 - 1.02	0.07
cutoff at 6 months (n=236), stratified	0.74	0.55 - 0.99	0.04
Censoring at start date of new cancer therapy			
cutoff at 4.8 months (n=198), unstratified	0.66	0.45 - 0.96	0.03
cutoff at 4.8 months (n=198), stratified	0.67	0.46 - 0.98	0.04
cutoff at 6 months (n=236), unstratified	0.70	0.50 - 0.98	0.04
cutoff at 6 months (n=236), stratified	0.71	0.50 - 1.00	0.05

Source: table 14 of the clinical overview version 2.0

An additional analysis supporting the difference between the long TFI and short TFI in the placebo group.

A TFI shorter than the median value of 4.8 showed a poor prognostic effect on OS in univariate and multivariate analyses (adjusted for baseline covariates, regardless of significance in univariate models and stratified for selected chemotherapy. The prognostic effect of TFI on OS by univariate and multivariate Cox analyse were assessed in the placebo group in Table 10.

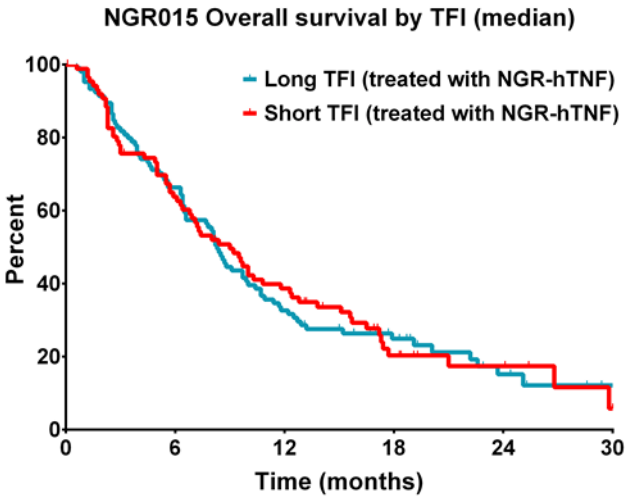
Table 10 Prognostic effect of TFI on OS by univariate and multivariate Co analyse in the placebo group (n=198)

Overall survival	N	Median (months)	Univariate			Multivariate*		
			HR	95% CI	p-value	HR	95% CI	p-value
Control arm								
TFI (median)								
Long (≥ 4.8 months) (ref.)	93	9.9	1	-	-	1	-	-
Short (< 4.8 months)	105	6.3	1.82	1.30 - 2.53	<0.0001	1.98	1.38 - 2.85	<0.0001
TFI (6 months)								
Long (≥ 6 months) (ref.)	79	13.4	1	-	-	1	-	-
Short (< 6 months)	119	6.4	2.15	1.51 - 3.06	<0.0001	2.45	1.66 - 3.61	<0.0001

Source table 11 of the clinical overview version 2.0

The overall survival KM curve for NGR-TNF did not show a difference in the treatment in the patients with a TFI <4.8 months and TFI ≥4.8 months (Figure 4). Such data for the TFI with cut–off value of 6 months and of the placebo group were not provided.

Figure 4 NGR015 overall survival by NGR-hTNF for the TFI <4.8 months and the TFI ⩾ 4.8 months



Source: figure 2D of the clinical overview version 2.0

Exploratory analyses

Additional exploratory analyses were conducted to support the outcomes in the short TFI subset. It was hypothesised that the improved NGR-hTNF effect in patients with short TFI would be related to an increased tumour hypoxia/neo-angiogenesis assessed by lactate dehydrogenase. A high LDH has been reported to predict poor prognosis, including mesothelioma, as well increased sensitivity to anti-VEGF monoclonal antibodies.

For categorising LDH in low and high levels, the first quartile (196 U/L was selected as cut off because it corresponds to site specific lower value of the upper limit of LDH (upper limit of normal (ULN) range 190-668).

- **ITT population**

In the NGR015 study, baseline serum LDH (median, 275 U/L; interquartile range, 196 to 389), assessed in 94% of patients (376 of 400; 189 and 187 for NGR-hTNF and placebo, respectively), did not differ between arms (median, 284 and 262 U/L, respectively; p=0.25).

In the subgroup with high LDH levels, defined by a LDH ≥ 1st quartile, NGR-hTNF showed a statistically significant improvement of 1.1 months in the mPFS over placebo, while a numerical improvement of 3 months was observed in the mOS (Table 11).

Table 11 PFS and OS in patients with high LDH levels in the ITT population (n=283)

LDH serum levels	N	HR for PFS	95% CI	Median PFS (months)		p-value
				NGR-hTNF	Placebo	
≥ 1st quartile (196 U/L)	283	0.78	0.61 - 1.00	3.8	2.7	0.05

LDH serum levels	N	HR for OS	95% CI	Median OS (months)		p-value
				NGR-hTNF	Placebo	
≥ 1st quartile (196 U/L)	283	0.81	0.61 - 1.06	9.7	6.7	0.12

Source: table 33 of the clinical overview version 2.0

- **Short-TFI population with TFI < 6 months**

In the short TFI subgroup <6 months, the observed improvements in PFS of NGR-hTNF over placebo were statistically significant in both subgroups according LDH ≥1st quartile and LDH ≥3rd quartile. The observed difference in mPFS over placebo was comparable (Table 12).

However, in the mOS differences between NGR-hTNF and placebo was larger in the subgroup with LDH ≥ 3rd quartile compared with the ≥ 1st quartile (the difference was 6 months compared to 3.6 months). Although the observed difference in the 3rd quartile was larger, it did not reach statistical significance (Table 12).

Table 12 PFS and OS according to baseline LDH levels for the subset with TFI shorter than 6 months (n=236).

LDH serum levels	N	HR for PFS	95% CI	Median PFS (months)		p-value
				NGR-hTNF	Placebo	
≥ 1st quartile (196 U/L)	181	0.62	0.45 - 0.84	3.2	1.7	0.002
≥ 3rd quartile (388 U/L)	66	0.44	0.24 - 0.80	3.0	1.7	0.004

LDH serum levels	N	HR for OS	95% CI	Median OS (months)		p-value
				NGR-hTNF	Placebo	
≥ 1st quartile (196 U/L)	181	0.68	0.49 - 0.96	9.6	6.0	0.03
≥ 3rd quartile (388 U/L)	66	0.66	0.37 - 1.20	12.8	5.8	0.17

Source: table 31 clinical overview version 2.0

- Outcome Complementary patients group TFI > 6 months

The current dossier fails to provide information for the main outcomes of the complementary subgroup with a TFI >6 months.

Summary of main efficacy results

Table 13 summarises the efficacy results from the main studies supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit/risk assessment (see later sections).

Table 13 Summary of efficacy for trial NGR015

Randomized double-blind phase III study of NGR-hTNF plus best investigator's choice (BIC) versus placebo plus BIC in previously treated patients with advanced malignant pleural mesothelioma (MPM)			
Study identifier	Study No. NGR015 (Internal Code: IPR/22.E) EudraCT No 2009-016879-29		
Design	International, multicentre, randomized double-blind phase III study, placebo controlled study on top of Best Investigator's Choice		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Until disease progression, no longer tolerated or at a patients/physician decision. Not applicable Not applicable
Hypothesis	Superior overall survival with a Hazard ratio of 0.726		
Treatments groups	NGR-hTNF		NGR-hTNF (0.8 µg/m^2) every week + BSC ± single-agent chemotherapy (n=200)
	Placebo		Placebo every week + BSC ± single–agent chemotherapy (n= 200)
Endpoints and definitions	Primary endpoint	Median Overall survival (mOS)	Time from the date of randomization until the date of death due to any cause.
	Secondary	PFS (median months)	Time from date of randomization until disease progression or death due to any cause
	Secondary	Disease control rate	The percentage of patients who have a best-response rating of complete response, partial response, or stable disease.
	Secondary	Disease duration control	In the subset of patients who achieve disease control, the duration of disease control will be measured from the date of randomization until disease progression, or death due to any cause.
Database lock	Not provided		
Results and Analysis			
Analysis description			
Analysis population and time point description	Post hoc defined subgroup with **TFI <6 months** based at the statistical significant interaction for the treatment free interval. Data cut of point 29 April 2014.		
Descriptive statistics and estimate	Treatment group	NGR-hTNF	Placebo
	Number of patients	117	119

variability	Median OS (months)	8.0	6.4
	95% CI	6.3-10.1	5.7-7.6
	PFS (months)	3.0	2.0
	95% CI	2.3-4.1	1.5-3.0
	Disease control rate (CR+PR+SD)/ number of patients	N=62 (53%)	N=57 (48%)
	Duration of disease control ≥ 6 months	38 % 26-50	26% 15-39
Effect estimate per comparison	Primary endpoint mOS	Comparison groups	NGR-hTNF vs. Placebo
		Stratified HR	0.70
		95% CI	0.52 - 0.95
		P-value	0.02
	Secondary endpoint PFS	Comparison groups	NGR-hTNF vs placebo
		Stratified HR	0.75
		95% CI	0.57-0.99
		P-value	0.04
	Secondary endpoint Disease control rate	Disease control rate	NGR-hTNF vs placebo
		Odds ratio	1.23
		95% CI	0.74-2.04
		P-value	0.44
	Secondary endpoint Duration of response	Duration of response	Data not provided
		95% CI	
		P –value	
Notes	Single arm chemotherapy included BIC : doxorubicin (60-75 m/m^2) every 3 weeks gemcitabine 100-1250 m/m^2 on days 1 and 8 every 3 week or vinorelbine 25 mg/m2 iv. Or 60 mg/m^2 per os on day 1 and 8 or oral vinorelbine 80 mg/m^2 weekly for 12 weeks		

Analysis description	Data provided were from the date of cut-off April 29th, 2014 when 75% of events had taken place. In the provided OS analyses the randomised patients who did not receive treatment were censored at the day of randomisation. An additional sensitivity analyses with the randomised patients who did not receive treatment and were followed-up till death or last day known to be alive shows a stratified HR of 0.74 (95% CI 0.55-0.99), p=0.04. The median OS data of this analysis were not provided. Data is not corrected for multiplicity

Source table 15, 21, 26 clinical overview version 0.2. Some slightly different outcomes are provided in version 0.0.

Clinical studies in special populations

No specific studies were conducted in special populations. The number and outcome of elderly patients in the clinical trials was not provided.

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	not reported	not reported	not reported
Non Controlled trials	not reported	not reported	nor reported

Supportive study

Study NGR 10

Title study: A phase II study of NGR-hTNF administered as single agent every 3 weeks or weekly in patients affected by advanced or metastatic malignant pleura mesothelioma previously treated with no more than one systemic therapeutic regimen

Study NGF-hTNF was an open label, single arm phase 2 trial evaluating the efficacy and safety of low dose NGR-hTNF at 0.8 µg/m^2 in patients affected by advanced or metastatic malignant pleural mesothelioma previously treatment first lime pemetrexed based therapy.

Study participants

The study included adult patients affected by malignant pleural mesothelioma previously treated with no more than one systemic therapeutic regiment. Patients could also be treated with other intra-pleural cytotoxic agents. The diagnosis of MPM was histologically of cytological confirmed epithelial, sarcomatous or mixed type. Patents must have had an ECOG performance status of 0-2 and adequate organ function. Patients with known cerebral metastases were excluded. Patients were also excluded with radiation therapy or chemotherapy within four weeks or having surgery within two weeks before treatment.

Treatments

The study included two cohorts. The patients were treated with NGR-hTNF 0.8 µg/m^2 during 60 minutes every 3 weeks (1st cohort) or every week (2nd cohort).

The treatment was discontinued in case of progressive disease, unacceptable toxicity or at the patients or treating physician's request.

Objective

The main objective of this study was to assess the anti-tumour activity of NGR-hTNF measured by the number of patients with PFS at week 12.

Secondary objectives included the Tumour Growth Rate control according to RECIST criteria (calculated as the sum of the CR/PR/SD), overall survival, DCE-MRI, PK, and safety.

Sample size

- Tri-weekly cohort

Sample size was calculated by the two stage Simon's optimal design assuming p0 = 35% (rate of PFS under the hypothesis that the treatment is not active), p1 = 60%, α = 10% and β=10%.

A total of 16 patients have to be enrolled in the first phase; the study would terminate with the number of PFS free patients were ≤7. The second stage would stop when the 27th patient was enrolled; further development would be initiated when at least 13 patients would be PFS free

- Weekly cohort

An additional cohort of 12 patients was planned to be consecutively enrolled and treated with a weekly schedule of NGR-hTNF. No formal size calculation was conducted for this patient group.

Results

Figure 5 Participant flow of the study NGR010

	Assessed for eligibility (n=)	Excluded from analyses because
Triweekly cohort		**Weekly cohort**
	Patients enrolled (N=57)	
Enrolled n=43	Patients receiving study medication (n=55)	Enrolled n=14
Patients treated with triweekly schedule (n=41) Not received medication (n=2) (worsening performance status n=1); Patients refusal (n=1)		Enrolled Patients treated with weekly schedule (n=14)

- Recruitment

The study was conducted between May 14th 2007 and September 14th 2010. The study enrolled 57 patients from four Italian sites.

Conduct of the study

The study was amended twice. The first amendment was initiated before the first patient was enrolled; the second amendment included the inclusion of an additional weekly schedule.

After completion of the first 27 in the tri-weekly cohort, an additional 16 patients were recruited, because of the lack of regulatory approved therapy and the high number of patients at screening.

Number analysed:

Tri-weekly cohort: a total of 43 patients were enrolled and a total of 41 treated. Two patients did not receive treatment.

Weekly cohort: a total of 14 patients were enrolled and treated.

Results

The outcome for the primary outcome measure, the proportion of patients with PFS at week 12, is not provided. Incomplete outcome measures for DCR, mPFS and OS were provided for the overall population and for the subgroup of patients with disease control. For an overview of the other outcome measures, please refer to Table 14.

Table 14 Summary of the overall provided results of study NGR010 – ITT analyses

Cohort	DCR (CR+PR+SD)	RR CR+PR	SD	mPFS	OS	PFS disease control N, (median, range)	OS Disease control. N(n0
Triweekly Cohort (n=43)	19 /43 (44%)	1/43 (2%)	18 /43(42%)	2.8 month (95% CI 2.3-3.3)months	NP	N=19 4.4 months (2.8-15.4)	13.3 months
Weekly cohort n=14	7/14 (50%)	0/14 0%	7/14 (50%)	NP	NP	N=7 9.1 months (3.1-15.8)	Not reached[a]

DCR= CR+PR+SD; RR= CR+PR; m= median, table made by assessor.

[a] the median follow up time for the weekly cohort is not provided.

Dynamic contrast enhanced resonance imaging (DCE-MRI)

The DCE-MRI analysis was not conducted.

Pharmacokinetics

PK analyses: insufficient data has been collected for assessment.

Overview of the comparative data of the phase II and Phase III study

The Phase II study NGR010 and the pivotal study NGR015 included almost the same study population. In study NGR010, the 0.8 µg/m^2 dose is administered tri-weekly or weekly. In study NGR015, the 0.8 µg/m^2 dose is administered weekly and compared to placebo. An overview of the primary and secondary outcome measures is provided in Table 15.

Table 15 Overview of the primary and secondary outcome measures of the study NGR010 and NGR015

Cohort	**NGR-hTNF (n)**	**DCR (n/%)**	**RR (n/%)**	**mPFS (95% CI)**	**mOS (95% CI)**
NGR010					
Triweekly	43	19 (44%)	1 (2%)	2.8 (2.3-3.3)	13.3
Weekly	14	7 (50%)	0	NP	NP
NGR015					
NGR-hTNF- ITT	200	114 (57%)	3 (2%)	3.4 (2.4-5.1)	8.5 (7.2-9.9)
NGR-hTNF-TFI < 6 months	117	62 (53%	2 (2%)	3.0 (2.3-4.1)	8.0 (6.3-10.1
Placebo – ITT	200	109 (55%)	6 (3%)	3.0 (2.3-3.7)	8.0 (6.6-8.9)
Placebo – TFI < 6 months	119	57 (48%)	3 (3%)	2.0 (1.5-3.0)	6.4 (5.7-7.6)

Source clinical AR and clinical overview version 02, tables 7, 15, 18, 21, 23 and 26

3.3.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Dose finding studies - tumour response

The dose finding studies were well designed studies in patients with refractory tumours. The primary endpoint in these studies was the anti-tumour effect by means of the disease control rate (DCR)(i.e. complete response (CR) + partial response (PR) + stable disease SD). The EMEA guideline recommends the use of the response rate (EMEA/CHMP/205/95 Rev 4 Guideline on the evaluation of anticancer medicinal), because spontaneous shrinkage of the tumour to a partial response is unlikely to occur. The DCR will probably overestimate the anti-tumour effect because patients with refractory tumours, but fit for additional therapy are selected patients, who suffer likely from an indolent tumour. Therefore, the anti-tumour effect is probably overestimated, in particular when a small number of patients are included and no comparison with placebo is made.

The dose finding studies were conducted with a tri-weekly dose administration, while the pivotal study is conducted with a weekly dosing administration. The only support for the applied weekly posology can be provided from a small cohort (n=14) of the phase II study NGR010 which is likely too limited.

Moreover, the provided data are incomplete and not supported with additional pharmacodynamic data or biomarkers. Therefore, the proposed posology is insufficiently supported with the dose finding studies unless a justification can be provided to bridge the data from the tri-weekly dosing schedule to the weekly dosing schedule.

Phase II and Phase III studies (NGR10 and NGR015)

General comment

The phase II study NGR010 and phase III study NGR015 studies were conducted in the target patient population, those with advanced malignant pleural mesothelioma after failure of pemetrexed. The in- and exclusion criteria were comparable facilitating comparison of the study results.

Both studies measured the response to treatment according to the modified RECIST criteria for Malignant Pleural Mesothelioma (Byrne, 2004). The use of this Modified RECIST criteria is agreed, because is defines the sites to be measured. Malignant pleural mesothelioma most commonly grows as a 'rind' around the pleural surface and without defining the selection of measurement sites, the tumour growth will likely be assessed differently by different investigators. With the modified criteria, the tumour response is thus better standardized.

Study NGR010 and study NGR015 were in principle well designed. However, both studies included amendments that may complicate the overall interpretation of the results.
In study NGR010, the weekly dose administration of study NGR010 is included as an amendment based on the assumption that the weekly dose schedule would maintain more constant vascular effects, thus potentially preventing tumour vasculature repair of regrowth that would happen in case of long treatment gaps. This argumentation is, however, not supported by additional, specific pharmacodynamic or biomarker data.
Unlike the tri-weekly cohort, the sample size (n=14) of the weekly cohort was not supported with a sample size calculation, or with decision rules to continue with this posology in the phase III trial. Limited pharmacodynamic data was collected in this small cohort, but the data apparently did not contribute to the decision to proceed with the weekly dose administration.
Study NGR015 included a substudy to investigate the combination of vinorelbine and NGR-hTNF. It is not clear what triggered the conduct of this substudy. The results of this substudy are also not presented and it cannot be determined whether the results from this substudy were included in the final analyses. Of concern is that the inclusion of this substudy may have jeopardized the integrity of the blinding of the study.

Specific comments Study NGR015

Design

The single pivotal study NGR015 is a randomised, double blind, placebo-controlled, multinational, parallel trial investigating NGR-hTNF on top of standard care in patients with advanced malignant mesothelioma after the failure of pemetrexed based chemotherapy. The study was stratified according to important prognostic factors and baseline chemotherapy according to best investigator's choice. The background chemotherapy was also defined in the protocol. The primary endpoint was the OS, with the PFS as a key secondary outcome measure. The protocol also included a quality of life measurement and predefined co-variates for the subgroup analyses. These factors make that, in principle, the study was well designed. There are some important comments:

a. The applicant described the applied background chemotherapy, but the choice of the chemotherapeutic drug, the advised posology and maximal cycle number by the protocol was not substantiated. For example, the number of cycles of vinorelbine i.v. was limited, but in other clinical studies, vinorelbine was given until no longer tolerated [Stebbing 2009]. Pemetrexed was not permitted as background therapy, although a rechallenge with pemetrexed can be considered when a treatment free interval of 6-12 months has been achieved. Also, guidelines do not recommend the use of doxorubicin.

b. Another concern is that patients could be included without a histological confirmation of MPM. The study did not include an external expert panel to confirm the diagnosis either, although both aspects are recommended by international guidelines for clinical trials in MPM.

c. The study included a quality of life measurement, i.e. the time to symptomatic improvement. This outcome measurement does not show an overall improvement in the quality of life. In a palliative setting, it is important to measure the overall quality of life because adverse events or frequent hospital visits may have a negative impact on the overall QoL.

d. Although the study protocol predefined the co-variates for the subgroup analyses, the protocol omitted to describe the expected direction of the observed effect by NGR-hTNF. Therefore, confirmation bias is likely to be introduced by post-hoc subgroup analyses.

e. The study did not include biomarkers like CD13 or VEGFR in the proposed study population. The inclusion of these biomarkers might have supported the proposed mode of action and provided support for the posthoc defined hypothesis.. A dose effect was observed for the baseline serum LDG levels.

Conduct of the study

The study included a total of 400 patients. This is a large number of patients for an orphan indication.

Included patient population

The study included a total of 41 sites in 12 different countries. Patients received best supportive care and chemotherapy according to local protocols and clinical use.

The background experience in the treatment of MPM varies between the study sides as the surgical staging was missing in > 40% patients.On the other hand, also experienced sites were included, because up to 40% of the patients received prior surgery, while surgery for MPM, with the exception thoracoscopy, is limited to experienced sites.
So, likely a heterogeneous patient population was included. A heterogeneous included population may enhance the external validity, but at the same time may undermine subgroup analyses as clustering of unknown prognostic baseline factors is more likely to occur. As a complete description of the baseline factors was not provided, interpretation of the results is hampered.

The exact staging and diagnosis of MPM is difficult as stated by the applicant. Patient

OS and PFS

The study included the OS and PFS as key primary endpoints as recommended by EMA guidelines and CHMP scientific advice. Concerns are raised in the analyses of the OS, PFS, DCR and RR.

For the OS, the randomised patients who did not receive treatment, were censored on the day of randomisation. However, according to the principles of ITT analyse, the outcome (overall survival) should be measured, regardless if treatment was given. An additional sensitivity analyse using this principle was also provided showing still statistically significant outcomes (stratified HR 0.74, 95%

0.55-0.99, p=0.04), but without providing the median OS values. The absences of these data hamper the clinical interpretation of results.

For the PFS, the analysis is impeded by a large number of missing post-baseline tumour assessments (n=77; 19%) in the ITT population and this number is unknown for the TFI <6 months. The large number of missing data undermines the validity of the PFS measurement. However, not only the PFS, but also the analyses of the DCR and RR are affected by this incomplete information.

Importantly, clarification is also needed for reported discrepancy (n=22) of the patients with non-assessable tumour response (n=55) (table 22 of the clinical AR, or table 23 of clinical overview version 2.0) and the reported patients with a lack of post baseline tumour assessment on measurable disease (n=77)(Figure 1).

Treatment Free Interval (TFI)

After the study had failed to meet the primary endpoint, a post-hoc, subgroup was defined based on the statistically significant interaction between treatment free interval (TFI) and overall survival. Several major methodological and clinical concerns are raised regarding the selection of the subgroup based on TFI:

a. The subgroup of interest, those with a TFI <6 months is based on a post-hoc, data driven baseline characteristic.

b. A total of 3 subgroups have been identified in the studied population based on differences between the start and end of the TFI measurements (see also Figure 6).

    1. The first group contains the patients that only received one first line pemetrexed treatment (*first line only-group; ITT population n=367*) – the TFI is defined by the last chemotherapy cycle till the start of the second line treatment. This group has the highest possibility of reaching a long TFI by definition as measuring TFI started directly after first line therapy.

    2. For patients that received maintenance treatment before study start (*maintenance group; ITT population n=12*), the TFI is defined by the end of the maintenance treatment till the start of the second line treatment. As a result, the TFI is only defined by logistical aspects.

    3. Patients that received a rechallenge with pemetrexed after first line pemetrexed treatment (ITT population *rechallenge group; n=21*). For the patients who received a rechallenge with pemetrexed, the TFI is defined by the end of first line treatment and the start of rechallenge. For these patients, the TFI is not followed by NGR-hTNF/or placebo, but by a rechallenge of pemetrexed. It is of concern that there might exist a long gap between the TFI and start of study treatment.

    It is unclear if and how the TFI measurements from these 3 subpopulations have affected the overall TFI results; the numbers included in the population of interest (TFI<6 months) is unknown.

c. The exact timing of TFI measurement needs to be clarified as well. The end of the TFI is defined by the start of NGR-hTNF/placebo. However, if treatment was started (several days/weeks?) *after* randomisation, then the time between randomization and treatment is unknown. The TFI is, therefore, not a disease- or patient characteristic and will be affected by logistical aspects, like the recovery/washout period of surgery/chemotherapy, which may

hamper the replication of this baseline characteristic. Also, it is also not known if the TFI is comparable following the different treatments.

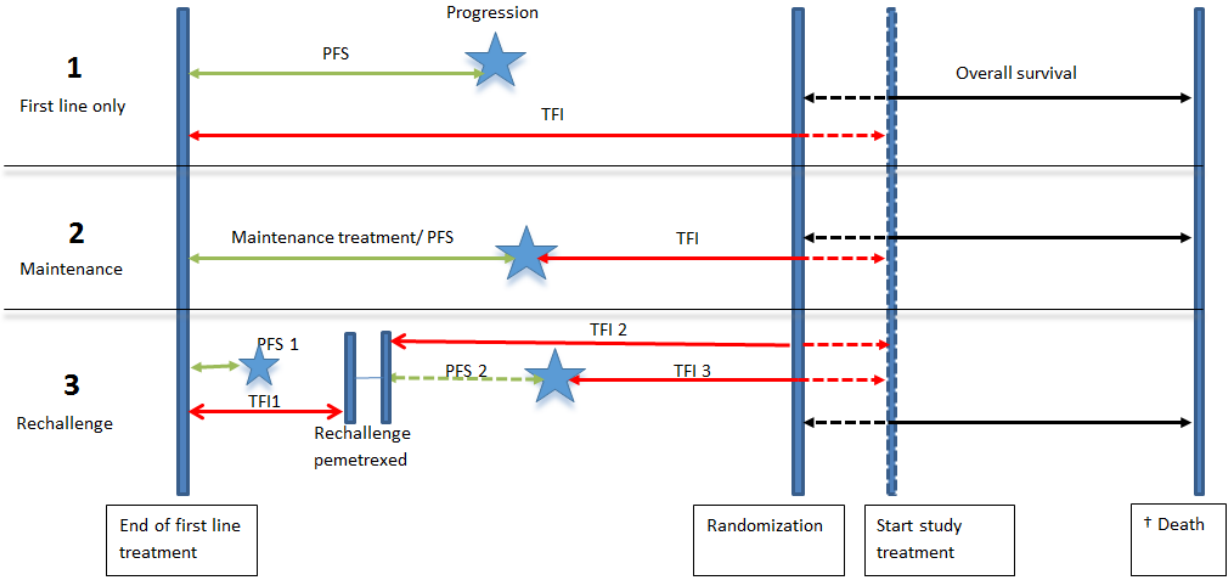
d. An overlap in time exists between the TFI and the PFS and OS measurements, because the starting point of the PFS and OS is the date of randomisation (see Figure 6). As a result, the PFS and OS might be affected depending on how the TFI was determined in each patient. For example, at least 14 randomised patients did not receive treatment. A TFI will be hard to establish for these patients. Clarification is needed, how these patients are allocated, i.e. to the short TFI or the long TFI, as this may affect the overall survival analysis and PFS analyses.

e. The identified subgroup with a TFI <6 months does not correspond with the intended target population. The proposed indication is for patients with a progression within 6 months since first line pemetrexed. However, "progression" is not synonymous with TFI, but more closely resembles PFS.

   Although the PFS and TFI might be comparable for the first line only-group, the PFS and TFI are different entities for the maintenance and rechallenge group (Figure 6). Patients who receive maintenance treatment may obtain a prolonged PFS on maintenance treatment, while the treatment free interval might be short (Figure 6). For the rechallenge group, PFS might overlap with measured TFI. However, it is of concern that PFS might also be measured until the end of rechallenge, i.e. PFS2, and the TFI might be measured from the end of the rechallenge period to the end of treatment.

f. Furthermore, the Progression Free Survival is an established entity in clinical practice, while the clinical relevance of TFI is less well established. Also, maintenance treatment is not an established therapy strategy in mesothelioma.

Figure 6 Illustration of the treatment free interval and overall survival measured in the pivotal study NGR015



Note: Schematic overview of possible Treatment Free Interval (TFI) subgroups in study NGR015. Subgroup 1 received only one first line pemetrexed treatment. Subgroup 2 received maintenance treatment prior to study start, subgroup 3 received first line pemetrexed and a rechallenge with pemetrexed (second line treatment). A star indicates progression. Green line: PFS, Red line: TFI, black line: overall survival. Dashed line indicates a possible additional or alternative interval length. Please note that the timeslines are not on scale.

Blinding

Concerns were raised whether the blinding of the study would have been sufficiently maintained because of the inclusion of a substudy with vinorelbine and the deblinding of 7 patients at one site. Moreover, as the majority of patients reported chills after infusion of NGR-hTNF, the risk of identifying the patients randomised to the study drug arm is potentially high, despite the double-blind design of the study.

Data presentation.

The data presentation of the clinical overview and study report is not complete and not always transparent. For example, the tables and figures make no references to the print-outs of the calculations. For the dose finding studies, these print-outs were often not provided. The missing of these print-outs hampers the assessment and validity of the provided data. Also, the statistical analyses plans and dates of data base locks were not provided for the dose finding groups.

Inconsistencies were observed in the presentation of the published data and the final study report and the reporting of data in the clinical overview version 0.0 and 2.0. These inconsistencies seem to indicate that the data base has been adjusted after the initial analyses.

Different subgroups analyses were provided according to a different cut off value for the treatment free interval. The subgroups are divided in a short TFI and the complementary long TFI. The subgroup of interest, those with a short TFI, refers to both the subgroup of patients with a TFI <4.8 months and the subgroup with a TFI <6 months. The use of the same wording – short TFI group- for these different subgroups is confusing. The overview presents data to support the subgroup with a TFI< 4.8 months, but incomplete and insufficient data are provided to support the subgroup of interest, those with a TFI <6 months, although we presume that that would be the subgroup that is proposed to substantiate the applied for indication.

Concerns are raised regarding the adherence to GCP, see also section 2.4.

Efficacy data and additional analyses

The dose response studies

The dose response studies showed the highest response rate (23%) for the 0.8/µg/m^2/3w dose administered at a 3 weekly interval, the other posologies showed response rates between 0-7%. Therefore, the choice for the 0.8/µg/m^2/3w appears to be justified, although the observed response rate is not in line with the other dosages and this is not understood. Also, the dose finding studies were conducted with a tri-weekly dosing scheme and it should be justified how the results of the tri-weekly dosing interval can be inferred to the weekly dosing interval.

Dose response malignant pleural mesothelioma: study NGR010 and NGR015

Study NGR010

Limited support for the weekly dosing interval could be provided by study NGR010. In study NGR010, the 0.8 µg/m^2/weekly and 0.8 µg/m^2/3w dosing schedule showed comparable response rates (0-2%) and disease control rates (44-50%). However, based on these similar results, no dose recommendation can be given, and the incomplete OS and PFS data as provided preclude a conclusion. The safety profile was in favour of the tri-weekly dose. The better safety profile for the weekly dosing interval provides an additional argument for the request to substantiate the weekly dose administration.

Study NGR010-NGR015 weekly dose administration.

The study NGR015 and NGR010 included a comparable patient population, facilitating the comparison of results for the weekly cohort. The studies showed a comparable DCR (50-57%) and RR (0-2%). In study NGR015, the DCR and RR was comparable for both the ITT and the TFI <6 month cohort.

The observed disease control rate with NGR-hTNF is comparable to other therapies in the second line treatment of malignant mesothelioma [Abel-Rahman, 2012]. However, the observed response rate (0-2%) was lower than observed with other treatments e.g. gemcitabine/vinorelbine combination or pemetrexed rechallenge (17%-18%), but comparable with gemcitabine (2%), where the response rates are measured according to the modified RECIST criteria [Abdel-Rahman, 2012].

Pivotal Study NGR 015

The single pivotal study showed similar results in the overall survival for NGR-hTNF and placebo for the ITT study population and failed to meet its primary endpoint (median OS 8.5 months (95% CI 7.2-9.9) vs 8.0 months (95% CI 6.6-8.9), respectively; HR 0.97 (p=0.79)). Based on the significant treatment by TFI interaction test, a data driven post hoc subgroup of interest was defined i.e. those with a Treatment Free Interval (TFI <6 months).

From a formal statistical view, no further confirmatory conclusion is possible, because of the high risk of selection bias. However, in case of exceptional circumstances like an orphan indication, careful assessment of the available evidence has to be performed. Careful consideration should also be given to the magnitude of effect, the external evidence that the subgroup is a well-defined and clinically relevant entity, the pharmacological rational or mechanism of action, replication of effect, and the internal validity of the trial (EMA/CHMP/539146/2013), as is done so below.

- Magnitude of effect in patient population TFI <6 months

In the first OS analyses in this subgroup, the randomised patients who did not receive treatment were censored at baseline. The observed improvement in OS of 1.6 month, if true, is considered of clinical relevance (stratified HR 0.70; p=0.02) in the context of an OS for placebo of 6 months.
The observed improvement in PFS for this subpopulation is considered marginal (1 month, stratified HR0.77; p=0.04). The effects are not supported by an improvement in QoL. According to this analysis, and again, if true, NGR-hTNF would be the first treatment showing a statistical significant and clinical relevant OS benefit in the second line treatment of mesothelioma on top of well-established treatments.
Additional sensitivity analyses for OS with including survival data of untreated patients (censored at randomization) showed a stratified HR of 0.74 (95% Ci 0.55-0.94) p=0.04. This analysis was not corrected for multiplicity. The overall median OS for NGR-hTNF and placebo were not provided for this analysis, which hampers further interpretation.

- TFI <6 months, weak external evidence for selection of subgroup

The subgroup of interest, TFI <6 months, is defined by the duration of the treatment free interval. Previous retrospective analyses showed that a prolonged PFS or TFI in response to first line chemotherapy was predictive for a prolonged OS following second line chemotherapy treatment in MPM. [Zucali 2014, Manegold 2005]. However, up till now, these observations have not been confirmed in prospective studies.

In contrast to previous findings, the current study shows a better effect with NGR-hTNF in patients with a short TFI. The Applicant refers to two studies to substantiate this effect by an anti-angiogenic product in the second line treatment of NSCLC. When added to chemotherapy, the VEGF-R2 inhibitor

ramucirumab showed a significant improved HR for mOS to placebo (HR 0.75; 95% CI 0.64-0.88) in the group with a short time since prior therapy (<9 months) compared with the group with a prolonged response since prior therapy (≥9 months) HR 0.95; 95% CI 0.75-1.20) [Garon, 2014]. The same observation was made for nintedanib, i.e. HR 0.75 (95% CI 0.60-0.92) vs. HR 0.89 (95% CI 0.66-1.92), respectively [Reck, 2014]. However, these results can only be considered to provide limited external support as it concerns other type anti-angiogenic products, in other tumour types, with a different duration of the treatment free interval in another cancer type.

- Uncertain robustness of TFI as parameter to select a patient population relative to PFS

TFI, as described above, might also be affected by logistic aspects like wash-out periods of the previous therapy, while PFS will be a clear pre-randomisation parameter in principle only affected by progressive tumour growth. Moreover, PFS is a well-established clinical entity, while the use of the TFI as defined in the current application have raised several concerns as mentioned before as it may also affect the OS and PFS. The use of the PFS to define the subgroup of interest would have been preferred, though it is not anticipated to resolve the currently identified concerns of this dossier.

- Mode of action – difference in effect size in overall population vs. subgroup of interest

To study the effect of NGR-hTNF on the short TFI group was not predefined. Post-hoc, the results were clarified by the heterogeneous course of MPM after first line therapy.

The proposed mode of action of NGR-hTNF is a targeted anti-angiogenetic effect. NGR-hTNF targets the tumour blood vessel by binding on the CD13. NGR-hTNF causes vascular leakage, which facilitated the penetration of chemotherapy in the tumour. NGR-hTNF is also hypothesised to have an anti-angiogenetic effect. Fast growing MPM tumours are more likely to express higher levels of CD13 and VEGF, the proposed targets for NGR-hTNF therapy. CD13 is upregulated by hypoxia, and the high LDH level of the exploratory analyses may confirm that these tumours have a higher level of hypoxia. The fast growing tumours are identified by the tumours with a short TFI <6 months as they are hypothesised to rely more on neovascularisation. The lack of benefit in the group with a TFI >6 months was explained that these tumours are proposed to rely less on neovascularisation.

We consider that although this effect might have been demonstrated in preclinical data, the provided evidence in humans is less clear for this product (see pharmacodynamic assessment). The current application does also not contain biomarkers to support the proposed hypothesis of increased neovascularisaton in tumours with a TFI <6 months.

Support for the potentiation of the anti-tumour effect by NGR-hTNF might have been provided by showing increased response rates because spontaneous tumour regression is unlikely to occur. However, the pivotal trial showed similar and very low response rates for NGR-hTNF and placebo, both in the ITT population (n=3, 2% vs. n=6, 3%) and in the TFI <6 months cohort (n=2, 2% vs. n=3, 3%). Other outcome measures that might provide limited support to the different mode of action in the overall population compared with the TFI<6 months might be the disease control rate or PFS. However, the use of these measurement is hampered by the large numbers of non-assessable disease (n=37 (16%) overall with n=25 (21%) NGR-hTNF and n=12 (14%) placebo).

In conclusion, the proposed different mode of action in the overall population compared to the subpopulation of interest is considered to be insufficiently proven.

- Replication of results

The current application is supported by one pivotal trial, which can only directly supported by the small cohort of n=14 patients from the phase II study NGR010. The DCR and RR are comparable, but the

PFS and OS have not been provided for the weekly cohort precluding a conclusion of the replication of effects. Also, the number might to too small to draw any conclusion.

Up till now, no treatments have been approved for the second line treatment of malignant pleural mesothelioma. Malignant pleural mesothelioma expresses high levels of VEGF, which could make the tumour sensitive to anti-angiogenetic therapy. However, the effect of anti-angiogenetic therapy in malignant mesothelioma is not well established. Recently, the VEGFR inhibitor bevacuzimab showed improved OS as a conjunct to cisplatin/pemetrexed in the first line therapy of mesothelioma [Zalcman, 2012], but a previous study conducted with cis/gemcitabine failed to show an OS improvement [Kindler 2012].

Malignant pleural mesothelioma is the first application of NGR-hTNF. No other products are approved in second line malignant pleural mesothelioma, also not bevacizumab. Therefore, replication of effect is limited to the small cohort n=14 included in the phase II study, a too small number to yield conclusive results. The included number of patients is too low (n=14) to make a comparison for the patients with a TFI <6 months or >6 months like in the pivotal study.

- Internal validity

In case an application is based on a post hoc defined subgroup, the study results must show strong internal validity. The provided outcome data for OS, PFS, and DCR, were concordant and showed all improvements for NGR-hTNF compared with placebo in the subgroup with a TFI <6 months. For the OS and PFS, also improvements in favour of NGR-hTNF were observed in the pre-specified subgroups. This observation supports the observed OS and PFS effect. However, major methodological and clinical concerns were raised regarding the included patient population and data analyses, most importantly the definition of the TFI population with a short treatment interval.

Included patient population
It is well recognised that MPM is a difficult diagnosis [ATS/ERS guidelines]. In the clinical study, the diagnosis was not confirmed by histology or an external review committee, and probably also ≥2 patients with metastasized mesenteric mesothelioma were included. Furthermore, the study also included many study centres (n=41) in different countries (n=12). The experience in the treatment of this relatively unknown tumour varied; while it also not clear, if the patients were initially staged according to the same staging system. The uncertainties in the diagnosis, staging system, and various experiences with this relatively unknown tumour may contribute to a heterogeneous patient population undermining the strength of subgroup analysis results.

Because of the lack of comparison of the baseline characteristics between NGR-hTNF and placebo in the subgroup with TFI <6 months, it cannot be excluded that the observed improvement in overall survival in the subgroup with a TFI <6 months was caused by an imbalance in prognostic factors.
In fact, too limited data has been provided for this subgroup. The data has to be completed before this subgroup can be considered to serve as support for the applied indication.

TFI and analyses of OS, PFS and DCR
The methodological concerns regarding the TFI and overall survival analyses and PFS/DCR have already been discussed. The replication of the identified subgroup based on the TFI is questioned; the PFS and OS might be affected depending on how the TFI was determined in each patient, while the provided OS analyses might show an overestimation of effect.

Please consider that the proposed indication is in patients with a progression within 6 months since first line pemetrexed. However, “progression” is not synonymous with TFI, but more closely resembles PFS.

The conclusion is that the internal validity of the study needs to be substantiated.

Additional concerns:

TFI near the cut of value of 6 months
We also consider that the overall survival failed to show an improvement in the initial analyses of the pivotal study. Therefore, if an improvement is shown in one group, the complementary group will show a detrimental effect. The TFI is a continuous baseline characteristic dichotomised at 6 months to define a subgroup that showed an improvement in OS. Therefore, for patients with a TFI value nearby the cut-off point, it will be hard to determine the expected effect and the patient population who will show a benefit. It is also noted that OS was reduced by over a month when expanding the cut-off from 4.8 months (median) to 6 months (due to reductions of the median OS in the NGR-hTNF arm only). An exact cut-off is therefore not well established.

3.3.7. Conclusions on clinical efficacy

The following indication is applied Zafiride, in the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy

The phase II and III studies do not show an anti-tumour effect of NGR-hTNF in malignant pleural mesothelioma as the observed response rate is very low (0-3%), both as monotherapy and in combination with chemotherapy, and is comparable to placebo (0-3%).

The results from the single pivotal study NGR015 showed no differences in overall survival between NGR-hTNF and placebo in the ITT and a post-hoc data driven subgroup was identified by the treatment free interval of <6 months in a heterogeneous MPM patient population. The currently presented data showed an improvement in OS of 1.6 months by NGR-hTNF 0.8 µ/m^2/weekly compared with placebo added to single agent chemotherapy. This effect might be of clinical relevance, given that the observed OS in placebo was 6.0 months. The effect on PFS is small, and the overall data is not supported by an improvement in the quality of life.

The use of the data driven subgroup identified by the TFI raise various major methodological and clinical concerns. The TFI is also not a well-established clinical entity. In fact, the applicant applies for a population characterised by showing progression within 6 months, but the PFS and TFI are distinct entities in this trial. Moreover, due to the lack of the comparison of the baseline characteristics, it cannot be excluded that the observed beneficial effect observed in the TFI <6 months is caused by an imbalance in prognostic factors.

The proposed mode of action is insufficiently supported with clinically pharmacodynamic data and lacks the support of biomarkers, this accounts for the ITT as well as for the subgroup of interest. This is the first application of NGR-hTNF and no other products are approved for the second line treatment of MPM. Therefore, the replication of the effect by NGR-hTNF 0.8 µg/m^2/w will be limited to the small cohort of the phase II study for which the incomplete data and the small number of patients involved preclude a definitive conclusion.

The provided data is insufficient and raises uncertainties regarding the magnitude of the effect, proposed mode of action, the identified subgroup of interest and the interval validity of the trial. Overall, the proposed beneficial effect is undetermined for NGR-hTNF for the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first line pemetrexed based therapy.

3.3.8. Clinical safety

The safety population consisted of 386 (97%) of 400 patients who were randomized and received treatment with a study drug (193 patients in each treatment group) in the pivotal, double-blind placebo-controlled Phase 3 study NGR015.

The total safety population consisted of patients with a short as well as a long treatment free interval (TFI) after first line treatment. A short TFI has been defined as <4.8 months (based on the observed median TFI in the study population), or <6 months (based on the proposed indication with the cut off at 6 months, see efficacy section).

In the short TFI subset <4.8 months, safety information was reported for 189 patients who underwent randomization and received treatment with a study drug (95%, n=189/198; n=87 in the NGR-hTNF arm and n=102 in the placebo arm).

In the short TFI subset <6 months safety information was reported for 125 patients (n=110 in the NGR-hTNF arm and n=115 in the placebo arm). Safety data in this overview will mainly focus on this short TFI subgroup, as this is the population brought forward in support for the applied for indication.

Baseline characteristics for the whole safety population and short TFI subset were generally comparable between the NGR-hTNF and placebo arm. Most patients were male (~75%), approximately 66 years of age, with 9-13% being >75 years of age and ~64% of patients had an ECOG performance score of 1 (~5% a score of 2). All patients received prior pemetrexed + cisplatin/carboplatin, and 35% of patients received prior surgery.

Patient exposure

Exposure data was provided for the total study population and short TFI subset <4.8 months. No exposure data for the subgroup with a TFI shorter than 6 months has been presented by the applicant**.**

Total safety population

Of the 386 treated patients in the pivotal Phase 3 trial, 367 (95%) received chemotherapy (184 NGR-hTNF and 183 placebo;

Table 16). In both arms, most patients received gemcitabine (~52%) or vinorelbine (~40%), and only a few patients received doxorubicin (3%).

The median duration of NGR-hTNF or matching placebo (scheduled every week) in the total safety population was 10 infusions in both treatment groups. The median duration of chemotherapy (scheduled every three weeks) was 4 cycles in the NGR-hTNF group (range 1-6) and 3 cycles in the placebo group (range 1-6). Of the patients in the NGR-hTNF arm, 42% completed 6 cycles of chemotherapy, compared to 32% in the placebo arm (p=0.05). In the NGR-hTNF arm, 36% received at least 18 infusions of study drug, compared with 28% of patients that received at least 18 infusions in the placebo arm (p-0.10).

Table 16. Summary of treatment exposure in the safety population of the NGR015 trial (n=386)

	NGR-hTNF plus BIC	Placebo plus BIC
NGR-hTNF or Placebo, n	193	193
Total number of weekly infusions	2,832	2,761
Mean	15	14
Median	10	10
(95% CI)	(7 - 14)	(7 - 12)
(range)	(1- 89)	(1- 91)
≥ 18 infusions, n (%)	69 (36%)	53 (28%)
Chemotherapy, n	184	183
Total number of triweekly cycles	707	674
Mean	4	4
Median	4	3
(95% CI)	(3 - 5)	(2 - 4)
(range)	(1 - 6)	(1 - 6)
6 cycles, n (%)	77 (42%)	58 (32%)

Adverse events

In the total safety population, almost all patients in both arms experienced at least one adverse event (AE) of any grade (Table 17). Slightly more grade 3 AEs (53% vs. 45%) and treatment discontinuations (16% vs. 12%) were reported with NGR-hTNF than with placebo. The rates of grade 4 and 5 AEs were similar between arms. In the subset with TFI shorter than 4.8 or shorter than 6 months, a similar safety profile was observed (Table 17).

Table 17. Summary of safety data in the total safety population of the NGR015 trial and short TFI subset classified as worst grade per patient

	Safety population		**Short TFI subset <4.8 months**		**Short TFI subset <6 months**	
Study emergent (S)AEs	**NGR-hTNF plus BIC (n=193)**	**Placebo plus BIC (n=193)**	**NGR-hTNF plus BIC (n=87)**	**Placebo plus BIC (n=102)**	**NGR-hTNF plus BIC (n=110)**	**Placebo plus BIC (n=115)**
Any grade	191 (99%)	185 (96%)	85 (98%)	99 (97%)	109 (99%)	112 (97%)
Grade 3	102 (53%)	86 (45%)	47 (54%)	40 (39%)	57 (52%)	47 (41%)
Grade 4	22 (11%)	19 (10%)	7 (8%)	8 (8%)	11 (10%)	9 (8%)
Grade 5	12 (6%)	13 (7%)	6 (7%)	10 (10%)	11 (10%)	10 (9%)
Grade 5 Treatment elated	-	-	-	-		
SAE	26%	24%	22%	26%		
Treatment discontinuation due to AEs	31 (16%)	24 (12%)	15 (17%)	12 (12%)	19 (17%)	13 (11%)

Short TFI subset <6 months

In the subset of patients with a TFI shorter than 6 months, the most common study emergent AEs of any grade reported for NGR-hTNF and placebo were similar to the short TFI subset <4.8 months. The adverse events included chills (50% vs. 10%), fatigue (47% vs. 50%), pain (45% vs. 44%), dyspnoea (37% vs. 29%) and pyrexia (29% vs. 23%; Table 18).

Study emergent AEs of any grade reported with ≥5% higher frequency in the NGR-hTNF arm were: chills (50% vs. 10%), dyspnoea (37% vs. 29%), pyrexia (29% vs. 23%) and leukopenia (12% vs. 5%). These AEs were highlighted in blue boxes in Table 18. A ≥5% higher frequency in the placebo arm was reported for decreased appetite (20% vs. 27%) and diarrhoea (6% vs. 13%).

Grade 3 to 4 study emergent AEs reported with ≥2% higher frequency in the NGR-hTNF arm compared to placebo were chills (5% NGR-hTNF vs.0% placebo), dyspnoea (6% vs. 3%), neutropenia (16% vs. 13%) and leukopenia (5% vs. 3%).

Table 18. Study emergent AEs in >10% of cases of patients with TFI shorter than 6 months (n=225), irrespective of treatment relationship, classified by preferred term and worst grade per patient.

Preferred term	NGR-hTNF plus BIC (n=110)				Placebo plus BIC (n=115)			
	All grades		Grade 3 to 4		All grades		Grade 3 to 4	
	No.	%	No.	%	No.	%	No.	%
Chills	55	50	6	5	12	10	-	-
Fatigue	52	47	4	4	57	50	9	8
Pain	50	45	7	6	51	44	10	9
Dyspnoea	41	37	7	6	33	29	4	3
Pyrexia	32	29	-	-	26	23	-	-
Nausea	29	26	-	-	32	28	-	-
Neutropenia	29	26	18	16	30	26	15	13
Anaemia	26	24	5	5	31	27	6	5
Decreased appetite	22	20	1	1	31	27	1	1
Constipation	21	19	-	-	26	23	1	1
Cough	21	19	1	1	19	17	-	-
Vomiting	17	15	2	2	18	16	1	1
Oedema	15	14	-	-	17	15	1	1
Thrombocytopenia	15	14	5	5	11	10	5	4
Leukopenia	13	12	5	5	6	5	3	3
Mucosal inflammation	8	7	-	-	12	10	-	-
Diarrhoea	7	6	2	2	15	13	1	1
Headache	7	6	-	-	12	10	-	-

Data include AEs reported between first dose and 28 days after last dose of study drug, with events termed according to consolidated AE category comprising synonymous MedDRA preferred terms. Safety analyses included all patients who received at least one dose of study drug. Some patients had more than one adverse event.

Adverse events of special interest

No adverse events of special interest have been monitored during the pivotal trial, since the applicant considered the toxicity profile observed in earlier phase I and II studies, given either as single agent or in combination with chemotherapy, favourable.

Serious adverse events and deaths

SAEs

In the total safety population, 147 SAEs were registered in 110 of 386 treated patients (28%) of the whole safety population, with 45 SAEs (31%) considered related in 37 of 386 patients (10%). The most commonly reported SAE was progressive disease (10%), while all the other SAEs had a frequency

below 10%. The SAE were not categorised according to system organ class, but were listed per patient in extended tables in appendices of the summary of clinical safety and final study report (starts at table 2.7.4.a; and section 14.3.2, respectively).

Deaths

Total safety population

At the time of the data cut off (29 April 2014), 19 deaths were reported: 12 deaths in the NGR-hTNF arm of the pivotal trial, and 7 in the placebo arm. None of the deaths were considered as treatment related by the investigator. The main cause of death in the NGR-hTNF arm was disease progression (n=4), followed by respiratory failure/insufficiency or dyspnoea (n=3), cardiac failure/cardiac event (n=2) or unknown (n=3). In the placebo arm, the main cause of death was disease progression (n=5), followed by cardiac arrest (n=1) and sepsis (n=1).

Short TFI subset <6 months

In the short TFI subset <6 months, 3 patients discontinued study treatment for death, all 3 deaths were reported in the NGR-hTNF arm and none with placebo. The causes were cardiac failure (n=1) and disease progression (n=2).

Laboratory findings

No clinically significant modifications related to NGR-hTNF were reported in the laboratory examinations. No clinically significant modifications of vital signs or physical findings related to NGR-hTNF were detected in the NGR015 study, except for two events of electrocardiogram QT prolongation. One event of QT prolongation was considered related and the second considered unrelated to NGR-hTNF. The outcome of the unrelated AE was unknown, while the related AE resolved.

Safety in special populations

No safety data in special populations has been provided. Most importantly, while the median age of the pivotal study population was 66, and 9-13% of the study population was over 75 years of age, no safety data separated by age have been provided.

Safety of NGR-hTNF in patients with renal or hepatic impairment has not been established.

No clinical data on exposed pregnancies and breast feeding are available. The safety of NGR-hTNF use during pregnancy or breast feeding has not been demonstrated.

Immunological events

According to the applicant, no immunological events, including the formation of antibodies directed to NGR-hTNF occurred in any patient treated. No safety data regarding immunological events, or details of anti-drug antibody measurement in clinical trials have been discussed.

Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies have been performed.

Discontinuation due to AES

In the total safety population, study treatment discontinuation due to AEs had been reported for 31 patients in the NGR-hTNF arm (16%) and 24 patients (12%) in the placebo arm. The most frequent AEs leading to treatment discontinuation were neutropenia (n=5, 16% NGR-hTNF vs. n=3, 13% placebo), condition aggravated (n=3, 10% vs. n=6, 25%), and disease progression (n=3, 10% vs. n=3, 12%). AEs leading to treatment discontinuation that occurred ≥5% more frequent in the NGR-hTNF arm were dyspnoea (n=2, 6% vs.0%) and respiratory failure (n=2, 6% vs. 0%).

Short TFI subset <6 months

In the safety population with a TFI shorter than 6 months, study treatment discontinuation due to AEs had been reported for 19 patients in the NGR-hTNF arm (17%) and 13 patients (11%) in the placebo arm. The most frequent AEs leading to treatment discontinuation in the short TFI subset were neutropenia (n=2, 11% NGR-hTNF vs. n=1, 8% placebo) and disease progression (n=3, 16% vs. n=3, 23%) (Table 19). All other AEs that led to discontinuation occurred in one patient each.

Table 19. Summary of AEs leading to treatment discontinuation in the subset of patients with TFI shorter than 6 months (n=225), classified by arm and BIC

Primary SOC – PT		BIC	NGR-hTNF (n=19)		Placebo (n=13)	
			No.	%	No.	%
Blood and lymphatic system disorders						
	Neutropenia	Vinorelbine	2	11	1	8
Cardiac disorders						
	Cardiac arrest	Vinorelbine	-	-	1	8
	Cardiac disorder	Vinorelbine	1	5	-	-
	Cardiac failure	None	1	5	-	-
	Pericardial effusion	Gemcitabine	1	5	-	-
General disorders and administration site conditions						
	Condition aggravated	Gemcitabine	1	5	2	15
		Vinorelbine	1	5	1	8
	Disease progression	Gemcitabine	2	11	2	15
		Vinorelbine	1	5	1	8
	Fatigue	Gemcitabine	-	-	1	8
		Vinorelbine	1	5	-	-
	Malaise	Vinorelbine	-	-	1	8
	Pain	Gemcitabine	1	5	-	-
Immune system disorders						
	Anaphylactic reaction	Vinorelbine	1	5	-	-
Infections and infestations						
	Respiratory tract infection	Gemcitabine	1	5	-	-
	Sepsis	Gemcitabine	1	5	-	-
Injury, poisoning and procedural complications						
	Hip fracture	Vinorelbine	1	5	-	-
Metabolism and nutrition disorders						
	Hypomagnesaemia	Gemcitabine	-	-	1	8
Nervous system disorders						
	Dizziness	Vinorelbine	-	-	1	8
	Simple partial seizures	None	1	5	-	-
Respiratory, thoracic and mediastinal disorders						
	Dyspnoea	None	1	5	-	-
	Pleural effusion	Vinorelbine	-	-	1	8
	Respiratory failure	Gemcitabine	1	5	-	-

3.3.9. Discussion on clinical safety

Study design

Main clinical safety data is derived from the pivotal randomized controlled Phase 3 study NGR015 investigating NGR-hTNF + best investigators choice (BIC) of chemotherapy versus placebo + BIC. In total 386 patients were randomized and received study treatment. Most safety data of study NGR015 was presented for the total safety population and two subgroups:

- The total safety population (n=193 in the NGR-hTNF arm vs. n=193 in the placebo arm).
- Patients with a treatment free interval (TFI) shorter than 4.8 months (n=87 vs. n=102).

Patients with a TFI shorter than 6 months (n=110 vs. n=115), this subset will be primarily discussed below as it represents the intended target population.

Baseline characteristics for the whole safety population and short TFI subsets were generally comparable between the NGR-hTNF and placebo arm. Most patients were male, approximately 66 years of age, and ~64% of patients had an ECOG performance score of 1. All patients received prior pemetrexed + cisplatin/carboplatin. These characteristics are representative for the intended target population.

Only adverse events with a clear relation with study medication were classified as treatment related events. Usually, also adverse events with a possible relationship with treatment are considered treatment related events.

Treatment exposure

In both the NGR-hTNF and placebo arm, most patients received gemcitabine (~52%) or vinorelbine (~40%) as best investigators choice (BIC) during the study, and only a few patients received doxorubicin (3%). The applicant should justify why the combination of NGR-hTNF with doxorubicin could be considered safe as only 3% of patients in the pivotal trial actually received this combination.

The median duration of NGR-hTNF or matching placebo treatment (scheduled weekly) was 10 infusions in both treatment groups. The median duration of BIC chemotherapy (scheduled every three weeks) was 4 cycles in the NGR-hTNF group and 3 cycles in the placebo group. It is reassuring that in both the total safety population, exposure to study drug as well as chemotherapy was similar in both treatment arms. A summary of treatment exposure in the short TFI subset <6 months was missing, and should be provided by the applicant, as this study population represents the intended target population.

Adverse events

Interpretation of safety data is hampered since limited summarizing tables were provided. From the data available, it seems as if most adverse events (AEs) occurred with similar frequencies between treatment arms. AEs of any grade reported with ≥5% higher frequency in the NGR-hTNF+BIC arm compared to placebo+BIC in the short TFI subset <6 months were: chills (50% vs.10%), dyspnoea (37% vs.29%), pyrexia (29% vs.23%), and leukopenia (12% vs.5%).

Grade 3 AEs were reported with a higher frequency in the NGR-hTNF arm compared to placebo (52% vs.41% in patients with a TFI shorter than 6 months). It is reassuring that apart from chills (5% vs.0%), no grade 3 AE occurred with ≥3% higher frequency in the NGR-hTNF arm compared to placebo. Grade 4 AEs occurred with almost similar frequency between treatment arms.

The number of SAEs in both arms of the short TFI subset <6 months is slightly higher in the NGR-hTNF arm compared to placebo (47 vs.39). Most SAEs in both arms were related to disease progression (6

events NGR-hTNF vs.5 events placebo, in the short TFI subset <6 months) or dyspnoea (6 events vs.1 event). Most other SAEs occurred in ≤3 patients.

A higher number of deaths was reported in the NGR-hTNF arm (n=12) compared to the placebo arm (n=7). These were not considered related to study treatment by the investigator. Nevertheless, it is questioned whether the deaths caused by respiratory events (n=3) in the NGR-hTNF arm, could be attributed to a hypersensitivity reaction related to the infusion. Furthermore, it is questioned what type of cardiac events were reported that caused two deaths.

Before final conclusions can be drawn regarding the safety of NGR-hTNF the following missing data should be submitted for all three safety populations:

- Percentages of treatment related AEs (all grade, grade 3-4, SAEs), and duration of AEs. From the study protocol, it seems as if only *clearly* related events were considered related to study treatment. The applicant is requested to provide the definition of a treatment related AE, and discuss whether *possible* related AEs were included in frequencies of "related AEs" as well. Otherwise, underreporting of events is likely to occur.
- A summarizing table according to the System Organ Class comparing frequencies of total and related SAEs in both treatment arms, for the overall patient population and the subgroup of interest. Apart from the total frequency, the number and frequency of all separate SAEs should be listed, categorized by system organ class (SOC) instead of individual patients. The use of MedDRA for the classification of adverse events is recommended.
- Summarizing tables according to SOC showing all grade AEs with ≥5% higher frequency and grade 3-4 AEs with ≥2% higher frequency in the NGR-hTNF arm compared to placebo (instead of AEs that occurred in more than 10% of patients in either treatment arm).
- A summarizing table with safety data per chemotherapy group, to facilitate comparison of safety data for the different NGR-hTNF-chemotherapy combinations. Possible differences in the type of AEs between the different chemotherapy agents should be discussed.
- Narratives of deaths, thoroughly describing the type of events to facilitate evaluation of a potential causal relationship between study treatment and death.
- Safety data of the Vinorelbine sub-study within the pivotal trial, and discussion regarding the selection of the lowest Vinorelbine dose. The applicant is requested to discuss why no recommendation regarding Vinorelbine dose has been included in the proposed SmPC, even though the amended study protocol advised the lowest Vinorelbine dose.

Immunogenicity

It is of concern that the immunogenicity of NGR-hTNF is insufficiently characterised. Anti-drug antibody (ADA) formation has not been investigated with weekly dosing, which is the proposed dosing frequency of Zafiride. However, a high frequency of immunological events has been observed in the weekly dosed pivotal trial. A higher frequency of chills (50% vs. 10%), dyspnoea (37% vs.29%), pyrexia (29% vs.23%) and treatment related anaphylactic reactions and hypersensitivity (together 4.7% vs.1%) has been observed with NGR-hTNF treatment compared to placebo. Moreover, dyspnoea and hypoxia were dose limiting adverse events in the dose finding studies. These observations raise additional concerns regarding the immunogenicity of weekly administrating Zafiride.

Adverse events of special interest

Infusion related reactions and allergic reactions have not been discussed as adverse event of special interest. Apart from chills, other AEs could be considered IRRs. It is, for example, questioned whether the higher frequency of dyspnoea (37% vs. 29%) and pyrexia (29% vs. 23%) AEs, and perhaps even the 3 deaths related to respiratory events could be considered immunological AEs. Note, that hypoxia and dyspnoea were the dose limiting toxicities in the phase I study. The applicant should discuss infusion related reactions as well as anaphylactic reactions as AE of special interest, The frequency of immunological AEs (overall and separated per AE) should be provided, as well as a discussion regarding grade, time to resolution, reappearance upon rechallenge, frequency with weekly dosing compared to dosing every three weeks (Phase I-II studies), and influence of infusion rate or additional measures taken to prevent recurrence.

No other adverse events of special interest have been discussed either. Cardiovascular toxicities are described for vascular targeting agents (including acute coronary and thrombophlebitic syndromes, alterations in blood pressure, heart rate, and ventricular conduction). According to the applicant, these events were not observed in Phase I studies, likely because of targeted delivery of the compound. Therefore the applicant decided not to discuss cardiovascular events as AE of special interest. However, the total patient exposure in the phase I-II studies is too limited to be conclusive and additional monitoring of these events would be mandatory for the safety of the included study patients in the pivotal trial. Indeed, several cardiotoxic adverse reactions (tachycardia, atrial fibrillation, hypo-/hypertension, cyanosis, QT prolongation) have been listed in table 4.8 of the SmPC, and were apparently considered treatment related. The applicant is asked to discuss cardiovascular toxicities as AE of special interest, including frequencies of the separate cardiovascular AEs.

More information regarding the two events of QT prolongation is requested. Case narratives should be provided, as well as detailed information regarding the measured ECG abnormalities including actual length of the QTc interval and the administered chemotherapy.

Special populations

No safety information separated by age has been provided, while the median age of the pivotal study population was 66, and 9-13% of the study population was >75 years of age.

A dose recommendation (or statement that this has not been investigated) for the elderly, and patients with renal or hepatic impairment should be included in section 4.2 of the SmPC. The warning regarding hepatic impairment in section 4.4 of the SmPC (“The safety and efficacy of Zafiride has not been studied in patients with moderate (Child Pugh B) or severe (Child Pugh C) hepatic impairment.”) could, therefore, be moved to a hepatic impairment paragraph in section 4.2.

Supportive data

The applicant is requested to provide safety data of supportive Phase II studies. Cumulative frequencies of AEs (all grades, grade 3-4 AEs, SAEs) should be provided, and the applicant should discuss whether the safety profile of weekly NGR-hTNF dosing was comparable to triweekly dosing.

3.3.10. Conclusions on clinical safety

Very limited safety data is provided making it difficult to draw final conclusions. The submitted data indicate that most adverse events occurred with similar frequencies between treatment arms. The addition of NGR-hTNF did not appear to exacerbate the known toxicities associated with the chemotherapy agents (hematologic AEs, fatigue, nausea and vomiting). However, a higher frequency of chills, pyrexia, anaphylaxis and cardiovascular events such as QT prolongation have been observed

with NGR-hTNF treatment compared to placebo. A discussion regarding infusion related reactions as well as anaphylactic reactions and cardiac toxicity as adverse events of special interest is needed. Moreover, since no ADA formation has been measured for the proposed weekly dosing regimen, reassurance should be provided regarding the immunogenicity of weekly NGR-hTNF.

3.4. Risk management plan

Safety concerns

Potential for medication errors

Due to usage of a syringe driver other than specified in the protocol and misinterpretation of handling rules 13 patients received insufficient treatment of NGR-hTNF or placebo. This incident happened at one site in UK and has been reported to ethics committee and MHRA as a serious breach report. NGR-hTNF will be available as prescription and its use is limited to hospital settings only, which reduces the potential for medication errors. However, multiple medication errors have occurred during the pivotal study. It is reassuring that this occurred at one site only, and seems to have been caused by not following the protocol correctly. Nevertheless, the applicant is asked to describe what preventive measures have been taken to prevent similar medication errors in clinical practice.

Summary of safety concerns

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

Summary of safety concerns	
Important identified risks	• Infusion related reaction (chills, fatigue, pyrexia) • Allergic reaction
Important potential risks	• Immunological events (antibody formation)
Missing information	• Use in paediatric patients • Use in pregnant women • Use in breast feeding women • Use in patients with renal impairment • Use in patients with hepatic impairment

Discussion on safety specification

The provided safety information was very limited and therefore the assessment of the safety specifications is premature. Additional safety information is requested, including a thorough analysis of adverse events of special interest. Based on the assessment of the available clinical PK and safety data, the applicant's proposal for the safety specification is not fully supported.

Comments on topics already included in the summary of safety concerns

- *Allergic reaction* as important identified risk: based on the outcome of the requested review in the clinical part of the dossier, this term could be amended to "*allergic reactions, including anaphylaxis*".

- *Use in pregnant women*, as missing information: there are no adequate data from the use of Zafiride in pregnant woman. Studies in animals have shown reproductive toxicity. Therefore, use during pregnancy should be upgraded to an important potential risk.

- *Immunological events (antibody formation)* as important potential risk: in the clinical part of the dossier additional safety information regarding immunogenicity and possibly antibody formation has been requested. Based on the assessment of additional data, further studies to further characterise this risk might be needed.

New topics that should be added to the summary of safety concerns

- *QT prolongation,* as important identified risk: two events of QT prolongation have been observed in the pivotal Phase III trial, including one event that was considered related to study treatment by the investigator. Therefore, a safety concern regarding the potential risk for QT prolongation should be added as important identified risk. Based on the outcome of the request for additional information, a thorough QT study could be needed.

- *Cardiac toxicity*, as identified/ potential important risk: cardiovascular toxicities are described for vascular targeting agents (including acute coronary and thrombophlebitic syndromes, alterations in blood pressure, heart rate, and ventricular conduction). According to the applicant, they did not appear in Phase I study, likely because of targeted delivery of the compound. Nevertheless, cardiac events have been observed in the Phase III study, and a potential risk for cardiovascular toxicities could not be excluded either. Moreover, some cardiovascular adverse events are listed in section 4.8 of the proposed SmPC. Depending on the clinical review of this topic, the applicant should discuss the need of inclusion of cardiac toxicity in the table with safety specifications as an important identified or potential risk.

- *Patients with cardiac impairment* and *immune compromised patients* should be added as missing information.

- *Hepatotoxicity, as important potential risk: s*ection 4.8 of the SmPC reports liver enzymes increased as 'common' and cases of hepatotoxicity have seemingly been observed in clinical and preclinical studies.

Conclusions on the safety specification

Having considered the data in the safety specification the CHMP considers that the following issues should be addressed:

- *Allergic reaction*: based on the outcome of the requested review in the clinical part of the dossier, this term could be amended to "*allergic reactions, including anaphylaxis*".
- *Use in pregnant women* should be upgraded to an important potential risk
- *Immunological events (antibody formation):* based on the assessment of additional data, further studies to characterise this risk might be needed.
- *QT prolongation* should be added as important identified risk

- The applicant should discuss the need of inclusion of *cardiac toxicity* as an important identified or potential risk.
- *Patients with cardiac impairment* and *immune compromised patients* should be added as missing information.
- *Hepatotoxicity,* should be added as important potential risk

Pharmacovigilance plan

As a part of the conditional marketing authorization, in order to obtain comprehensive efficacy and safety data, MolMed is fully committed to perform an additional confirmatory phase 3 study in mesothelioma patients to be defined during the marketing authorization process.

Having missed the primary endpoint in trial NGR015, MolMed plans to initiate an additional confirmatory phase 3 study to confirm safety and efficacy in mesothelioma patients.

Populations not studied, including pregnant women, breastfeeding women and patients with severe concomitant diseases do not constitute an efficacy issue for NGR-hTNF, but rather present certain safety issues which are discussed elsewhere.

Summary of planned additional PhV activities from RMP

On-going and planned studies in the post-authorisation pharmacovigilance development plan				
Study / activity type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status (planned or started)**	**Date for submission of interim or final reports (planned or actual)**
Confirmatory phase 3 trial (3)	Assess safety in adult patients	Infusion related reactions (chills, fatigue, pyrexia) Allergic reaction	Planned	

Overall conclusions on the PhV Plan

The proposed post-authorisation PhV development plan is not considered sufficient to identify and characterise the risks of the product and the applicant should provide further information on the clinical trial proposed as a PhV study. More specifically, the applicant is requested to provide details with regards to the estimated number of patients to be enrolled in the above study, endpoints and the list of countries where the study will be conducted.

The summary of clinical safety (module 2.7 of the application dossier) reports 3.1% events (6/193) of grade 3 'Gamma-glutamyltransferase increased' [severe or medically significant] and 1.6% (3/193) of grade 3 events of 'transaminase increased' which are also listed as 'common' in section 4.8 of the SmPC. Moreover, preclinical studies also states that "*Histological examination showed, in the liver of treated animals, a non-dose dependent bile duct proliferation and/or peribiliar mononuclear inflammation, associated with peribiliar fibrosis and/or centrilobular or even nodular multifocal*

hepatocellular fibrosis. These findings were still present in animals allowed the four-week recovery period". 'Transaminases increased' is also listed as 'common' in section 4.8 of the proposed SmPC.

Based on the above, it is considered that liver function should be monitored in the proposed study. The pharmacovigilance activities for this safety concern should include causality assessment of any events of hepatotoxicity and discussion of the outcome of any eventual de-challenge/re-challenge.

The pharmacovigilance plan should also monitor the important potential risk of 'immunological events (antibody formation)'. For every subject enrolled in the study should be included laboratory data, causality assessment, eventual de-challenge/re-challenge and outcome.

Risk minimisation measures

The proposal from the applicant for risk minimisation measures is detailed below:

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Infusion related reaction (chills, fatigue, pyrexia)	Listed as undesirable effects in section 4.4 and 4.8 of the SmPC and section 2 and 4 of the PIL To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None
Allergic reaction	Listed as undesirable effects in section 4.3 and 4.8 of the SmPC and section 2 and 4 of the PIL To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None
Immunological events (antibody formation)	To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None
Use in paediatric patients	A warning is placed in section 4.2 of the SmPC and section 2 of the PIL: To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None
Use in pregnant women	Use of NGR-hTNF is contraindicated in pregnant women, SmPC 4.3. A warning is placed in section 4.6 of the SmPC and section 2 of the PIL: To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None
Use in breast feeding women	Use of NGR-hTNF is contraindicated in breast feeding women, SmPC 4.3. A warning is placed in section 4.6 of the SmPC and section 2 of the PIL: To be used in hospital under the supervision of health care providers experienced in the treatment of cancer patients.	None

Overall conclusions on the risk minimisation measures

Based on the data submitted, the proposed risk minimisation measures are considered insufficient to minimise the risks of the product in the proposed indication(s). More specifically, the applicant is requested to submit an updated table addressing the 'list of questions to be addressed by the applicant'.

In particular, the applicant has included infusion-related reactions as an important identified risk in the summary of safety concerns and proposed routine risk minimization measures based on the inclusion of this safety issue in section 4.4 and 4.8 of the SmPC. The applicant should therefore discuss whether the proposed recommendations for administration are optimal for this risk and whether any reduction in infusion rate might be considered as measure to be added to the safety concern above in order to minimize the risk posed by this safety issue.

Also, section 4.8 of the SmPC reports liver enzymes increased as 'common' and cases of hepatotoxicity have seemingly been observed in clinical and preclinical studies. The applicant should discuss an appropriate risk minimisation measure for the risk of hepatotoxicity (e.g. routine monitoring of liver function test in every patient).

Conclusion

The CHMP and PRAC considered that the risk management plan could be acceptable if the applicant implements the changes to the RMP as detailed in the endorsed rapporteur assessment report.

3.5. Pharmacovigilance system

The CHMP considers that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

4. Orphan medicinal products

Orphan designation

Malignant pleural mesothelioma (MPM) is a rare and incurable disease. In Europe, about 1.6 per 100,000 inhabitants corresponding to 8,000 new diagnoses annually. Based on a 5-year survival of 5%, the complete prevalence is 1.9 per 100,000 corresponding to 10,000 prevalent cases [Gatta, 2001]; thereby fulfilling the criteria for an orphan disease

NGR-hTNF is human tumour necrosis factor coupled with CNGRC peptide intended for the treatment of malignant pleural mesothelioma. NGR-hTNF was designated as an Orphan Medicinal Product in the European Union on 03 June 2008 - EU/3/08/549.

Similarity

The application did not contain critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products. This is accepted as currently no approved products exist in the acclaimed indication.

Derogation(s) from market exclusivity

Not applicable as currently no approved products exist in the acclaimed indication.

5. Benefit risk assessment

5.1. Therapeutic Context

5.1.1. Disease or condition

The proposed target indication is for *the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy.*

The proposed posology is 0.8 micrograms/m^2 every week as long as clinical benefit is observed or until unacceptable toxicity occurs.

The aim of this new treatment is to prolong overall survival and progression free survival.

5.1.2. Available therapies and unmet medical need

Gemcitabine, vinorelbine or retreatment with pemetrexed are recommended by the ATS/ERS guideline in the treatment of second line malignant pleural mesothelioma (MPM). These treatments can be considered as well established, although none of these therapies are approved for the treatment of second line MPM.

Patients with recurrent MPM after first line treatment with pemetrexed based therapy, have a median survival of about 6-7 months if left untreated. The overall survival might be prolonged to 8.4 months with second line chemotherapy.

5.1.3. Main clinical study

The main evidence to support this application is obtained from a subgroup from a single pivotal, phase III, multinational, randomised, stratified, placebo controlled, double blinded study comparing NGR-hTNF + Best investigators choice with Placebo + Best Investigator's choice (BIC) in 400 previously pemetrexed treated patients with advanced malignant pleural mesothelioma with radiologically documented progressive disease.

Best investigator's choice (BIC) included either supportive care alone or combined with a single-agent chemotherapeutic agent (gemcitabine, vinorelbine or doxorubicin).

The study failed to meet its primary endpoint OS after 75% of events have been analysed (median OS 8.5 months (95% CI 7.2-9.9) vs 8.0 months (95% CI 6.6-8.9), respectively; HR 0.97 (p=0.79)). The current application is based on the results of the data driven, identified subject population of interest, the patients (n=236) with a treatment free interval (TFI) of <6 months after first line or maintenance therapy. The TFI is defined by the time between the last treatment and initiation of the second line treatment.

5.2. Favourable effects

- The post hoc identified subgroup of interest, i.e. patients with a short treatment free interval (TFI) <6 months (n=236), showed an improvement in median overall survival (mOS) of 1.6 months with NGR-hTNF compared with placebo (mOS NGR-TNF 8.0 (95% CI 6.3-10months), placebo 6.4 (95% CI 5.7-7.6) months, stratified HR 0.70 (95% CI 0.52-0.95, p=0.02).
- The observed improvement in mOS is supported with an improved median progression free survival (mPFS) of 1 month (mPFS NGR-hTNF 3.0 months [95% 2.3-4.1] vs. placebo 2.0 months [.5-3.0], stratified HR 0.75 [05% 0.57-0.99] p=0.04).
- Subgroup analyses also showed a favourable effect of the covariates of interest, including ECOG score.

5.3. Uncertainties and limitations about favourable effects

Quality

Manufacturing process and quality of clinical batches

Three processes have been developed: processes A, B and C. Process A batches were used for non-clinical and phase I and II clinical trials. The process as well as the quality of the batches differ between Process A and B. Process B batches have been used for Phase III trials. Process C is the proposed commercial drug substance (DS) manufacturing process, there is no clinical experience with process C batches. Process C is as yet not validated and not well-controlled. Characterisation data are very limited. Consistency, comparability and stability cannot be assessed. No drug product (DP) batches according to commercial process have yet been manufactured. Therefore, comparability with clinical batches has not been demonstrated.

Definition of the drug substance, establishment of release specifications and assessment of potency

The drug substance is described as consisting of four forms, the “native form”, which is the intact molecule, and three “isoforms”. All three isoforms have changes in the N-terminal 6 amino acid moiety, which contains the NGR sequence which is pivotal for the claimed specific binding to CD13. No information was provided whether the three isoforms have the same action as the native compound and the same is the case for deamidated forms which seem to be included in the release specification for isoforms. The isoforms and deamidated forms could be expected not to bind to CD13 (or with different affinity/specificity) with the TNF domain still retaining its cytotoxicity. As the potency is measured with an *in-vitro* assay, which measures the cytotoxicity of the TNF domain, not the functionality of the NGR domain, the potency test will not detect possible differences in functionality of the isoforms/deamidated forms vs. the native form. With the specifications as proposed at present, even the whole material could exist of isoforms and deamidated forms, which might lack the CD13 selectivity, but retain normal cytotoxicity. This represents potential risks for both efficacy (decreased or absent selectivity) and safety (increased toxicity).

Pharmacokinetics

Overall, due to the lack of key information on the analysis of the samples for NGR-hTNF in the various clinical studies, the reliability of the NGR-hTNF pharmacokinetic data reported in this assessment report cannot be scrutinized. Therefore, at this stage, the pharmacokinetic data cannot be used for support of the current application, and cannot be used e.g., for basic description of the pharmacokinetics of NGR-hTNF, nor as support for extrapolation of the clinical data obtained in the

clinical studies to other patient populations. In addition, no pharmacokinetic data were provided for the to be marketed formulation C, which appears markedly different, e.g., with respect to the relative amount of isoforms present in the formulation, from the process A formulation used in the pharmacokinetic studies.

Pharmacodynamic effects

The proposed posology is insufficiently supported by the data of the phase I-II studies, because:

a. the pharmacodynamic results to support the application (Ktrans of the Dynamic contrast enhanced (DCE) -MRI, tumour responses, soluble-TNF-RI and TNF-RII receptors) have only been obtained in patients administered with a 3-weekly dosing schedule. Although it can be agreed that the effects after the first dose are similar, it needs to be justified, how the results obtained in a tri-weekly schedule of NGR-TNF can be inferred to a weekly schedule.

b. the phase II study NGR010 shows no differences in the disease control rate and response rate between the tri-weekly and weekly dose of NGR-hTNF 0.8 µg/m^2 administration in patients with relapsed malignant pleural mesothelioma. The provided mPFS and OS data is incomplete, and the patient population very small (n=14), precluding a definitive conclusion.

The proposed mode of action is a targeted treatment by NGR-hTNF. TNF alfa is believed to be an anti-angiogenic effect. However, this is clinically insufficiently supported and no clinical biomarkers have been identified to support the claim that the effect of the drug relies on a anti-angiogenic effect. These aspects need clarification.

The response rate might provide evidence of an anti-tumour effect, because spontaneous shrinkage of tumour is unlikely to occur. However, NGR-hTNF showed a very low response rate of 0-3% that is comparable to placebo (3%) and the response rates did not differ between ITT population and the TFI < 6 months.

Pivotal study

After the study had failed to meet the primary endpoint, a post-hoc subgroup was defined based on the statistically significant interaction between TFI and OS. Several major methodological and clinical concerns were raised regarding the selection of the subgroup based on TFI as discussed in the clinical efficacy section (see also Figure 6). Importantly, the TFI and PFS appear to be different entities in this application, while reference to progression is made in the applied for indication. TFI might be affected by logistic aspects of MPM treatment and as a consequence affect the primary and secondary outcome measure. Moreover,

a. The clinical relevance of the primary endpoint of TFI is less well known.

b. The subgroup of interest, those with a TFI <6 months, is based on a post-hoc, data driven analysis.

c. The method of measuring the TFI differed largely between individual patients and resulted in sub-subgroups of patients based on treatment stage, being first line treatment only, maintenance therapy and rechallenge with pemetrexed.

d. The time between randomization and treatment is unknown and no comparison was made between NGR-hTNF and placebo.

e. As an overlap in time exists between the TFI and the PFS and OS measurements, the PFS and OS might be affected depending on how the TFI was determined in each patient.

f. The identified subgroup with a TFI <6 months does not correspond with the intended target population.

g. The patients in the TFI <6 months subgroup who did not receive treatment to the day of randomisation were excluded in the OS analyses, but this is not an analyses resembling ITT. Sensitivity analyses for OS that were conducted according to the ITT principle showed a smaller effect in terms of HR (HR 0.74 (95% CI 0.55-0.99) p=0.04), but the median OS for NGR-hTNF and placebo were not provided hampering the interpretation of the result.

h. The interpretation of the PFS, Disease control rate (DCR) and Response Rate (RR) results is hindered by the large number of patients of whom post-treatment assessments of disease was not available. It is not clear how of many patients data are missing, as there are also inconsistencies in the reported number of patients that did not receive a post-baseline tumour assessment, in both the ITT and in the TFI <6 months group.

Due to a lack of information on prognostic factors at baseline, it is not known whether the subgroups TFI <6 months and the complementary group with TFI ≥6 months are different. The primary and secondary outcome measures for the long TFI ≥ 6 months are also lacking.

It can also not be excluded that the observed improvement in OS for NGR-hTNF in the subgroup of TFI< 6 months is driven by an imbalance in prognostic factors. The subgroup of TFI <6 months needs also be further characterised by the patients flow and to be compared with the TFI >6 months patients.

The external validity to support the observed benefits is very limited as this application is the first application of NGR-hTNF for an indication without approved therapy. Some external evidence for the applied subgroup could be provided by showing the replication of the observed effect in OS and PFS in the small cohort (n=14) of the phase II study. However, the cohort is too small and the provided data is incomplete precluding a definitive conclusion.

The pivotal study showed no differences in overall survival between NGR-hTNF and placebo. The baseline characteristic TFI is a continuous baseline characteristic, dichotomised at 6 months to define a subgroup that showed an improvement in OS. This indicates that in the complementary subgroup a detrimental effect will be shown. For patients with a TFI near the cut-off value, it will be hard to determine the expected benefit. OS was reduced by over a month when expanding the cut-off from 4.8 months (median) to 6 months (due to reductions of the median OS in the NGR-hTNF arm only). An exact cut-off is therefore not well established.

The trial did not request histological confirmation of the MPM diagnosisby an independent expert panel.

The included patient population can be regarded as heterogeneous, due to e.g. differences is standard of care and applied staging systems which hampers interpretation of subgroup results.

The background treatment included best supportive care with or without additional single dose chemotherapy. There is uncertainty on the suitability of the choice of the type of chemotherapy. The used posologies and treatment cycles need to be substantiated.

The data presentation is not transparent. The tables and figures do not contain a reference to the printouts of the calculations or listings. The printouts of these calculations are not provided for the phase I-II studies. Importantly, differences exist in the presentation of the baseline characteristics and outcomes presented in the clinical overview version 0.0 and 2.0.This implies that changes have been made in the database after the initial presentation and this needs clarification.

Also, other uncertainties have been raised regarding the adherence to GCP and the study protocol during the conduct of the studies and the adherence to the protocol (see section 2.4).

5.4. Unfavourable effects

The total safety population consisted of 386 patients of the pivotal Phase 3 trial NGR015, that were randomized and received NGR-hTNF + BIC (n=193), or placebo + BIC (n=193). The main focus here will be on the subgroup of patients with a TFI shorter than 6 months (n=110 NGR-hTNF vs. n=115 placebo), which represents the intended target population.

Short TFI subset <6 months

TEAEs were observed in >96 % of patients in both arms of the pivotal trial. Study emergent AEs of any grade reported with ≥5% higher frequency in the NGR-hTNF+BIC arm compared to placebo+BIC were: chills (50% vs 10%), dyspnoea (37% vs 29%), pyrexia (29% vs 23%), and leukopenia (12% vs 5%).

Grade 3 AEs were reported with a higher frequency in the NGR-hTNF arm compared to placebo (52% vs. 41%). Grade 4 AEs were reported for approximately 10% in both treatment arms. Grade 3 to 4 study emergent AEs reported with ≥2% higher frequency in the NGR-hTNF arm compared to placebo were chills (5% NGR-hTNF vs. 0% placebo), dyspnoea (6% vs. 3%), neutropenia (16% vs. 13%) and leukopenia (5% vs, 3%).

The number of SAEs occurred was higher in the NGR-hTNF arm compared to placebo: 47 vs. 39. Most events in both arms were related to disease progression (~5 events with NGR-hTNF) or dyspnoea (~6 events).

Deaths were reported more frequently in the NGR-HTNF arm (n=12) compared to placebo (n=7) of the total study population. The main cause of death in the NGR-hTNF arm was disease progression (n=4), followed by respiratory failure/insufficiency or dyspnoea (n=3), cardiac failure/cardiac event (n=2) or unknown (n=3). None were considered related to study treatment. In the short TFI subset <6 months: 3 deaths were reported in the NGR-hTNF arm compared to none in the placebo arm. The causes were cardiac failure (n=1) and disease progression (n=2).

Two events of QT prolongation were reported, of which one was considered treatment related.

Study treatment discontinuation had been reported for a slightly higher percentage of patients in the NGR-hTNF arm (17%) compared to placebo (11%) of the short TFI subset <6 months. The most frequent AEs leading to treatment discontinuation in the short TFI subset were neutropenia (n=2: 11% NGR-hTNF vs. n=1: 8% placebo) and disease progression (n=3: 16% vs. n=3: 23%).

5.5. Uncertainties and limitations about unfavourable effects

Interpretation of safety data is severely hampered since only very limited safety data has been presented. Summarizing tables of SAEs, treatment related AEs and (S)AEs per chemotherapy group were not included. Furthermore, detailed information on AEs of special interest, the vinorelbine sub-study, causes of death, and safety data in the elderly population as well as in supportive Phase I-II trials is missing.

Anti-drug antibody formation has not been investigated with the proposed weekly dosing regimen. Anti-drug antibody (ADA) formation has been investigated in Phase I-II clinical studies with dosing every three weeks, and the Applicant states that no ADA positive samples have been detected. However, method validation and bioanalytical reports of the immunogenicity assessment were not submitted, preventing interpretation of the observed results.

Infusion related reactions and allergic reactions have not been discussed as adverse event of special interest. It is uncertain, whether apart from chills, other AEs were considered IRRs. Only limited safety data is provided in summarized tables, which hampers interpretation of the immunogenicity of NGR-hTNF.

No other adverse events of special interest have been discussed (or monitored?) either. Cardiovascular toxicities are described for vascular targeting agents. Several cardiotoxic adverse reactions (tachycardia, atrial fibrillation, hypo-/hypertension, cyanosis, QT prolongation) have been listed in table 4.8 of the SmPC, and were apparently considered treatment related.

The applicant should justify why the combination of NGR-hTNF with doxorubicin as best investigators choice (BIC) could be considered safe as only 3% of patients in the pivotal trial received this combination.

Effects Table

Table 20. Effects Table for Zafiride Zafiride is indicated for the treatment of adult patients with advanced malignant pleural mesothelioma who have progressed within six months after a first-line pemetrexed-based therapy. Data cut-off 29 April 2014.

The effect table applies to patients with a TFI <6 months, the subpopulation of interest.

Effect	Short Description	Unit	NGR-hTNF	Placebo	Uncertainties / Strength of evidence	References
Favourable Effects						
Overall survival	Time from randomisation to death, any cause	months	8.0 (95% CI 6.3-10.0)	6.4 95% CI 5.7-7.1)	The HR 0.70 (0.52-0.95). The effect is observed in a post-hoc defined data driven subgroup analyses after the pivotal study failed. The subgroup analyses is based on the baseline characteristic treatment free interval (TFI) This baseline characteristic Treatment free interval is affected by logistical procedures and analyses; it is not a distinct patient or disease characteristic. The PFS and OS might be affected depending on how the TFI was determined in each patient. The OS analyses censored the randomised patients who did not receive treatment at randomisation; these patients were not followed up till death or last known date to be alive. This analyses may overestimate the efficacy The TFI is a continuous variable dichotomised at 6 months. Therefore, it is difficult to determine the value of the benefit or detriment between the patient subgroups defined by a TFI cut of point of 6 months.	
Progression free survival	Time from date of randomization until disease progression or death due to any cause	Months	3.0 95% CI 2.3-4.1	2.0 95% CI 1.5-3.0	HR 0.77 (95% CI 0.59-0.99) p=0.04 An unidentified number of patients (n=77 or n=55) in the ITT population had no post baseline tumour assessment; this number is also unknown for the TFI < 6 months	

Effect	Short Description	Unit	NGR-hTNF	Placebo	Uncertainties / Strength of evidence	References
Disease control rate	The percentage of patients who have a best-response rating of complete response, partial response, or stable disease.	%	53	48	Odds ratio 1.23 (95% CI 0.74-2.01) Disease control rate includes CR+PR+SD and may overrate the drug activity. A more sensitive outcome measure to measure a direct anti-tumour effect would be the response rate (CR+PR). Also, an unidentified number post-baseline tumour assessments are missing (n=77 or n=55).	
Unfavourable Effects						
TEAEs	TEAEs that occurred with ≥5% higher frequency in the NGR-hTNF+BIC arm (TFI <6 months) Chills Dyspnoea Pyrexia Leukopenia	%	 50% 37% 29% 12%	 10% 29% 23% 5%	Interpretation of safety data is hampered by missing information, a.o. percentages of treatment related AEs (all grade, grade 3-4, SAEs), and duration of AEs is unknown. Data for patients with a TFI >6 months is missing. Uncertainties regarding immunogenicity of NGR-hTNF: ADA formation not measured for weekly dosing. Method validation and bioanalytical reports of the immunogenicity assessment with triweekly dosing were not submitted, hampering thorough evaluation of the observed results. High frequency of immunological events with NGR-hTNF compared to placebo. Furthermore, it is unknown whether cardiovascular toxicities were related to study treatment.	

Effect	Short Description	Unit	NGR-hTNF	Placebo	Uncertainties / Strength of evidence	References
Grade 3 AEs	The percentage of patients that experienced at least one grade 3 TEAE (TFI <6 months)	%	52%	41%	Interpretation of safety data hampered by missing data (see above).	
Deaths	The number of deaths at the time of the data cut off April 2014 in the total safety population (and TFI <6 months)	Number	12 (3)	7(0)	Case narratives too limited. Uncertain whether the deaths caused by respiratory events (n=3) in the NGR-hTNF arm, could be attributed to a hypersensitivity reaction related to the infusion. Furthermore, it needs to be specified which cardiac events were reported that caused two deaths.	
Discontinuation due to AEs	The percentage of patients that discontinued study treatment due to AEs.	%	17%	11%	Time to resolution of AEs leading to discontinuation unknown.	

Abbreviations: AE: adverse event, TEAEs: treatment emergent adverse events

5.6. Benefit-risk assessment and discussion

5.6.1. Importance of favourable and unfavourable effects

There is an unmet medical need in the setting of second line treatment in advanced MPM. The overall survival after first line pemetrexed treatment is limited to approximately 8.4 months [This study, Jassem, 2008]. Up till now, no second line treatment is approved for the treatment of MPM, although the use of vinorelbine, gemcitabine, or pemetrexed is recommended in guidelines [ATS/ERS, ESMO].

The proposed indication is second line treatment in patients who showed progression within six months after first line pemetrexed based therapy. This application is based on a post-hoc, data driven subgroup from a failed single pivotal study. From a formal statistical view, no further conclusion is possible, as the potential of selection bias is considerable.Even so, after careful assessment of the information, multiple major deficiencies have been identified in the quality, non-clinical and clinical parts of the dossier that undermine the validity of the obtained data and prohibit interpretation of the results. These have to be resolved as the current dossier cannot be considered of sufficient quality to support the application.

For the quality, non-clinical and clinical pharmacology aspects, these refer amongst others to insufficient characterisation of the DS, DP, potency assays, uncertainty on the comparability of the three production processes and insufficient data on pharmacokinetics and pharmacodynamics.

Some of the most important clinical concerns will be addressed here. First, the subgroup data do not support the applied for indication in various aspects. The proposed indication includes a post-hoc identified subgroup of MPM, patients that progressed within 6 months after first line pemetrexed based therapy. However, the data presented in the dossier concerned the subgroup defined by a Treatment Free Interval (TFI) of 4.8 months, with lesser data for the subgroup with a TFI <6 months. The data of the subgroup with a TFI <6 months most likely resembles the applied indication, although “Progression” is not synonymous with TFI. TFI is a less well established clinical entity compared to PFS. Moreover, TFI is also influenced by additional logistic or methodological aspects a.o. related to MPM treatment, while PFS is not. Therefore, caution should be used when interpreting the data from this post-hoc, data driven subgroup analysis.

Second, the method of measuring the start of the TFI differed between patients and resulted in identification of three subgroups, i.e. first line therapy only, those who received maintenance treatment and those who received rechallenge with pemetrexed, of which the number of patients involved within the population with a TFI <6 months is not known. The assessment of the end of the TFI seemed to be different between patients as well, due to a possible gap between randomization and start of study treatment. Moreover, the PFS and OS provided by NGR-hTNF might be affected depending on how the TFI was determined in each patient.

Third, it can also not be excluded that the effect of NGR-hTNF in the observed subgroup with TFI <6 months might be driven by an imbalance in prognostic favourable factors, because no comparison has been provided for the baseline characteristics between NGR-hTNF and placebo in this subgroup. As a consequence the improvement in OS of 1.6 months compared to placebo may be overestimated, also due to the various methodological constraints in the assessment of TFI.

Fourth, the small OS effect is supported by a small improvement in PFS, but the analysis of PFS is hampered by an unknown number of missing post-tumour assessments. No supporting data have been provided for the quality of life.

Regarding the proposed indication, the applicant applies for an indication as a single agent in the treatment of advanced malignant mesothelioma. However, as in the pivotal study at least >95% of patients received background chemotherapy with gemcitabine, vinorelbine or doxorubicin, the use of NGR-hTNF in conjunction with chemotherapy needs to be reflected in the indication.

As stated above, the subgroup of interest is data driven. The validity of the subgroup might be increased if the application was supported with biomarkers supporting the effects observed in the applied subgroup. However, no biomarkers have been provided. Moreover, there are insufficient pharmacodynamic (PD) data to support the proposed mode of action of NGR-hTNF, a targeted anti-angiogenetic effect, and the posology. The PD needs to be substantiated and clarified, particularly as for the applied weekly dosage no data were submitted.

Importantly, the anti-tumour effect of the product is uncertain because the pivotal trial showed a comparable response rates in the study arm both in the ITT population (n=3, 2% vs. n=6, 3%) and in the TFI <6 month cohort (n=2, 2% vs. n=3, 3%). The large number of non-assessable data makes comparisons with other endpoints like progressive disease or stable disease inconclusive. Therefore, the proposed (supportive) anti-tumour effect is (clinically) insufficiently proven, making a chance finding in the subgroup analyse more likely, particularly when obtained in such a heterogeneous population.

The risk of a chance finding would be diminished, if replication of the observed effect has been observed. However, currently, no treatments are approved for the applied second line treatment in MPM, limiting the replication of results. This application is the first application for NGR-hTNF. The external validity is limited to the replication of the observed effects in the small cohort (n=14) included phase II study. However, the cohort is too small and the provided supportive data is incomplete precluding a conclusion.

The submitted safety data indicate that most adverse events occurred with similar frequencies between treatment arms. The addition of NGR-hTNF did not appear to exacerbate known toxicities associated with the different chemotherapy agents. However, it is of major concern that the interpretation of safety data is currenty hampered due to the very limited availability of the safety data. Numerous additional analyses have to be provided before final conclusions can be drawn.

It is of concern that the immunogenicity of NGR-hTNF is insufficiently characterized. Method validation and bioanalytical reports were not submitted for ADA testing in Phase I-II studies, hampering evaluation of the observed results. Moreover, ADA formation has not been investigated with weekly dosing, which is the proposed dosing frequency of Zafiride. However, a high frequency of immunological events has been observed in the weekly dosed pivotal trial. Moreover, dyspnoea and hypoxia were dose limiting adverse events in the dose finding studies. These observations raise additional concerns regarding the immunogenicity of weekly administration of Zafiride.

No other adverse events of special interest have been discussed either, despite the proposed anti-angiogenic effect of the drug. Cardiovascular toxicities are described for vascular targeting agents. Indeed, several cardiotoxic adverse reactions (tachycardia, atrial fibrillation, hypo-/ hypertension, cyanosis, QT prolongation) have been listed in table 4.8 of the SmPC, and were apparently considered treatment related. The applicant is asked to discuss cardiovascular toxicities as AE of special interest, including frequencies of the separate cardiovascular AEs and a discussion regarding the risk of QT prolongation. The safety profile of NGR-hTNF treatment remains to be elucidated in the elderly.

Although the treatment appeared to be well tolerated, NGR-hTNF treatment was not associated with a symptomatic improvement, while, more importantly, the overall effect on the quality of life was not measured.

5.6.2. Balance of benefits and risks

Based on the current dossier, several major objections in the quality, non-clinical, clinical pharmacology and clinical section were raised leading to a negative benefit/risk. The dossier is deficient in multiple aspects, undermining the validity of the obtained quality and (pre)clinical data. The data has to be completed and the currently identified uncertainties have to be adequately addressed in order to allow for a proper B/R assessment in relation to the applied for indication being the treatment of second line malignant mesothelioma.

5.6.3. Additional considerations on the benefit-risk balance

Since a conclusion on the efficacy of treatment cannot be drawn, and safety assessment is limited, the benefit-risk is negative. As a result, it cannot be determined whether the unmet medical need in MPM can be fulfilled by NGR-hTNF. Therefore, the main requirement for a conditional approval has not been met. Also, the proposed clinical studies to provide the confirmatory data for the applied conditional approval for the proposed target population do not all match the therapeutic setting of the current pivotal trial. The planned study with a similar target population has not started yet. The recruitment of this study should have been finalized to support the conditional approval, because once Zafiride has obtained a conditional approval, the trial will be hard to conduct and ethical feasibility can be questioned.

The applicant applies for an indication as a single agent in the treatment of advanced malignant mesothelioma. However, the effect of NGR-hTNF has been investigated in conjunction with chemotherapy in the second line treatment of advanced or metastatic MPM. Therefore, the concomitant use of chemotherapy needs to be reflected in the indication.

5.7. Conclusions

The overall B/R of Zafiride is negative and the application is not approvable.