22 May 2025

EMA/CHMP/169210/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Nidlegy

International non-proprietary name: bifikafusp alfa / onfekafusp alfa

Procedure No. EMEA/H/C/005651/0000

Note

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

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List of abbreviations

ADA	Anti-Drug Antibody
AE	Adverse Event
AESI	Adverse Event of Special Interest
AJCC	American Joint Committee on Cancer
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AlphaLISA	Amplified luminescent proximity homogeneous assay
AST	Aspartate Aminotransferase
AUC	Area Under the concentration vs time Curve
AUC (0-t)	Area Under the Curve up to last quantifiable time-point
BICR	Blinded Independent Central Review
BMI	Body Mass Index
BRAF	Gene encoding B-Raf protein
CFU	Colony Forming Units
CI	Confidence interval
CIP	Cleaning In Place
CK	Creatine Kinase
CL	Clearance rate
Cmax	Peak serum concentration
CMV	Human cytomegalovirus
CoA	Certificate of Analysis
CPP	Control process parameter
CR	Complete Response
CTCAE	Common Terminology Criteria of Adverse Events
CTL	Cytotoxic T lymphocytes
DCR	Disease control rate
DELFIA	dissociation-enhanced lanthanide fluorescence immunoassay
DMFS	Distant Metastasis-Free Survival
DS	Drug Substance
ECACC	European Collection of Cell Cultures
ECG	Electrocardiogramm
EDB	Extradomain B of fibronectin
ELISA	Enzyme-linked immunosorbent assay
FDG PET-CT	Fluoro-deoxyglucose Positron Emission Tomography/Computed Tomography
FFPE	Formalin-Fixed Paraffin-Embedded

FU	Follow-up
HAFA	Human Anti-Fusion Antibodies
HR	Hazard ratio
HRP	Horseradish Peroxidase
ICI	Immune Checkpoint Inhibitor
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL2	Interleukin-2
IPC	In process Control
IRAE	Immune-Related Adverse Event
IU	International Unit
i.v.	Intravenous infusion
L19	Human recombinant antibody fragment (scFv format) directed against the ED-B domain of Fibronectin
L19TNF	Antibody-cytokine fusion protein of the L19 and human TNF
L19IL2	Antibody-cytokine fusion protein of the L19 and human IL2
LAL	Limulus Amoebocyte Lysate
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantification
Lpm	Liter per minute
LRFS	Local Recurrence-Free Survival
MedDRA	Medial Dictionary for Regulatory Activities Terminology
MTD	Maximum Tolerated Dose
MVM	Minute Virus of Mice
NK cells	Natural Killer cells
OD	Optical Density
ORR	Objective Response Rate
OS	Overall Survival
PCR	Polymerase chain reaction
PFS	Progression-Free Survival
PK	Pharmacokinetics
PPCB	Postproduction Cell Bank
PR	Partial Response
PT	Preferred Term
PV	Process Validation
RCC	Renal Cell Carcinoma

RECIST	Response evaluation criteria in solid tumours
RD	Recommended Dose
RFS	Recurrence-Free Survival
RH	Room Humidity
Rpm	Revolution per minute
RT	Room Temperature
RT-PCR	Real Time PCR
SAE	Serious Adverse Event
scFv	single chain Fv
SD	Stable disease
SOC	System Organ Class
SPR	Surface Plasmon Resonance
T ½	Half-life time
TEM	Transmission Electron Microscopy
Tmax	Time to peak serum concentration (Cmax)
TNF	Tumour Necrosis Factor
TOV	Turn Over Volume
ULOQ	Upper limit of quantification
QA	Quality Assurance
QC	Quality Control
VH	heavy chains
VL	light chains
WFI	Water For Injection
ZK 269136	L19IL2
ZK 342019	L19TNF

1. Rapporteurs CHMP recommendations

Based on the review of the data on quality, safety, efficacy, the application for Nidlegy in the treatment of adult patients with locally advanced fully resectable melanoma is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions (see section VI).

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

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

- Insufficient description of the DS and DP manufacturing processes, their controls and validation
- In order to identify QA which are important to ensure safety and efficacy of the molecules a thorough characterisation of the molecules needs to be provided, which is insufficient for the two molecules.
- Control of DS/DP, additional quality attributes may need to be included in the specifications depending on the outcome of the characterisation and certain acceptance criteria should be revised.
- In 2019, the manufacturing process was transferred from the applicant´s facility in Montarioso to a new facility in Rosia (Siena, Italy). It is stated that the drug product manufacturing process remains unchanged – with the exception of a new filling machine. For L19TNF DS and DP comparability between pre- and post-change drug product batches has currently not been demonstrated. The impact of changes between the clinical and commercial scale drug product batches on quality, safety and efficacy needs to be discussed by the applicant.
- The evidence that has been provided to support the requested indication, which is based on a sole pivotal study, PH-L19IL2TNF-02/15, is not sufficiently compelling to support a marketing authorisation application. The RFS analysis is not considered to be a suitable endpoint to evaluate the benefit of treatment with Nidlegy. Provided Event-free survival results are not considered to be clinically meaningful and the robustness is questioned. Moreover, based on information provided, the study integrity appears to have been affected, as data-driven decision-making during the study cannot be excluded. Overall, the clinical benefit of Nidlegy cannot be determined.

1.1. Questions to be posed to additional experts

Currently not applicable.

1.2. Inspection issues

1.2.1. GMP inspection(s)

GMP status of testing laboratories should be clarified.

1.2.2. GMP status of testing laboratories should be clarified. GLP inspection(s)

N/A

1.2.3. GCP inspection(s)

A routine GCP inspection for Nidlegy was adopted by the CHMP on 5 August 2024. The GCP inspection has been carried out at two clinical sites in Italy and Poland, and at the sponsor site. The inspection's conclusion is that the study has generally been conducted in sufficient compliance with GCP.

1.3. New active substance status

The applicant conducted protein search runs on three different search engines (commercial and academic) confirmed the novelty and uniqueness of both bifikafusp alfa and onfekafusp alfa. The results support the conclusion that both bifikafusp alfa and onfekafusp alfa contained in the medicinal product Nidlegy are to be qualified as new active substances.

1.4. Additional data exclusivity / marketing protection

Not applicable.

1.5. Similarity with authorised orphan medicinal products

N/A

1.6. Derogation(s) from market exclusivity

N/A

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

The applicant proposes the following indication:

> *Nidlegy is indicated for the neoadjuvant treatment of adult patients with locally advanced fully resectable melanoma.*

According to the current definition for melanoma staging by the American Joint Committee on Cancer (AJCC) (8th edition) this includes stage IIIB/C/D melanoma.

The product will be given as intratumoural injection prior to surgery.

2.1.2. Epidemiology and risk factors

Locally advanced melanoma refers to stage III melanoma, which includes patients with regional lymph node metastases and/or in-transit/satellite metastases.

Skin melanoma accounts for 4% of all new cancer diagnoses in EU-27 countries in 2020 and 1.3% of all deaths due to cancer, according to the European Cancer Information System. The incidence of melanoma has been rising in most countries worldwide. Over 100,000 new melanoma cases were reported in 2022 in the EU, leading to over 15,000 deaths.

There are approximately 18,000 newly diagnosed and 24.000 recurrent (most progressing from stage I/II) stage III melanoma patients every year in the European Economic Area (EEA), Switzerland (CH), United Kingdom (UK) and United States. Incidence is greater among geriatric populations with most of new melanoma diagnosis in adults of ages 45-69. Men are more likely to develop melanoma than women. Melanoma is disproportionally reported among fair-skinned Caucasian populations.

About 10,000 newly diagnosed and 13.000 recurrent stage III melanoma patients present the disease every year in EEA, CH and UK. Approximately 80% present a resectable disease. Hence, there are about 8.500 newly diagnosed and 11,000 recurrent stage III melanoma patients every year in EEA, CH and UK, who may be candidate for curative surgery.

Melanoma is considered as a multi-factorial disease arising from an interaction between genetic susceptibility and environmental exposure. The most important risk factors include UVA and UVB exposure as well as the number of melanocytic nevi, familiar history and genetic susceptibility.

2.1.3. Biologic features

Stage III melanoma frequently exhibits specific genetic alterations including BRAF mutations (approx. 40-50% of melanomas; most commonly V600E) or NRAS mutations (15-20%). Tumour-associated antigens are recognised by the immune system triggering an immune response involving the activation of innate immune cells like natural killer (NK) cells and dendritic cells as well as antigen-specific T cells, particularly CD8+ cytotoxic T lymphocytes. The presence of immune lymphocyte infiltrate within the primary lesion is associated with improved prognosis.

2.1.4. Clinical presentation, stage and prognosis

The tumour stage at the diagnosis is the main predictor of survival rate, accounting for 98.3% at 5 years for localised melanoma and 16% for metastatic disease. Tumour thickness (Breslow score), lymph node involvement, and the presence of metastasis are at the basis of melanoma staging and prognosis. If left untreated, melanoma can quickly spread to other parts of the body, such as lymph nodes or visceral organs and require systemic treatments.

At stage III, has already spread beyond the original tumour site to nearby small areas of skin, lymph nodes, or lymph vessels. The cancer has not spread to distant parts of the body or organs (absence of distant metastases). The 5-year survival rates for stage III melanoma range between 40 to 78 percent. However, these rates largely depend on the substages (i.e.. IIIA, IIIB, IIIC, or IIID). Patients with stage IIIB disease have a reported 10-year survival rate of 77%. The majority of the patients in the pivotal trial had stage IIIC disease. Patients with stage IIIC disease have a reported 10-year survival rate of 60% (CA Cancer J Clin 2017; 67(6):472-492).

Treatment of advanced melanoma is still challenging due to the high mutational rate and immune evasion. The introduction of immunotherapies, targeted therapies vaccines, small molecules, and combination therapies have advanced melanoma management, bust still the mortality rate is not yet neglectable.

2.1.5. Management / unmet medical need

According to the most recent ESMO guideline (Michielin et al.; 2019), current standard treatment for locally advanced resectable melanoma consists of upfront surgery followed by adjuvant systemic therapy which includes the approved PD-1 inhibitors (nivolumab and pembrolizumab) as well as targeted therapies including the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib in patients with a BRAF V600 mutation.

Patients with lymph-node involvement (stage III) and/or in-transit/satellite metastases, have a high risk of local and distant recurrence after surgery, with 5-year survival dropping down to 39-70% (Balch et al.; 2009). Patients with stage IIIB disease have a five-year survival between 20-59%, with certain subgroups (number of involved nodes, extracapsular extension, iliac involvement, groin metastases) showing even worse survival rates of around 5% at 5 years (van Akkooi et al.; 2007, Balch et al.; 2009, van der Ploeg et al.; 2011). Even with adjuvant therapy, relapse rates remain high ranging between 30% and 90% (Michielin et. al.; 2020; Rutkowski et al.; 2022). There is an unmet medical need for additional therapies to improve outcomes for patients with locally advanced, fully resectable melanoma.

The International Neoadjuvant Melanoma Consortium Informs that "patients with clinical stage III disease remain at a high risk of recurrence even with these adjuvant therapy advances. Therefore, improving existing therapies, innovating new therapeutic drugs, and investigating new combination regimens is greatly needed in the neoadjuvant setting (i.e., drug is given before definitive resection) for patients with high-risk clinical stage III melanoma" (Amaria et al. Lancet Oncol. 2019).

Recent studies have shown a potential role for neoadjuvant therapy in the treatment of locally advanced fully resectable melanoma. The rationale for neoadjuvant immunotherapy stems from the concept that the stimulation of tumour-infiltrating lymphocytes, while the tumour is still present, may result in a robust antitumour immune response and in the induction of memory T cells important for achieving a long-term systemic benefit. This approach may also lead to a reduction of the tumour mass prior to surgery. Several approaches have already been investigated, such neoadjuvant immunotherapy (e.g., pembrolizumab, the combination of relatlimab and nivolumab, and the combination of nivolumab and ipilimumab) and intralesional treatment (e.g., T-VEC). Currently, no neoadjuvant therapies for locally advanced, resectable melanoma are authorised by the European Commission, but encouraging results have recently been published for nivolumab plus ipilimumab as neoadjuvant therapy (Blank et al. NEJM. 2024) and already referred to in literature as the new standard of care (O'Leary. Nature Medicine. Research Highlight. 2024).

2.2. About the product

Nidlegy is a product consisting of the combination of two individual immunocytokine molecules, bifikafusp alfa (also denominated L19IL2) and onfekafusp alfa (also denominated L19TNF), which are mixed together immediately prior to administration.

The product will be given as intratumoural injection (also referred as intralesional injection) prior to surgery in patients with locally advanced fully resectable melanoma.

Nidlegy is provided as a combination pack including two individual single use vials of 1 ml each which contain, respectively:

- 2.17 mg/ml of bifikafusp alfa

- 0.40 mg/ml of onfekafusp alfa

Bifikafusp alfa is a recombinant fusion protein composed of two moieties L19, a human monoclonal antibody fragment in single chain variable fragment (scFv) format and IL2, the human cytokine Interleukin-2. Similarly, onfekafusp alfa is a recombinant fusion protein composed of two moieties L19 and TNF, the human cytokine Tumour Necrosis Factor

The antibody moiety (L19) in both pharmaceutical ingredients binds with high affinity to the alternatively spliced EDB domain of fibronectin, which is a marker of neoangiogenesis and is abundantly expressed in the perivascular stroma of most solid tumours (and metastases from several hematologic malignancies).

The antitumoural mechanism of action of the product is based on the specific activities of the two cytokine moieties, IL-2 or TNF. Intratumoural administration of rIL2 can augment the activity of cytotoxic T lymphocytes and also induces specific T helper cells, natural killer and lymphokine activated killer (LAK) cells. On the other hand, TNF administration activates several pathways, which ultimately lead to extravasation of erythrocytes and lymphocytes that provoke haemorrhagic necrosis of the tumour.

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

A pre-submission meeting was held on 27 February 2024. It was to present the clinical data package and to receive feedback regarding whether the primary efficacy analysis should be based on the Blinded Independent Central Review (BICR), supported by the investigators' assessments.

No scientific advice has been sought from CHMP with regard to the clinical development of L19IL2/L19TNF in the targeted indication and the overall melanoma development program. No documents have been submitted by the applicant within the dossier.

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

GMP

The manufacturer of the drug substance L19TNF onfekafusp alfa and for drug substance L19IL2 Bifikafusp alfa (PHILOGEN S.P.A., Loc. Bellaria, n. 35 - 53018 SOVICILLE (SI)) is located inside the EEA. An actual certificate of GMP compliance of this manufacturer was provided (Certificate No: IT-API/133/H/2024). The actual QP-declaration (12.06.2024) for drug substance L19TNF - onfekafusp alfa and for drug substance L19IL2 bifikafusp alfa confirm that the active substance has been manufactured in compliance with the EU GMP rules.

The drug product is manufactured, tested, packaged and released by Philogen S.p.A (Loc. Rosia – Via Bellaria, 35, 53018 Sovicille (SI), Italy). Proof of GMP compliance has been demonstrated. MIAs and GMP certificates for the aforementioned manufacturer can be retrieved from the EudraGMP database. A GMP compliance certificate is also provided in Annex 59 to the eAF.

GLP

A table containing declaration of GLP compliance was included as Annex V to the cover letter of this application. Regarding the documentation of protocols and reports uncertainties aroused and the topic might be covered in the requested combined GMP/Review triggered inspection.

GCP

It is described in the CSR that the pivotal trial was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent revisions and is consistent with the Good Clinical Practice (GCP), as well as the applicable regulatory requirement(s) for the countries in which the trial was conducted according to International Conference on Harmonisation (ICH) guidelines. A GCP inspection was adopted by the CHMP in August 2024. The inspection's conclusion is that the study has generally been conducted in sufficient compliance with GCP.

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

2.5.1. Legal basis

The legal basis for this application refers to:

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

2.5.2. PRIME

N/A

2.5.3. Accelerated assessment

N/A

2.5.4. Conditional marketing authorisation

N/A

2.5.5. Marketing authorisation under exceptional circumstances

N/A

2.5.6. Additional data exclusivity/marketing protection

N/A

2.5.7. New active substance status

The applicant requested the active substances bifikafusp alfa and onfekafusp alfa contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

The applicant conducted protein search runs on three different search engines (commercial and academic) confirmed the novelty and uniqueness of both bifikafusp alfa and onfekafusp alfa. The results support the conclusion that both bifikafusp alfa and onfekafusp alfa contained in the medicinal product Nidlegy are to be qualified as new active substances

2.5.8. Orphan designation

N/A

2.5.9. Similarity with orphan medicinal products

N/A

2.5.10. Derogation(s) from orphan market exclusivity

N/A

2.5.11. Information on paediatric requirements

Pursuant to Article 25 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) on the granting of a class waiver (EMA/PDCO/664578/2012 Corr).

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

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The antibody moiety (L19) in both pharmaceutical ingredients binds with high affinity to the alternatively spliced EDB domain of fibronectin, which is a marker of neoangiogenesis and is abundantly expressed in the perivascular stroma of most solid tumours (and metastases from several hematologic malignancies).

The antitumoral mechanism of action of the product is based on the specific activities of the two cytokine moieties, IL-2 or TNF. Administration of rIL2 in vivo augments the activity of cytotoxic T lymphocytes and also induces specific T helper cells, natural killer and lymphokine activated killer

(LAK) cells. On the other hand, TNF administration activates several pathways, which ultimately lead to extravasation of erythrocytes and lymphocytes that provoke haemorrhagic necrosis of the tumour.

3.1.2. Active substance (bifikafusp alfa, L19IL2)

3.1.2.1. General information

L19 Interleukin-2 (L19IL2) is a recombinant fusion protein composed of L19, a human monoclonal antibody fragment in the single chain Fv (scFv) format and human cytokine Interleukin-2.

3.1.2.2. Manufacture, process controls and characterisation

Manufacturer(s)

L19IL2 Drug Substance is manufactured, tested for release and released by Philogen S.p.A (Italy). The bifikafusp drug substance (DS) manufacturing and quality control takes place at Philogen S.p.A, Loc. Rosia, Sovicille, Italy. Manufacturing and control of cell banks, testing on post-production cell bank, testing on crude harvest, and microbiological identification testing are outsourced. GMP status of these laboratories should be provided.

Description of the manufacturing Process and Process controls

The L19IL2 Drug Substance (DS) is a clear, colourless liquid solution containing the L19IL2 fusion protein which is produced in mouse hybridoma host cells cultured in bioreactors.

The manufacturing process begins with the thawing of a vial from a cell bank, followed by expansion and fermentation in single use bioreactors. After a defined production period, the fermentation bioreactor is harvested, and the harvest material is clarified to remove residual biological material.

The purification process includes viral inactivation/clearance steps, nanofiltration, final formulation and final filtration.

While the description of each manufacturing process step, including its purpose and execution, has been improved, further clarification and additional information are still required.

Flow charts accompany the process description, outlining process parameters, controls, and their acceptance criteria. Although hold times between steps are provided, the maximum allowable hold durations used during manufacturing should be validated.

Regarding the purification phase, the collection criteria should be clearly described, and an updated dossier should be submitted.

The L19IL2 Drug Substance (DS) is a clear, colourless liquid solution containing the L19IL2 fusion protein produced in mouse hybridoma host cells, cultured in bioreactors.

Control of materials

Materials used throughout the manufacturing process, steps where they enter the process, and reference to quality standard are provided in tabular formats. Culture media and additives used for cell growth and fermentation in the current manufacturing process do not contain any material of human/animal origin. The materials used during manufacturing process are received with a Certificate of analysis by the supplier, which is acceptable. All materials were indicated and all buffer solutions used in the manufacturing process, along with their composition, are included.

The generation of the L19IL2 fusion protein cell bank system, their characterisation and testing are now sufficiently described. L19IL2 fusion protein is produced in mouse hybridoma host cells.

The cell bank was characterised in accordance with ICH Q5A and ICH Q5D requirements

However, a protocol for qualification of future cell banks should be provided including the genetic stability studies.

Control of critical steps and intermediates

A summary of the controls of critical steps and intermediates of the manufacturing process of L19IL2 is provided. The CQA and their control strategy outlined. The acceptance criteria for the CQA have been established based on historical manufacturing experience in Philogen's Montarioso facility. All critical steps and intermediates have acceptance criteria and no action limits. Analytical Methods used for In Process Control (IPC) are sufficiently described. Testing of the crude harvest for absence of mycoplasma and adventitious agents is acknowledged. The justification of the process parameters monitored, and pre-defined acceptance criteria are based on a process parameter risk analysis and small scale-testing of critical process parameters, as provided in process validation section.

Process validation

The L19IL2 process validation studies were performed by producing three consecutive PV batches at Philogen Rosia site, Località Bellaria, n.35 - 53018 Sovicille (SI) ITALY. Process parameters and acceptance criteria have been identified based on the outcome of the risk assessment and results of the process development studies. These batches were produced under standard operating conditions and met the established control limits. However, a comprehensive justification and discussion are required for each identified Critical Control Parameters (CCPs), including the specific quality attributes potentially impacted. Additionally, a detailed classification and definition of the relevant Critical Quality Attributes (CQAs) must be provided

The validation of the L19IL2 manufacturing process is not considered sufficient since some validation reports were not submitted. Additional documents should be provided in the dossier to support the validation process assessment.

Further assessment is needed to confirm the viral clearance capacity of the purification process. Clarification regarding the purification step’s yield and performance capabilities should also be provided. Process-and product-related impurities were defined. The clearance of host cell-derived impurities as well as other process-related impurities should be monitored across the purification process and measured using validated methods. The absence of product related impurities in the validation batches and in the intermediates should be demonstrated using a suitably validated assay.

The nitrosamine risk assessment together with the provided FMEA analysis supports the conclusion that the risk for entry of generation of nitrosamines is low. The elemental impurities Risk Assessment on the DS manufacturing process was conducted and it was concluded that there is a low risk to transfer EI from raw materials, packaging materials and process equipment into the DS. Additional data on EI levels in the AS should be provided.

Manufacturing process development

Philogen produced the monoclonal antibody L19IL2 at its Montarioso manufacturing site until 2022. The L19IL2 material produced at the site was used for all clinical studies conducted with L19IL2 alone or in combination with other drugs. In 2019, the manufacturing process of L19IL2 was transferred from

Philogen's facility in Montarioso to Philogen's facility in Rosia (Siena, Italy) which is intended for the production of commercial batches. Of note, these batches have not been used in clinical trials. The manufacturing process of L19IL2 performed at the Philogen Rosia plant is the same (same phases and same steps) as the manufacturing process of L19IL2 performed at the Philogen Montarioso plant. The differences between the two manufacturing processes are related to the use of different equipment necessary to carry out the required scale-up. For the comparability assessment, three process validation DS and DP batches manufactured at Rosia site are compared with historical data of DS and DP clinical batches manufactured at Montarioso. The comparison includes DS and DP release testing results and in-process control data. Additionally, comparative extended characterisation, stability studies and forced degradation studies have been performed for the finished product.

Characterisation

L19IL2 is an antibody-cytokine fusion protein consisting of the L19 antibody fused to human interleukin-2 (IL-2). L19 is a single-chain variable (scFv) antibody fragment that selectively binds to a splice variant of fibronectin containing the extra-domain B (EDB). The IL-2 payload stimulates a potent anti-cancer immune response. The characterisation comprises physical, chemical and biological methods. However it is not clear which batches and which conditions are used for the various characterisation studies.

The applicant should perform additional characterisation with other sensitive methods to ensure the identity and purity control across the manufacturing process, and for the release/stability testing. An alignment with ICH Q1A and Q5C is requested.

The immunoreactivity assay is performed to test the potency of the active substance.

The IL-2 bioactivity assay is performed to test the biological activity (potency) of the active substance and is based on a colorimetric method. The results lead to dose-response curve and define the EC50 of the L19IL2 product and IL2 standard, respectively. Product related substances and product-related impurities were not investigated. Additional studies are requested.

Process-related impurities are identified and are adequately controlled.

However, the overall information provided on the characterisation of the drug substance and product is still too limited to provide sufficient basis for a control strategy. In line with guidance from ICH Q1A and Q5C, appropriate stress stability studies with a broad panel of sensitive and specific state of the art analytical methods should be performed.

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

Control of drug substance

Specifications

The DS specification includes visual appearance, general tests, protein quantity, identity, purity, potency, product- and process-specific impurities, and safety attributes.

Acceptance criteria for most DS specifications set were justified using the three-sigma approach based on data historical clinical DS batches. However, the current specifications are not considered sufficient to fully ensure product quality.

During the assessment, the applicant was asked to justify the absence of certain specification attributes, to tighten acceptance criteria for several quality attribute (bacterial endotoxin and purity) and to set up a method for the identity control.

Control of product related substances and product-related impurities were not included.

Analytical methods are described.

The applicant should update the specifications, define the acceptance criteria providing adequate justification based on batch data and product and process characterisation, and update the method descriptions and the method validations, such that it can be ensured that the control of drug substance is sufficient to guarantee the quality of the DS.

A new analytical method to quantify the content of stabilizing agent was implemented at the Rosia site. The new method confirmed the same stabilising agent content range for both process validation baches produced at the Rosia facility and for clinical batches produced at the Montarioso facility. Moreover, the method in Rosia showed reduced analytical variability compared to the previously established method in Montarioso, supporting its suitability for routine use.

Reference material

Information on the reference material was included in the dossier. Analysis data and CoA of the internal L19IL2 reference material (RM) was provided. This material was produced, qualified and used at the Philogen Montarioso plant and was also used during the L19IL2 process validation (PV) batches in Rosia for all release and stability tests. Further information on the history of reference standard batches used during development were provided too.

The applicant also generated new primary reference material at the Rosia plant (mix of the three process validation batches) but confirmed that this has not been used. Clarification on this point is requested.

The test panel used to characterise the reference material, as well as the analytical panel for requalification of the primary reference standard, are currently not acceptable due to missing quality attributes.

A like-vs-like reference standard (L19IL2) should be established and used as calibration curve material in the routine assay unless it can be demonstrated that IL2 behaves similarly as L19IL2 (e.g. comparable dose-response curves)

Container closure system

The L19IL2 drug substance is stored in a sterile bag system with a semi-rigid structure composed of a film and protective shell. The bag consists of two polyester plates and a bag assembly encased in a rigid external support. Data concerning the biocompatibility and extractable profile of the film used in the bag system were provided. The film has been tested according to USP <87> and USP <88>, and complies with the European Pharmacopoeia monograph 3.1.7 for polyethylene-vinyl acetate used for containers and tubing for total parenteral nutrition. The extractable profile from the bag provider was presented. However, the risk analysis of the extractables observed from the provider and the risk assessment for the DS Nidlegy are missing. The applicant should perform a theoretical risk analysis for the extractable amounts obtained and the doses given with Nidlegy. If this assessment indicates an extractable with risk, a leachable study with the DS Nidlegy should be performed accordingly

Information's and CoA concerning the bags used during the manufacturing process are provided. The CoA are controlled internally upon receipt to verify the compliance.

3.1.2.4. Stability

Stability studies under the proposed storage condition are currently ongoing on the three process validation batches from Rosia plant. A shelf life is proposed based on real time stability data of the three Process Validation batches from Rosia plant, commercial scale batches. This could be accepted but the study is ongoing and the applicant is invited to provide additional data and the final shelf life to be granted will depend on the data set available during the review process. Additionally, discrepancies have been observed between the data from the PV batches and the corresponding stability batches. To clarify the situation, the applicant should report the timeline with dates in a tabular format for each PV batch. This should include the manufacturing date, test date for each parameter, batch release date and inclusion (starting date) of the stability study should be reported. The applicant should either remove the delayed analysis results, or substantiate that these values are scientifically valid

3.1.3. Finished Medicinal Product (bifikafusp alfa, L19IL2)

3.1.3.1. Description of the product and pharmaceutical development

Description and Composition of the Drug Product

Nidlegy is a combination pack composed of L19IL2 and L19TNF drug product. L19IL2 drug product is presented as solution for injection for intra-tumoral administration in a glass vial with a bromobutyl rubber stopper.

The formulation contains 2.17 mg of L19IL2 in a sodium/potassium buffer with stabilisers (mannitol, polysorbates, glycerol). The qualitative and quantitative composition of the L19IL2 drug product is provided.

Pharmaceutical development

Components of the drug product

Components of the L19IL2 drug product were briefly described. Excipients are listed and their function are stated.

Formulation development

The formulation of the L19IL2 drug product was developed by testing the stability of the product under different conditions, using different buffers. However, the information provided is considered limited and the investigation performed to assess the formulation at the proposed storage condition is unclear. The applicant should propose a storage condition, based on adequately designed, performed and documented pharmaceutical development and stability studies.

Manufacturing process development

The L19IL2 manufacturing process is a standard aseptic filling process used for this type of products. In 2019, the manufacturing process of L19IL2 was transferred from Philogen's Montarioso facility to the Rosia site (Siena, Italy). The process remained unchanged, except for the introduction of a new filling machine.

A comparability study has been conducted and includes comparison of release and in-process controls, extended characterisation of the final product, stability and forced degradation studies, and additional analytical tests. For DP, only a comparison of pre- and post-change drug product release data is provided in Module S.2.6. Historical data from Montarioso batches and new data from Rosia batches are compared.

No adequate comparability acceptance criteria were defined, and the batches were tested against current release specifications. However, since specifications, analytical methods and their validation raise concerns, comparability between Montarioso and Rosia DP remains not demonstrated.

Information on process characterisation studies is not provided. Process characterisation activities are expected in the scope of process validation and respective study results should be provided in the dossier.

Container Closure System

The primary container closure system used for L19IL2 drug product consists of a glass vial with a rubber stopper and flip-off cap. Information on the container closure system is insufficient and the suitability of the container closure system is not documented in the dossier.

Microbiological Attributes

L19IL2 drug product is a sterile medicinal product obtained through aseptic filling. Container closure integrity is ensured through leak detection method (vacuum decay method). The validation of the vacuum decay method should be performed with a different filter.

Compatibility

L19IL2 and L19TNF are presented as combination pack. Both are administered intra-lesional using the same syringe. The method of administration is described in more detail in the accompanying SmPC and has now also been added to the dossier. Before mixture, the vials are kept at room temperature for at least 1 hour. The desired amount of L19TNF is drawn from the vial into a syringe. Subsequently, the whole amount of the L19IL2 vial is drawn into the same syringe. The mixture is stable for up to 48 hours at room temperature or at 2-8°C. For injection either a 30-gauge needle (superficial injections) or a 27-gauge needle (deep injections) is to be used.

An in-use stability/compatibility study of the mixed products has been conducted and is presented in the dossier for the combination pack. The mixture of both products has been analysed after storage of 24 or 48 hours at RT or refrigerated. The batch release specifications were used to assess the stability of the mixture at different temperature. In the conducted study, the L19IL2 and L19TNF DP mixture was confirmed to be stable at the proposed condition.

However, the applicant should substantiate compatibility of L19IL2 and L19TNF with a properly designed study and employing appropriate (compatibility and stability) indicating methods.

3.1.3.2. Manufacture of the product and process controls

Manufacturer(s)

L19IL2 drug product is manufactured, tested, packaged and released by Philogen S.p.A (Loc. Rosia – Via Bellaria, 35, 53018 Sovicille (SI), Italy). Proof of GMP compliance has been demonstrated. MIAs and GMP certificates for the aforementioned manufacturer can be retrieved from the EudraGMP database.

Batch formula

A batch formula resembling the drug product composition is provided.

The claimed batch size for L19IL2 is not supported by current validation data, as fewer vials have been filled so far. The full batch size must be validated, and the relevant dossier sections should be updated accordingly.

Description of Manufacturing Process and Process Controls

The L19IL2 drug product manufacturing process includes the following steps: drug substance conditioning, aseptic filling, vial stoppering, vial crimping, vial inspection, labelling and packaging. A process flow chart has been provided, outlining process parameters, including hold times, and in-process testing. Reprocessing is not considered and no DS pooling is foreseen for Rosia-derived material.

A narrative of the DP manufacturing process has been provided describing all steps and controls. Sampling and testing steps are clearly indicated. Filters have been sufficiently characterised. Nitrogen (sterile) is purged into the vials before and after filling.

However, it is noted that the narrative description and the flow-chart lack sufficient detail. Each process step, process control and process parameter should be appropriately defined.

Flow-chart and narrative should be updated, accordingly.

Controls of Critical Steps and Intermediates

Process parameters and in-process testing are provided for sterility steps and filling operations. Product purity monitoring should be approached.

A risk assessment should be conducted to define CPP and IPC.

Process Validation and/or Evaluation

The DP manufacturing process has been validated using three consecutive L19IL2 commercial scale drug product batches Information on the actual results observed for drug substance preparation, sterile filtration, filling, vial stoppering should be reported in the dossier. However the process control of drug substance preparation should be detailed.

Information on sterilisation method and sterilisation validation needs to be provided for all product-contact materials used during filling.

Extractables/leachables: Extractables and leachables studies are planned for the L19IL2 primary container closure system and the identified high-risk single-use systems used during DP manufacture.

Transport validation: According to the applicant, transport validation is ongoing and results might be available by mid-2025. The question remains and results from (simulated) transport validation studies should be provided.

Process characterisation: Process characterisation activities are expected in the scope of process validation strategy and respective study results should be provided in the dossier

3.1.3.3. Product specification, analytical procedures, batch analysis

Specification(s)

The release specification for L19IL2 drug product include tests for visual appearance, general tests, identity, purity and impurities, biological activity, total protein, sub-visible particles, extractable volume, endotoxin, sterility and container closure integrity testing. The above mentioned parameters are also tested for stability studies. The majority of the test parameters (specifications set, analytical methods and their validation) are identical to the DS section, thus the same assessment applies, and concerns raised on test methods and specifications for DS are also applicable to the DP. As a consequence of this and data lacking on DS process development and validation, purity and potency of the DP are not ensured.

Analytical procedures

The analytical methods have been adequately described and non-compendial methods appropriately validated to demonstrate their suitability for their intended use, according to the ICH guidelines. A drug product-specific analytical procedure for container closure integrity testing has been conducted. Bridging studies are currently ongoing to support a robust comparability assessment.

Validation of analytical procedures

Improved analytical method description and validation reports are submitted for all methods. Method descriptions and validation reports are still inadequate in certain respects. Based on those issues the control of identity, potency and purity is still considered insufficient.

The sterility test for the L19IL2 drug product has been verified. For the container closure integrity testing reference is made to the compendial status. Since this method is not listed in the European Pharmacopoeia, method validation/verification results were provided. A revalidation with a different filter is requested.

Batch analyses

Batch analyses data are provided for L19IL2 clinical and process validation batches. Batch analysis data for the clinical drug product lots manufactured at Montarioso manufacturing facility are provided. Furthermore, batch analyses data is provided for three process validation batches manufactured at Rosia site. All batches comply with the pre-established specifications valid at the time of testing.

A summary table with the batch information/batch history (i.e. batch size (number of vials), batch size (volume), manufacturing date (dd/mm/yyyy), manufacturing site, DS batch number, batch use) should also be added in the dossier.

Characterisation of impurities

No new impurities have been introduced during the L19IL2 drug product manufacturing process and reference is made to Module S.3.2 of the dossier. This approach can be considered acceptable.

Evaluation of elemental impurities is currently ongoing. The applicant should update the dossier accordingly.

The applicant has provided a risk evaluation concerning the presence of nitrosamine impurities in the finished product. Based on the evaluation, the risk was considered low. The applicant should update the dossier, accordingly.

Justification of specifications

The acceptance criteria identification for L19IL2 DP tests are based on a statistical analysis (three sigma approach) of historical release data from batches produced in Montarioso site. This approach was used for visual appearance, identity, purity and impurities, pH, osmolality, biological activity, total protein, sub-visible particles, extractable volume, endotoxin, sterility and container closure integrity testing. These parameters are also monitored during stability studies.

However, during the evaluation procedure, the applicant was requested to tighten certain specification acceptance criteria, while broadening the range of others, in order to ensure appropriate control of the product's quality attributes.

Control of Excipients

The development and control sections of DP do not include any information on how the quantitative composition of the DP with respect to excipient composition is under control. General tests alone do not provide sufficient assurance that the composition is consistent among batches. The applicant’s justifications on the control of stabilising compounds are accepted

Reference standards or materials

The same reference standard is used for both L19IL2 drug substance and drug product testing. For specific comments on the L19IL2 internal reference standard see respective Module S.5. Overall, information regarding the L19IL2 reference standard is considered insufficient and not in line with the requirements of ICH Q6B guideline.

In addition to the L19IL2 reference standard, a tabulated summary of all commercial reference materials used for all assays is provided

Container closure system

The primary container closure selected for use with the L19IL2 drug product consists of a 3 mL glass vial closed with a rubber stopper and a white flip-off cap Specifications for the primary container closure system (vial and rubber stopper) and the flip-off seal were added to the dossier. The product-contact material complies with compendial requirements. Technical drawings for the vial components are provided. Inconsistencies in the description of the container closure system and rubber stopper are noted in some sections of the dossier. A harmonised description of the primary container closure system should be envisaged and respective dossier Modules should be corrected.

Additionally, information regarding the suppliers and sterilisation of the container closure systems (i.e. rubber stoppers and glass vials) should also be included in the dossier.

Extractables/leachables studies for the L19IL2 drug product primary container closure system are ongoing. Results are expected in the coming months for extractables and at a later stage for leachables. Results from these studies will need to be provided to demonstrate that the selected primary container closure system is compatible with the L19IL2 drug product over the entire shelf-life. Detailed information should be included in the dossier.

L19IL2 drug product is part of a combination pack composed of L19IL2 (bifikafusp alfa) and L19TNF (onfekafusp alfa) drug product. The secondary packaging for the L19IL2/L19TNF combination pack consists of a temperature resistant and water-repellent carton box. Inside the carton box, the vials are securely placed in a thermoformed PVC tray. Schematic pictures are provided. The information has

been added to the updated dossier for the combination pack. Reference to the secondary container closure system should also be added to individual relevant sections for L19TNF and L19IL2.

3.1.3.4. Stability of the product

An ongoing long term stability study has been provided to determine the stability of L19IL2. The dataset includes historical data from clinical batches manufactured at Philogen S.p.A Montarioso and process validation batches manufactured at Philogen.

However, no stability data under alternative storage conditions have been presented, and therefore the justification for the claimed storage condition cannot remain insufficient. The design of the registration stability studies is in line with ICH Q5C guideline. The selected stability assays and specifications resemble the parameters selected for release specifications.

The selection of the stability assays was not justified and limited information on the stability-indicating properties of the methods has been provided in the dossier. It is unknown which tests are stability indicating.

The container closure system used for L19IL2 drug product stability studies is representative. For clinical batches manufactured at Montarioso site, 3 mL glass vials with a bromobutyl stopper were used. For PPQ batches manufactured at Rosia site, 3 mL glass vials with a coated bromobutyl stopper were used.

At the recommended storage condition, all results were within specifications for the available timepoints.

However, given the uncertainties regarding the manufacturing process transfer, analytical methods and comparability, supportive data from clinical development batches for shelf-life setting is not considered acceptable.

Additional stability studies were conducted under stress conditions: a short-term excursion study at refrigerated and room temperature conditions, and a freeze/thaw study. In the short-term excursion study, the absence of the results at certain time points prevents the conclusions presented by the applicant from being supported

The stress conditions applied through repeated freeze/thaw cycles have demonstrated that the product is stable: no protein precipitation, degradation, proteolysis of the product or loss of potency have been detected up to three freeze/thaw cycles.

A photostability study in line with the requirements of ICH Q1B guideline has been conducted. Results demonstrate that the samples are light sensitive. However the variability in the results should be further investigated.

Section 6.4 of the SmPC contains the statement "Store in the original carton in order to protect from light", which is acceptable.

3.1.3.5. Adventitious agents

No TSE risk materials have been identified. FBS was used at initial cell cultivation, cloning and selection steps. However, a serum-free cell bank has been established. In summary, compliance with TSE-Guideline EMEA 410/01 rev03 has been demonstrated.

Cell banks have been tested according to ICH Q5A. The virus testing program for unprocessed bulk and drug substance is in line with ICHQ5A (R2). Testing of unprocessed bulk on MVM is in line with recommendation from ICHQ5A(R2). A brief summarising description of the methods for routine testing of unprocessed bulk was provided in the dossier. The estimated particles/dose is acceptable.

The choice of model viruses in considered in line with ICHQ5A. Controls for cytotoxicity and interference of test materials with virus detection have been performed. Three steps of the manufacturing process have been evaluated for virus reduction. The last step of purification has not been evaluated for virus reduction; additional studies are requested. Finally, clarification is requested on virus carry-over runs.

Adequate summary reports on down-scaling and virus reduction experiments have been provided.

End-of-lifetime resin studies were performed with X-MuLV and MVM for other purification steps and showed comparable inactivation capacity. Raw data should be included in the dossier.

3.1.3.6. GMO

Not applicable.

3.1.4. Active substance (L19TNF, onfekafusp alfa)

3.1.4.1. General information

3.1.4.2. The L19TNF product is a recombinant fusion protein composed of L19, a human monoclonal antibody fragment in the single chain Fv (scFv) format and human cytokine TNF (Tumour Necrosis Factor). Manufacture, process controls and characterisation

Manufacturer(s)

The manufacturer of the active Substance onfekafusp alfa (PHILOGEN S.P.A., Loc. Bellaria, n. 35 - 53018 SOVICILLE (SI)) is located inside the EEA. An actual certificate of GMP compliance of this manufacturer was provided. The actual QP-declaration for drug substance L19TNF - onfekafusp alfa - confirm that the active substance has been manufactured in compliance with the EU GMP rules. Manufacturing and control of cell banks, testing on postproduction cell bank, TEM and mycoplasma testing on crude harvest, and microbiological identification are outsourced to certified laboratories. GMP status should be provided.

Description of the manufacturing Process and Process controls

The L19TNF drug substance (DS) is a clear, colourless liquid solution containing the fusion protein L19TNF produced in Chinese Hamster Ovary host cells (CHO), cultured in bioreactors The L19TNF Drug Substance (DS) is a clear, colourless liquid solution containing the fusion protein L19TNF, produced in mammalian host cells using a fed-batch fermentation process in single-use bioreactors.

The manufacturing process consists of a cultivation process with nutritive feeds. One vial from the cell bank is thawed and the cell culture is expanded and fermented in single use bioreactors. The fermentation bioreactor is harvested after a defined production period and the harvest bulk is clarified with the aim to remove residual biological material.

The purification process includes viral inactivation/clearance steps, nanofiltration, final formulation and final filtration.

The description of the manufacturing process steps is accompanied by flow charts indicating the process parameters, process controls and their acceptance criteria.

Hold time between process steps are provided but the maximum time limit used during the manufacturing process should be further refined.

Control of materials

The strategy for the material qualification program was outlined. All materials are sourced from approved suppliers. Materials used throughout the manufacturing process, steps where they enter the process, and reference to quality standard are provided in tabular formats. Culture media and additives used for cell growth and fermentation in the current manufacturing process do not contain any material of human/animal origin. The material used during manufacturing process are received with a Certificate of analysis by the supplier, which is acceptable. For non-compendial grade material specifications and tests are provided. L19TNF is produced in mammalian host cells transfected with a plasmid containing the genetic information required for expression of the L19TNF polypeptide chain.

The gene encoding the L19 single-chain antibody fragment was fused to the sequence encoding the mature human TNFα peptide via a linker and inserted into an expression plasmid. The resulting construct was transfected into a mammalian host cell line for recombinant expression.

The cell was characterised in accordance with ICH Q5A and ICH Q5D requirements. A protocol for qualification of future cell banks should be provided.

Control of critical steps and intermediates

A summary of the controls of critical steps and intermediates of the manufacturing process of L19TNF is provided. Testing of the crude harvest for absence of mycoplasma and adventitious agents is acknowledged. The analytical methods used for in process control (IPC) testing are the same as those used for the release of the L19TNF DS. All the methods have been validated. The results of the comparability study for each method are reported and can be considered comparable.

Process validation

The L19TNF process validation studies have been performed by producing three consecutive PV batches at Philogen Rosia site, Località Bellaria, n.35 - 53018 Sovicille (SI) ITALY. Process parameters and acceptance criteria have been identified based on the outcome of the risk assessment and results of the process development studies. However, a comprehensive justification and discussion are required for each identified Critical Control Parameters (CCPs), including the specific quality attributes potentially impacted, their classification and definition.

The validation of the L19TNF manufacturing process is not considered sufficient since some validation reports were not submitted. Hold time between process steps are provided but the maximum time limit used during the manufacturing process should be further defined at the proposal storage condition.

The clearance of impurities originating from the host cells and other process related impurities should be monitored across the purification process and the data should be presented in the dossier. Process Validation and/or Evaluation and the relevant methods should be validated.

Manufacturing Process Development

Philogen has manufactured L19TNF for safety tox studies and for use in clinical trials in the Montarioso facility (Siena, Italy) until 2023. Continued process development activities during this period have been documented. However, the applicant is requested to clarify which batches were used in the clinical trials. In November 2023 manufacturing process was transferred from Montarioso facility to Rosia site to enable production scale-up and prepare for the commercial launch. Three consecutive process validation batches of L19TNF have been produced in the Rosia Facility (Siena, Italy). Of note, these batches have not been used in clinical trials and thus sufficient comparability of these batches with batches used in the clinic needs to be demonstrated.

The manufacturing process has undergone changes over time, and certain limitations—such as the absence of stability data for earlier process versions—reduce the depth of available comparisons. Additionally, the set of analytical methods applied to support comparability is relatively limited.

During the site transfer, a new bioassay method was introduced and validated, and was later replaced with the method previously used, maintaining the same acceptance criteria. The results obtained were compliant.

Some differences were observed at the intermediate purity level between batches, likely due to variations in operating conditions and materials. However, these differences do not appear to impact the final product. Further clarification is requested regarding the acceptance criteria applied across the different process phases. The currently used bioactivity method aligns with the approach from the original site and has been applied successfully to all batches from the new facility.

Comparative studies under stress conditions indicate similar degradation behaviour across sites, though some initial differences were noted. Further clarification may help support overall comparability. During the process performance comparison, a difference in product-related quality attributes was observed at an intermediate stage. This variation was investigated through comparative studies, confirming that it is attributable to the implementation of the revised process configuration.

The difference is not considered to impact the final product quality, as the main control over this attribute is achieved in a subsequent purification step. After this step, the attribute levels are aligned across both processes.

Nevertheless, justification for applying the proposal acceptance criteria is needed.

Overall, the panel of analytical methods used in the comparability exercises is considered sparse. An extensive characterisation of L19TNF is expected including different orthogonal methods. With regard to the biological activity, a clear outline of method development process and the different stages of method validation should be provided to demonstrate that the assay now transferred from the Montarioso to the Rosia facility is sensitive and provides reliable results. The method is rather variable and the validation is not considered appropriate/successful. The method should be improved and adequately validated.

Characterisation

L19TNF is a fusion protein consisting of the L19 antibody moiety fused to human tumour necrosis factor (TNF). L19 is a single-chain variable (scFv) antibody fragment that binds selectively to a splice variant of fibronectin containing the extra-domain B (EDB). TNF is a pro-inflammatory cytokine that stimulates immune cells against cancer. The characterisation comprises physical, chemical and biological methods.

However, the applicant should perform additional characterisation with other sensitive methods to ensure the identity and purity control across the manufacturing process, and for the release/stability testing.

Biological activity (TNFalpha activity)

The biological activity of L19TNF is assessed by cytotoxic assay. It is a quantitative test which measures cell viability by observing the colour change of the MTT substrate from yellow to purple. Immunochemical properties of L19TNF have been extensively characterised by means of Biacore, Immunofluorescence and FACS analyses. In line with guidance from ICH Q1A and Q5C, appropriate stress stability studies with a broad panel of sensitive and specific state of the art analytical methods should be performed.

Potential product- and process-related impurities in onfekafusp DP are theoretically named. Product-related impurities are controlled by specifications. Further clarifications are needed on how the impurities are removed through the manufacturing process.

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

Control of Drug Substance

Specifications

Specifications are set in accordance with ICH Q6B. The DS specification covers appearance, general tests, identity, purity, potency, product- and process-specific impurities and safety.

Specifications and proposed limits are in general not considered adequate. The identity control is insufficient since the tests lack specificity. A sufficiently specific identity test should be implemented. The TNF bioactivity assay is validated. During the assessment, the applicant was requested to introduce a suitable method for the control of impurities. Furthermore, the applicant was requested to tighten the acceptance criteria for Bacterial endotoxin and purity.

The applicant should update the specifications, define the acceptance criteria providing adequate justification based on batch data and product and process characterisation, and update the method descriptions and the method validations, such that it can be ensured that the control of drug substance is sufficient to guarantee the quality of the DS.

Reference material

The L19TNF reference material (RM) is used as internal control in several analytical methods and was prepared using a pool of the 3 consecutive process validation (PV) batches, which is acceptable. An adequate container closure system should be considered. All analytical methods at the Philogen Rosia plant have been validated using the same reference material produced, qualified and used at the Philogen Montarioso plant. This reference material was employed during the L19TNF Process Validation (PV) batches in Rosia for all release and stability tests. A CoA is provided. Comparability between the reference materials from Montarioso and Rosia has been established. A new Rosia reference material has been generated. To date, the primary reference material generated at the Rosia plant has not been used. It is stated that before using the primary Reference Material generated at Rosia plant, a bridging analysis will be performed. A protocol for bridging of current to future RM should be included in the dossier. The proposed analytical panel for primary reference standard re-qualification and also working

Reference Material re-qualification is not considered adequate. It is expected that re-qualification is supported by the same panel of analytical techniques as the initial qualification

Container Closure System

The L19IL2 drug substance is stored in a sterile bag The bag-in plate concept is a semi-rigid bag composed of 2 polyester plates and a bag assembly protected with a surrounding shell. The applicant should provide a risk assessment with regard to extractables and potentially leachables. It should be clarified whether product-specific leachable studies are considered and if not, this should be justified. Test procedures and acceptance criteria for control of incoming material are provided. The CoA are controlled internally upon receipt to verify the compliance, and the release is performed by the QC department of Philogen S.p.A.

3.1.4.4. Stability

Stability studies at the proposed storage condition are ongoing on three Process validation batches from Rosia Plant. A shelf life proposed based on real time stability data for the three Process Validation, commercial scale batches The applicant is invited to provide additional data and the final shelf life to be granted will depend on the data set available during the review process. The interpretation of these studies is hampered by the lack of data regarding some quality attributes at some time-points and uncertainties regarding analytical techniques.

To confirm validity of these results, date of initiation of the stability study, and of each individual analysis should be provided.

3.1.5. Finished Medicinal Product (onfekafusp alfa, L19TNF)

3.1.5.1. Description of the product and Pharmaceutical Development

Description and Composition of the Drug Product

Nidlegy is a combination pack composed of L19IL2 (bifikafusp alfa) and L19TNF (onfekafusp alfa) drug product. Each pack contains two vials (one vial of L19IL2 and one vial of L19TNF drug product).

L19TNF drug product is presented as solution for injection for intra-tumoral administration in a 1 glass vial with a bromobutyl rubber stopper. L19TNF (0.4 mg/1 ml/vial) is formulated with a sodium/potassium buffer, stabilisers (mannitol, polysorbates, glycerol) and the chelating agent EDTA. The qualitative and quantitative composition of the L19TNF drug product is provided.

Pharmaceutical development

Components of the drug product

Components of the L19TNF drug product were briefly described. Excipients are listed and the function of the individual excipients are stated.

Formulation development

The formulation of the L19TNF drug product has been developed by testing the stability of the product under different conditions, using different formulation buffers Phosphate-based buffers were

considered the most appropriate. The information provided is considered sufficient. The development of a combination pack was already accepted by EMA and will not be further discussed here.

Manufacturing process development

The L19TNF manufacturing process is a standard aseptic filling process used for this type of products. In 2019, the manufacturing process of L19TNF was transferred from the applicant´s facility in Montarioso to a new facility in Rosia (Siena, Italy). Drug product manufacturing process remains unchanged – with the exception of a new filling machine.

A comparability study has been conducted and includes comparison of release and in-process controls, extended characterisation of the final product, stability and forced degradation studies, and additional analytical tests. For DP, only a comparison of pre- and post-change drug product release data is provided in the dossier. The study was carried out on historical Montarioso batches and Rosia batches. A graphical summary is provided in the dossier however the scale of parameters, the full range evaluated and the batch genealogy should be clearly presented to ensure comprehensive understanding and traceability.

Some concerns remain regarding the adequacy of specifications, analytical methods and their validation. The comparability between Montarioso and Rosia DP remains not demonstrated.

Information on process characterisation studies should be provided in the eCTD format.

Container Closure System

The primary container closure system used for L19TNF drug product consists of a glass vial with a rubber stopper and flip-off cap. Information on the container closure system and the suitability of the container closure system are not provided in eCTD format.

Microbiological Attributes

It is described that the L19TNF drug product is a sterile medicinal product obtained through aseptic filling. Container closure integrity is ensured through leak detection method (vacuum decay method). The validation of the vacuum decay method should be performed with a different filter.

Compatibility

L19IL2 and L19TNF are presented as combination pack. Both are administered intra-lesional using the same syringe. The method of administration is described in more detail in the accompanying SmPC, and has now also been added to Module 3. Before the mixture, both vials should be kept at room temperature for about 1 hour. The desired amount of L19TNF is drawn from the vial into a syringe. Subsequently, the whole amount of the L19IL2 vial is drawn into the same syringe. After thawing, the mixed product is stable for up to 48 hours at room temperature or at 2-8°C. For injection either a 30-gauge needle (superficial injections) or a 27-gauge needle (deep injections) is to be used.

An in-use stability/compatibility study of the mixed products has been conducted and is presented in Module P.2.2 for the combination pack. The mixture of both products has been analysed after storage of 24 or 48 hours at RT or refrigerated. The batch release specifications were used to assess the stability of the mixture at different temperature. In the conducted study, the L19IL2 and L19TNF DP mixture stored at both RT and at 2-8°C was stable at the proposed storage condition.

However the applicant should substantiate compatibility of L19IL2 and L19TNF with a properly designed study and employing appropriate (compatibility and stability) indicating methods.

3.1.5.2. Manufacture of the product and process controls

Manufacturer(s)

L19TNF drug product is manufactured, tested, packaged and released by Philogen S.p.A (Loc. Rosia – Via Bellaria, 35, 53018 Sovicille (SI), Italy). Proof of GMP compliance has been demonstrated. MIAs and GMP certificates for the aforementioned manufacturer can be retrieved from the EudraGMP database.

Batch formula

A batch formula resembling the drug product composition is provided. The full anticipated batch size should be supported by process validation activities, and the relevant sections of the dossier should be updated accordingly.

Description of Manufacturing Process and Process Controls

The L19TNF drug product manufacturing process includes drug substance conditioning, aseptic filling, vial stoppering, vial crimping, vial inspection, labelling and packaging. A process flow chart with process parameters, including hold times, and in-process testing has been provided. Reprocessing is not considered.

No information on batch numbering is provided. An explanation of the batch numbering system, including information regarding any pooling of harvests or intermediates and batch size or scale should be provided.

The current narrative description and the flow-chart do not sufficiently describe the L19TNF drug product manufacturing process. Some materials added during the process are not described. In the flow-chart, and in-process controls for each step are incomplete. Only "filled volume" and release testing is mentioned. An update of the process flow-chart with the respective information is requested. Information on process parameters is also incomplete. All process parameters with respective ranges should be added to the dossier. In the narrative description of the manufacturing process the individual process steps are not described. An updated, more detailed description of the manufacturing process is requested.

Several aspects of the drug product manufacturing process require clarification. These include how excipient composition is controlled, how solution homogeneity is ensured during filling, and alignment between batch volumes and the number of filled units. The description of the filling process requiring clarification, and details on sterilisation methods, use of process gases, primary packaging, filter integrity testing, and visual inspection procedures are either missing or incomplete. Additionally, the aseptic process simulation is only described in general terms without specific data. Further information is requested to ensure full process understanding and compliance.

The manufacturer is asked to address this and clearly confirm that no blending of DS batches for a DP batch will occur. Appropriate specification for some materials should be set in 3.2.P.4 and the method of sterilisation of the gasses during production be indicated. The applicant should describe and report results of the aseptic process simulation of their manufacturing process.

Overall, the information for the L19TNF manufacturing process is considered limited and does not allow for thorough assessment.

Controls of Critical Steps and Intermediates

Process parameters and in-process testing are provided for sterility steps and filling. Product purity monitoring should be also approached

A risk assessment should be conducted to define PP, IPC and CQA.

Process Validation and/or Evaluation

The DP manufacturing process has been validated using three consecutive L19TNF commercial scale drug product batches. The validation results for sterile filtration and filling should be provided in the eCTD format. A narrative description of the preparation steps of DS should be documented.

Sterilisation validation: All single-use systems, vials, stoppers and flip-off caps are sterilised by their respective suppliers. The information provided on these components can be accepted. All removable stainless-steel components (such as hoppers, chutes, and vibrating cups) are cleaned and sterilised through a validated cycle before each use. Information on sterilisation method and sterilisation validation needs to be provided for all product-contact materials used during filling.

Extractables/leachables: Before approval, results from extractables/leachables studies for the DS and DP primary container closure system and the identified high-risk single-use equipment used during manufacturing need to be provided – demonstrating that the material is compatible with L19IL2.

Transport validation: transport validation is ongoing.

3.1.5.3. Product specification, analytical procedures, batch analysis

Specification(s)

The release specification for L19TNF drug product include tests for visual appearance (visible particles, turbidity, colour), identity (ELISA), purity and impurities (SDS-PAGE, SEC), pH, osmolality, biological activity (immunoreactivity, TNF bioactivity), total protein, sub-visible particles, extractable volume, endotoxin, sterility and container closure integrity testing. The aforementioned parameters are also tested for stability studies. The majority of the test parameters specification, analytical methods, analytical procedures and their validation are identical to the DS section, thus the same assessment applies, and concerns raised on test methods and specifications for DS are also applicable to the DP. As a consequence of this and data lacking on DS process development and validation, purity and potency of the DP are not ensured.

Analytical procedures

Validation of analytical procedures

Improved analytical method description and validation reports are submitted for all methods. Method descriptions and validation reports are still inadequate in certain respects. Based on those issues the control of identity, potency and purity is still considered insufficient.

Together with the site transfer from Montarioso to Rosia, almost all analytical methods were changed. Hence adequate method bridging results should be provided.

Regarding bioactivity method: It is acknowledged that the "old" potency method has been transferred from Montarioso to Rosia site. A transfer validation summary and report are provided. The bioactivity

test was simultaneously run at both sites to measure inter-laboratory precision. The following parameters were verified: specificity, linearity, precision (repeatability, intermediate precision and reproducibility), robustness, and range. According to the validation report, the assay has been successfully transferred. However, the method is rather variable and the validation is not considered appropriate/successful. The method should be improved and adequately validated.

The sterility test for the L19TNF drug product has been verified. For the container closure integrity testing reference is made to the compendial status. Since this method is not listed in the European Pharmacopoeia, method validation/verification results were provided. A revalidation with a different filter is requested.

Batch analyses

Batch analyses data are provided for L19TNF clinical and process validation batches. Batch analysis data for the clinical drug product lots manufactured at Montarioso manufacturing facility are provided Furthermore, batch analyses data is provided for three process validation batches (manufactured in 2023. All batches comply with the pre-established specifications valid at the time of testing.

A summary table with the batch information/batch history (i.e. batch size (number of vials), batch size (volume), manufacturing date (dd/mm/yyyy), manufacturing site, DS batch number, batch use) should also be added in the eCTD format.

Characterisation of impurities

No new impurities have been introduced during the L19TNF drug product manufacturing process and reference is made to Module S.3.2 of the dossier. This approach can be considered acceptable.

Evaluation of elemental impurities is currently ongoing. The dossier needs to be updated with results from the elemental impurities evaluation before approval.

The applicant has provided a risk evaluation concerning the presence of nitrosamine impurities in the finished product. The risk was considered low.

Justification of specifications

Acceptance criteria for L19TNF DP are based on an analysis of historical release data (three sigma approach) on batches produced in Montarioso This approach was used for impurity, immunoreactivity, total protein, bioactivity, and HCP.

During the evaluation procedure, the applicant was requested to tighten certain specification acceptance criteria, while broadening the range of others, in order to ensure appropriate control of the product's quality attributes.

Control of Excipients

The development and control sections of DS and DP do not include any information on as to how the quantitative composition of the DP with respect to excipient composition is under control. Controls on pH and osmolality alone do no provide sufficient assurance that the composition is consistent among batches. The applicant refers to standard manufacturing steps such as weight checks to justify that excipient composition is under control and refrains from factual control of stabilising compounds apparently as they would not consider this relevant for the safety of the product. While this reasoning can be contended, it is proposed not to further pursue on this issue.

Reference standards or materials

The same reference standard is used for both L19TNF drug substance and drug product testing. Information regarding the internal reference standard is included in Module S.5 (Reference Standards or Materials). Overall, information regarding the L19TNF internal reference standard is considered insufficient and not in line with the requirements of ICH Q6B guideline.

Container closure system

The primary container closure selected for use with the L19TNF drug product consists of a 3 mL glass vial closed with a bromobutyl rubber stopper and a light blue flip-off cap. Specifications for the primary container closure system (vial and rubber stopper) and the flip-off seal were added to section P.7 of the dossier. The product-contact material complies with compendial requirements. Technical drawings for the vial components are provided. Inconsistencies in the description of the container closure system and rubber stopper are noted in in the dossier. A harmonised description of the primary container closure system should be envisaged and respective dossier Modules should be corrected.

Information on the suppliers should be provided in the eCTD format.

Extractables/leachables studies for the L19IL2 DP primary container closure system are ongoing. Detailed information should be included in Module P.2 and a short summary or reference in Module P.7.

L19TNF drug product is part of a combination pack composed of L19IL2 (bifikafusp alfa) and L19TNF (onfekafusp alfa) drug product. The secondary packaging for the L19IL2/L19TNF combination pack consists of a temperature resistant and water-repellent carton box. Inside the carton box, the vials are securely placed in a thermoformed PVC tray. Schematic pictures are provided. The information has been added to the updated dossier for the combination pack. Reference to the secondary container closure system should also be added to individual sections P.7 for L19TNF and L19IL2.

3.1.5.4. Stability of the product

An ongoing long term stability study has been provided to determinate the stability of commercial batches of L19TNF solution for injection at the proposed storage condition. The proposed long-term storage condition for L19TNF drug product is -80°± 5°C. A shelf-life of 60 months is proposed, based on long-term stability data generated from historical data manufactured at Philogen S.p.A Montarioso and data from three process validation batches manufactured at Philogen S.p.A. Rosia (Siena, Italy).

Studies at other conditions were not presented then the claimed storage condition cannot be justified.

The selected stability assays resemble the parameters selected for release specifications.

The selection of the stability assays was not justified and limited information on the stability-indicating properties of the methods has been provided in the dossier. From the results provided in the dossier, it is unknown which tests are stability indicating.

The container closure system used for L19TNF drug product stability studies is representative. For clinical batches manufactured at Montarioso site, 3 mL glass vials with a bromobutyl stopper were used. For PPQ batches manufactured at Rosia site, 3 mL glass vials with a Fluorotec-coated bromobutyl stopper were used.

At the recommended storage condition, all results were within specifications. Purity is slightly decreasing over time. However, given the uncertainties regarding the manufacturing process transfer, analytical methods and comparability, the approach for shelf-life setting is not considered acceptable.

Accelerated stability study was carried out and stopped at that time point since an "out of specification" result was obtained for TNFα bioactivity and purity.

Additional stability studies were conducted under stress conditions: a short-term excursion study at refrigerated and room temperature conditions, and a freeze/thaw study. The short-term excursion study was conducted with the aforementioned clinical batches. It was demonstrated that L19TNF drug product, is stable up to 48 hours at 5°± 3°C or up to 6 hours if stored at room temperature. A freeze/thaw stability study has been conducted for L19TNF. A summary of results and actual data should be included in the dossier.

A photostability study in line with the requirements of ICH Q1B guideline has been conducted. The analytical methods used in this study include visual appearance, purity by SEC, SDS-PAGE, total protein assay, bioactivity, identity by ELISA and immunoreactivity. No further information on the photostability set-up is provided.

Results demonstrate that the samples are light-or temperature-sensitive.

Section 6.4 of the SmPC contains the statement "Store in the original carton in order to protect from light".

3.1.5.5. Adventitious agents

No TSE risk materials have been identified. FBS was used at initial cell cultivation, cloning and selection steps. However, a serum-free cell bank has been established. In summary, compliance with TSE-Guideline EMEA 410/01 rev03 has been demonstrated. The TSE statement is provided and acceptable.

Cell banks have been tested according to ICH Q5A(R2). Detection of endogenous retrovirus like particles is expected for the CHO cell line.

The virus testing program for unprocessed bulk and drug substance is in line with ICHQ5A (R2). Testing of unprocessed bulk on MVM is in line with recommendation from ICHQ5A(R2).) "Three detector cell line" report assay needs to be provided.

The choice of model viruses in considered in line with ICHQ5A(R2). Controls for cytotoxicity and interference of test materials with virus detection have been performed. 3 Steps of the manufacturing process have been evaluated for virus reduction. Adequate summary reports on down-scaling and virus reduction experiments have been provided.

Clarification is requested on virus carry-over runs.

End-of-lifetime resin studies were performed with X-MuLV and MVM for other purification steps and showed comparable inactivation capacity. Raw data should be included in the dossier.

A sufficient excess capacity of the manufacturing process to remove retrovirus-like particles has been demonstrated.

3.1.5.6. GMO

Not applicable.

3.1.6. (L19IL2/L19TNF Drug product combination pack)

The MAH presented updated information in separate Modules P.1/2/3/7. This includes, but is not limited to, unequivocal information regarding the exact contents (one vial of each individual DP), secondary packaging, site of manufacture ('Rosia'), and compatibility.

Description and Composition of the Medicinal Product:

The proposed medicinal product consists of L19IL2 (bifikafusp alfa) and L19TNF (onfekafusp alfa) presented as two separate drug products (DP).

The L19IL2 DP is a liquid, sterile, nonpyrogenic preparation for intra-tumoral administration, containing the L19IL2 immunocytokine as an active substance.

The L19TNF DP is a liquid, sterile, nonpyrogenic preparation for intra-tumoral administration, containing the L19TNF immunocytokine as an active substance.

Both L19IL2 and L19TNF DPs are intended for use in combination. The Nidlegy combination pack is presented in a carton box containing two 3mL clear glass vials: one for L19IL2 identified by a white flip-off seal cap and one for L19TNF identified by a light blue flip-off seal cap.

Pharmaceutical Development

Compatibility:

An in-use stability/compatibility study covering the mixed products has been conducted and is presented in the dossier for the combination pack. The mixture of both products has been analysed after storage of 24 or 48 hours at RT or refrigerated. Analytical procedures include visual appearance, protein concentration, general test, purity, identity, immunoreactivity (L19IL2 and L19TNF), and bioactivity (L19IL2 and L19TNF). In this study, the L19IL2 and L19TNF DP mixture stored at both RT and at 2-8°C was confirmed to be stable and within the defined specifications for up to 48 hours. Overall, compatibility is insufficiently addressed. The applicant relies on data which have been collected as part of in-use stability, to also substantiate compatibility. However, these data do not sufficiently support compatibility and in-use stability. The MAH should substantiate compatibility of L19IL2 and L19TNF with a properly designed study and employing appropriate (compatibility and stability) indicating methods.

Manufacture

Manufacturer(s):

Nidlegy combination pack is packaged at Philogen S.p.A (Loc. Rosia – Via Bellaria, 35, 53018 Sovicille (SI), (Italy)). Respective proof of GMP compliance has been provided.

Container Closure System:

The secondary packaging for the L19IL2/L19TNF combination pack consists of a temperature resistant and water-repellent carton box. Inside the carton box, the vials are securely placed in a thermoformed PVC tray. Reference to the secondary container closure system should be in eCTD format

Stability

The applicant has provided additional data and clarification regarding the in-use shelf-life. Apparently, the same data are also used to address compatibility, although this is not explicitly discussed. Given

the uncertainties regarding the manufacturing process transfer, analytical methods and comparability, the approach for shelf-life setting is not considered acceptable.

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

Nidlegy is a product consisting of the combination of two individual immunocytokine molecules, bifikafusp alfa (also denominated L19IL2) and onfekafusp alfa (also denominated L19TNF), which are mixed together immediately prior to intralesional injection.

In order to facilitate the review and to ease comments, two separate quality assessment reports are prepared. The overview and conclusion herein cover both molecules as well as the combination package. The structure of the dossier and the dossier content follow the requirements as outlined the NTA Volume 2B but major gaps are still identified regarding the information provided by the applicant resulting in multiple major objections for both molecules.

Module 3 is not approvable in its current form, as it lacks correctness and scientific consistency. Therefore, descriptions (like e.g. process descriptions), claims (like e.g. specifications and storage periods), and supporting information cannot be reliably assessed and interpreted. The applicant must carefully review the Module 3 with respect to correct relevant information presented in an integrated manner including the correctness of multiple hyperlinks.

Major gaps are identified regarding the information provided by the applicant resulting in multiple major objections.

The manufacturing process descriptions of the drug substances lack essential details that are needed to assess their quality and their manufacturing process consistency and information critical for the quality assessment has to be provided. The information provided on manufacturing process development is inadequate and does not give confidence that the proposed manufacturing process can deliver a consistent product with an adequately controlled drug substance quality. Similarly, due to inadequate history of manufacturing process development it is not possible to assess comparability of the preclinical and clinical batches with the proposed commercial manufacturing process. The information provided by the applicant on the characterisation of the drug substance/ product and its degradation is too limited to provide sufficient basis for a control strategy.

The L19IL2 Drug Substance (DS) is produced in mouse hybridoma host cells whereas the L19TNF Drug Substance (DS) is a produced in Chinese Hamster Ovary host cells (CHO), cultured in bioreactors. The cell culture starts from one vial of the cell bank, which is extended until the bioreactor is inoculated. Overall, the description of the manufacturing process and process controls provides an overview but still lacks important details. For process validation three batches were manufactured according to the pre-defined conditions. Results obtained during manufacture of the three validation batches demonstrate consistency, but certain aspects were not addressed yet during validation, e.g. impurity clearance. The applicant followed a traditional approach regarding process validation and it is acceptable.

Philogen has been producing the fusion proteins in the Montarioso manufacturing site up to 2022. The L19TNF material produced in Montarioso has been used for all clinical studies conducted with L19TNF alone or in combination with other drugs. The clinical development batches manufactured during this period should be clarified. In 2019, the manufacturing process of L19TNF was transferred from Philogen's facility in Montarioso to Philogen's new facility in Rosia (Siena, Italy). For L19TNF the material manufactured at the new Rosia plant cannot be considered sufficiently comparable to the

material manufactured at the Montarioso plant and used in clinical studies and thus major objections remain.

In order to identify QA which are important to ensure safety and efficacy of the molecules a thorough characterisation of the molecules needs to be provided, which is missing for the two molecules. Usually characterisation is focused on the intended molecule and further characterisation of isoforms are performed if warranted. Remaining limitations of the characterisation also led to a major concern regarding the control of the drug substances. Additional quality attributes may need to be included in the specifications depending on the outcome of the characterisation and certain acceptance criteria should be revised. The analytical control of Drug Substances for identity, potency and purity is considered inadequate. Insufficient information is provided in method descriptions and method validations. Several acceptance criteria are not well defined and the justification of these criteria is lacking.

The L19TNF and L19IL2 reference materials is used as internal control in several analytical methods and were prepared using a pool of the 3 consecutive Process Validation (PV). The information concerning the reference standards is sufficient.

The proposed shelf life of both drug substances is currently not acceptable but the applicant is invited to submitting additional (final) date with the responses. The final shelf life to be granted depends on the data provided during the review process. The shelf-life claim of drug substances is not supported since assessment of the stability data is not possible due to the lack of data on potential degradation, inadequate control of potency and purity, uncertainties regarding analytical techniques and stability data provided.

Nidlegy is a combination pack composed of L19IL2 and L19TNF drug product. L19IL2 drug product is presented as solution for injection for intra-tumoral administration in a glass vial with a bromobutyl rubber stopper. L19TNF drug product is presented as solution for injection for intra-tumoral administration in a glass vial with a bromobutyl rubber stopper. L19TNF (0.4 mg/1 ml/vial) is formulated with a sodium/potassium buffer, stabilisers (mannitol, polysorbates, glycerol) and the chelating agent EDTA.

In 2019, the manufacturing processes of both L19IL2 and L19TNF were transferred from the applicant´s facility in Montarioso to a new facility in Rosia (Siena, Italy). The impact of changes between the clinical and commercial scale drug product batches on quality, safety and efficacy needs to be discussed by the applicant.

Although the DP product manufacture is rather straightforward significant lack of information in the process description and its validation has been identified. The drug product manufacturing processes are standard aseptic filling processes used for this type of products. The aseptic filling process has been validated by media fill simulations. Overall, the information for the DP manufacturing processes lacks some important information. Therefore, considering the number of OCs this topic is still raised as MO. The process steps are controlled by process parameters and in-process testing. The strategy to control the processes is incompletely described.

Appropriate control of DP is not guaranteed. As issues on specification, analytical methods and their validation are identical to the DS section the same assessment applies and concerns raised on test methods and specifications for DS are also applicable to the DP. In line with the concerns raised on these test parameters for drug substance, the drug product section should also be updated.

The design of the stability study is criticised. The proposal to store at -80 °C is not corroborated by information on degradation of the product. The shelf-life claim for both drug products should be based on the stability data obtained with the representative commercial scale batches. Again, the applicant is invited to submitting additional (final) date with the responses. The final shelf life to be granted depends on the data provided during the review process.

Nidlegy is a product consisting of the combination of two individual immunocytokine molecules, bifikafusp alfa (also denominated L19IL2) and onfekafusp alfa (also denominated L19TNF), which are mixed together immediately prior to intralesional injection. Nidlegy is indicated for the treatment of adult patients with locally advanced fully resectable melanoma. Nidlegy is provided as a combination pack including two individual single use vials of 1 ml each which contain, respectively:

- 2.17 mg/ml of bifikafusp alfa
- 0.40 mg/ml of onfekafusp alfa

Updated information is provided for the combination pack of L19TNF and L19IL2 in a separate drug product section which is acknowledged. The MAH should substantiate compatibility of L19IL2 and L19TNF with a properly designed study and employing appropriate (compatibility and stability) indicating methods.

In conclusion, from quality point of view the application for Nidlegy in the treatment of adult patients with locally advanced fully resectable melanoma is not approvable since numerous "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Outstanding Issues (see section VI). In addition, satisfactory answers must be given to the "other concerns" as detailed in the List of Outstanding Issues.

3.2. Non-clinical aspects

3.2.1. Introduction

Nidlegy is a product consisting of two individual immunocytokine molecules, bifikafusp alfa and onfekafusp alfa, which are mixed immediately prior to intralesional injection.

Bifikafusp alfa (L19IL2 = ZK269136) is a recombinant fusion protein composed of two portions: L19, a human monoclonal antibody fragment in single chain variable fragment (scFv) format, and IL2, the human cytokine Interleukin-2. Bifikafusp alfa is produced in mouse hybridoma host cells by recombinant DNA technology.

Onfekafusp alfa (L19TNF = ZK342019) is a recombinant fusion protein composed of two portions: L19, a human monoclonal antibody fragment in the scFv format, and TNFα, the human cytokine Tumour Necrosis Factor alpha. Onfekafusp alfa is produced in Chinese hamster ovary cells (CHO) by recombinant DNA technology.

The antibody moiety (L19) in both pharmaceutical ingredients binds with high affinity to the alternatively spliced EDB domain of fibronectin, which is a well-characterised marker of neoangiogenesis and is abundantly expressed in the perivascular stroma of most solid tumours.

The antitumoural mechanism of action of the product is based on the specific activities of the two cytokine moieties, IL-2 or TNF.

3.2.2. Pharmacology

3.2.2.1. Primary pharmacodynamic studies

The applicant has provided study reports R063 and R064 to show that L19IL2 and L19TNF have binding affinity and specificity for ED-B protein in different species. These studies indicated full alignment of 89 amino acids of ED-B between human, mouse, rat, rabbit, dog and cynomolgus monkey. In addition, L19IL2 and L19TNF showed reproducible binding kinetics to recombinant ED-B across six batches, as evaluated with Surface Plasmon Resonance. Using ELISA, specificity of L19IL2 and L19TNF for ED-B was shown in comparison to 20 other proteins, including ED-A. Here, the applicant states that specificity of L19IL2 and L19TNF for ED-B was shown by ELISA in comparison to 20 "randomly picked available recombinant proteins", including the ED-A splice variant (which is the target of the F8 fusion domain used in in vivo studies). Besides showing specificity, in order to generate meaningful safety-relevant non-clinical in vitro data, it would have been more appropriate to rationally select off-targets for binding experiments based on similarity (structure, location and level of expression, protein family, etc.) rather than on availability. Nevertheless, specificity of L19 for ED-B has been shown.

With flow cytometry, it was further shown that L19IL2 could bind to four cell lines transduced with membrane-bound ED-B but not to non-transduced cell lines. The binding affinity of L19IL2 to ED-B was 2.5 nM, for L19TNF this affinity was 1.5 nM. In study report R077, the binding affinity of three concentrations of L19IL2 and L19TNF to ED-B antigen was evaluated. In this study, using Biacore X100, > 10-fold difference in binding affinity was observed compared to the data in study reports R063 and R064. This has been further discussed in the Quality dossier; the applicant states that this is because of changes in the experimental setup and presents supportive data, which is acceptable.

A cytokine (IFN-γ) release study has been conducted, in which human PBMCs were incubated with different concentrations of L19IL2, L19TNF or the combination, in the presence or absence of ED-B antigen coated on the culture plates (i.e. study report R065). In the absence of ED-B, no IFN-γ expression was observed. In the presence of ED-B, treatment with high concentrations of L19IL2 or L19TNF resulted in some IFN-γ expression, which was increased when conjugated cytokines were used in combination. These data indicate that the cytokines would only be able to induce immune cell activation when L19 would bind to the plates (to ED-B). This is not in agreement with the general (literature-described) effects of IL2 and TNF on immune cells. The applicant did not explain this, for example whether the lack of an IFN-γ response in the absence of ED-B was due to low concentrations of IL2 and TNF. Other markers of immune cell activation (e.g. CD69 expression, proliferation) or other potentially upregulated cytokines/chemokines have not been studied. Although OKT3 was used as a general positive control for TCR-induced IFNγ secretion, the unconjugated recombinant cytokines IL2 and TNFα were unfortunately omitted from the experiments. Altogether, the results from the PBMC study indicate some potentially synergistic effect of the L19IL2 and L19TNF combination, but need to be treated with caution regarding safety/efficacy-related in vivo effects.

In the same study report, the murine cell lines GL-261 (glioma) and SMA-560 (astrocytoma) were treated with increasing concentrations of L19mTNF. Viability of both cell lines decreased in a comparable dose range. No human cell line with L19TNF treatment was used here, the reason for this was not explained by the applicant.

Although the PBMC data indicate that there is a synergistic activity between L19IL2 and L19TNF in the presence of ED-B antigen, this study does not provide additional insight in the binding to or direct

effects of L19IL2 and/or L19TNF on certain immune cells, as requested. It was only shown that the murine version of TNF conjugated to L19 had a direct cytotoxic effect on murine tumour cells, but a similar experiment with IL2 or with human L19TNF was not conducted. The aim of the raised question was to determine whether IL2 and TNF (when conjugated to L19) can have direct effects on immune cells and tumour cells. Therefore, the question remains and the applicant is asked to provide (*in vitro*) data on the binding of IL2 and/or TNF, when conjugated to L19, to specific immune cells and tumour cells and the related pharmacodynamic effects, or justify the absence of such data.

The in vivo PD program was quite extensive and studied L19IL2 and L19TNF or combination of both in tumour bearing mice. Results from several models are compiled in very short study reports, but are derived from different publications, which are included in the dossier. Despite extensive usage of in vivo models to demonstrate the anti-tumour activity of Nidlegy, only one model was employed reflecting the intended indication and mode of administration described in the MAA.

In the provided report R066, expression profiles of ED-A and ED-B 14 primary human tumours and in five murine tumours were determined. There was comparable expression of ED-A and ED-B in the tumour types, based on visual assessment of microscopic slides only (no quantitative data provided). Therefore, the applicant stated that the F8 antibody (targeting ED-A) and L19 antibody (targeting ED-B) ca be used interchangeably or as surrogate for each other, depending on the chosen cell line. Only one (1) tumour model has been studied in K1735M2 melanoma-bearing mice receiving 1 intralesional injection of untargeted recombinant human IL2 and recombinant murine TNFα as single agents or combination of both, as well as targeted (F8) immunocytokines.

As such, the study from Pretto et al. (2014) can be regarded relevant, as the F8 moiety (binding to ED-A on murine cells) could be seen as a surrogate for L19 binding to ED-B on human tissue. This study could therefore be considered the PoC study for Nidlegy, using the (surrogate) conjugated product combination and the intratumoural route of administration in melanoma. However, report R066, also mentioned that the mouse melanoma cell line K1735M2 (which is also used in Pretto et al., 2014) showed a higher ED-A expression compared to ED-B expression. Since no other melanoma cell lines or primary/metastatic melanoma samples were investigated with this respect, the question remains whether melanomas in general express more of the EDA than EDB antigen, and how expression levels of EDB are correlated with observed efficacy. This has not been addressed non-clinically, it is therefore unclear how much ED-B expression would be needed to retain the cytokines (conjugated) longer in the tumour, and whether this retaining in the tumour would improve efficacy. Taken together, the value of L19 for targeting IL2 and TNF to ED-B expressing tumours and retaining these cytokines in the lesions for a longer time to increase their anti-tumour effect remains unclear. See also assessment of OC341 below.

Moreover, based on the data provided in Pretto et al., higher uptake of F8-IL2 in murine tissues (including SC injected mouse melanoma cells) following IV administration was observed compared to IV-administered L19-IL2. Therefore, available F8 data could overestimate the L19 effect. This was also observed in the *in vivo* data following intratumoural administration (Figure 3 of the publication, see below). In a mouse melanoma model with high ED-A expression (K135M2), F8-conjugated IL2+TNF were more effective (figure b below) than unconjugated cytokines (figure a below), although data are not presented in same graph, making direct comparison more difficult. In a mouse fibrosarcoma model with high ED-B expression, L19-conjugated TNF alone or L19-conjugated IL2+TNF resulted in complete tumour remission in all treated mice (figure d below), but this was also the case with the combination of unconjugated cytokines (figure c below), suggesting no added value of L19 conjugation in this tumour setting.

Based on literature data, the presence of a functional immune system seems to be essential for the efficacy of Nidlegy treatment, although the type of immune cells attributing to the immunocytokine-induced anti-tumour response seems to be dependent on the tumour type. The applicant has not described which cell type(s) will be important in the response against melanoma at different stages. However, we consider that no additional non-clinical discussion on the cytokine mechanisms is necessary. Kinetic data regarding e.g. retention and potential release of the cytokines from the tumour (melanoma) should come from clinical studies.

Systemic use of L19-conjugated cytokines

The presented literature studies indicate that the growth of several tumour types (either SC injected or orthotopically) in mice could be reduced/delayed with systemic L19IL2 and/or L19(m)TNF, while rhIL2 or rmTNF alone or in a mix with L19 (without conjugation) had less or no effect on the tumour growth. As such, the applicant has provided a proof-of-concept that (tumour-)targeted delivery of intravenous IL2 and TNF can result in enhanced anti-tumour effects compared to non-targeted recombinant IL2 and TNF, and that L19IL2 and L19TNF could therefore be used to treat solid or haematological tumours. However, the tumours used in the studies are not all considered equally relevant for melanoma, which is a solid tumour derived from melanocytes (i.e. neuroectodermal cells). The applicant has not described what level of EDB/B-FN is present in melanoma tissue in the primary PD section. Moreover, the animal models (largely immunocompromised, with different tumour burdens) do not fully reflect the clinical situation, and treatment regimens (dose, route-of-administration, duration/cycles, co-medication) used in the studies are considerably different from the clinical protocol. In studies with immunocompetent animals, it is not clear whether antibodies against the human L19 have been induced that could reduce anti-tumour activity of the immunocytokines over time. As such, based on the submitted literature data, no conclusion can be drawn on the combination of L19IL2 and L19TNF regarding an improved anti-tumour effect in melanoma as compared to the combination of unconjugated recombinant IL2 and TNF. This should be addressed in clinical trials.

Intratumoural use of L19-conjugated cytokines

Except for the study from Schwager et al. (2023), all non-clinical studies presented have been conducted with systemic injection of the immunocytokine(s). When IV administration would show antitumour efficacy, it is very likely that intratumoural injection would show antitumour activity. Indeed, intratumoural administration of L19IL2 and/or L19TNF resulted in tumour growth delay or even cure in tumour-bearing mice. Nevertheless, the effect of conjugation of the cytokines to L19 for intratumoural treatment is not clear, as no non-clinical comparison of the efficacy of the unconjugated TNF + IL2 combination versus the L19-conjugated TNF + IL2 combination upon intratumoural administration was conducted. In the melanoma model from Pretto et al., F8-IL2 could be detected in the tumour for at least 5 days, while unconjugated IL2 was generally present for < 3 days within the tumour. From the paper it is not clear whether the used doses (30 µg F8-IL2 and 10 µg unconjugated IL2) can be considered alike with regard to the cytokine level, but the applicant mentioned that equimolar doses were used. Residence time of (un)conjugated TNF could not be determined. L19-conjugated versus unconjugated cytokine persistence was not analysed in the fibrosarcoma model. In the PK data, it was shown that L19TNF has a longer residence time, but L19IL2 was not analysed. As such, the statement of the applicant that L19-conjugated cytokines have a longer residence time when injected intratumourally when compared to IV administration could not be verified non-clinically.

Taken together, the applicant has justified the absence of data generated with L19 conjugated cytokines by referring to F8-conjugated cytokine data. It can be agreed with the applicant that the

concept of anchoring cytokines at the tumour site with a tumour-targeting antibody (either with F8 or L19) is a general one, it is not agreed that F8-related data can be considered fully supportive for L19 use. Thus, the data from Pretto et al. (2014) are considered a general proof-of-concept for tumour-targeting antibodies, but are not sufficient to indicate the value of L19 conjugation in the setting of intratumoural administration in human melanomas. No toxicity endpoints were reported from this study, which could have contributed – in absence of higher efficacy – to establish evidence for an improved safety profile of the conjugated cytokines compared with unconjugated counterparts. The absence of relevant non-clinical PD data must be taken into consideration in the benefit-risk assessment.

Clinically, it appears that L19 conjugation can result in longer retention of the cytokines within the tumour tissue when compared to IV administration. This may lead to reduced dosing frequency in patients (although this seems not to be investigated clinically). However, it is not clear whether the longer retention time is due to the more direct administration route or due to conjugation to L19. In addition, whether this provides more benefit compared to intratumoural injection of both unconjugated cytokines is not clear and is further discussed/assessed in the Clinical section below.

Taken together, the applicant has shown that conjugation of IL2 and TNF to L19 increases efficacy of the cytokines after IV administration. This can be explained by the targeting effect of L19 towards the tumours. However, no (non-clinical) comparison of the efficacy of the unconjugated IL2 + TNF combination versus L19-conjugated IL2 + TNF combination upon intratumoural administration was conducted. It may be argued that, because the cytokines are already injected at their target site, targeting via L19 is no longer necessary. According to the applicant, clinical data show a longer residence time of conjugated IL2 and TNF when injected intratumourally when compared to IV administration. It is however not clear whether this is due to conjugation to L19 or to the different route of administration. The applicant is requested to discuss the added value of L19 for intratumoural administration.

Results of the TCR studies:

In cross-reactivity studies with human tissues using three healthy donors, L19 (either conjugated to IL2 or TNF) primarily bound to connective tissue in active female reproductive tissue and to some immune cells. This distribution is in accordance with existing knowledge on the presence of ED-B expression. No evaluation of tissue binding of IL2 or TNF alone was evaluated, as Nidlegy should only contain conjugated cytokines (see assessment of degradation products in DP, as described in the Quality part).

Data related to L19 binding to its target (ED-B) has been evaluated in the non-GLP IHC study presented in the secondary PD section of the dossier, although this could have been part of the primary PD. We have the following concerns (the first OC is a combination between non-clinical and clinical):

Using 24 primary and 29 metastatic melanoma samples, low intensity L19 staining in the basal lamina of the (primary) tumours was observed. In metastatic lesions, clear L19 (diffuse) staining of interstitial stroma was noted, which was not only related to the vasculature. No staining was observed in five normal skin samples nor with a negative control antibody.
The applicant explained that in study 1530, both primary and metastatic melanoma lesions were investigated and that most lesions expressed ED-B protein. However, in the immunofluorescence analysis (Figure 11.1 of the report) the two primary melanoma examples are not (visually) staining positive for L19, in contrast to the two metastatic melanoma examples. Moreover, the analysis of the

full sample panel (Figure 11.2 of the report) indicates that around 95% of the primary lesions and around 70% of the metastatic lesions show no or only weak L19 staining. Non-clinical *in vivo* data in animals (e.g. Pretto et al., 2014) used conjugated cytokines in tumours with strong target antigen expression to show the proof-of-concept. The applicant considers that the level of antigen may not be that important when the intratumoural route of administration is used, in contrast to e.g. systemic injection. This argumentation can be followed, but the reasoning from the applicant is fully based on expectations/assumptions. It would have been of value when the residence/efficacy of L19-conjugated cytokines was shown to be superior to unconjugated cytokines in e.g. the murine melanoma model K1735M2 (used in Pretto et al., 2014), as this cell line had low ED-B expression.

At least non-clinically, the applicant has not actually shown that IL2 and TNF reside longer in tumours with weak ED-B expression when cytokines are conjugated to L19. As such, whether L19 conjugation is of value in tumours with low ED-B expression and whether the target population has sufficient ED-B expression for the cytokine anchoring concept of L19 remains unclear. Reference is made to the assessment of the clinical data on efficacy of intratumorally administered L19IL2 and L19TNF in melanomas from patients.

The applicant has not evaluated the binding of L19 to wound healing tissue or (non-tumour) tissues with extensive remodelling. Based on literature, it is very likely that ED-B is expressed in such tissues, because of the presence of angiogenesis.
ED-A and ED-B can be expressed in adult tissues with wound healing or tissue remodelling (next to solid tumour tissues). In addition, in other non-oncological pathologies ED-B expression can also occur. However, literature regarding this expression is sparse. The applicant has evaluated the available literature in report R064. However, this report contains only results from the binding kinetics and specificity of six batches of L19TNF to recombinant ED-B tested by Surface Plasmon Resonance and ELISA. The applicant likely refers to report R067 (in the Toxicology section of the submitted CTD). In this report, six studies are cited. The applicant further mentions that there are several publications on ED-B expression in mouse and rat that were somehow related to inflammation, tissue remodelling and angiogenesis.

Together, these data indicate that ED-B can be expressed in non-cancerous tissue in humans. Nevertheless, the applicant did not discuss the level of ED-B expression in wound healing tissue and in tissue remodelling diseases (in comparison to expression levels in solid tumours). This may not be feasible, as the ED-B expression in cancerous and non-cancerous may be dependent on several patient and disease factors. The applicant is asked to discuss potential on-target off-tumour toxicity when using Nidlegy in tumour patients with wounds or diseases in which ED-B will be expressed.

3.2.2.2. Secondary pharmacodynamic studies

The secondary pharmacology program comprised two (2) GLP-compliant tissue cross reactivity (TCR) studies (report no. 530830 and 530867) in which binding to human tissue was assessed individually with L19IL2-FITC or L19TNF-FITC, respectively. Despite the declaration of GLP compliance given in the study report, these studies were not tabulated in Annex V to the cover letter, and they should be added.

As a general comment, TCR studies should usually be included in eCTD sections 2.6.6.8 and 4.2.3.7.7

A non-GLP IHC study in human tumour issue from melanoma patients used the clinical L19 mAb (described in a publication by Pini et al., 1998, included in the dossier) to assess binding in primary and metastatic lesions, and is discussed above.

3.2.2.3. Safety pharmacology programme

Safety pharmacology studies investigated effects of L19IL2 or L19TNF as monotherapies, but not in combination, in rats. Safety pharmacology endpoints were also included in the toxicity studies in rats and cynomolgus. Effects were recorded on Cardiovascular system, CNS, respiratory system and renal function. The overall safety pharmacology program is acceptable.

Previous findings from non-clinical studies and clinical experience with IV administration of (immuno)cytokines could be reproduced, and could therefore be attributed to the cytokine moieties of the IMPs. During the in-life phase of study no. A34242 (cardiovascular effects of L19TNF on unrestrained, conscious and catheterised telemetered rats exposed to TNFalpha), two (2) animals in the high dose group (1.25 mg/kg) died 24 and 48 h post-dose, respectively. The involvement of TNF receptors 1 and 2 in cardiomyocyte differentiation, signalling and function has been established recently, and suggests differential contributions of the two receptors. The mechanism underlying this effect was not further investigated and remains unclear, also because functional binding of L19TNF to the rat TNFR2 has not been shown. However, no deaths occurred in the other safety studies at the same dose level.

The studies no. A30354, A31036 and A31373 have been conducted in 2005-2006, but reports are marked as DRAFT reports. However, a generic signature page for the final report is attached, dated 2017. The applicant is asked to provide final signed versions of the Safety pharmacology reports A30354, A31036 and A31373 and delete the generic study authentication page.

Since information on IL2 and TNF safety is available in literature, we consider that the conducted safety PD studies are mainly relevant for investigation of any L19-associated effects. No respiratory study was presented for L19IL2, and no hERG studies were presented for both compounds. Based on the biotechnological nature of Nidlegy and the absence of any cardiovascular, respiratory and renal adverse events of special interest in the clinical studies, the absence of these studies can be accepted.

All safety pharmacology studies have been conducted using IV administration route. A non-clinical PK question is proposed (see below) to discuss the relevance of IV dosing studies versus the intended route of administration. Moreover, a toxicology OC is proposed regarding the exposure margins (see below).

With regard to the findings from these studies, it is expected that the applicant will mention all relevant safety findings relevant for the human situation in the RMP, in line with the relevant guideline (Guidance on the format of the risk management plan (RMP) in the EU – in integrated format, EMA/164014/2018 Rev.2.0.1 accompanying GVP Module V Rev.2).

3.2.2.4. Pharmacodynamic drug interactions

No studies were conducted, which is acceptable for biotherapeutics.

3.2.3. Pharmacokinetics

For use in pivotal studies, product-specific validated analytical methods for the detection of L19IL2 and L19TNF in monkey serum have been developed, based on AlphaLISA (L19TNF, Val.rep. R PL 048) and DELFIA methods (L19IL2, Val.rep. R PL 049). Two (2) validated ELISA assays have been developed for detection of anti-L19TNF antibodies (ADA) in monkey serum (Va.rep. R PL 050) or anti-L19IL2 ADA in monkey serum (Val.rep. R PL 051).

Absorption

Pharmacokinetics studies in tumour-bearing mice showed a biphasic profile for L19IL2 and L19mTNF. Both immunocytokines had a T1/2α in the range of 40 – 55 minutes and a T1/2β of few hours.

The pharmacokinetics of L19IL2 and L19TNF were also investigated during the toxicology studies. In rats, the maximum concentration of L19IL2 (Cmax) was reached around 1-2 h after the start of dosing and the terminal elimination half-life (T1/2el) was estimated to be around 1.35 to 2.12 hours. In Cynomolgus monkeys, the maximum concentration of L19IL2 was reached 5-30 min after the end of the infusion with a complete clearance from the circulation within 24 h, which is in line with the observations in rodents. Pharmacokinetics of L19TNF in Cynomolgus monkeys were challenging due to the low dose administered during the toxicology studies which often resulted in serum concentrations below the limit of detection. In one study, L19TNF was quantifiable up to 6 h post administration. The peak concentration is reached immediately after the end of administration and complete clearance is observed within 24 h.

Single-dose TK parameters are reported only for L19TNF after IV infusion into cynomolgus monkeys. The test item seems to distribute only in the centralised compartment, but exhibits a biphasic elimination (slow initially, faster after 8-12 hours) which is below LOD after 24 h. Estimated terminal half-life is approx. 1.7 – 1.8 hours. The male animal showed slightly higher exposure than the two females. At the dose of 0.4 mg/kg no toxicities were observed.

Biodistribution

Biodistribution studies were conducted comparing targeted vs. non-targeted variants of L19IL2 (Report no. 896) and L19mTNF (Report no. 899) in tumour bearing mice. The results showed selective accumulation of radio-iodinated targeted cytokines in the tumour vasculature of EDB-FN expressing tumours via the L19 moiety.

The tumour/blood ratio of L19IL2 and L19mTNF is shown, and a microautoradiography of IMPs in an F9 teratocarcinoma is provided. Data is identical to data published in the included manuscripts by Carnemolla et al., 2002 and by Borsi et al., 2003.

In report r075 radio-labelled L19TNF (~6 ug) was injected into the lateral tail vein of 129/SvEv mice bearing subcutaneously implanted F9 lesions. Biodistribution was analysed after 24 hours in resected tumour and organs (liver, lung, spleen, heart, kidney, intestine, and blood) using a gamma counter. 26.9% of the injected dose per gram of tissue was retrieved in the tumour (not corrected for tumour growth), versus 0.9-3.54%/g in the other tissues, indicating a tumour-specific uptake of L19TNF.

In report r076 radio-labelled L19IL2 (~30 ug) was injected into the lateral tail vein of 129/SvEv mice bearing subcutaneously implanted F9 lesions. Biodistribution was analysed after 24 hours in resected tumour and organs (liver, lung, spleen, heart, kidney, intestine, and blood) using a gamma counter. 3.23% of the injected dose per gram of tissue was retrieved in the tumour (not corrected for tumour growth), versus 0.21-0.65%/g in the other tissues, indicating a tumour-specific uptake of L19IL2.

The results show that L19 coupled to TNF and IL2 accumulate in F9 lesions following intravenous administration, indicating that targeting is possible for certain tumour types. Since uncoupled IL2 and TNF were not included in this study, it is not clear whether anchoring to L19 results in increased tumour uptake. In addition, results in F9 lesions (with high EDB expression) may not be translatable to other tumour types, especially melanomas with low EDB expression. The results of these studies are

considered a general proof-of-concept for tumour-targeting antibodies, but are not sufficient to indicate the value of L19 conjugation in the setting of human melanomas.

No further data are provided regarding the dose response part of study 899. The applicant is therefore requested to provide the results of the dose response part of study 899.

The applicant has provided 2 additional studies with intralesional administration of L19IL2 and L19TNF.

Report r066 describes the expression profiles of EDA and EDB in various primary human tumours and murine tumour models and compares the efficacy of the intratumoural administration of, amongst others, recombinant IL2, recombinant TNF, recombinant IL2 + recombinant TNF, L19IL2, L19TNF and L19IL2 + L19TNF. The expression pattern of EDA and EDB is virtually identical in all primary human tumours and murine tumour models, except for the K1735M2 mouse melanoma model that showed a significantly higher expression of EDA compared to EDB (EDB expression was low).

Unfortunately, L19IL2, L19TNF and L19IL2 + L19TNF were not tested in the (for the human indication more relevant) murine K1735M2 melanoma model, but only in Wehi-164 tumour-bearing mice. In this model, intratumoural injection of recombinant TNF alone already cured 3 out of 4 mice while the combination of non-targeted IL2 + TNF cured 4 out of 4 mice. This study therefore is not able to show a superior performance of (the combination of) L19-targeted cytokines over the non-targeted combination (see also discussion above "Intratumoural use of L19-conjugated cytokines").

Report r079 describes a biodistribution study using a radiolabelled formulation of L19TNF to evaluate its *in vivo* tumour-residence time upon intratumoural administration. Radio-labelled L19TNF or TNF was injected intratumorally in mice bearing subcutaneously implanted CT26 carcinoma lesions. Radioactive signals in tumour and blood were measured after 30 minutes, 2 hours and 6 hours. Results show that L19TNF has an extended tumour residence time compared to the untargeted recombinant TNF. The EDB expression of CT26 carcinoma cells is unknown and therefore, it is unclear whether the increased tumour-residence time may also be translated to tumours with weak ED-B expression (see also Q334). Nevertheless, the study provides some evidence that conjugation of TNF to L19 can increase tumour-residence time. Unfortunately, such proof was not provided for IL2.

Metabolism

No studies on metabolism were conducted, which is acceptable for biotherapeutics.

Excretion

This section is normally not applicable for biotherapeutics. However, the monomeric size of the molecules (42 - 44 kDa) would in principle allow for renal clearance mechanism. Also, the applicant states that: *"Further studies on the pharmacokinetics of L19IL2 and L19TNF in rats and Cynomolgus monkeys confirmed a kidney-mediated clearance and a biphasic blood clearance profile with a rapid α phase of high amplitude and a slow β phase which expected for systemically administered L19IL2 and L19TNF."* Reports R073 and R074 were provided, which describe a distribution/excretion study of L19TNF and L19IL2, respectively. In this setting, IV injection of radio-iodinated L19TNF into the tail vein of Balb/c mice was followed by euthanisation and microdissection after 30 min post-injection. The radioactivity taken up into liver, spleen, kidney, feces and urine was determined and showed similar distribution of L19TNF (mean 9-12% ID/g) in all organs besides feces, where no significant amounts of radioactivity were measured. In contrast, following administration of L19IL2, around 65% ID/g of radioactivity was detected in urine, and less than 6% ID/g in all other organs. Since the L19 moiety is present in both compounds, the differential uptake of L19TNF especially into liver and spleen indicates a substantial contribution of TNF-binding activity in vivo. For substances

targeting TNF receptors, the liver is a known target organ of toxicity. Consequently, hepatotoxicity was detected for L19TNF in the non-clinical toxicity study 8417185 (increased liver enzymes), and this is corroborated by the findings of the distribution study.

Pharmacokinetic Drug Interactions
No studies were conducted, which is acceptable for biotherapeutics. A general remark was introduced regarding the ability of cytokines to regulate CYP-proteins, which could theoretically lead to effects on PK of other (small) molecules.

Discussion on Pharmacokinetics
Pharmacokinetics of L19-scFv, L19IL2 and L19TNF have been evaluated during tissue biodistribution studies in mice using radiolabelled preparations. L19IL2 TK parameters were determined based on serum pharmacokinetics sampling during the toxicology studies in Sprague-Dawley rats and Cynomolgus monkeys. The TK properties of L19TNF were investigated only in toxicology studies in cynomolgus monkeys.
For all pharmacokinetics and tissue distribution studies L19IL2 and L19TNF (or L19mTNF murine surrogate) was administered intravenously in analogy to what was done during early clinical trials. Analytical procedures to detect the test items were validated (reports are provided) and deemed suitable for the intended purpose.
The distribution of radioactive tracer-labelled 125I-L19IL2, 125I-L19TNF and 125I-L19(scFv) was only evaluated following intravenous administration in tumour-bearing mice, more importantly teratosarcoma bearing mice. This study only shows targeted expression to a tumour type with high EDB-FN expression (which could be expected). Results can, therefore, not be translated to every other tumour type, especially not to tumour types with lower EDB-FN expression. Also for these (non-GLP) studies, only very minimal study details are provided (no individual data, no results of the dose response part of study 899, no description of organs analysed nor distribution profile to these organs in study 896). The applicant should be requested to provide these data, if available (OC already proposed).

Most importantly, Nidlegy is to be administered by intralesional injection into cutaneous, subcutaneous, and/or nodal lesions. Intratumoural administration of Nidlegy in melanoma is expected to be associated with a long residence time. It is described in Pretto 2014, that the antibody moiety mediates a prolonged residence time of cytokines in the injected lesions (derived from a melanoma cell line). However, this has only been described for F8-IL2 vs IL2, but not for L19-IL2 and L19-TNF. Additional preclinical data are only available after intravenous administration. No justification for the absence of intratumoural (or subcutaneous) studies has been provided. Moreover, no discussion has been provided on the meaning of the results following intravenous administration for (repeated) intratumoural (melanoma)administration.

The PK/TK properties of bifikafusp alfa (L19IL2) and onfekafusp alfa (L19TNF) have been investigated in one single-dose toxicity study in rats, and one single-dose study in cynomolgus monkeys (L19TNF), both as single agents. Several repeat-dose toxicity studies in rats and cynomolgus monkeys were conducted to test L19IL2 and L19TNF also as single agents. Only one study assessed the TK of the combination. For all pharmacokinetics and tissue distribution studies L19IL2 and L19TNF was administered intravenously in analogy to what was done during early clinical trials.

Only the TK sample analysis after a single dose of L19IL2 in rats (study report no. 665294) provided enough data for calculation of TK parameters. Data from studies of L19IL2 in cynomolgus (reported in

reports nos. 665315 and 665320) were not sufficient for TK determination, mostly attributable to insufficient assay sensitivity.

TK assessment after a single IV dose of 0.4 mg/kg of L19TNF was done as part of an acute toxicity study in cynomolgus monkeys (study report no. 666565). At this dose level, in both animals (1M/1F) no toxicities were observed. TK samples were collected up to 24 hours post-dose.
The calculated elimination half-life of 1.7-1.8 hours is within the expected range for a molecule of the given size. The estimated clearance rate of 9 mL/h/kg is considered low by the applicant, and attributed to suspected saturation of elimination mechanisms and hepatic/renal clearance. These speculations are not supported by data.

TK data of the combination of bifikafusp alfa and onfekafusp alfa were collected in the pivotal GLP toxicology study in cynomolgus monkeys (study report no. 8417185). The mean elimination half-lives of L19IL2 and L19TNF alone or in combination were ranging from 1.8 – 2.9 hours (males and females) and 0.6 – 1.65 hours (males and females), respectively. No to slight accumulation was observed between day 1 and day 43 in all groups, which is expected for drugs with very short half-lives. The relatively shorter half-life of L19TNF in the GLP study compared with the single-dose acute tox study might be attributable to the strongly reduced dose and indicate non-linear PK of L19TNF, presumably caused by TMDD. However, this could not be proven because no study was conducted using several dose levels within one study. The formation of ADA was observed, but did not seriously affect exposure of the animals. Other TK assessments conducted on the single agents in several cynomolgus studies were inconclusive.

Conclusion on Pharmacokinetics
The preclinical PK/TK assessment of Nidlegy was based on IV infusion (slow bolus or 1h infusion) of the single agents in tumour-bearing mice, rats and cynomolgus monkeys, which is not the clinical ROA.

Only very limited PK/TK analyses have been performed, partly since in most studies, assay sensitivity was insufficient. In several of the study reports (IL2: DRF, 665315; 4 cycle, 665320; TNF: prelim inf, 666172; 4 cycle, 665839) samples needed to be diluted 80-160 times, resulting in many samples with concentrations <LOD.

On the other hand, the concentration of L19-IL2 and L19-TNF in majority of samples measured in the 10 week 4 cycle study (in the range of 100-10.000 ng/ml) was far above the ULOQ of the methods of analysis that were used (both with ULOQ of 10.000 pg/ml).

In addition, no data have been provided on the clearance or volume of distribution of L19-IL2. Such basic PK parameters should be provided. Also no adequate data have been provided following repeated dosing of L19-IL2 and/or L19-TNF (i.e. from repeated dosing within 1 cycle: plasma levels were only analysed on day 1 of cycle 1 and 3, but not after multiple dosing within 1 cycle). Nevertheless, considering the short T1/2 and the suggested dosing interval, accumulation is not expected.

The limited data suggest relatively short serum half-lives upon systemic administration, in accordance with the molecular size, and non-linear PK due to TMDD. Considering the actual clinical route of application of Nidlegy (intralesional injection), the submitted PK/TK data are rated not very informative and altogether not very relevant.

3.2.4. Toxicology

3.2.4.1. Single dose toxicity

The toxicity profiles of L19IL2 and L19TNF following IV administration have been investigated in dose range finding (DRF) studies in rat and cynomolgus monkeys.

In the DRF studies, generally, L19IL2 was better tolerated and up to 10.56 mg/kg/dose in rats there were no deaths, although AEs were noted already at 1.32 mg/kg/dose. In cynomolgus, doses of 0.57 and 2.85 mg/kg/dose were associated with transient AEs.

In contrast, L19TNF administration was toxic to rats at doses of 0.77 and 1.54 mg/kg/dose, and at 0.16 mg/kg/dose in cynomolgus. No toxicities were observed at 0.307 mg/kg/dose in rats and up to 0.12 mg/kg/dose in cynomolgus.

3.2.4.2. Repeat dose toxicity

Cynomolgus monkeys treated IV with L19IL2 or L19TNF in 4 cycles over a period of 10 weeks had transient AEs at L19IL2 doses of 0.057 to 0.57 mg/kg/dose, which increased with dose, but did not lead to premature deaths. The highest tested dose of 0.08 mg/kg/dose of L19TNF was well tolerated in monkeys.

Based on the findings of the single agent studies, dose levels of the combination of both test items were defined for the pivotal toxicity study. Low dose combination of L19IL2/L19TNF at 0.15/0.03 mg/kg/dose and high dose combination of 0.57/0.08 mg/kg/dose were compared against same dose levels of the single agents. While the single agents were well tolerated at all dose levels, 1 dose of 0.57/0.08 mg/kg was not tolerated by one female monkey, leading to dose reduction in the following administrations (0.3/0.06 mg/kg/dose). Repeated dose cycles of a combination of 0.15/0.03 mg/kg L19IL2/L19TNF administered twice in each dosing cycle or 0.30/0.06 mg/kg administered once in each dose cycle were also tolerated, but were associated with transient severe clinical observations and impaired movement, increased liver enzymes, decreased red blood cell counts, increased absolute B and T cell counts, and decreased blood pressure and microscopically observed lymphocytic or mixed inflammatory cell infiltrates at the injection sites of most animals at the terminal sacrifice. The effect of the combination of L19IL2 and L19TNF on cytokine secretion (IFNg and IL-6) was considered synergistic. A four-cycle single-dose of 0.30/0.06 mg/kg L19IL2/L19TNF is considered the NOAEL under the condition of the study.

Due to the different route and frequency of administration in the cynomolgus study, no clear translation can be made with regard to the expectedness of described AEs at the clinical exposure.

3.2.4.3. Genotoxicity

No studies were conducted, which is acceptable for biotherapeutics.

3.2.4.4. Carcinogenicity

No studies were conducted, which is acceptable for biotherapeutics.

3.2.4.5. Reproductive and developmental toxicity

Nidlegy is intended for the treatment of patients with advanced cancer. Therefore, in accordance with ICH S9, dedicated studies to assess the fertility and early embryonic development to implantation and the effects on pre- and postnatal development including maternal function were not conducted.

However, assessment of male and female reproduction was conducted with Nidlegy as part of the 10-week toxicity study in cynomolgus monkeys. In cynomolgus monkey, the reproductive organs were assessed as part of the histological examinations. No abnormalities or findings considered related to the test article were observed.

Similarly, reproductive organs of rats were analysed. No morphological lesions were observed that were considered to be related to the administration of the test article.

The effects of L19IL2 and L19TNF on embryofetal development were studied individually in SD rats. Simultaneous administration was not studied. Assessments of the foetuses were carried out at end of the study (GD 20). No NOAEL for fetal development could be set for both test items. Both compounds were found to be teratogenic. L19TNF was also considered embryolethal. Although no information is available regarding EFD effects from studies of the combination of L19IL2 and L19TNF, additive embryofetal toxicity can be expected. A warning should be included in the SmPC of Nidlegy.

Other studies were not conducted.

3.2.4.6. Toxicokinetic data

Exposure to both test items could be shown in all animals and across all dose groups in the pivotal study (study report no. 8417185). ADAs, although frequently observed, did not have a great impact on exposure or elimination rates. Accumulation or sex differences were not observed. The lower AUC and Cmax values in the high dose combo group at day 43 are also attributable to the reduced dose (toxicity of the first high dose led to death of one female monkey). The combination of L19IL2 with L19TNF clearly decreased the systemic tolerability of the test items compared with single compound administration.

TK/PK data comparison rat vs. human and monkey vs. human indicate a Cmax ratio (cyno:human) between 33.2 – 139.4 (L19IL2) and 268 - 1240 (L19TNF), and an AUC ratio (cyno:human) between 25.3 – 655 (L19IL2) and 138.1 – 1279 (L19TNF). Clinical data do not indicate accumulation or reduction of exposure between day 1 and day 22. In the non-clinical studies, a reduced exposure due to production of ADAs is observed on day 43 compared with day 1. As a conclusion, the safety margins for systemic exposure between the doses applied in the pivotal non-clinical studies (after IV injection) and the clinical doses (after intratumoural injection) appear to be sufficiently high to suggest a manageable safety profile.

Local Tolerance

Local tolerance was assessed in three (3) studies, a dedicated local tolerance study in rat using the L19IL2/L19TNF mixture and SC administration, and as part of the repeat-dose toxicity studies in cynomolgus with the single agents administered IV. Local up to severe reactions were observed in all studies. Severe necrosis of the tail tissue after IV application of L19TNF led to premature sacrifice of 5 monkeys. Thus, the potential for injection site reactions during intralesional administration must be considered high, and has been observed in the clinical studies.

3.2.4.7. Other toxicity studies

Antigenicity
The formation of anti-drug antibodies (ADA) was investigated in the toxicology studies and has been discussed in the sections above. Overall, the incidence of ADA formation following repeated dosing was high (48% of animals receiving L19TNF and 77% of animals receiving L19IL2). Despite high incidence of ADA, exposure to the test article was not significantly impaired. It is recognised that ADA occurrence in animals is not predictive for the antigenic potential in humans.

Immunotoxicity
The two test items of this study (L19IL2 and L19TNF) are two immunocytokines that are being developed for the use as a neoadjuvant in resectable metastatic melanoma.

Due to the nature and MoA of the immunocytokines, effects on immune cells are expected, findings on lymphocyte populations were considered part of the pharmacological mechanism of L19IL2 or L19TNF, and none of these findings was considered adverse. Most parameters measured did not indicate a synergistic effect of the combination of the test items, compared with single agent administration.

Only IFNγ and IL-6 concentrations after combined administration of 0.30/0.06 mg/kg L19IL2/L19TNF largely exceeded concentrations after administration of L19IL2 or L19TNF alone, clearly indicated a synergistic effect of the combination on cytokine release in serum. In the setting of cynomolgus monkey toxicity studies, this effect might be considered adverse if put in relationship with the clinical signs observed.

No additional cytokines or chemokines were reported (e.g. IL-8, IL-10, MCP-1), and only after the first dose a 6-h sample was measured, which is usually a post-dose time where many cytokines reach their serum Cmax. The effects of subsequent doses on cytokine release was only assessed after 24 h post-dose. The overall cytokine profile after administration of L19IL2/L19TNF is therefore not considered comprehensive. However, no signs of CRS were reported from clinical studies, which outweighs the missing non-clinical data.

3.2.4.8. Discussion on Toxicity Studies

It needs to be stated that in the pivotal study (in which L19IL2 and L19TNF were administered together) a 4-cycle treatment regimen was used (i.e. IV administration once every 22 days, in contrast to clinically: intralesional administration once every week) and that most adverse effects were at least partially reversible.

Due to the different administration schedule and route of administration in the animals versus patients, safety margins based on dose level have low meaning. Systemic exposure is expected to be very different between the IV and intratumoural administration routes. Clinical data indicate that there is limited leakage of L19IL2/L19TNF into the circulation upon intratumoural injection, though it is unclear how long systemic exposure remains.

TK/PK data comparison rat vs. human and monkey vs. human indicate a Cmax ratio (cyno:human) between 33.2 – 139.4 (L19IL2) and 268 - 1240 (L19TNF), and an AUC ratio (cyno:human) between 25.3 – 655 (L19IL2) and 138.1 – 1279 (L19TNF). Clinical data do not indicate accumulation or reduction of exposure between day 1 and day 22. In the non-clinical studies, a reduced exposure due to production of ADAs is observed on day 43 compared with day 1. As a conclusion, the safety margins for systemic exposure between the doses applied in the pivotal non-clinical studies (after IV injection)

and the clinical doses (after intratumoural injection) appear to be sufficiently high to suggest a manageable safety profile..

Reproductive toxicity

It is stated in the SmPC that both L19IL2 and L19TNF can harm the embryofoetal development. According to Guideline EMEA/CHMP/203927/2005, where data indicate the potential for an increased risk to the developing embryo/foetus when using the medicinal product, such a risk (including the anticipated exposure margin between IV administration in animals and intralesional administration in patients) must be weighed against the potential benefit of the treatment for the pregnant woman, before a contraindication is considered. The contraindications for pregnancy and lactation have been removed by the applicant. The wording of the labelling is not yet fully agreed and adaptations are proposed in the SmPC document.

Risk Management Plan (RMP)

In part II module SII of the RMP, the applicant has provided an overview of the toxicity studies, with quite some detail.

The applicant has now updated the RMP and included a tabulated summary. The provided information is considered too detailed. In line with the RMP guidance, only a high-level summary of significant findings should be given in the table (combining similar studies and without specific study setup information). The tabulated summary on non-clinical safety findings in the RMP should be revised and made more concise. The section below table SII.1 contains unnecessary information and should be deleted.

3.2.5. Ecotoxicity/environmental risk assessment

A justification for not conducting ERA studies was provided by the applicant and is acceptable.

Following the phase I assessment using EMEA/CHMP/SWP/4447/00 Rev. 1, both active substances L19IL2 and L19TNF, consisting of natural amino acids, are unlikely to pose a risk to the environment due to their physico-chemical nature. Therefore, Nidlegy is not expected to pose a risk to the environment.

In their ERA, the applicant made use of the revised EMA guideline on ERA (EMEA/CHMP/SWP/4447/00 Rev. 1). The Phase I decision tree in Fig. 2 (p. 12) of the GL applies. Question 1 of this tree answers with "NO" as both active ingredients are non-natural peptides since they are recombinant fusion peptides. As the applicant does not refer to article 10 of directive 2001/83/EC (Q2), Q3a should then be answered: 'Is the active substance a non-natural peptide/protein?'. The explanation to question Q3a (p. 14) states that 'Peptides and proteins that have been structurally modified using non-natural amino acids to increase biostability are considered non-natural.' Both proteins, bifikafusp alfa and onfekafusp alfa, are recombinant fusion peptides, but they consist of only natural amino acids. This renders them 'natural proteins' in the light of Q3a and following the decision tree Q4 should follow. This seems to be erroneous in this case, the exception for non-natural peptides consisting of natural amino acids should in our opinion already have been given at Q1 as the explanation for Q3a indicates that the answer for Q1 should have been "YES" after all. Nevertheless, in this case it cannot be concluded that the product is unlikely to alter the concentration or distribution of the active substances in the environment (since they are not natural) but it can be stated that, as indicated in the explanation of Q1, due to the

physico-chemical nature of the active substances this product, consisting of natural amino acids, they are unlikely to pose a risk to the environment.

3.2.6. Discussion on non-clinical aspects

This section is integrated in the respective non-clinical subsections of the Overview.

3.2.7. Conclusion on non-clinical aspects

Overall, the **pharmacology** studies provided some evidence that the active substances of Nidlegy are pharmacodynamically active *in vivo*, when administered systemically and intratumourally. Regarding the characterisation of the *in vitro* and *in vivo* mode-of-action and efficacy, several deficiencies were identified, which should be addressed in writing by the applicant. In addition, questions remain regarding the (added) value of L19 fusion when using Nidlegy intratumourally. The applicant has provided a proof-of-concept that (systemic administration of) Nidlegy could result in an anti-tumour response in solid tumours, and that such a response would be more efficacious as compared to unconjugated IL2 and TNF. However, based on the submitted primary and secondary pharmacology data, it is not clear whether Nidlegy also has higher efficacy in melanoma, especially in primary lesions (considering the ED-B expression levels) as compared to a combination of unconjugated IL2 and TNF. Evidence for efficacy specifically in melanoma patients and the added value of L19 conjugation when using intratumoural administration should come from clinical studies and is assessed in the Clinical part.

From a **pharmacokinetic** point of view, the most relevant species for non-clinical safety studies was the cynomolgus monkey. However, only limited PK/TK data are available for L19IL2 and L19TNF, which was at least in part attributable to problems with the TK assays Although a preliminary PK of Nidlegy after systemic (IV) administration could be derived from the pivotal toxicity study. Due to the different (intratumoural) clinical route of application the actual relationship of measured exposure levels with observed toxicities could not be established.

The overall **toxicology** programme revealed several toxicities related to already known side effects of the clinically approved recombinant IL-2 and TNFa cytokines (e.g. Proleukin®, Beromun®). These were mainly related to the immunostimulatory pharmacological actions of IL2 and TNF. The clinical relevance of the non-clinical findings upon IV administration is uncertain. Both immunocytokines were found to be teratogenic, L19TNF was also found embryolethal. The teratogenicity of L19IL2 and L19TNF was in line with the expected L19 target binding in foetal tissues.

In conclusion, from a non-clinical point of view, the application for Nidlegy in the treatment of adult patients with locally advanced fully resectable melanoma could be approvable provided that applicant adequately addresses several other concerns detailed in the list of outstanding issues.

3.3. Clinical aspects

- **Tabular overview of clinical studies**

Besides the initial exploratory trial of L19IL2 as single agent for the intralesional treatment of melanoma (Phase II study NCT01253096, PH-L19IL2-03/09), the clinical development plan of

L19IL2/L19TNF included an exploratory Phase II study in patients with stage III/IV melanoma (PH-L19IL2TNF-02/12), and two phase III studies (PH-L19IL2TNF-01/18 and PH-L19IL2TNF-02/15). No dedicated studies have been performed in special populations.

Additional clinical trials to explore the pharmacology of L19IL2 and L19TNF alone or as combination have been performed in melanoma and non-melanoma patients (see Section 3.3.1).

Table 1. Clinical trials involving intralesional application of L19IL2 or L19IL2/L19TNF in melanoma

Title of the study	Phase	ClinicalTrials.gov Identifier	Status
Phase II, non-randomised, multicenter, prospective study designed to test the efficacy and safety of intratumorally administered L19IL2 in patients suffering from metastatic melanoma.	II	NCT01253096 (Other Study ID Numbers: PH-L19IL2-03/09, EudraCT No. 2009-014799-23)	Completed
A Phase II Study of Intratumoral Application of L19IL2/L19TNF in Melanoma Patients in Clinical Stage III or Stage IV M1a With Presence of Injectable Cutaneous and/or Subcutaneous Lesions	II	NCT02076633 (Other Study ID Numbers: PH-L19IL2TNF-02/12, EudraCT No. 2012-001991-13)	Completed
A Pivotal Phase III, Open label, Randomised, Controlled Multi-Center Study of the Efficacy of L19IL2/L19TNF Neoadjuvant Intratumoral Treatment Followed by Surgery Versus Surgery Alone in Clinical Stage III B/C Melanoma Patients	III	NCT02938299 (Other Study ID Numbers: PH-L19IL2TNF-02/15, EudraCT No. 2015-002549-72)	Ongoing
An Open Label, Randomised, Controlled Multi-Center Study of The Efficacy of Daromun (L19IL2 + L19TNF) Neoadjuvant Intratumoral Treatment Followed by Surgery and Adjuvant Therapy Versus Surgery and Adjuvant Therapy in Clinical Stage IIIB/C Melanoma Patients	III	NCT03567889 (Other Study ID Numbers: PH-L19IL2TNF-01/18, EudraCT No. 2015-002549-72)	Ongoing

3.3.1. Clinical pharmacology

The clinical pharmacology of Nidlegy (mixture of the two immunocytokines L19IL2 and L19TNFa) as intratumoural combination therapy as well as systemic and intratumoural monotherapy has been investigated in cancer patients in different clinical trials (see Table 3-3-1 and Table 3-3-2).

With respect to systemic administration of either immunocytokine, the pharmacokinetic and immunogenicity profile of L19IL2 and of L19TNFa were characterised in two open-label Phase I/II studies, PH-L19IL2-01/05 (NCT01058538) and PH-L19TNFalpha-02/07 (NCT01253837), respectively.

As to the intratumoural administration, the pharmacokinetic profile of the combination therapy of L19IL2 and L19TNFa was analysed in a randomised, controlled, open-label Phase III study (PH-L19IL2TNF-01/18; NCT03567889) of neoadjuvant treatment of locally advanced melanoma.

It should be noted that in clinical studies with intratumoural injection of Nidlegy, there were uncertainties regarding the actually injected volume, and thus the dose administered. For the intravenous studies, the concerns are less, but not completely absent. In study PH-L19IL2TNF-02/15, there were also uncertainties whether the volume, and thus dose, injected per tumour was equal. This also raises uncertainties for study PH-L19TNFalpha-01/18. In addition, the size of the lesions within a patient, and also the amount and size of lesions between patients, probably differed in study PH-L19TNFalpha-01/18, and this has not been taken into account. Therefore, the PK data presented in this Overview, especially after intratumoural administration, should be regarded with caution.

Table 2. Clinical studies investigating pharmacokinetic properties of L19IL2 and L19TNFa as single agents and in combination therapy

Clinical Trial	Study Title	Assay	Size of PK population	Route of Administration
L19IL2 Pharmacokinetic Analysis				
PH-L19IL2-01/05	*A Dose Finding Pharmacokinetic Study of the Tumour-targeting Human L19IL2 Monoclonal Antibody-cytokine Fusion Protein in Patients with Advanced Solid Tumours*	Pharmacokinetic analysis of L19IL2 in human serum from patients in Phase I Pharmacokinetic and HAFA analysis of L19IL2 in human serum from patients in Phase II	21	Systemic IV infusion over 1 h on Days 1, 3 and 5 per cycle (21 days), max. 6 cycles
L19TNF Pharmacokinetic Analysis				
PH-L19TNFalpha-02/07	*Phase I/II study of the tumour-targeting human L19TNFα monoclonal antibody-cytokine fusion protein in patients with advanced solid tumours*	Bioanalysis of TNFalpha in human serum Bioanalysis of L19TNFalpha in human serum	30	Systemic IV infusion over 1 h on Days 1, 3 and 5 per cycle (21 days), max. 6 cycles

L19TNFa + L19IL2 Pharmacokinetic Analysis				
PH-L19IL2TNF-01/18	*An Open-Label, Randomised, Controlled Multi-Center Study of The Efficacy of Daromun (L19IL2+L19TNF) Neoadjuvant Intratumoural Treatment Followed by Surgery and Adjuvant Therapy Versus Surgery and Adjuvant Therapy in Clinical Stage IIIB/C Melanoma Patients.*	Pharmacokinetic analysis of L19IL2 and L19TNFa in human serum from patients treated with Daromun (L19IL2 + L19TNFa) in clinical Study PH-L19IL2TNF-01/18 – Phase 3	19	Intralesional Injection Q1W for 4 weeks

Source: Table 1 SCP

Characterisation of immunogenicity of Nidlegy was performed in an open-label, Phase II (PH-L19IL2TNF-02/12; NCT02076633) study in metastatic disease and in a randomised, controlled, open-label Phase III study (PH-L19IL2TNF-02/15; NCT029382999) in the neoadjuvant setting. Immunogenicity of L19IL2 and L19TNFa was also evaluated in single agent studies (see Table below).

Table 3. Clinical studies investigating immunogenic properties of L19IL2 and L19TNFa as single agents and in combination therapy

Clinical Trial	Study Title	Assay	Size of HAFA population	Route of Administration
L19IL2 Immunogenicity Analysis				
PH-L19IL2-01/05	*A Dose Finding Pharmacokinetic Study of the Tumour-targeting Human L19IL2 Monoclonal Antibody-cytokine Fusion Protein in Patients with*	HAFA analysis of L19IL2 in human serum by SPR	33	Systemic
		HAFA analysis of L19IL2 in human serum by ELISA		
		Pharmacokinetic and HAFA analysis of		

	Advanced Solid Tumours	L19IL2 in human serum from patients in Phase II		
PH-L19IL2-03/09	*A Phase II study of intratumoural application of L19IL2 in patients with stage III/IV melanoma.*	Analysis of Systemic Distribution and HAFA responses in patients treated with L19IL2	22	Intratumoural
L19TNFa Immunogenicity Analysis				
PH-L19TNF-02/07	*Phase I/II study of the tumour-targeting human L19TNFα monoclonal antibody- cytokine fusion protein in patients with advanced solid tumours*	HAFA analysis of L19TNFa in human serum by SPR and ELISA	40	Systemic
		HAFA analysis of L19TNFa in human serum by SPR		
		Bioanalysis of circulating antibodies against L19TNFa in human serum samples by bridging immunoassay		
L19TNFa + L19IL2 Immunogenicity Analysis				
PH-L19IL2TNF-02/12	*Phase II study of intratumoural application of L19IL2/L19TNF in melanoma patients in clinical stage III or stage IV M1a with presence of injectable cutaneous and/or subcutaneous lesions.*	HAFA analysis of L19IL2 and L19TNFa in human serum by SPR and ELISA	19	Intratumoural

PH-L19IL2TNF-02/15	*A pivotal Phase III, open label, randomised, controlled multi-center study of the efficacy of L19IL2/L19TNF neoadjuvant intratumoural treatment followed by surgery versus surgery alone in clinical stage III B/C melanoma patients.*	BIAcore analysis of HAFA responses in patients treated with L19IL2 and L19TNFa	113	Intratumoural

Source: Table 2 SCP

As L19IL2/L19TNFa is developed as solution for intratumoural injection, bioavailability and systemic concentration of L19IL2/L19TNFa are not predictive of pharmacodynamic activity. Indeed, the intended mode of action foresees a long-lasting residence at the tumour site, with minimal leakage into peripheral blood.

Quantification of L19IL2 and L19TNFa concentrations

The quantification of L19IL2 and L19TNFa in human serum was performed using validated methods based on the DELFIA and AlphaLISA technology.

L19IL2

L19IL2 in the sample was captured by a biotinylated EDB fragment bound to a streptavidin coated plate (Method A32422). Detection was performed by an Europium-labelled anti-human IL2 mAb. Throughout the clinical development, the methodology of the analytical method used to quantify the concentration of L19IL2 did not change, despite the analyses being conducted in different laboratories (see Table 3-3-5).

L19TNF

In the initial clinical studies, L19TNF concentration was measured in serum by a ligand binding assay (A56430) where the biotinylated antigen EDB was captured to streptavidin plates, the analyte was then bound and detected by a sulfo-tagged goat anti-human TNF antibody and electrochemiluminiscent readout. In the subsequent method, RPL019 v0 (used in study PH-L19IL2TNF-01/18), quantitative measurements of L19TNF in human serum samples were made by an AlphaLISA bead-based immunoassay. A sandwich assay approach using the anti-TNF antibody conjugated to AlphaLISA acceptor beads and a second biotinylated anti-TNF antibody as detection components, has been developed. The assay detects only the TNF portion of the L19TNF fusion protein.

During clinical development of Nidlegy, methods were repeatedly transferred to and validated at different laboratories.

Bioanalytical methods were generally shown to perform with acceptable accuracy and precision. Examined validation parameters mostly met the acceptance criteria outlined in the EMA guideline (EMA/CHMP/ICH/172948/2019).

Table 4. Validation parameters for quantification of L19IL2 and L19TNF

	A32422 (L19IL2)	0138-2007-R (L19IL2)	A56430 (L19TNF)	A56468 (L19TNF)	R PL 003 (L19IL2)	R PL019 (L19TNF)
*AC/RE (Between run)	AC 86-103 %	AC -3.4-7.6 %	AC 99.3-103%	AC 97.4-102 %	RE -0.76-11.92 %	RE -15.52 -3.47 %
**Precision (between run)	2.6-15.9%	5.9 – 18.9 %	< 7.69%	< 3.94 %	7.28-14.34 %	5.48 – 16.20%
Within run accuracy*	AC 65 – 106 %	RE -16.1 – 23.4 %	RE -16.8 – 5.98 %	AC 96.8-101 %	RE -13.95 -20.97 %	RE -22.4 – 21.02 %
Within run precision**	1.1 – 12.8 %	4.6 – 13.7 %	2.10 – 6.67 %	4.55-20.2 %	5.21 – 18.72 %	0.88 – 17.09 %
Short-term stability (r.t.)	19 hours	N/A	2 hours (37°C)	2 hours (37°C)	4 hours (R PL056)	4 hours (R PL055)
Short-term stability (4°C)	N/A	N/A	6 hours	N/A	N/A	N/A
Long term stability	97 days (-20°C)	N/A	25 months (<-10°C)	23 months (<-10°C)	14 months (-80 °C) (R PL056)	12 months (-80 °C) (R PL055)
Freeze-thaw stability	4 cycles	N/A	4 cycles	3 cycles	3 cycles (R PL056)	3 cycles (R PL055)
Dilution range	1:2 to 1:1000	1:2 to 1:1000	1:10 to 1:50	N/A	1:2-1:1000	N/A
Recovery / Matrix effect*	AC 102-95 %	RE -12.1 – 19.6 %	RE -22.9 – 16.2 %	RE -0.81 – 10.66 %	RE < 20%	RE -22.60-22.66 %

*Accuracy (AC) is reported as $\frac{mean}{nominal\ value}$ x 100 or as Recovery (RE%) $\frac{mean-nominal}{mean}$ x 100; ** precision is reported as CV % $\frac{Standard\ Deviation}{mean}$ x100

Source: Table 3, Summary of Biopharmaceutic Studies, updated after D120 LoQ

To evaluate the mutual interference of L19TNF and L19IL2 quantification, the DELFIA and AlphaLISA methods were used to measure the concentrations of the analytes in QC solutions prepared by spiking L19IL2 and L19TNF in human serum.

Table 5. Validation criteria for quantification of L19IL2 and L19TNF

	A32422 (L19IL2)	0138-2007-R (L19IL2)	A56430 (L19TNF)	A56468 (L19TNF)	R PL 003 (L19IL2)	R PL019 (L19TNF)
*AC/RE (Between run)	AC 86-103 %	AC -3.4-7.6 %	AC 99.3-103%	AC 97.4-102 %	RE -0.76-11.92 %	RE -15.52 -3.47 %
**Precision (between run)	2.6-15.9%	5.9 – 18.9 %	< 7.69%	< 3.94 %	7.28-14.34 %	5.48 – 16.20%
Within run accuracy*	AC 65 – 106 %	RE -16.1 – 23.4 %	RE -16.8 – 5.98 %	AC 96.8-101 %	RE -13.95 - 20.97 %	RE -22.4 – 21.02 %
Within run precision**	1.1 – 12.8 %	4.6 – 13.7 %	2.10 – 6.67 %	4.55-20.2 %	5.21 – 18.72 %	0.88 – 17.09 %
Short-term stability (r.t.)	19 hours	N/A	2 hours (37°C)	2 hours (37°C)	4 hours (R PL056)	4 hours (R PL055)
Short-term stability (4°C)	N/A	N/A	6 hours	N/A	N/A	N/A
Long term stability	97 days (-20°C)	N/A	25 months (<-10°C)	23 months (<-10°C)	14 months (-80 °C) (R PL056)	12 months (-80 °C) (R PL055)
Freeze-thaw stability	4 cycles	N/A	4 cycles	3 cycles	3 cycles (R PL056)	3 cycles (R PL055)
Dilution range	1:2 to 1:1000	1:2 to 1:1000	1:10 to 1:50	N/A	1:2-1:1000	N/A
Recovery / Matrix effect*	AC 102-95 %	RE -12.1 – 19.6 %	RE -22.9 – 16.2 %	RE -0.81 – 10.66 %	RE < 20%	RE -22.60-22.66 %

None of the PK assay validations included the determination of selectivity in lipemic or haemolysed samples. Incurred sample reanalyses have obviously not been conducted.

Assays used for analysis of immunogenicity

To assess the immunogenicity of L19IL2 and L19TNFa, the applicant described that a three-step approach was applied: Detection of possible antibodies in a first screening assay; confirmation of the antibodies specificity to L19IL2 or L19TNFa by competition with free L19IL2 or L19TNFa, or rabbit anti-serum; characterisation of positive and specific antibodies with regard to isotype (i.e., IgG and/or IgM) and/or titre. Throughout the clinical trials conducted, ELISA in different assay formats (sandwich, competition) with colorimetric readout and Surface Plasmon Resonance (SPR) as orthogonal technology were used (see Table below).

Immunogenicity of L19IL2

The immunogenicity of L19IL2 in human serum was initially carried out by ELISA for the analysis of samples collected in the Study PH-L19IL2-01/05 (5.3.1.4; ADA-method-valid - 0498-2007-R). The serum collected from the patients were incubated on a L19IL2 derivatised plate and the presence of bound antibodies was detected by Anti-human IgG.

Analogously an ELISA method (R PL 007 and R PL 005 for confirmatory) relying on the same analytical procedure of the method 0498-2007, was validated to measure the immunogenic response after treatment with L19IL2 in the studies PH-L19IL2-03/09 and PH-L19ILTNF-02/12.

Later, an SPR method (5.3.1.4, R PL004) was developed that directly measures the interaction between the L19IL2 immobilised and the reactive antibodies present in the serum. This method allowed to further characterise the antibodies as IgG or IgM and define the subunit specificity (L19 or IL2). The method was validated and used for PH-L19IL2-01/05, PH-L19IL2-03/09, PH-L19IL2TNF-02/12 and PH-L19ILTNF-02/15.

Immunogenicity of L19TNF

Likewise, immunogenicity generated by the treatment of L19TNF was assessed by ELISA and/or SPR methods. Both methods rely on the interaction of the antibodies generated by the patients with an immobilised L19TNF. In the case of the ELISA the interaction is then detected by an anti-human IgG (R PL 009 and R PL 010 for confirmatory), used in the PH-L19TNF-02/07 and PH-L19ILTNF-02/12. The SPR method determines also the subunit specificity (L19 or TNF) and isotype of the generated antibodies (R PL006). The method was validated and used in the PH-L19TNF-02/07, PH-L19IL2TNF-02/12 and PH-L19ILTNF-02/15.

In order to evaluate the neutralising capacity of the antibodies against L19IL2 and L19TNF an SPR method was developed (5.3.1.4 R PL062, - ADA-method-valid) and used in the PH-L19IL2TNF-02/15. In this method the natural substrate of L19TNF and L19IL2 (i.e. EDB) is coated on an SPR chip. Then separately L19TNF and L19IL2 with and without the serum of the ADA positive patients are analysed on the EDB coated chip. The neutralising effect of the ADA are detected by the degree of inhibition of binding between L19TNF or L19IL2 with EDB.

Validation results of assays for determination of immunogenicity are presented below:

Table 6. ADA assay validation parameters

	0498-2007-R	R PL 004	R PL 006	R PL 007	R PL 009
Chip stability	N/A	L19IL2 58 cycles scFvL19 33 cycles IL2 31 cycles	L19TNF 58 cycles scFvL19 33 cycles TNF 57 cycles	N/A	N/A
Cut off	0.166 OD	F.CP = NC.IS + CF* (CF = 15.8 RU)	F.CP = NC.IS + CF* (CF = 30.6 RU)	0.099 OD	0.306 OD
Precision intra-assay**	1.5-6.6 %	0.56 %	1.91 % - 2.11%	0.14-12.98 % High 0.69-35.8 % Low	1.15-18.64 %
Precision inter-assay**	14.9 %	0.56-1.61 %	4.42 %	15.9-36.86 %	8.76-9.69 %
Cut off confirmatory (Percentage of Inhibition)	N/A	50 %	50 %	26.2 % (R PL005) 30 % (R PL 007 v1)	19.9% (R PL010)
Specificity	Positive control compliant	Positive and negative control compliant	Positive and negative control compliant	Positive and negative control compliant	Conforms
Sensitivity (µg/mL)	N/A	LOD_{L19IL2} 0.146 LOD_{IL2} 0.0625 $LOD_{scFvL19}$ 0.232	LOD_{L19TNF} 0.324 LOD_{TNF} 0.03125 $LOD_{scFvL19}$ 0.232	N/A	N/A
Linearity range (µg/mL)	N/A	L19IL2: 9.34-0.146 IL2: 1-0.0625 scFvL19: 14.848-0.232	L19TNF: 20.72-0.324 TNF: 2-0.03125 scFvL19: 14.848-0.232	N/A	Humira 0.001-0.040 (µg/mL)
Recovery /Selectivity*	AC 101.4-108.1%	AC_{L19IL2} 79.3-111% AC_{IL2} 87.9-110.5% $AC_{scFvL19}$ 94.2-124.7 %	AC_{L19TNF} 101.2-104.1% AC_{TNF} 93.4-107.2% $AC_{scFvL19}$ 94.2-124.7 %	AC 74.7-98 %	AC 84.23-106.75%
Stability short term	4 hours r.t.	N/A	N/A	4 hours r.t., 3 freeze thaw	4 hours r.t., 3 freeze thaw
Dilution linearity	1:100-1:3200	N/A	N/A	1:500-1:16000	1:500-1:16000

*Accuracy (AC) is reported as $\frac{mean}{nominal\ value}$ x 100 ** precision is reported as CV % $\frac{Standard\ Deviation}{mean}$ x100

Drug tolerance level was not determined in any of the ADA assays listed above. No cross-validation of the assays has been conducted.

The table below provides a summary of all bioanalytical methods used in the respective studies.

Table 7. Summary of bioanalytical methods used to analyse L19IL2 and L19TNF concentration in human plasma and immunogenic response

Analyte	Matrix	Method	Validation Reports No (eCTD location)	LLOQ -ULOQ ng/mL	Clinical study No	Bioanalytical Report No (eCTD location)
L19IL2	Serum	DELFIA	A32422 (5.3.1.4)	0.2 - 100	PH-L19IL2-01/05- Phase I	A35021; REP453 (5.3.3.2)
L19IL2	Serum	DELFIA	0138-2007-R (5.3.1.4)	5.16 - 233.33	PH-L19IL2-01/05- Phase II	0111-2009-R (5.3.3.2); REP142(5.3.5.3)
L19TNF	Serum	ECL	A56430 (5.3.3.2)	0.5 - 200	PH-L19TNF-02/07	A56430; R080 (5.3.3.2)
TNF	Serum	ECL	A56468 (5.3.3.2)	0.138 - 50	PH-L19TNF-02/07	A56468 (5.3.3.2)
L19IL2	Serum	DELFIA	R PL 003 V0-3 (5.3.1.4)	5.16 - 233.33	PH-L19IL2TNF-01/18	R045 v1(5.3.5.1-App16.5)
L19IL2	Serum	DELFIA	R PL 044 V0, R PL056 V0 (5.3.1.4)	N/A*	PH-L19IL2TNF-01/18	R045 v1(5.3.5.1)
TNF/L19TNF	Serum	AlphaLISA	R PL019 V0 (5.3.1.4)	0.01 - 22.5	PH-L19IL2TNF-01/18	R045 v1(5.3.5.1)
TNF/L19TNF	Serum	AlphaLISA	R PL045 V0, R PL055 V0 (5.3.1.4)	N/A*	PH-L19IL2TNF-01/18	R045 v1(5.3.5.1)
HAFA L19IL2	Serum	ELISA	0498-2007-R/ R PL 005 V.0 (5.3.1.4)	N/A	PH-L19IL2-01/05 Phase II	0087-2008-R/ REP 142 (5.3.5.3)
HAFA L19IL2	Serum	SPR	R PL 004 V. 0 (5.3.1.4)	N/A	PH-L19IL2-01/05 Phase I-II	REP 197 (5.3.5.3)
HAFA L19TNF	Serum	ELISA	R PL 009 V. 0 & R PL 010 (5.3.1.4)	N/A	PH-L19TNF-02/07	REP 514 (5.3.5.3)
HAFA L19TNF	Serum	SPR	R PL 006 V. 0 (5.3.1.4)	N/A	PH-L19TNF-02/07	REP 233 (5.3.5.3)
HAFA L19IL2	Serum	ELISA/SPR	R PL 007 & R PL 005/ R PL004 V.0 (5.3.1.4)	N/A	PH-L19IL2-03/09	REP 629 (5.3.5.3)
HAFA L19TNF	Serum	ELISA	R PL 009 V. 0 & R PL 010 (5.3.1.4)	N/A	PH-L19IL2TNF-02/12	REP 807 (5.3.5.3)
HAFA L19TNF	Serum	SPR	R PL 006 V. 0 (5.3.1.4)	N/A	PH-L19IL2TNF-02/12	REP 807 (5.3.5.3)
HAFA L19IL2	Serum	ELISA	R PL 007 V. 1 (5.3.1.4) & R PL 005 V.0	N/A	PH-L19IL2TNF-02/12	REP 807 (5.3.5.3)
HAFA L19IL2	Serum	SPR	R PL 004 V. 0 (5.3.1.4)	N/A	PH-L19IL2TNF-02/12	REP 807 (5.3.5.3)
HAFA L19TNF	Serum	SPR	R PL 006 V. 1-2 (5.3.1.4)	N/A	PH-L19IL2TNF-02/15	R 050 v2 (5.3.5.1-App16.5)
HAFA L19IL2	Serum	SPR	R PL 004 V. 0-1 (5.3.1.4)	N/A	PH-L19IL2TNF-02/15	R 050 v2 (5.3.5.1)
HAFA Neutralising antibodies L19IL2 L19TNF	Serum	SPR	R PL 062 V.0 (5.3.1.4)	N/A	PH-L19IL2TNF-02/15	R 050 v2 (5.3.5.1)

* Stability of L19TNF and L19IL2 and mutual interference studies.

Source: Table 2, Summary of Biopharmaceutic Studies, updated after D120 LoQ

3.3.1.1. Pharmacokinetics

Absorption

In Studies 01/05 and 02/07, L19IL2 and L19TNFa were administered by short IV infusion over 60 minutes. In these cases, bioavailability was 100%.

In Study 01/05, mean Cmax of L19IL2 ranged between 622.8 ng/mL and 970.3 ng/mL at the recommended dose (RD) of 22.5 Mio IU L19IL2. In Study 02/07, mean Cmax of L19TNFa at the recommended dose of 13.0 µg/kg at Day 3 was 56324 ng/L in Phase I and 67254 ng/L in Phase II (L19TNFa assay, Assay A) or 43108 ng/L in Phase I and 44799.8 ng/L in Phase II (TNFa assay, Assay B).

In Study 01/18, intratumoural administration of 13 Mio IU of L19IL2 and 400 µg of L19TNFa resulted in Cmax of 111.11 ng/mL at Day 1 and 116.35 ng/mL at Day 22 for L19IL2 and Cmax of 2.5 ng/mL at Day 1 and 2.25 ng/mL at Day 22 for L19TNFa.

Systemic leakage of L19IL2 and L19TNFa from the tumour lesions into the systemic circulation was assessed. Theoretical values for Cmax and AUC were estimated and compared to the observed values at day 1 in the PH-L19IL2TNF-01/18 study. For Cmax values, this was based on the assumption that all of the active substance would leak into the systemic circulation and that the volume of distribution can be calculated as 0.08L/kg body weight, where 55% is plasma. The theoretical Cmax value was calculated for each patient of the PH-L19IL2TNF-01/18 PK population and the percentage of the observed Cmax value compared to the theoretical Cmax value was determined. The percentage of the average Cmax observed (at day1) with respect to the average of the theoretical one (at day 1) was 26.6% and 3.2% for L19IL2 and L19TNF, respectively. The theoretical values for AUC (0-t) were interpolated from values obtained from the single agent intravenous studies, PH-L19IL2-01/05 and PH-L19TNF-02/07. Similarly, the observed AUC (0-t) values at day 1 were determined to be 20.6% and 24.3% of the theoretical AUC (0-t) values calculated for L19IL2 and L19TNF, respectively.

Distribution

In Study 01/05, Vd of L19IL2 at a dose of 22.5 Mio IU ranged between 4.63 – 5.73 L. In Study 02/07, Vss after a dose of 13 µg/kg L19TNFa was described to be 0.01 L in Assay A and 0.02 L in Assay B.

For Study 01/18, Vd of L19IL2 and L19TNF for the overall PK population are still missing.

Elimination

In Study 01/05, mean CL and t1/2 of L19IL2 ranged between 769.6 mL/h – 1639 mL/h and 2.74 h – 4.55 h at the recommended dose of 22.5 Mio IU, respectively. In Study 02/07, mean CL and t1/2 of L19TNFa (Assay A) were determined to be 6.33 L/h and 0.64 h at the recommended dose of 13.0 µg/kg in Phase II. Mean CL and t1/2 of L19TNFa in Assay B were determined to be 20 L/h and 1.04 h, respectively.

For Study 01/18, values for CL of the overall PK population are still missing. Half-life of L19IL2 (at 13 Mio IU) was 8 h (± 11 h) at Day 1 and 16.1 h (± 25.3 h) at Day 22. Half-life of L19TNFa (at 400 µg) was 4 h (± 3 h) at Day 1 and 6.4 h (± 5.9 h) at Day 22.

No clinical metabolism or excretion studies were conducted with L19IL2 and L19TNFa, as these were not considered necessary or relevant for biologics such as Nidlegy (CPMP/ICH/302/95). Like other

therapeutic proteins, Nidlegy was expected to be metabolised primarily by proteolytic catabolism. However, in a preclinical quantitative biodistribution study it was shown that L19IL2 was preferentially excreted via the urinary pathway, suggesting similar behaviour in humans. No conclusive data on human excretion pathways is available.

Dose proportionality and time dependencies

Approximately dose proportional increases of Cmax and AUC were observed after systemic administration of L19IL2 (Study PH-L19IL2-01/05). L19TNFa Cmax and AUC also increased with increasing dose in an approx. dose-proportional manner after systemic administration (Study PH-L19TNF-02/07).

No accumulation of L19IL2 occurred after repeated systemic administration. Instead, L19IL2 concentrations in Study 01/05 seemed to decrease with repeated dosing. No information on time dependency of L19TNFa from Study 02/07 is available.

In Study PH-L19IL2TNF-01/18, no accumulation of L19IL2 and L19TNFa is indicated, Cmax and AUC values are comparable between Day 1 and Day 22 (see Table 7).

Pharmacokinetics in the target population

In Study PH-L19IL2TNF-01/18, the pharmacokinetic profile of L19IL2 and L19TNFa as combination therapy was evaluated at day 1 and 22.

L19IL2/L19TNF doses were administered according to a dose algorithm depending on the size of the lesion:

Table 8. Dosing algorithm in Study PH-L19IL2TNF-01/18

Lesion diameter (cm)	Volume to be injected (mL)	Dose L19TNF (µg)	Dose L19IL2 (MioIU)
> 3.0	2.0 (or max. volume)	400	13
> 2.0 to 3.0	1.5	300	9.75
> 1.0 to 2.0	1	200	6.5
> 0.5 to 1.0	0.5	100	3.25
> 0.1 to 0.5	0.25	50	1.625

For each treatment, 2 mL of the study drug are prepared corresponding with 400 µg L19TNF and 13 Mio IU L19IL2. According to the dose algorithm outlined above, a specific volume of this preparation will be injected. Therefore, each patient receives a dose tailored to the lesion diameter or total lesion diameter, respectively (multiple lesions).

The summary of PK results is presented in the table below.

Table 9. Summary of pharmacokinetic parameters for PH-L19IL2TNF-01/18 study

Study ID	Study Objective	Study Design	Treatment (Dose, Route & Regimen)	PK population			Mean PK Parameters			
							C_{max} [ng/mL]	T_{max} [h]	$AUC_{(0-t)}$ [ng*h/mL]	$T_{1/2}$ [h]
PH-L19IL2TNF-01/18 (intratumoral)	Primary: - Efficacy of L19IL2/L19TNF as neoadjuvant treatment followed by surgery and adjuvant therapy (improve RFS) Secondary: - Improve OS - Improvement of RFS as determined by local investigator - Assessment of pathological responses at time of surgical resection - Safety and tolerability Exploratory: - LRFS	Open-label, randomized, controlled multi-center Phase III study in patients with resectable Stage IIIB/C melanoma	Intratumoral administration into injectable cutaneous, subcutaneous, and nodal tumors once weekly for up to 4 weeks Dose per visit: 13 Mio IU of L19IL2 and 400 µg of L19TNF in combined total volume of approximately 2 mL The largest lesion is treated first and all other lesions are then prioritized for	19	L19IL2	Day 1	111.11 ± 113.39	2.2 ± 2.1	496.13 ± 550.56	8.0 ± 11.0
						Day 22	116.35 ± 126.10	1.8 ± 2.1	460.62 ± 412.07	16.1 ± 25.3
					L19TNF	Day 1	2.50 ± 2.70	2.3 ± 1.5	7.93 ± 7.53	4.0 ± 3.0
	- DMFS - Study of blood biomarkers Further: - PK and Population PK		injection based on lesion size until no lesion is left uninjected or until no volume is left to inject (whichever occurs first)			Day 22	2.25 ± 2.65	2.4 ± 1.8	7.69 ± 8.55	6.4 ± 5.9

Source: Table 5, updated SCP after D120 LoQ

Special populations

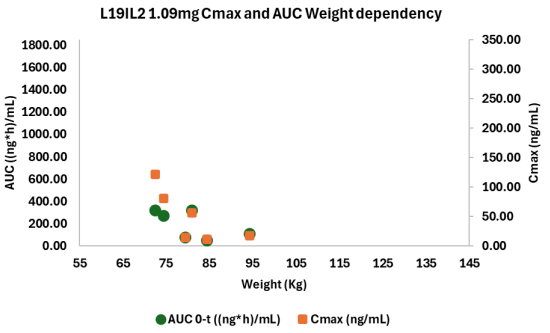
Hepatic and renal impairment

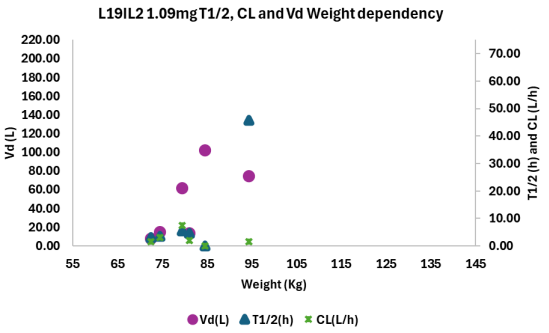
No patients with hepatic impairment have been included in arm 1 of Study 01/18 and thus, no PK data for patients with hepatic impairment are available. Two patients with renal impairment have been included in Arm 1 of Study 01/18, while only for 1 patient dense PK sampling has been conducted. No data have been presented and data from population PK modelling are not yet available.

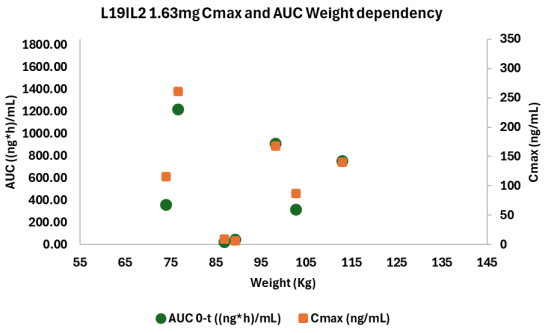
Weight

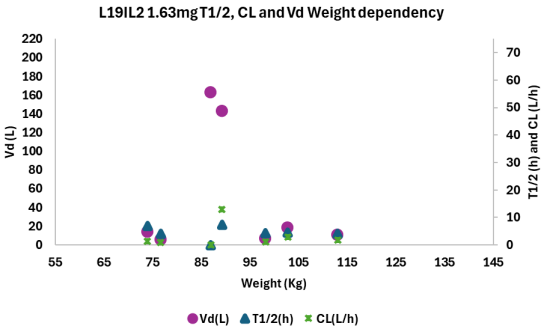
PK data by body weight have been provided for Study 01/18 and are based on a limited number of patients. PK parameters were plotted against body weight for each dose level received by patients separately.

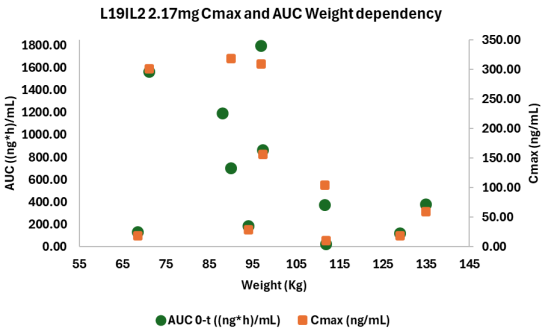
Figure 1. PK data by body weight for each dose level (Study 01/18)

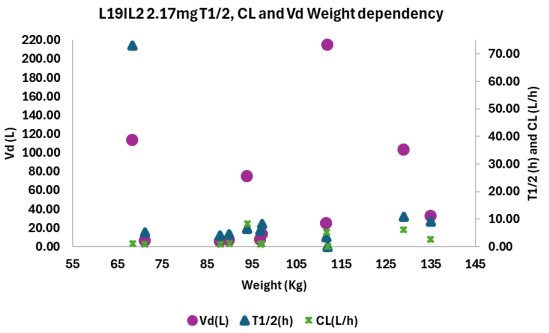

L19IL2 1.09mg Cmax and AUC Weight dependency
AUC ((ng*h)/mL)
Cmax (ng/mL)
Weight (Kg)
AUC 0-t ((ng*h)/mL)
Cmax (ng/mL)

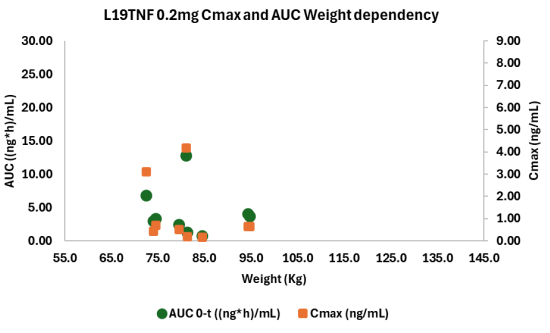

L19IL2 1.09mg T1/2, CL and Vd Weight dependency
Vd (L)
T1/2 (h) and CL (L/h)
Weight (Kg)
Vd(L)
T1/2(h)
CL(L/h)

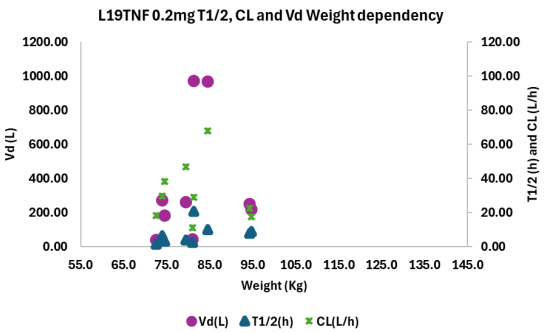

L19IL2 1.63mg Cmax and AUC Weight dependency
AUC ((ng*h)/mL)
Cmax (ng/mL)
Weight (Kg)
AUC 0-t ((ng*h)/mL)
Cmax (ng/mL)

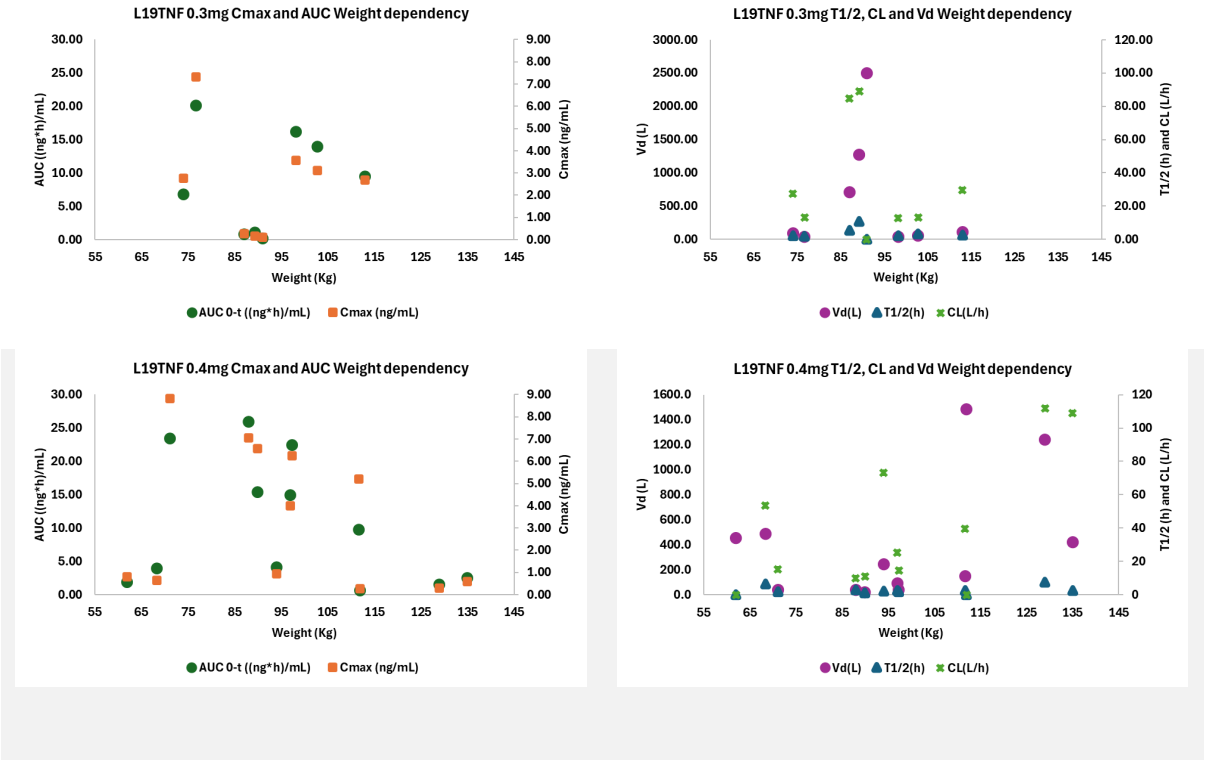

L19IL2 1.63mg T1/2, CL and Vd Weight dependency
Vd (L)
T1/2 (h) and CL (L/h)
Weight (Kg)
Vd(L)
T1/2(h)
CL(L/h)


L19IL2 2.17mg Cmax and AUC Weight dependency
AUC ((ng*h)/mL)
Cmax (ng/mL)
Weight (Kg)
AUC 0-t ((ng*h)/mL)
Cmax (ng/mL)


L19IL2 2.17mg T1/2, CL and Vd Weight dependency
Vd (L)
T1/2 (h) and CL (L/h)
Weight (Kg)
Vd(L)
T1/2(h)
CL(L/h)


L19TNF 0.2mg Cmax and AUC Weight dependency
AUC ((ng*h)/mL)
Cmax (ng/mL)
Weight (Kg)
AUC 0-t ((ng*h)/mL)
Cmax (ng/mL)


L19TNF 0.2mg T1/2, CL and Vd Weight dependency
Vd (L)
T1/2 (h) and CL (L/h)
Weight (Kg)
Vd(L)
T1/2(h)
CL(L/h)



Age

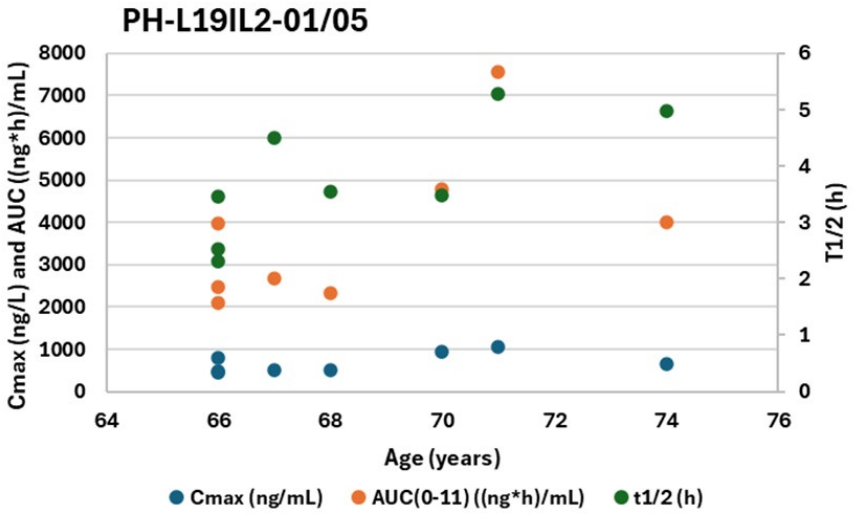
The number of patients included in the PK analysis in the studies PH-L19IL2-01/05, PH-L19TNF-02/07 and PH-L19IL2TNF-01/18 are summarised in the table below.

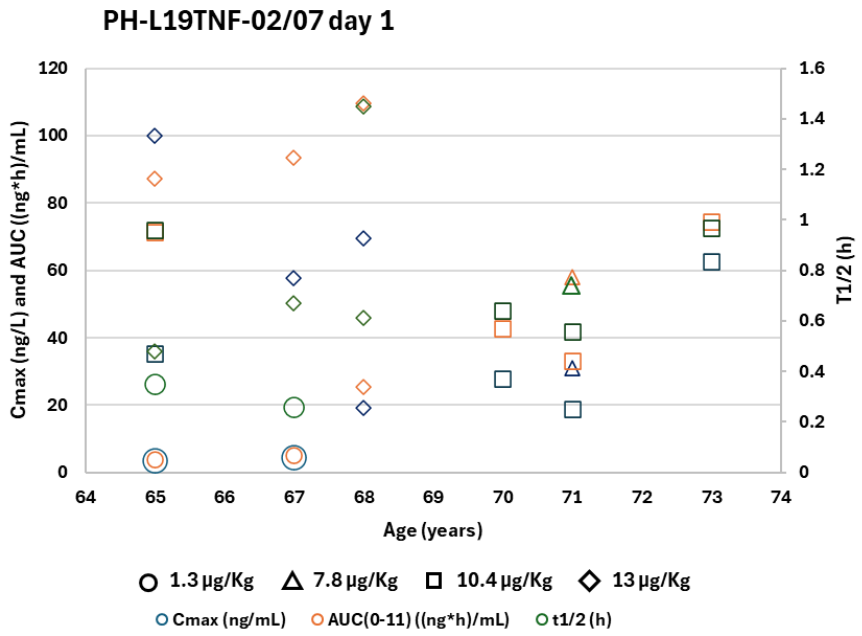
Table 10. Overview of elderly patients in clinical studies

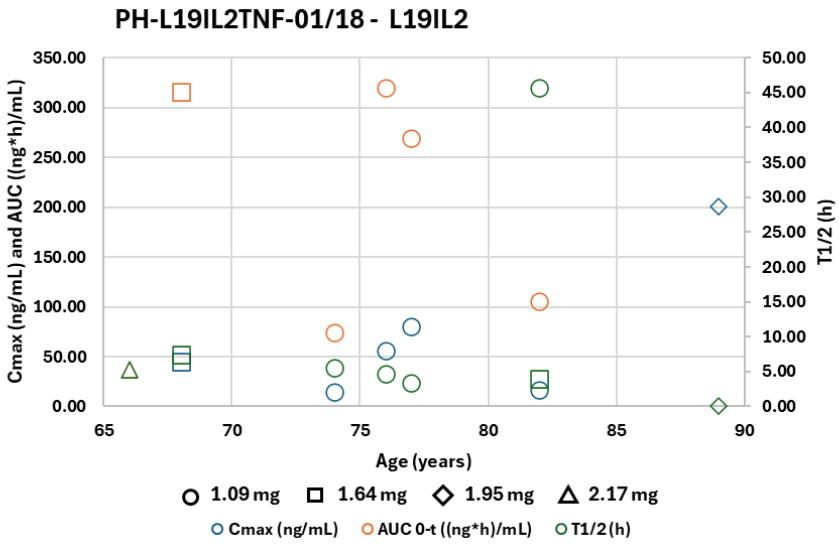
	Age 65-74 (Older subjects number/total number)	Age 75-84 (Older subjects number/total number)	Age 85+ (Older subjects number /total number)
PH-L19IL2-01/05 Phase I/II	8/33	0/33	0/33
PH-L19TNF-02/07 Phase I/II	12/34	0/34	0/34
PH-L19IL2TNF-01/18 Phase III	3/19	4/19	1/19

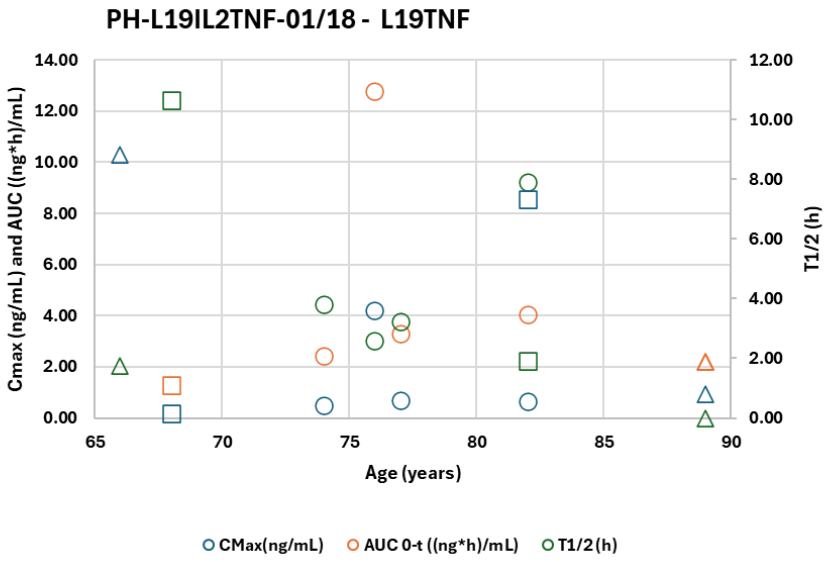
Key PK parameters (Cmax, AUC and t1/2) from studies PH-L19IL2-01/05, PH-L19TNF-02/07 and PH-L19IL2TNF-01/18 by age are presented below.

Figure 2. Cmax, AUC and t1/2 parameters by age (studies PH-L19IL2-01/05, PH-L19TNF-02/07 and PH-L19IL2TNF-01/18)









Sex

Female patients demonstrate approximately 25% reduced mean Cmax of L19IL2 compared to male patients, while this was comparable for L19TNFa. Similarly, Tmax of L19IL2 was reached in female patients after approximately 3.1h, while in male patients this was reached after approximately 1.6h. Tmax of L19TNFa was comparable between female and male patients.

Table 11. Comparison of pharmacokinetic parameters in female and male patients in the PH-L19IL2TNF-01/18 study

		L19IL2					L19TNF				
		Number of patients	Cmax [ng/mL]	Tmax [h]	AUC (0-t) [(ng*h)/mL]	T1/2 [h]	Number of patients	Cmax [ng/mL]	Tmax [h]	AUC (0-t) [(ng*h)/mL]	T1/2 [h]
Female	Day 1	N = 7 (T½ only for N=4)	93.45 ± 110.81	3.14 ± 2.55	430.12 ± 590.95	4.56 ± 1.07	N = 7 (T½ only for N = 6)	2.46 ± 3.14	2.64 ± 1.6	7.45 ± 8.92	4.15 ± 3.25
	Day 22	N = 3	96.59 ± 70.97	2.5 ± 3.04	451.20 ± 377.11	29.45 ± 37.80	N = 4	2.34 ± 2.72	2.25 ± 1.5	8.54 ± 9.36	6.07 ± 5.47
Male	Day 1	N = 11 (T½ only for N= 10)	122.35 ± 118.89	1.59 ± 1.64	538.26 ± 548.30	9.31 ± 12.90	N = 12 (T½ only for N =10)	2.53 ± 2.55	2.04 ± 1.41	8.20 ± 7.01	3.85 ± 3.00
	Day 22	N = 4	131.16 ± 166.61	1.25 ± 1.19	467.68 ± 494.61	6.16 ± 3.40	N = 7	2.11 ± 2.81	2.79 ± 1.87	7.04 ± 8.82	7.34 ± 6.43

Ethnicity

The patients included in the dense PK study of PH-L19IL2TNF-01/18 so far were 100% Caucasian. Similarly, the PK population of the study PH-L19IL2-01/05 was 100% Caucasian.
No PK data for other ethnicities are available or have been presented in further detail.

Pharmacokinetic interaction studies

An in vitro enzyme inhibition assay on the major isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 has been performed to evaluate a potential inhibition of human CYP450 enzymes by L19IL2 and L19TNF. Neither L19IL2 nor L19TNF was found to inhibit the activity of these six major isoforms at concentrations of at least 1640 ng/mL L19IL2 and 352 ng/mL L19TNF, which is at least 10x higher than the Cmax observed in the clinical trials.

Table 12. P450 inhibition data

	CYP1A2	CYP2C19	CYP2D6	CYP3A4	CYP2C9	CYP4A5
	EOMCC	EOMCC	EOMCC	BOMCC	BOMCC	BOMCC
COMPOUND	Mean (± SD.) IC_{50}					
L19IL2	>>1640ng/mL	>>1640ng/mL	>>1640ng/mL	>>1640ng/mL	>>1640ng/mL	>>1640ng/mL
L19TNF	>>352ng/mL	>>352ng/mL	>>352ng/mL	>>352ng/mL	>>352ng/mL	>>352ng/mL

Results are expressed as Mean ± S.D., n=3

Table 13. P450 inhibition data on standard inhibitors

Cytochrome	Standard	IC50 (uM)
1A2	Naftoflavone	0.021±0.001
2C9	Suphaphenazole	0.33±0.008
2C19	Miconazole	0.052±0.003
2D6	Quinidine	0.0055±0.0007
3A4	Ketoconazole	0.039±0.008
3A5	Ketoconazole	0.062±0.001

Results are expressed as Mean ± S.D., n=2

Pharmacokinetics using human biomaterials

N.A.

Exposure relevant for safety evaluation

Systemic concentrations at the claimed intralesional posology are below the systemic exposure of L19IL2 and L19TNF achieved with IV monotherapy administration, where a dose of 22.5 Mio IU for L19IL2 and 13 µg/kg for L19TNF was found to be safe and determined as recommended dose (RD). Mean AUC for L19IL2 (Study 01/05) and L19TNF (Study 02/07) is described with 5272 ng*h/mL and 90.36 ng*h/mL, respectively. By contrast, the maximum AUC of L19IL2 and L19TNF in study PH-L19IL2TNF-01/18 was 1797.14 ng*h/mL and 25.96 ng*h/mL, respectively.

3.3.1.2. Pharmacodynamics

Mechanism of action

Nidlegy (L19IL2/L19TNFa) is a product consisting of the combination of two individual immunocytokines, bifikafusp alfa (also denominated L19IL2) and onfekafusp alfa (also denominated L19TNFa), which are mixed immediately prior to intralesional injection.

L19 is an antibody fragment in single-chain variable (scFv) format, which selectively binds to the splice variant of fibronectin containing the Extra-Domain B (EDB), a tumour-associated antigen that is found in the majority of aggressive tumour types, but is virtually undetectable in normal tissues (Carnemolla et al. 1989). IL2 is a proinflammatory cytokine that boosts the activity of cytotoxic T lymphocytes, as well as T Helper and Natural Killer cells (Rosenberg et al. 1989, Lotze 1995). TNFa is also a proinflammatory cytokine stimulating cell-based immunity, but which also increases vascular permeability, leads to tumour necrosis and that can directly kill tumour cells expressing the cognate TNFa receptor. The L19 antibody moiety is intended to allow stable "anchoring" of the cytokine payloads at the site of disease.

Primary and Secondary pharmacology

Immunophenotyping

To assess the pharmacodynamic properties of L19IL2/L19TNFa, subpopulations of lymphocytes were quantified by flow cytometry or semiquantitative analysis after staining of surgical specimens.

In the flow cytometry-based study about peripheral PBMCs populations, a significant increase in regulatory T cells in patients of arm 1 (who received L19IL2/L19TNFa followed by surgery) compared to patients of arm 2 could be observed. A reduction of MDSC between baseline and day of surgery in arm 1 could be observed but not in a statistically significant manner. No significant changes in frequency were observed for CD4+ T cell, NK cell, and CD8+ T cell subpopulations.

In the study about tumour-infiltrating lymphocyte populations, a majority of patients, i.e., 89%, who received neoadjuvant L19IL2/L19TNFa, showed a high or intermediate amount of infiltrating lymphocytes and only 11% of patients exhibited a low infiltrate. Conversely, 59% of patients, who only received surgery, showed a low degree of infiltration, an intermediate amount of lymphocytes was present in 32% of patients, while a high amount of infiltrating lymphocytes was only detected in 9% of patients.

In another preliminary study in 32 patients (15 from L19IL2/L19TNFa arm, 17 from control arm) surgical specimens were used to quantify subpopulations of lymphocytes and their states of activity. CD4 (T helper lymphocytes), CD8 (cytotoxic T lymphocytes), CD56 (natural killer cells), and FoxP3 (regulatory T cells) staining of histological slides prepared from Formalin-Fixed Paraffin Embedded (FFPE) blocks was performed. The amount of lymphocytes and the immunoreactivity were scored semiquantitatively by a dermatopathologist into four categories each and evaluated; in addition to this a digitally assisted analysis was performed.

In the visual analysis, no significant differences in percentage of CD4 T cells, CD8 T cells, FoxP3+ Treg cells and CD56+ NK cells over the total tumour-infiltrating lymphocyte population could be observed between the two study arms. In the digital analysis, CD4 T cells, CD8 T cells, FoxP3+ Treg, and NK cells were observed to be more abundant in patients with neoadjuvant L19IL2/L19TNFa treatment, than in patients who only received surgery.

Table 14. Summary of the immunophenotyping analysis in the study PH-L19IL2TNF-02/15

Study ID	Immuno-phenotyping population	Assay		CD4 T cells	CD8 T cells	NK cells	Regulatory T cells
PH-L19TNF-02/15 (intratumoral)	32	CD4, CD8, CD56, and FoxP3 stainings of histological slides prepared from FFPE blocks assessed by visual, semiquantitative analysis		No significant differences between patients from the two treatment arms	More abundant in patients treated with L19IL2/L19TNF than in controls (difference not statistically significant, p = 0.0693 in an unpaired, one-tailed t-test, 95% CI)	No significant differences between patients from the two treatment arms	More abundant in patients treated with L19IL2/L19TNF (marginally significant)
		CD4, CD8, CD56, and FoxP3 stainings of histological slides prepared from FFPE blocks assessed digitally-assisted analysis	L19IL2/L19TNF + Surgery Arm (Arm 1, N = 15)	52.9 ± 25.7%	41.5 ± 18.5%	7.4 ± 15.9%	17.9 ± 19.1%
			Surgery Arm (Arm 2, N = 17)	35.4 ± 23.3%	13.7 ± 16.4%	6.5 ± 10.5%	11.9 ± 22.1%
	Immuno-phenotyping population	**Assay**	**CD4 T cells**	**CD8 T cells**	**NK cells**	**Regulatory T cells**	**Myeloid-derived suppressor cells**
	100	Flow cytometry of blood purified immune cells with two antibody panels: (a) CD3/CD4/CD8/HLADR/ CD11b/CD56/CD14/CD16 (b) CD25/CD4/CD8/FoxP3	No significant differences between patients from the two treatment arms			Significant increase in patients treated with L19IL2/L19TNF	Significant decrease in patients treated with L19IL2/L19TNF

Source: Table 11 SCP

Immunogenicity

In study PH-L19IL2TNF-02/12 study, the overall incidence rate of HAFA was 26.3% (5 out of 19 evaluable patients). None of these positive samples in ELISA assay were able to compete with reference rabbit antiserum in a competitive ELISA. No neutralising antibody assay was performed in this study.

In Study PH-L19IL2TNF-02/15, the immunogenic profile of L19IL2 and L19TNFa as combination therapy at day 8 and day 29 after intratumoural administration as neoadjuvant treatment as well as at the first follow-up visit was evaluated. In addition, a blood sample taken at screening was tested for premature presence of HAFA for each patient.

In the treatment arm (Arm 1), L19IL2/L19TNFa was administered as intratumoural injection in an approximate volume of 2 mL (13 Mio IU L19IL2 and 400 µg of L19TNFa) once every week for up to 4 weeks. Samples from 113 patients are included in the HAFA analysis.

Only one out of 113 patients (0.9%) showed a positive signal for HAFA at baseline. SRP assay revealed presence of HAFA against L19IL2 in 39 patients (34.5%) and in 6 patients (5.3%) for L19TNFa. HAFA signals were typically low, transient and never exceeded more than 25.4 % for L19IL2 and 23.6% for L19TNFa of the positive control signal. Moreover, only 6 patients (5.3%) were found positive at the follow up visit at three months' time for HAFA against L19IL2 and only 2 patients (1.8%) for HAFA against L19TNFa.

Only 4 patients had antibodies with a neutralising effect (3.4%): one patient with confirmed HAFA against L19IL2 with a neutralising effect toward L19 moiety and 4 patients with confirmed HAFA against L19TNF with neutralising effect toward L19 moiety.

For both immunocytokines, the response was generally attributable to IgG antibodies specific to L19 subpart.

Table 15. Summary of HAFA analysis in the studies PH-L19IL2TNF-02/12 and PH-L19IL2TNF-02/15

Study ID	HAFA population	Type of assay	Number of positive patients	Type of response	Comments
PH-L19IL2TNF-02/12 (intratumoral)	19	SPR	2	n/a	IgG
		ELISA	3	n/a	IgG
		ELISA competition	0	-	None of the antibodies was strong enough to compete with antibodies from rabbit antiserum
PH-L19IL2TNF-02/15 (intratumoral)	113	SPR (L19IL2)	39	Transient	The titer of L19IL2 HAFA was determined to be 822 ± 479 (range 500-2000). Most HAFA were characterised as IgG isotypes specific to the L19 subunit. 1 patient had HAFA with neutralizing effect (titer 100)
		SPR (L19TNF)	6	Transient	The titer of L19TNF HAFA was determined to be 300 ± 112 (range 250-500). Most HAFA were characterised as IgG isotypes specific to the L19 subunit. 4 patients had HAFA with neutralizing effect (titer 100-250)

Source: Excerpt from Table 7 SCP

Since in study PH-L19IL2TNF-02/12 and study PH-L19IL2TNF-02/15 no PK sampling has been conducted in parallel to ADA sampling, the impact of ADA positivity on PK has not been analysed.

Table 16. Impact of presence of ADA on safety

The table on the number of patients experiencing AEs by HAFA-positivity for L19TNF and L19IL2 for studies PH-L19IL2TNF-02/12 and PH-L19IL2TNF-02/15 is presented below:

	OVERALL (N=139)*							
	L19TNF				L19IL2			
	Negative/ N/E (N=131)		Positive (N=8)		Negative/ N/E (N=99)		Positive (N=40)	
ANY	**128**	**97.7%**	**8**	**100.0%**	**96**	**97.0%**	**40**	**100.0%**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	9	6.9%	0		5	5.1%	4	10.0%
LEUKOCYTOSIS	4	3.1%	0		2	2.0%	2	5.0%
EOSINOPHILIA	3	2.3%	0		2	2.0%	1	2.5%
ANAEMIA	2	1.5%	0		1	1.0%	1	2.5%
THROMBOCYTOPENIA	1	0.8%	0		1	1.0%	0	
THROMBOCYTOSIS	1	0.8%	0		1	1.0%	0	
CARDIAC DISORDERS	12	9.2%	0		6	6.1%	6	15.0%
TACHYCARDIA	8	6.1%	0		4	4.0%	4	10.0%
ARRHYTHMIA	1	0.8%	0		1	1.0%	0	
CARDIOVASCULAR DISORDER	1	0.8%	0		0		1	2.5%
PALPITATIONS	1	0.8%	0		1	1.0%	0	

SINUS TACHYCARDIA	1	0.8%	0		0		1	2.5%
EAR AND LABYRINTH DISORDERS	8	6.1%	1	12.5%	6	6.1%	3	7.5%
VERTIGO	7	5.3%	1	12.5%	6	6.1%	2	5.0%
EAR DISCOMFORT	1	0.8%	0		0		1	2.5%
ENDOCRINE DISORDERS	1	0.8%	1	12.5%	0		2	5.0%
HYPOTHYROIDISM	1	0.8%	0	0.0%	0		1	2.5%
IMMUNE-MEDIATED THYROIDITIS	0	0.0%	1	12.5%	0		1	2.5%
EYE DISORDERS	1	0.8%	0		1	1.0%	0	
DIPLOPIA	1	0.8%	0		1	1.0%	0	
GASTROINTESTINAL DISORDERS	43	32.8%	4	50.0%	30	30.3%	17	42.5%
NAUSEA	28	21.4%	1	12.5%	16	16.2%	13	32.5%
VOMITING	15	11.5%	2	25.0%	10	10.1%	7	17.5%
DIARRHOEA	11	8.4%	0		8	8.1%	3	7.5%
ABDOMINAL PAIN UPPER	4	3.1%	0		2	2.0%	2	5.0%
GASTROOESOPHAGEAL REFLUX DISEASE	2	1.5%	0		2	2.0%	0	
TOOTHACHE	2	1.5%	0		1	1.0%	1	2.5%
ABDOMINAL DISTENSION	1	0.8%	0		1	1.0%	0	

ABDOMINAL PAIN	0		1	12.5%	0		1	2.5%
ANAL INCONTINENCE	1	0.8%	0		1	1.0%	0	
CONSTIPATION	1	0.8%	0		1	1.0%	0	
DYSPHAGIA	1	0.8%	0		0		1	2.5%
GASTRITIS	0		0		0		0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	125	95.4%	8	100.0%	94	94.9%	39	97.5%
INJECTION SITE REACTION	94	71.8%	6	75.0%	68	68.7%	32	80.0%
PYREXIA	69	52.7%	6	75.0%	46	46.5%	29	72.5%
CHILLS	59	45.0%	3	37.5%	35	35.4%	27	67.5%
FATIGUE	20	15.3%	1	12.5%	15	15.2%	6	15.0%
INFLUENZA LIKE ILLNESS	18	13.7%	1	12.5%	12	12.1%	7	17.5%
INJECTION SITE PAIN	6	4.6%	2	25.0%	5	5.1%	3	7.5%
ASTHENIA	7	5.3%	0		5	5.1%	2	5.0%
OEDEMA PERIPHERAL	6	4.6%	0		5	5.1%	1	2.5%
PAIN	4	3.1%	0		3	3.0%	1	2.5%
AXILLARY PAIN	3	2.3%	0		3	3.0%	0	
SWELLING	3	2.3%	0		2	2.0%	1	2.5%
FEELING COLD	2	1.5%	0		1	1.0%	1	2.5%

INFLAMMATION	2	1.5%	0		2	2.0%	0	
INJECTION SITE ERYTHEMA	2	1.5%	0		2	2.0%	0	
OEDEMA	2	1.5%	0		2	2.0%	0	
CHEST PAIN	1	0.8%	0		0		1	2.5%
IMPAIRED HEALING	1	0.8%	0		1	1.0%	0	
INJECTION SITE INFLAMMATION	0		1	12.5%	0		1	2.5%
INJECTION SITE NECROSIS	0		1	12.5%	1	1.0%	0	
LOCALISED OEDEMA	1	0.8%	0		1	1.0%	0	
MALAISE	1	0.8%	0		1	1.0%	0	
PERIPHERAL SWELLING	1	0.8%	0		1	1.0%	0	
IMMUNE SYSTEM DISORDERS	8	6.1%	0		4	4.0%	4	10.0%
DRUG HYPERSENSITIVITY	8	6.1%	0		4	4.0%	4	10.0%
INFECTIONS AND INFESTATIONS	20	15.3%	0		14	14.1%	6	15.0%
NASOPHARYNGITIS	5	3.8%	0		4	4.0%	1	2.5%
URINARY TRACT INFECTION	3	2.3%	0		2	2.0%	1	2.5%
ERYSIPELAS	2	1.5%	0		1	1.0%	1	2.5%
INJECTION SITE INFECTION	1	0.8%	0		1	1.0%	0	
POSTOPERATIVE WOUND INFECTION	2	1.5%	0		2	2.0%	0	

ACARODERMATITIS	1	0.8%	0		0		1	2.5%
BACTERIURIA	1	0.8%	0		0		1	2.5%
EYELID INFECTION	1	0.8%	0		1	1.0%	0	
HERPES ZOSTER	1	0.8%	0		1	1.0%	0	
ORAL HERPES	1	0.8%	0		1	1.0%	0	
PNEUMONIA	1	0.8%	0		0		1	2.5%
RASH PUSTULAR	1	0.8%	0		1	1.0%	0	
SEPSIS	1	0.8%	0		0		1	2.5%
SUBCUTANEOUS ABSCESS	1	0.8%	0		1	1.0%	0	
UPPER RESPIRATORY TRACT INFECTION	1	0.8%	0		1	1.0%	0	
WOUND INFECTION	1	0.8%	0		1	1.0%	0	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	19	14.5%	1	12.5%	14	14.1%	6	15.0%
SEROMA	7	5.3%	0		5	5.1%	2	5.0%
PROCEDURAL PAIN	4	3.1%	0		2	2.0%	2	5.0%
WOUND DEHISCENCE	1	0.8%	1	12.5%	2	2.0%	0	
WOUND SECRETION	2	1.5%	0		2	2.0%	0	
ANAEMIA POSTOPERATIVE	1	0.8%	0		0		1	2.5%
INJURY	1	0.8%	0		1	1.0%	0	

LIGAMENT SPRAIN	1	0.8%	0		1	1.0%	0	
POST PROCEDURAL HAEMATOMA	1	0.8%	0		0		1	2.5%
PROCEDURAL NAUSEA	1	0.8%	0		1	1.0%	0	
RADIATION SKIN INJURY	1	0.8%	0		0		1	2.5%
THERMAL BURN	1	0.8%	0		1	1.0%	0	
INVESTIGATIONS	31	23.7%	2	25.0%	19	19.2%	14	35.0%
ALANINE AMINOTRANSFERASE INCREASED	13	9.9%	1	12.5%	8	8.1%	6	15.0%
GAMMA-GLUTAMYLTRANSFERASE INCREASED	10	7.6%	1	12.5%	6	6.1%	5	12.5%
ASPARTATE AMINOTRANSFERASE INCREASED	8	6.1%	0		4	4.0%	4	10.0%
LIPASE INCREASED	3	2.3%	1	12.5%	2	2.0%	2	5.0%
BLOOD ALKALINE PHOSPHATASE INCREASED	3	2.3%	0		2	2.0%	1	2.5%
BLOOD CREATININE INCREASED	2	1.5%	0		2	2.0%	0	
BODY TEMPERATURE INCREASED	3	2.3%	0		2	2.0%	1	2.5%
C-REACTIVE PROTEIN INCREASED	2	1.5%	0		1	1.0%	1	2.5%
S100 PROTEIN INCREASED	2	1.5%	0		1	1.0%	1	2.5%
TRANSAMINASES INCREASED	2	1.5%	0		1	1.0%	1	2.5%
AMYLASE INCREASED	1	0.8%	0		0		1	2.5%

BLOOD CREATINE PHOSPHOKINASE INCREASED	1	0.8%	0		0		1	2.5%
BLOOD GLUCOSE INCREASED	1	0.8%	0		0		1	2.5%
BLOOD LACTATE DEHYDROGENASE INCREASED	1	0.8%	0		0		1	2.5%
BLOOD POTASSIUM INCREASED	1	0.8%	0		1	1.0%	0	0.0%
EJECTION FRACTION DECREASED	1	0.8%	0		0		1	2.5%
GLOMERULAR FILTRATION RATE DECREASED	1	0.8%	0		0		1	2.5%
HEART RATE INCREASED	1	0.8%	0		1	1.0%	0	
LYMPH NODE PALPABLE	0		1	12.5%	0		1	2.5%
LYMPHOCYTE COUNT INCREASED	1	0.8%	0		1	1.0%	0	
NEUTROPHIL COUNT DECREASED	1	0.8%	0		0		1	2.5%
PLATELET COUNT DECREASED	1	0.8%	0		1	1.0%	0	
PLATELET COUNT INCREASED	1	0.8%	0		0		1	2.5%
METABOLISM AND NUTRITION DISORDERS	13	9.9%	2	25.0%	8	8.1%	7	17.5%
DECREASED APPETITE	8	6.1%	2	25.0%	4	4.0%	6	15.0%
HYPOKALAEMIA	3	2.3%	0		3	3.0%	0	
HYPOPHOSPHATAEMIA	2	1.5%	0		0		2	5.0%
HYPERURICAEMIA	1	0.8%	0		1	1.0%	0	0.0%

HYPONATRAEMIA	1	0.8%	0		1	1.0%	0	
IRON DEFICIENCY	1	0.8%	0		1	1.0%	0	
TYPE 1 DIABETES MELLITUS	0		0		0		0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	20	15.3%	2	25.0%	14	14.1%	8	20.0%
PAIN IN EXTREMITY	7	5.3%	0		5	5.1%	2	5.0%
BACK PAIN	5	3.8%	0		2	2.0%	3	7.5%
ARTHRALGIA	3	2.3%	1	12.5%	4	4.0%	0	0.0%
MYALGIA	2	1.5%	0		0		2	5.0%
FLANK PAIN	1	0.8%	0		1	1.0%	0	
GROIN PAIN	0		1	12.5%	0		1	2.5%
NECK PAIN	1	0.8%	0		1	1.0%	0	
POLYARTHRITIS	1	0.8%	0		1	1.0%	0	
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	1	0.8%	0		1	1.0%	0	
TUMOUR PAIN	1	0.8%	0		1	1.0%	0	
NERVOUS SYSTEM DISORDERS	38	29.0%	2	25.0%	27	27.3%	13	32.5%
HEADACHE	26	19.8%	1	12.5%	17	17.2%	10	25.0%
SYNCOPE	2	1.5%	1	12.5%	2	2.0%	1	2.5%

DIZZINESS	1	0.8%	1	12.5%	1	1.0%	1	2.5%
PARAESTHESIA	2	1.5%	0		1	1.0%	1	2.5%
CARPAL TUNNEL SYNDROME	1	0.8%	0		0		1	2.5%
DYSAESTHESIA	1	0.8%	0		0		1	2.5%
DYSGEUSIA	1	0.8%	0		1	1.0%	0	
HYPERTONIA	1	0.8%	0		1	1.0%	0	
MENINGISM	1	0.8%	0		1	1.0%	0	
MIGRAINE	1	0.8%	0		1	1.0%	0	
MUSCLE CONTRACTIONS INVOLUNTARY	1	0.8%	0		1	1.0%	0	
MYOCLONUS	1	0.8%	0		1	1.0%	0	
PRESYNCOPE	1	0.8%	0		1	1.0%	0	
SCIATICA	1	0.8%	0		0		1	2.5%
SENSORY LOSS	1	0.8%	0		1	1.0%	0	
TREMOR	1	0.8%	0		1	1.0%	0	
PSYCHIATRIC DISORDERS	4	3.1%	0		4	4.0%	0	
ANXIETY	1	0.8%	0		1	1.0%	0	
DEPRESSED MOOD	1	0.8%	0		1	1.0%	0	
DEPRESSION	1	0.8%	0		1	1.0%	0	

RESTLESSNESS	1	0.8%	0		1	1.0%	0	
RENAL AND URINARY DISORDERS	2	1.5%	0		2	2.0%	0	
POLLAKIURIA	1	0.8%	0		1	1.0%	0	
URINARY INCONTINENCE	1	0.8%	0		1	1.0%	0	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	1	0.8%	0		1	1.0%	0	
VULVOVAGINAL PRURITUS	1	0.8%	0		1	1.0%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	7	5.3%	1	12.5%	5	5.1%	3	7.5%
COUGH	4	3.1%	0		1	1.0%	3	7.5%
DYSPNOEA	3	2.3%	1	12.5%	4	4.0%	0	
PULMONARY MICROEMBOLISM	1	0.8%	0		1	1.0%	0	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	35	26.7%	2	25.0%	25	25.3%	12	30.0%
ERYTHEMA	7	5.3%	2	25.0%	7	7.1%	2	5.0%
NIGHT SWEATS	6	4.6%	0		2	2.0%	4	10.0%
RASH	6	4.6%	0		5	5.1%	1	2.5%
PRURITUS	4	3.1%	0		2	2.0%	2	5.0%
ACTINIC KERATOSIS	3	2.3%	0		3	3.0%	0	
HYPERHIDROSIS	3	2.3%	0		2	2.0%	1	2.5%
DERMATITIS CONTACT	2	1.5%	0		1	1.0%	1	2.5%

ALOPECIA	1	0.8%	0		1	1.0%	0	
COLD SWEAT	1	0.8%	0		1	1.0%	0	
DERMATITIS	1	0.8%	0		1	1.0%	0	
DRY SKIN	1	0.8%	0		0		1	2.5%
PEMPHIGOID	1	0.8%	0		1	1.0%	0	
RASH ERYTHEMATOUS	1	0.8%	0		1	1.0%	0	
RASH MACULO-PAPULAR	1	0.8%	0		1	1.0%	0	
SARCOID-LIKE REACTION	1	0.8%	0		0		1	2.5%
URTICARIA	1	0.8%	0		1	1.0%	0	
VASCULAR DISORDERS	21	16.0%	1	12.5%	12	12.1%	10	25.0%
HYPERTENSION	6	4.6%	1	12.5%	5	5.1%	2	5.0%
HYPOTENSION	6	4.6%	0		3	3.0%	3	7.5%
LYMPHOEDEMA	5	3.8%	0		2	2.0%	3	7.5%
FLUSHING	3	2.3%	0		1	1.0%	2	5.0%
AORTIC STENOSIS	1	0.8%	0		0		1	2.5%
EMBOLISM	1	0.8%	0		1	1.0%	0	
HYPERTENSIVE CRISIS	1	0.8%	0		1	1.0%	0	
SUPERFICIAL VEIN THROMBOSIS	1	0.8%	0		1	1.0%	0	

3.3.2. Discussion on clinical pharmacology

Pharmacokinetics

Clinical pharmacokinetics of Nidlegy (L19IL2 and L19TNFa) was characterised in studies in which the L19IL2 and L19TNFa were administered systemically as single agents (Study 01/05 and Study 02/07) and in Study 01/18, in which the combination of L19IL2 and L19TNFa was administered at the claimed posology (up to 13 Mio IU L19IL2 and 400 µg L19TNFa, depending on the size of the lesions) via the intratumoural route of administration. No thorough dose finding has been conducted and doses were not selected based on any exposure-response relationships. PK data supporting this submission are generally considered limited, and data are solely available for 19 patients in Study 01/18.

It should further be noted that in clinical studies with intratumoural injection of Nidlegy, there were uncertainties regarding the actually injected volume, and thus the dose administered. For the intravenous studies, the concerns are less, but not completely absent. In study PH-L19IL2TNF-02/15, there were also uncertainties whether the volume, and thus dose, injected per tumour was equal. This also raises uncertainties for study PH-L19TNFalpha-01/18. In addition, the size of the lesions within a patient, and also the amount and size of lesions between patients, probably differed in study PH-L19TNFalpha-01/18, and this has not been taken into account. Therefore, the PK data presented in this Overview, especially after intratumoural administration, should be regarded with caution.

Bioanalytical methods

For the quantification of L19IL2 and L19TNFa concentrations in human serum, a variety of different assay methods was utilised. In the early studies with systemic administration, L19IL2 was quantified in a DELFIA time-resolved fluoroimmunoassay (TR-FIA), in which L19IL2 in the sample was captured by a biotinylated EDB fragment (Study 01/05). Two distinct methods for the quantification of the intact L19TNFa antibody molecule (L19TNFa assay) and both the free TNFa and L19TNFa molecules (TNFa assay) were used in Study 02/07. In the L19TNFa assay, L19TNFa was captured by an immobilised EDB fragment and detected using an anti-human TNFa antibody. In the TNFa assay, TNFa was captured by an immobilised monoclonal anti-human TNFa antibody followed by binding of a second anti-human TNF antibody for detection. For quantification of TNFa in Study 01/18, a commercial kit representing an AlphaLISA bead-based immunoassay method was used. During clinical development of Nidlegy, methods were repeatedly transferred to and validated at different laboratories. Overall, PK methods appear acceptable, although some parameters did not always meet acceptance criteria as per ICH M10 and not all relevant parameters were included in the validation exercise. Of note, for method R PL 019 (L19TNF) used in Study 01/18, no results are available for dilutional linearity and hook effect. The applicant is asked to comment and provide results for these validation parameters. The applicant should further provide an analysis of PK results across studies, as far as the data are available, and a confirmation that the analytical methods are sufficiently comparable.

Immunophenotyping was performed either by flow cytometry of blood purified PBMCs (2 antibody panels in Study 02/15: (a) CD3/CD4/CD8/HLADR/CD11b/CD56/CD14/CD16 and (b) CD25/CD4/CD8/FoxP3) or IHC stainings (CD4, CD8, CD56, and FoxP3) of histological slides prepared from FFPE blocks. Since these methods were obviously not validated, any results are solely considered as exploratory evidence.

Human anti-fusion protein antibodies (HAFA) against L19IL2 and L19TNFa were determined using ELISA in different assay formats (sandwich, competition) with colorimetric readout and Surface Plasmon Resonance (SPR) as orthogonal technology. Throughout the studies, numerous different assay methods have been developed and validated, while it seems as if no cross-validation of the assays was performed, which requires justification. It is further indicated that sensitivity has not been validated for HAFA methods R PL 007 and 009. The applicant should discuss and provide respective results. Drug tolerance has not been part of the validation exercise of ADA assays used for Nidlegy. The applicant argues that timepoints for HAFA sampling were spaced in time (day1, day8, day 29 and three months after first injection) in order to minimise the presence of the drug in the sample, which is generally endorsed. However, since no trough sampling has been conducted in Study 01/18 and no PK sampling was done in Study 02/15 (the study for which most immunogenicity results are available), it is uncertain whether residual L19TNF or L19IL2 concentrations could still present in study samples. The absence of Nidlegy was verified in a subset of HAFA samples from Study 01/18 (N=8), that were subjected to L19IL2 and L19TNF quantification. The concentration of L19TNF and L19IL2 in the HAFA samples was below LLOQ. Accounting for the half-life reported for L19IL2 and L19TNFa in Study 01/18 (below 24 h), this finding seems plausible and would have been expected. On the other hand, since ADA interference with quantification of L19IL2 and L19TNF in the respective PK assays was not determined, it is also uncertain whether ADA present in PK samples could potentially interfere with detection of L19IL2 and L19TNF. Nevertheless, given that available results overall indicate that interference of L19IL2 or L19TNF in HAFA samples is unlikely, the issue is not further pursued.

In response to the D120 LoQ, the applicant provided a method description and validation results for a NAb assay that was used in Study 02/15 (method R PL 062). Table 4 of the Summary of Biopharmaceutic Studies (updated after D120 LoQ) should be revised by addition of HAFA validation criteria for method R PL 062.

Pharmacokinetic data analysis

For studies PH-L19IL2-01/05 and PHL19TNFalpha-02/07, the approach for PK analysis is not fully clear and relevant appendices for these studies are missing. The applicant is requested to clarify.

A population PK study of L19IL2 and L19TNF is foreseen by protocol in the PH-L19IL2TNF-01/18 study. No report is available yet as the study is still ongoing and the population PK samples will be bulk evaluated at the end of the study.

The applicant is however requested to commit to a PAM to submit the population PK analysis, including the investigation of PK in special populations (body weight, organ impairment, age, etc.), after authorisation (if applicable).

ADME

In Study PH-L19IL2TNF-01/18, patients received a dose tailored to the lesion diameter or total lesion diameter in case of multiple lesions (see dosing algorithm depicted Table 3-3-6). Thus, the recommended dose of 13 Mio IU of L19IL2 and 400 µg of L19TNFa (corresponding to the dose claimed in the current submission) was not administered to all patients. For the algorithm-based dosing regimen, Cmax was described to be 111.11 ng/mL at Day 1 and 116.35 ng/mL at Day 22 for L19IL2, and 2.5 ng/mL at Day 1 and 2.25 ng/mL at Day 22 for L19TNFa. The mean Cmax values for L19IL2 and L19TNFa after dosing were described to be reached around 2.2h and 2.3h after the first administration (corresponding to Tmax) of L19IL2 and L19TNF, respectively.

Bioavailability after intratumoural administration cannot be reliably determined. Dose levels, administration schedules and PK assays used in the studies with systemic administration of L19IL2 (Study 01/05) and L19TNFa (Study 02/07) were different from those used in study 01/18 with intratumoural administration. The applicant however conducted a calculation of systemic leakage of L19IL2 and L19TNFa, yielding an estimate of observed AUC(0-t) values being 20.6% and 24.3% of the theoretical AUC(0-t) values calculated for L19IL2 and L19TNFa. There are still uncertainties regarding the calculation of systemic leakage, see **LoOI**. Ultimately, considering that L19TNF and L19IL2 had been previously administered at higher dose levels via IV route of administration without raising significant safety concerns (RD of 22.5 MioIU of L19IL2 weekly and 13 µg/kg L19TNF), it can be assumed that the dose levels selected for the approval of Nidlegy should be equally safe, even if higher or even complete systemic leakage would occur.

As anticipated, half-life of L19IL2 and L19TNFa is longer with intratumoural administration (Study PH-L19IL2TNF-01/18) as compared to systemic administration, indicating sustained absorption after intratumoural injection. Given that almost all measured serum concentrations at day 22 pre-dose were <LLOQ, it seems that 1 week after administration, in general no more drug substance is present in the tumour. It is however unknown whether some residual drug is present in the tumour. No studies evaluating the tumour residence time of intralesionally administered L19IL2 and L19TNF have been conducted in human melanoma patients. The applicant claims that based on preclinical biodistribution studies and studies with systemically administered, radiolabelled L19 antibody, a residence time of several days can be expected for Nidlegy at the tumour. However, this is solely agreed on for L19TNF, but was not proven for L19IL2.

No metabolism studies were conducted. According to the applicant, L19IL2 and L19TNFa are expected to be eliminated via proteolytic catabolism. IL2 is however normally eliminated via rapid renal excretion. In line with this, a preferential excretion of L19IL2 via the urinary pathway was shown in a preclinical quantitative biodistribution study (Report R074, 4.2.2.5). Considering this, absence of conclusive data on human excretion pathways and data in patients with renal impairment is considered a limitation and should be adequately reflected in the SmPC.

Pharmacokinetics of L19IL2 and L19TNFa appeared to be approx. dose proportional after i.v. administration.

In Study PH-L19IL2TNF-01/18, AUC(0-t) determined for L19IL2 was 496.13 ng*h/mL on Day 1 and 460.62 ng*h/mL on Day 22. AUC(0-t) determined for L19TNFa was 7.93 ng*h/mL on Day 1 and 7.69 ng*h/mL on Day 22 (see Table 3-3-7). Overall, there is no indication of drug accumulation after intratumoural administration of Nidlegy in Study 01/18 (accumulation ratios for Cmax and AUC comparing Day 22 with Day 1 are below 1).

Additional PK data analyses and clarifications for Study PH-L19IL2TNF-01/18 are requested from the applicant (see **LoOI**). In general, it is unclear whether the values for AUC and Cmax presented in the SCP and SmPC were dose-normalised for the intratumoural administration, and why the values for systemic administration were not dose-normalised. The applicant should clarify this and present dose-normalised AUC and Cmax. The dose-normalisation should be equal for the data after intratumoural and after systemic administration so a meaningful comparison can be made.

Intra- and inter-individual variability

Inter-individual variability seems to be high, i.e. RSDs in most cases >100%, which may have been caused by the low number of subjects, different number of tumours per subject, and different locations of the tumour.

Intra-individual variability after intratumoural administration in Study 01/18 was low on average (the median of the ratio of concentrations of L19IL2 and L19TNF comparing Day 8, Day 15 and Day 22 with Day 1 administrations was 1).

Pharmacokinetic interactions

An in vitro enzyme inhibition assay has been conducted in response to the D120 LoQ. It was shown that neither L19IL2 nor L19TNF significantly inhibited the activity of six major CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5). Concentrations tested were up to 10x higher than the Cmax observed in the clinical trials. The respective information was included in section 4.5 of the SmPC.

PK in special populations

No data for patients with hepatic impairment are available. In Study PH-L19IL2TNF-01/18, 2 patients with renal impairment were included in Arm 1, while dense PK sampling has only been conducted in 1 patient. Regarding this patient, the applicant is requested to clarify the severity of renal impairment, and provide the plasma concentration-time curve in comparison to the other patients in the study. In addition, the applicant should provide any data available for patients with hepatic and renal impairment (e.g. from other studies than 01/18). The statement in section 4.2 of the SmPC should be further justified and revised accordingly. Furthermore, a paragraph on special populations should be included in section 5.2 of the SmPC, in which it is described that no dedicated studies have been conducted in patients with hepatic or renal impairment and, if so, that no PK data are currently available for these patients.

PK data by body weight have solely been provided for Study 01/18 and are based on a limited number of patients. No clear trend is indicated for the PK parameters of L19IL2 and L19TNF based on body weight. This observation could be due to the low number of subjects. The applicant did not elaborate whether the effect of body weight will be investigated in the popPK model. This is highly recommended, given that data from Study 01/18 presented above are derived from a small sample size and difficult to interpret. The applicant is asked to comment. In addition, the exposure plots (Cmax and AUC by body weight) should be provided for the full PK population by using dose-normalised Cmax and AUC.

Differences in L19IL2 exposure were indicated in female as compared to male patients in Study 01/18, with Cmax and AUC(0-t) being lower in female patients (see Table 3-3-8). Slight differences were also observed for L19TNFa. The applicant argues that the difference in half-life in study PH-L19IL2TNF-01/18 (intratumoural administration) is attributed to the high variability. However, in studies PH-L19IL2-01/05 and PH-L19TNFalpha-02/07, the variability is lower, but the half-life also differs, but the other way around. Based on the data, no definitive conclusion can be drawn.

No clear trend towards a different PK in the elderly is apparent based on the available data. Still, the applicant is requested to present the results of study PH-L19IL2-01/05 and study PH-L19TNF-02/07 using dose-normalised values, based on absolute doses, versus age. The applicant states that the

influence of age on PK parameters will be further investigated when additional data become available from the ongoing PH-L19IL2TNF-01/18, which also includes population PK. This is endorsed.

All subjects included in study PH-L19IL2TNF-01/18 so far were Caucasian. This means that at this time, no conclusions can be drawn on effect of ethnic factors at this time. This may be possible at the end of the trial, provided that patients of other ethnicities will also be included. Also in study PH-L19IL2-01/05, 100% of the subjects was Caucasian. The applicant is requested to clarify the ethnicity of the subjects in study PH-L19TNF-02/07.

In general, PK data for above mentioned subgroups should be provided as soon as Study PH-L19IL2TNF-01/18 has been completed and population PK analysis results are available (see **PAM**).

Pharmacodynamics

Nidlegy (L19IL2/L19TNFa) consists of the combination of two individual immunocytokines, bifikafusp alfa (also denominated L19IL2) and onfekafusp alfa (also denominated L19TNFa), which are mixed immediately prior to intralesional injection. The two individual cytokines IL2 and TNFa of fully human sequence are cloned and expressed as fusion proteins with the human antibody fragment L19. L19 is an antibody fragment in single-chain variable (scFv) format, which selectively binds to the splice variant of fibronectin containing the Extra-Domain B (EDB), a tumour-associated antigen that is found in the majority of aggressive tumour types, but is virtually undetectable in normal tissues (Carnemolla et al. 1989). Due to selective binding of L19 to EDB in the tumour, the pro-inflammatory action of the cytokines IL2 and TNFa can be induced at the site of disease; the L19 antibody moiety is intended to allow stable "anchoring" of the cytokine payloads in the tumour. IL2 is known to boost the activity of cytotoxic T lymphocytes, as well as T Helper and Natural Killer cells. TNFa is known to stimulate cell-based immunity, increase vascular permeability, and induce tumour necrosis, thereby directly killing tumour cells expressing the cognate TNFa receptor.

Immunophenotyping

The change in immune cell levels and composition after four intratumoural L19IL2/L19TNFa administrations in blood of patients taken at baseline, surgery, and follow-up visit, as well as in surgical specimen, was evaluated in the open-label, Phase III PH-L19IL2TNF-02/15 study.

Flow cytometry and IHC methods were used for immunophenotyping. As discussed before, applied methods were obviously not validated and therefore, reliability of results is considered questionable.

Only a limited number of patients was included for IHC analysis (N=32 corresponding to those patients enrolled at the coordinating center of University Hospital Schleswig-Holstein, Campus Kiel).

In the flow cytometry-based study using PBMC populations, a significant increase in regulatory T cells and a significant decrease in myeloid-derived suppressor cells (MDSCs) in patients who received L19IL2/L19TNFa followed by surgery as compared to patients who only underwent surgery could be observed.

In the visual analysis of IHC stainings, no significant differences were observed in the percentage of CD4+ T cells, CD8+ T cells, FoxP3+ Treg cells and CD56+ NK cells over the total tumour-infiltrating lymphocyte population between the two study arms. However, differences were observed in the digitally assisted analysis: CD4 T cells, CD8 T cells, FoxP3+ Treg, and NK cells were shown to be more abundant in patients with neoadjuvant L19IL2/L19TNFa treatment, than in patients who only received surgery. In line with this, a high or intermediate amount of infiltrating lymphocytes was determined by H&E staining in 89% of patients who received neoadjuvant L19IL2/L19TNFa, while high and

intermediate amount of infiltrating lymphocytes was only detected in approx. 40% of patients who only underwent surgery.

Overall, immunophenotyping data indicate that the treatment with L19IL2/L19TNFa is able to elicit an immunostimulatory and thereby anti-tumoural action.

Immunogenicity

In Study 02/12, HAFAs were solely analysed at screening and on Day 43. The overall incidence rate of HAFA was 26.3% (5 out of 19 evaluable patients), of which only IgG HAFA were identified for two patients by SPR and for three additional patients by ELISA.

In Study 02/15, the immunogenic potential of L19IL2 and L19TNFa was analysed on day 8 and day 29 as well as at the first follow-up visit at 3 months. HAFA against L19IL2 were detected in 39 patients (34.5%) and HAFA against L19TNFa were detected in 6 patients (5.3%). HAFA response appeared to be mainly transient, given that only 6 patients (5.3%) were found positive for HAFA against L19IL2 and only 2 patients (1.8%) for HAFA against L19TNFa at the follow-up visit. Although this may be agreed, it is noted that the initial immunogenic response a few weeks after start of treatment with Nidlegy appears relatively high (at least for HAFA against L19IL2). Study 02/15 was the only study in which neutralising HAFA were investigated. In only 4 patients (3.4%), a neutralising effect was detected.

The applicant provided an analysis of adverse events (SOC and PT) by ADA status (positive or negative for L19IL2 or L19TNF). Referring to table 9 in the updated SCP (2.7.2), a trend towards more frequent occurrence of some disorders (e.g. cardiac disorders, GI disorders, investigations, vascular disorders) or specific PTs (e.g. injection site reactions, pyrexia, headache, decreased appetite) in patients positive for L19IL2 antibodies is indicated. The sample size of patients with anti-L19TNF antibodies was too low to draw reliable conclusions. For a better overview, the applicant should further provide a table on overall adverse events (all-cause and related AEs, all-cause and related SAEs, ≥ grade 3 AEs, AEs leading to treatment discontinuations and interruptions) by ADA status in studies PH-L19IL2TNF-02/12 and PH-L19IL2TNF-02/15.

Since in Study 02/15, no PK sampling was conducted in parallel with HAFA sampling, the impact of ADA positivity on PK cannot be analysed. Because the applicant claims in the SmPC that no evidence of ADA impact on pharmacokinetics was observed, the applicant is requested to present PK data of ADA-positive versus ADA-negative patients from study PH-L19IL2-01/05 and PH-L19TNF-02/07 in order to substantiate this claim.

The impact of the presence of HAFA in systemic circulation on the efficacy of L19IL2/L19TNFa is overall assumed to be minor, given that patients will be treated with a maximum of 4 doses/4 weeks via the intratumoural route of administration.

3.3.3. Conclusions on clinical pharmacology

The available information on pharmacokinetics of Nidlegy after intratumoural administration is sparse. Further PK data and analyses are requested and clarification on some outstanding issues is required. The applicant is requested to commit to submit an update of the PK data, including population PK modelling analysis with investigation of PK in special populations (body weight, organ impairment, age, etc.), when study PH-L19IL2TNF-01/18 has been completed **(PAM)**.

The immunogenic potential of Nidlegy after intratumoural administration was characterised in a larger patient population in the pivotal study 02/15, indicating a considerable and potentially clinically relevant presence of HAFA against L19IL2. Additional data of the impact of HAFA positivity on safety are requested.

Finally, PK/PD data presented in the SmPC should be revised, see LoOI and comments included in the SmPC.

3.3.4. Clinical efficacy

Table 17. Clinical studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
PH-L19IL2TNF-02/12	Completed Start date: 05-Dec-2012 End date: 23-Mar-2015 Enrolled: n=22 Planned: n=20	Phase 2 (exploratory) Single-arm, uncontrolled, multicenter (2 sites)	Mixture of: L19IL2: 10 Mio. IU L19TNF: 312 µg (dose adjustments between 78-312 µg possible for L19-TNF) Intratumoural injection once every week for up to 4 weeks Follow-up: up to 52 weeks 1 year FU for OS	▪ Stage III or stage IV M1a melanoma (AJCC 7th Ed.) ▪ Presence of measurable and injectable cutaneous and/or subcutaneous lesions ▪ Males or females, age > 18 years ▪ Life expectancy of at least 12 weeks
PH-L19IL2TNF-02/15	Ongoing Enrolment completed Start date: 29-Jun-2016 Enrolled: n= 246 Planned: n= 214	Phase 3 (pivotal) Open-label, randomised (1:1), controlled, multicenter	Mixture of: L19IL2: 13 Mio. IU L19TNF: 400 µg (dose adjustments between 100-400 µg possible for L19TNF) Arm 1: Intratumoural injection once every week for up to 4 weeks followed by surgery	▪ Melanoma locally advanced disease (III B and III C; according to AJCC 7th Ed.) eligible for complete surgical resection ▪ Adult patients with an age ≥18 years ▪ At least one injectable cutaneous,

			Arm 2: Surgery of all lesions within 4 weeks Follow-up: up to 36 months 5 years FU for OS	subcutaneous, or nodal melanoma ▪ Post-surgery EMA-approved adjuvant therapy allowed at discretion of the treating physician ▪ Life expectancy of at least 24 months
PH-L19IL2TNF-01/18 *No clinical efficacy data are available.*	Ongoing Enrolment not completed Enrolled: 107 (cut-off: 29-Nov-2024). Planned: 186 Start: September 2018	Phase 3 Open-label, randomised (1:1), controlled, multicenter	Mixture of: L19IL2: 13 Mio. IU L19TNF: 400 µg Arm 1: Intralesional injections) followed by SoC (surgery and adjuvant therapy) Arm 2: SOC	▪ Stage IIIB/IIIC melanoma

3.3.4.1. Dose-response studies

No dose response studies with the combination of L19L2 and L19TNF have been performed.

In the Phase II study of L19IL2/L19TNF in Stage IIIB, IIIC and IVM1a melanoma patients, L19TNF at the dose of 312 µg (approximately 31% of the systemic RD) was added to L19IL2 10.0 Mio IU for combined intralesional administration. No PK exposure data are available from this trial.

In the Phase III studies in the neoadjuvant setting, slightly higher doses of both L19IL2 and L19TNF (13 Mio IU L19IL2 and 400 µg L19TNF, 57.8% or approximately 40% of the systemic RD of L19IL2 and L19TNF, respectively) than those tested in the previous phase II trial were used, to promote a rapid mount of the systemic effect before the surgical removal of the injected lesions.

See Section 3.3.1 for additional details.

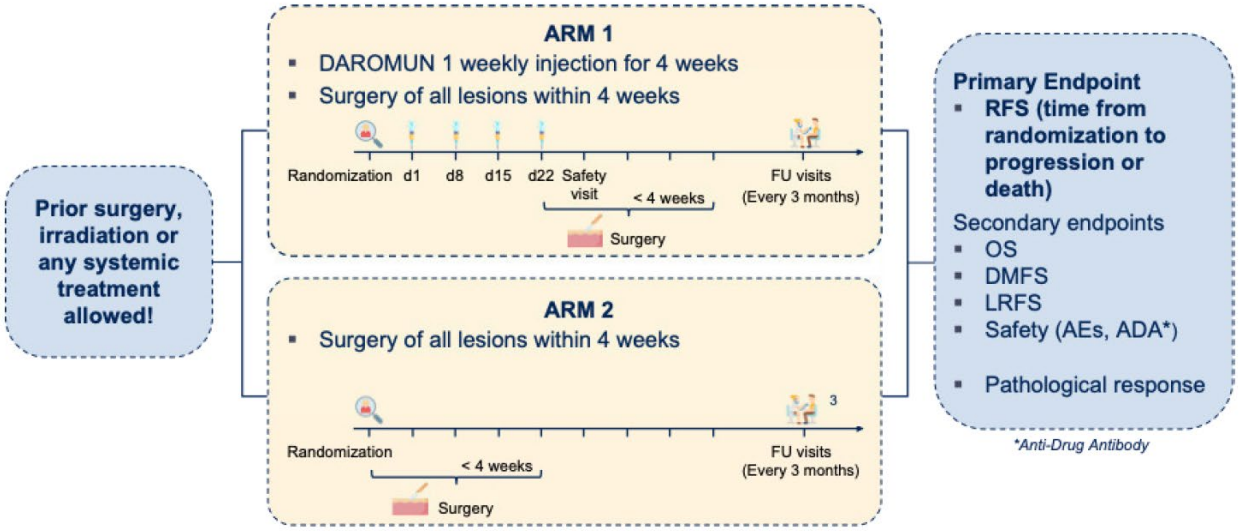
3.3.4.2. Main study

PH-L19IL2TNF-02/15

Methods

Study PH-L19IL2TNF-02/15 was a pivotal Phase III, open label, randomised, controlled multi-center trial to evaluate the efficacy of L19IL2/L19TNF neoadjuvant intratumoural treatment followed by surgery versus surgery alone in patients with clinical stage III B/C melanoma with/without prior therapy and presence of injectable cutaneous and/or subcutaneous or nodal metastases. Post-surgery treatment with EMA-approved adjuvant therapies were allowed, at the discretion of the treating physician in both arms of the study.

Figure 3. Study schema PH-L19IL2TNF-02/15



Study Participants

Key inclusion criteria:

- Adult patients with an age ≥18 years
- ECOG Performance Status/WHO Performance Status ≤ 1
- Diagnosis of malignant melanoma of the skin with locally advanced disease as defined by clinical stage III B and III C according to AJCC 7th Ed., eligible for complete surgical resection
- Eligible subjects must have measurable disease and must be candidate for intralesional therapy with at least one injectable cutaneous, subcutaneous, or nodal melanoma lesion (≥ 10 mm in longest diameter) or with multiple injectable lesions that in aggregate have a longest diameter of ≥ 10 mm
- Prior anti-tumour treatment for the primary melanoma lesion, including surgery and approved adjuvant treatments (e.g. radiotherapy, immune checkpoint inhibitors, BRAF/MEK inhibitors, etc.) was allowed
- Post-surgery EMA-approved adjuvant therapy was allowed in both Arms at discretion of the treating physician
- Life expectancy of at least 24 months

Key exclusion criteria:

- Uveal melanoma, mucosal melanoma or melanoma with unknown primary
- Evidence of distant metastases at screening
- Previous or concurrent cancer that is distinct in primary site or histology from the cancer being evaluated in this study except cervical carcinoma in situ, treated basal cell carcinoma, superficial bladder tumours (Ta, Tis & T1), second primary melanoma in situ or any cancer curatively treated ≥5 years prior to study entry
- Anti-tumour therapy (except allowed treatments listed at point 3 of Inclusion criteria) within 4 weeks before enrolment

- Previous in vivo exposure to monoclonal antibodies for biological therapy (except allowed treatments listed at point 3 of Inclusion criteria) in the 6 weeks before enrolment
- Planned administration of growth factors or immunomodulatory agents (except allowed treatments listed at point 3 of Inclusion criteria) within 7 days before enrolment
- Patient requiring or taking corticosteroids or other immunosuppressant drugs on a long-term basis. Limited use of corticosteroids to treat or prevent acute hypersensitivity reactions is not considered an exclusion criterion
- Presence of active infections (e.g., requiring antimicrobial therapy) or other severe concurrent disease, which, in the opinion of the investigator, would place the patient at undue risk or interfere with the study
- Active autoimmune disease.

Treatments

As per study protocol, patients in the treatment Arm (Arm 1) should receive a mixture of L19IL2 (13 Mio IU) and L19TNF (400 µg), administered intratumourally into all injectable cutaneous, subcutaneous, and nodal tumours once weekly for up to 4 weeks. The volume of the prepared mixture was 2 ml. The dose of L19TNF could be adjusted between 100 and 400 µg (i.e., 100 µg, 200 µg, 300 µg or 400 µg) for each of the 4 administrations according to size and number of lesions and based on the patient's tolerability to the treatment at the investigator's discretion. No dose adaptions for the L19IL2 component were foreseen.

Patients were treated once weekly until all lesions disappeared or for maximal 4 weeks. A tolerance of +/- 3 days was acceptable. If new regional lesions occurred during these 4 weeks, they were treated together with the pre-existing metastases.

At each treatment visit, the investigator had to evaluate all known melanoma lesions as injectable or non-injectable. The total daily dose was distributed between all injectable cutaneous, sub-cutaneous and nodal metastases until no lesion was left un-injected or until no volume was left to inject, whichever occurred first.

If the procedure of injection of a specific lesion, due to any reason, posed a significant risk for the health of the patient, the physician could spare the specific lesion from injection, and documented it as "non-injectable lesion". If indicated, injections were guided by sonography for deep soft tissue metastases to ensure the intratumoural route.

Patients in Arm 2 received directly surgical resection of melanoma tumour lesions within 4 weeks after randomisation without any intertumoural administrations.

Objectives

The primary objective was to demonstrate superior efficacy of L19IL2/L19TNF neoadjuvant treatment followed by surgery compared to surgery alone in terms of recurrence-free survival (RFS) of patients with locally advanced and fully resectable melanoma. Post-surgery adjuvant treatment was allowed in both arms.

The secondary objective of the study was to demonstrate superior efficacy of L19IL2/L19TNF neoadjuvant treatment followed by surgery compared to surgery alone in terms of overall survival (OS).

Other secondary objectives were to demonstrate

- Improvement in terms of local recurrence-free survival (LRFS) in L19IL2/L19TNF+surgery compared to surgery alone
- Improvement in terms of distant metastasis-free survival (DMFS) in L19IL2/L19TNF+surgery compared to surgery alone
- Pathological responses (i.e., pathological Complete Response, pathological near-Complete Response, pathological Partial Response, pathological Non Response) assessed on the surgical specimen after tumour removal in L19IL2/L19TNF+surgery
- Safety and tolerability of L19IL2/L19TNF treatment

Outcomes/endpoints

Primary Endpoint:

The primary endpoint was defined as RFS, which is the time between the date of first recurrence (local, regional, distant or primary melanoma, whichever comes first) or death, and the date of randomisation.

Secondary Endpoints:

Secondary endpoints were defined as:

- Overall survival (OS) in the treatment arm (L19IL2/L19TNF plus surgery followed by adjuvants; Arm 1) versus control arm (surgery followed by adjuvants; Arm 2)
- Local recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS) in the treatment arm (L19IL2/L19TNF plus surgery - Arm 1) versus control arm (Arm 2)
- Proportion of patients with pathological responses (defined as the proportion of patients with a pathological CR, pathological near-CR, or pathological PR) in Arm 1
- Safety of intratumoural administration of L19IL2/L19TNF
- Biomarker studies (both arms): immunophenotypic characterisation of PBMCs for changes in absolute counts and relative percentages of lymphocytic subpopulations (e.g., Tregs, MDSCs, etc.) over time (only for patients recruited in German centres; the data will be collected for at least 50 patients)
- Assessment of the formation of human anti-fusion protein antibodies (HAFA) against L19IL2 and L19TNF

Sample size

The sample size was calculated assuming a two-sided log-Rank test for the RFS Kaplan-Meier curves comparison between subjects randomised to receive L19IL2/L19TNF plus surgery (Arm 1) and surgery

(Arm 2). A Sequential Design with a two-sided overall alpha of 0.05 and power of 0.85 was implemented. Approximately 158 (79+79) subjects were needed to observe the required 95 recurrence events assuming a 25% rate for permanent early censoring.

Investigation of Overall Survival was considered a key secondary objective. The overall two-sided alpha level was set to 0.05 with approximately 85% of power. Approximately 214 (107+107) subjects were needed to observe the required 104 death events, assuming a 10% rate for permanent early censoring.

Randomisation and blinding (masking)

This was an open-label study. A randomisation list was prepared for each study site using permuted block randomisation and equal treatment allocation to either receive L19IL2/L19TNF plus surgery or surgery alone. The block sizes were chosen randomly from two different pre-specified sizes (2 and 4) and within each block the order of treatments was randomly permuted.

No stratification was used.

Statistical methods

Analysis Population

Primary Analysis Population (PAS): all patients enrolled and randomised to the two different arms of the study (ITT population). Patients lost during the study were considered as censored to the last available assessment date. Efficacy objectives were investigated in the PAS population.

Efficacy analysis

Primary endpoint analysis: a two-sided log rank test with alpha equal to 5% was used to test equality between RFS in the L19IL2/L19TNF plus surgery arm and RFS in the surgery arm alone. Kaplan Meier estimators were planned to be provided for both arms.

Table 18. Censoring Scheme for RFS

Situation	Date of Event or Censoring	Outcome for RFS
Recurrence (local, regional, distant, new primary melanoma)	Date of first recurrence	Event
Death without recurrence before the last RFS follow-up	Date of death	Event
Death without recurrence after the last RFS follow-up	Date of last RFS follow-up	Censored
No recurrence and no death	Date of last RFS follow-up	Censored
Patient discontinuation for any reason other than recurrence or death	Date of last available visit	Censored
New anticancer treatment started (except allowed adjuvant therapies)	Date of last tumor assessment	Censored
Patient not-NED at last surgery	Date of Randomization	Censored
Patient did not have surgery	Date of Randomization	Censored

Multiplicity

Conflicting information were provided throughout the study protocol and CSR on the testing of multiple endpoints (RFS and OS) or specifically whether OS was planned to be tested confirmatory.

- Protocol section 6.9.1: Secondary endpoints such as OS were analysed for explorative purpose and no multiplicity adjustment was foreseen.
- Protocol section 6.9.3: At the time of RFS primary analysis, an interim analysis for OS was planned including Lan-DeMets alpha spending function to adjust for an interim look. In case of significant result, OS monitoring would have been stopped.

Interim analyses

Two interim analysis of RFS were planned: at first interim analysis approximately 24 events were to be considered (25% information fraction), while at second about 48 events (50% information fraction).

One interim analysis for OS was planned at the time of the primary analysis (95th recurrence event). Lan-DeMets alpha spending function were to be implemented to determine the required nominal alpha level considering the death information fraction at the time of the analysis (Number of observed death events / Expected number of death events [104]). In case of significant result, the OS monitoring may be stopped. Final decision was left to the DSMB.

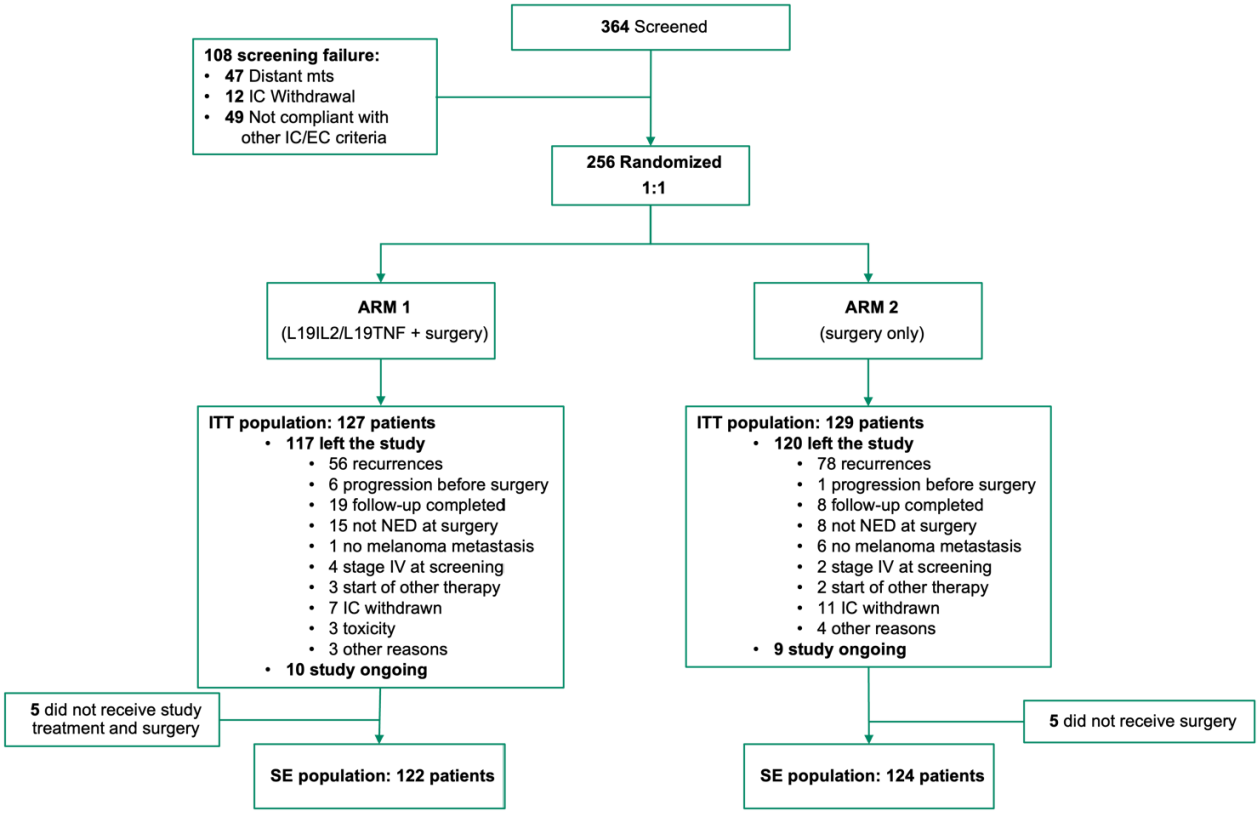
Changes to the statistical methods

Initially, a different primary endpoint and fixed study design was planned, which was changed in several protocol amendments:

- Protocol version 4 (21.12.2018): Primary endpoint remained RFS rate at 1 year as per initial protocol. Two interim analyses at 25% and 50% of expected number of events (95) were introduced. Lan-DeMets alpha spending function with O'Brien-Fleming boundaries were planned to adjust for the interim analyses. The sample size calculation remained approximately 214 patients.
- Protocol version 5 (02.02.2021): Primary endpoint was changed to RFS in general. According to the CSR, this change has been discussed and agreed with the European Medicines Agency. No documentation on this discussion was provided by the applicant. Furthermore, an interim analysis for OS was specified at the of RFS primary analysis. The use of EMA-approved adjuvant therapy after surgery was allowed.

Participant flow

Figure 4. Flow chart and patient disposition as per BICR assessment



Recruitment

First patient enrolled:	29-Jun-2016
Primary Data cut-off:	03-May-2023
Secondary Data cut-off:	29-Nov-2024

Recruitment:	Completed
Trial status:	Ongoing

Conduct of the study

Please refer to Statistical methods.

Protocol amendments

The attached table below shows the version and date of approval by CA and EC for the various amendments of the clinical protocol approved in Germany (the first country in chronological order to which each amendment was submitted). The table also indicates the number of patients included at the date when each protocol version went into force.

Table 19. Protocol amendments

	Approval date		Patients enrolled
Protocol version	CA	EC	
2 dated 27/11/2015	22/12/2015	02/06/2016	0
3 dated 26/05/2016	05/07/2016	25/07/2016	1
4 dated 12/12/2018	07/01/2019	28/01/2019	77
5 dated 22/01/2021	19/02/2021	11/03/2021	158
6 dated 08/02/2022	17/02/2022	23/03/2022	236
7 dated 10/01/2024	19/03/2024	16/04/2024	257

Protocol deviations

Table 20. Major protocol deviations

Protocol deviation	Total	Arm 1	Arm 2	Both arms
MPD-GCP	22	17	0	5
MPD	187	119	55	13
Minor	1628	1104	524	0

Table 21. Major protocol deviations

Major Protocol deviation	Total (N=187)	Arm 1	Arm 2	Both arms
IC/EC criteria	68	31	37	0
Skipping of dose admin.	17	17	0	0
Study procedures not compliant	24	13	6	5
Wrong/Missing HAFA sampling	22	17	5	0

Wrong storage of IMP	18	18	0	0
Skipping scheduled visits	4	2	2	0
Wrong IMP dose	9	9	0	0
Wrong IMP administration	1	1	0	0
Development of withdrawal criteria w/o withdrawal	6	3	3	0
Consent procedures not in compliance	6	2	2	2
Data breaches	5	-	-	5
Not compliant IMP management	5	5	-	0
Wrong eCRF data entry/management	1	0	0	1
Wrong SAE reporting	1	1	0	0

Baseline data

Table 22. Summary of demographics and baseline characteristics in ITT population

	PH-L19IL2TNF-02/15		
	Arm 1	Arm 2	Total
	(n =127)	(n=129)	(n=256)
Age , years Median (range)	(n=126) 64 (23-88)	(n=129) 63 (22-91)	(n=255) 64 (22-91)
Age < 65 yr Median (range)	(n=65) 56 (23-64)	(n=68) 52 (22-64)	(n=133) 53 (22-64)
65⩽Age<75 yr Median (range)	(n=33) 69 (65-74)	(n=32) 69 (65-74)	(n=65) 69 (65-74)
75⩽Age<85 yr Median (range)	(n=24) 79 (75-843)	(n=23) 78 (75-84)	(n=47) 79 (75-84)
Age ≥ 85 yr Median (range)	(n=4) 87 (85-88)	(n=6) 86 (85-91)	(n=10) 86.5 (85-91)
Gender — no. (%)			
Females	54 (42.5%)	55 (42.6%)	109 (42.6%)
Males	73 (57.5%)	74 (57.4%)	147 (57.4%)
ECOG PS – no. (%)			
0	118 (92.9%)	125 (96.9%)	243 (94.9%)
1	95 (7.1%)	4 (3.1%)	13 (5.1%)
LDH level — no. (%)			
Low or normal	110 (86.6%)	106 (82.2%)	216 (84.4%)
High	17 (13.4%)	23 (17.8%)	40 (15.6%)
BRAF status — no. (%)			
Mutated	49 (35.6%)	44 (34.1%)	93 (36.3%)
Wild type	43 (33.9%)	50 (38.8%)	93 (36.3%)
Unknown	35 (27.6%)	35 (27.1%)	70 (27.3%)
Stage (AJCCv7) no. (%)			

III B	38 (29.9%)	39 (30.2%)	77 (30.1%)
III C	66 (52.0%)	69 (53.5%)	135 (52.7%)
III X	8 (6.3%)	15 (11.6%)	23 (9.0%)
IV	7 (5.5%)	2 (1.5%)	9 (3.5%)
ND	8 (6.3%)	4 (3.1%)	12 (4.7%)
Ulceration* — no. (%)			
Yes	57 (44.9%)	68 (52.7%)	125 (48.8%)
No	53 (41.7%)	48 (37.2%)	101 (39.4%)
Unknown	17 (13.4%)	13 (10.1%)	30 (11.7%)
De novo/Recurrent			
De novo	6 (4.7%)	4 (3.1%)	10 (3.9%)
Recurrent	121 (95.3%)	125 (96.9%)	246 (96.1%)
Median time from first diagnosis of melanoma	35.5 months (95% CI: 20.0 – 52.0)	32 months (95% CI: 14.0 – 55.0)	22.5 months (95% CI: 15.0 – 29.0)
Number of LN - no. (%)			
0	61 (48.0%)	62 (48.1%)	123 (48.0%)
1	47 (37.0%)	46 (35.7%)	93 (36.3%)
2	12 (9.5%)	12 (9.3%)	24 (9.4%)
3	4 (3.1%)	6 (4.6%)	10 (3.9%)
4	2 (1.6%)	3 (2.3%)	5 (1.9%)
5	1 (0.8%)	0 (0%)	1 (0.8%)
Anatomical localisation			
Head and neck	13 (10.2%)	15 (11.6%)	28 (10.9%)
Lower limb and hip	55 (43.3%)	63 (48.8%)	118 (46.1%)
Upper limb and shoulder	45 (35.4%)	40 (31.0%)	85 (33.2%)
Trunk	22 (17.3%)	17 (13.2%)	39 (15.2%)
Under revision	1 (0.8%)	3 (2.3%)	4 (1.6%)

Baseline characteristics of study participants who were and were not censored for non-administrative reasons

Table 23. Baseline characteristics of study participants who were censored for non-administrative reasons

Table 1/ITT: Summary of Baseline Characteristics - Non Administrative

		Arm 1 N (37)	Arm 1 (%)	Arm 2 N (29)	Arm 2 (%)	Total N (66)	Total (%)
ECOG[1] (Freq.)	0	34	(91.89%)	27	(93.10%)	61	(92.42%)
	1	3	(8.11%)	2	(6.90%)	5	(7.58%)
Number of Lymph Nodes	0	18	(48.65%)	15	(51.72%)	33	(50.00%)
	1	12	(32.43%)	10	(34.48%)	22	(33.33%)
	2	4	(10.81%)	2	(6.90%)	6	(9.09%)
	3	2	(5.41%)	2	(6.90%)	4	(6.06%)
	4	1	(2.70%)			1	(1.52%)
Prior Surgeries	0	3	(8.11%)			3	(4.55%)
	1	7	(18.92%)	4	(13.79%)	11	(16.67%)
	2	12	(32.43%)	12	(41.38%)	24	(36.36%)
	>=3	15	(40.54%)	13	(44.83%)	28	(42.42%)
Number of Lesions	1	16	(43.24%)	18	(62.07%)	34	(51.52%)
	11			1	(3.45%)	1	(1.52%)
	12	1	(2.70%)			1	(1.52%)
	2	8	(21.62%)	2	(6.90%)	10	(15.15%)
	3	8	(21.62%)	5	(17.24%)	13	(19.70%)
	4	2	(5.41%)	2	(6.90%)	4	(6.06%)
	5	1	(2.70%)			1	(1.52%)
	7	1	(2.70%)			1	(1.52%)
	9			1	(3.45%)	1	(1.52%)
Current Stage AJCC 7th Ed. (Freq.)[2]	3B	6	(16.22%)	7	(24.14%)	13	(19.70%)
	3C	22	(59.46%)	17	(58.62%)	39	(59.09%)
	3X	2	(5.41%)	4	(13.79%)	6	(9.09%)
	IV	5	(13.51%)	1	(3.45%)	6	(9.09%)
	UNK	2	(5.41%)			2	(3.03%)
Current Stage AJCC 8th Ed. (Freq.)	3B	5	(13.51%)	4	(13.79%)	9	(13.64%)
	3C	21	(56.76%)	20	(68.97%)	41	(62.12%)
	3D	4	(10.81%)	1	(3.45%)	5	(7.58%)
	IV	5	(13.51%)	1	(3.45%)	6	(9.09%)
	UNK	2	(5.41%)	3	(10.34%)	5	(7.58%)
Details Localization*	Upper limb and shoulder	12	(32.43%)	9	(31.03%)	21	(31.82%)
	Head and neck	4	(10.81%)	3	(10.34%)	7	(10.61%)
	Lower limb and hip	18	(48.65%)	12	(41.38%)	30	(45.45%)
	Trunk	6	(16.22%)	6	(20.69%)	12	(18.18%)

Race (Freq.)	Caucasian	36	(97.30%)	27	(93.10%)	63	(95.45%)
	White	1	(2.70%)	2	(6.90%)	3	(4.55%)
De Novo/Recurrent Disease	DeNovo	3	(8.11%)			3	(4.55%)
	Recurrent	34	(91.89%)	29	(100.00%)	63	(95.45%)
Gender (Freq.)	Female	20	(54.05%)	13	(44.83%)	33	(50.00%)
	Male	17	(45.95%)	16	(55.17%)	33	(50.00%)
Lactate Dehydrogenase	HIGH	5	(13.51%)	3	(10.34%)	8	(12.12%)
	NORMAL-LOW	32	(86.49%)	26	(89.66%)	58	(87.88%)
Localization*	Lymph Node	19	(51.35%)	14	(48.28%)	33	(50.00%)
	Skin	24	(64.86%)	16	(55.17%)	40	(60.61%)
	Soft Tissue	1	(2.70%)	1	(3.45%)	2	(3.03%)
Age (years)	Max	85		85		85	
	Mean	64.3		59.7		62.3	
	Median	67		60		65	
	Min	31		22		22	
	N	37		29		66	
	SD	14.32		14.42		14.44	
18<=Age<65 (years)	Max	62		60		62	
	Mean	50.4		48.5		49.5	
	Median	50		49		50	
	Min	31		22		22	
	N	16		15		31	
	SD	8.97		10.34		9.54	
65<=Age<75 (years)	Max	74		74		74	
	Mean	69.6		68.8		69.2	
	Median	69		68.5		69	
	Min	65		65		65	
	N	11		10		21	
	SD	3.11		3.12		3.06	
75<=Age<85 (years)	Max	84		78		84	
	Mean	80		77		79.3	
	Median	79		78		79	
	Min	76		75		75	
75<=Age<85 (years)	N	9		3		12	
	SD	2.45		1.73		2.6	
Age>=85 (years)	Max	85		85		85	
	Mean	85		85		85	
	Median	85		85		85	
	Min	85		85		85	
	N	1		1		2	
	SD					0	
BRAF Mutation	Mutated	14	(37.84%)	13	(44.83%)	27	(40.91%)
	UNK	13	(35.14%)	10	(34.48%)	23	(34.85%)
	Wild Type	10	(27.03%)	6	(20.69%)	16	(24.24%)
Ulceration	No	10	(27.03%)	9	(31.03%)	19	(28.79%)
	UNK	5	(13.51%)	2	(6.90%)	7	(10.61%)
	Yes	22	(59.46%)	18	(62.07%)	40	(60.61%)
Prior Radiotherapies	No	35	(94.59%)	29	(100.00%)	64	(96.97%)
	Yes	2	(5.41%)			2	(3.03%)
Prior Antineoplastic Therapies	No	26	(70.27%)	21	(72.41%)	47	(71.21%)
	Yes	11	(29.73%)	8	(27.59%)	19	(28.79%)

ECOG[1] (Eastern Cooperative Oncology Group) Performance Status:
0 = Able to carry out all normal activity without restriction
1 = Restricted in physically strenuous activity but ambulatory and able to carry out light work;
2 = Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours;
3 = Capable of only limited self-care; confined to bed or chair more than 50% of waking hours;
4 = Completely disabled; cannot carry on any self-care; totally confined to bed or chair; 5= Death;
Current Stage AJCC 7th Ed. (Freq.)[4]: UNK = data under revision;
III x = stage III b/III c with pathological T or N subcategories not available

Median Sum Diameters of Target Lesions (mm)	36.75 (95% CI: 29.80 - 44.20)	26 (95% CI: 20.67 - 36.00)	35 (95% CI: 25.50 - 37.00)
Median Time from First Diagnosis of Melanoma (months)	27 (95% CI: 13.00 - 53.00)	22 (95% CI: 11.00 - 40.00)	22 (95% CI: 14.00 - 37.00)
Median number of Prior Surgeries	2 (1.00 - 12.00)	2 (1.00 - 12.00)	2 (1.00 - 12.00)

Table 24. Baseline characteristics of study participants who were not censored for non-administrative reasons

Table 1/ITT: Summary of Baseline Characteristics - Other

		Arm 1 N (85)	(%)	Arm 2 N (95)	(%)	Total N (180)	(%)
Age (years)	N	84		95		179	
	Mean	63.2		62.5		62.8	
	SD	14.18		14.6		14.37	
	Min	23		25		23	
	Max	88		91		91	
	Median	64		64		64	
18<=Age<65 (years)	N	44		50		94	
	Mean	53		51.2		52	
	SD	11.45		9.77		10.57	
	Min	23		25		23	
	Max	64		64		64	
	Median	57		53		55	
65<=Age<75 (years)	N	22		22		44	
	Mean	69.6		69		69.3	
	SD	2.4		1.84		2.14	
	Min	65		66		65	
	Max	74		73		74	
	Median	69.5		69		69	
75<=Age<85 (years)	N	15		19		34	
	Mean	78.7		79.3		79	
	SD	2.77		2.84		2.79	
	Min	75		75		75	
	Max	84		84		84	
	Median	79		79		79	
Age>=85 (years)	N	3		4		7	
	Mean	87.3		87.5		87.4	
	SD	0.58		2.38		1.72	
	Min	87		86		86	
	Max	88		91		91	
	Median	87		86.5		87	
Gender (Freq.)	Female	33	(38.82%)	42	(44.21%)	75	(41.67%)
	Male	52	(61.18%)	53	(55.79%)	105	(58.33%)
Race (Freq.)	Caucasian	84	(98.82%)	92	(96.84%)	176	(97.78%)
	Not Available	1	(1.18%)	2	(2.11%)	3	(1.67%)
	White			1	(1.05%)	1	(0.56%)
ECOG[1] (Freq.)	0	79	(92.94%)	93	(97.89%)	172	(95.56%)
	1	6	(7.06%)	2	(2.11%)	8	(4.44%)
Lactate Dehydrogenase	HIGH	11	(12.94%)	19	(20.00%)	30	(16.67%)
	NORMAL-LOW	74	(87.06%)	76	(80.00%)	150	(83.33%)
Current Stage AJCC 7th Ed. (Freq.)[2]	3B	30	(35.29%)	30	(31.58%)	60	(33.33%)
	3C	42	(49.41%)	51	(53.68%)	93	(51.67%)
	3X	6	(7.06%)	11	(11.58%)	17	(9.44%)
	IV	2	(2.35%)			2	(1.11%)
	UNK	5	(5.88%)	3	(3.16%)	8	(4.44%)
BRAF Mutation	Mutated	33	(38.82%)	28	(29.47%)	61	(33.89%)
	UNK	22	(25.88%)	25	(26.32%)	47	(26.11%)
	Wild Type	30	(35.29%)	42	(44.21%)	72	(40.00%)
Ulceration	No	41	(48.24%)	38	(40.00%)	79	(43.89%)
	UNK	12	(14.12%)	10	(10.53%)	22	(12.22%)
	Yes	32	(37.65%)	47	(49.47%)	79	(43.89%)
De Novo/Recurrent Disease	DeNovo	3	(3.53%)	4	(4.21%)	7	(3.89%)
	Recurrent	82	(96.47%)	91	(95.79%)	173	(96.11%)
Number of Lesions	1	50	(58.82%)	57	(60.00%)	107	(59.44%)
	2	15	(17.65%)	19	(20.00%)	34	(18.89%)
	3	8	(9.41%)	7	(7.37%)	15	(8.33%)
	4	10	(11.76%)	9	(9.47%)	19	(10.56%)
	5	1	(1.18%)	1	(1.05%)	2	(1.11%)
	6	1	(1.18%)	1	(1.05%)	2	(1.11%)
	7			1	(1.05%)	1	(0.56%)
Number of Lymph Nodes	0	39	(45.88%)	45	(47.37%)	84	(46.67%)
	1	34	(40.00%)	36	(37.89%)	70	(38.89%)
	2	8	(9.41%)	8	(8.42%)	16	(8.89%)
	3	2	(2.35%)	4	(4.21%)	6	(3.33%)
	4	1	(1.18%)	2	(2.11%)	3	(1.67%)
	5	1	(1.18%)			1	(0.56%)
Localization*	Skin	48	(56.47%)	49	(51.58%)	97	(53.89%)
	Lymph Node	46	(54.12%)	50	(52.63%)	96	(53.33%)
	Other	1	(1.18%)	1	(1.05%)	2	(1.11%)

Details Localization*	Head and neck	9	(10.59%)	8	(8.42%)	17	(9.44%)
	Lower limb and hip	37	(43.53%)	50	(52.63%)	87	(48.33%)
	Upper limb and shoulder	30	(35.29%)	31	(32.63%)	61	(33.89%)
	Trunk	15	(17.65%)	11	(11.58%)	26	(14.44%)
	Under Revision			3	(3.16%)	3	(1.67%)
Prior Radiotherapies	No	80	(94.12%)	90	(94.74%)	170	(94.44%)
	Yes	5	(5.88%)	5	(5.26%)	10	(5.56%)
Prior Antineoplastic Therapies	No	53	(62.35%)	58	(61.05%)	111	(61.67%)
	Yes	32	(37.65%)	37	(38.95%)	69	(38.33%)
Prior Surgeries	0	3	(3.53%)	4	(4.21%)	7	(3.89%)
	1	16	(18.82%)	22	(23.16%)	38	(21.11%)
	2	32	(37.65%)	29	(30.53%)	61	(33.89%)
	>=3	34	(40.00%)	39	(41.05%)	73	(40.56%)
	Under Revision			1	(1.05%)	1	(0.56%)
Current Stage AJCC 8th Ed. (Freq.)	3B	14	(16.47%)	18	(18.95%)	32	(17.78%)
	3C	58	(68.24%)	64	(67.37%)	122	(67.78%)
	3D	6	(7.06%)	3	(3.16%)	9	(5.00%)
	IV	2	(2.35%)			2	(1.11%)
	UNK	5	(5.88%)	10	(10.53%)	15	(8.33%)

ECOG[1] (Eastern Cooperative Oncology Group) Performance Status:
0 = Able to carry out all normal activity without restriction
1 = Restricted in physically strenuous activity but ambulatory and able to carry out light work;
2 = Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours;
3 = Capable of only limited self-care; confined to bed or chair more than 50% of waking hours;
4 = Completely disabled; cannot carry on any self-care; totally confined to bed or chair; 5= Death;
Current Stage AJCC 7th Ed. (Freq.)[2]: UNK = data under revision;
III x = stage III b/III c with pathological T or N subcategories not available

Median Sum Diameters of Target Lesions (mm)	21 (95% CI: 17.00 - 27.00)	26 (95% CI: 21.00 - 30.00)	23 (95% CI: 20.00 - 27.00)
Median Time from First Diagnosis of Melanoma (months)	25 (95% CI: 19.00 - 35.00)	26 (95% CI: 20.00 - 34.00)	25 (95% CI: 21.00 - 33.00)
Median number of Prior Surgeries	2 (1.00 - 7.00)	2 (1.00 - 12.00)	2 (1.00 - 12.00)

Table 25. Summary of medical history abnormalities in the SE population

	ARM 1 n=122	ARM 2 n=124	Total n=246
Any system	111 (91.0%)	116 (93.5%)	227 (92.3%)
Allergies or drug sensitivities	24 (19.7%)	29 (23.4%)	53 (21.5%)
Cardiovascular system	69 (56.6%)	70 (56.5%)	139 (56.5%)
Collagen	1 (0.8%)	-	1 (0.4%)
Endocrine system	34 (27.9%)	39 (31.5%)	73 (29.7%)
Gastrointestinal system	31 (25.4%)	37 (29.8%)	68 (27.6%)
Hemopoietic lymphatic system	14 (11.5%)	20 (16.1%)	34 (13.8%)
Hearing and vestibular – special sense	6 (4.9%)	1 (0.8%)	7 (2.8%)
Infections and infestations	1 (0.8%)	-	1 (0.4%)
Injury, poisoning and procedural complications	1 (0.8%)	-	1 (0.4%)
Liver and biliary system	15 (12.3%)	12 (9.7%)	27 (11.0%)
Metabolic and nutritional	40 (32.8%)	31 (25.0%)	71 (28.9%)
Musculoskeletal and connective tissue disorder	1 (0.8%)	-	1 (0.4%)
Musculoskeletal system	47 (38.5%)	42 (33.9%)	89 (36.2%)
Nervous system	20 (16.4%)	23 (18.5%)	43 (17.5%)
Other	18 (14.8%)	12 (9.7%)	30 (12.2%)
Psychiatric	10 (8.2%)	15 (12.1%)	25 (10.2%)
Renal genitourinary system	37 (30.3%)	43 (34.7%)	80 (32.5%)
Respiratory system	15 (12.3%)	19 (15.3%)	34 (13.8%)
Skin and appendages	37 (30.3%)	39 (31.5%)	76 (30.9%)
Vision	14 (11.5%)	11 (8.9%)	25 (10.2%)
Under revision	9 (7.4%)	7 (5.6%)	16 (6.5%)

Source: Appendix 14.1, Table 11.2.2

Prior therapies

Systemic therapies

The table below describes the type, frequency and setting of prior systemic therapies received by patients (SE population, data cutoff date of November 29th, 2024) before inclusion in the study PH-L19IL2TNF-02/15, separated by arm.

Table 26. Prior systemic therapies received by patients (SE population)

	Arm 1 (n=122)	Arm 2 (n=124)
Prior systemic therapy TYPE – n (%)*	**43/122 (35.2)**	**43/124 (34.7)**
Immunotherapy only	**36 (83.7) $**	**30 (69.8) $**
Targeted Therapy only	**4 (9.3)**	**5 (11.6)**
Immunotherapy and Targeted Therapy	1 (2.3)	3 (7.0)
Immunotherapy and Chemotherapy	1 (2.3)	2 (4.7)
Chemotherapy only	-	2 (4.7)
Immunotherapy and Clinical Trial	1 (2.3)**	1 (2.3)***
Prior systemic therapy SETTING – n (%)*	**43/122 (35.2)**	**43/124 (34.7)**
Adjuvant only	**37 (86.0)**	**34 (79.1)**
Not Specified	5 (11.6)	8 (18.6)
Adjuvant and Neoadjuvant	-	1 (2.3)
Adjuvant and Not Specified	1 (2.3)	-
Median cumulative duration of prior ICI systemic therapy (95% CI) – months	**5.8 (4.8 - 11.0)**	**8.5 (3.9 - 11.3)**
Median time from last prior ICI systemic therapy to study entry (95% CI) – months	9.5 (6.6 - 25.8)	14.2 (3.9 - 25.9)

* Total patients with prior systemic treatment n=43 // $ of which ICI – n (%): DAROMUN + Surgery 19/43 (44.2); Surgery 15/43 (34.9)
** Pembrolizumab versus placebo // *** SGI-110 (DNMT inhibitor)

Surgeries

Table 27. Summary of prior surgeries

Table 1/ITT: Summary of Baseline Characteristics
*: For these categories there is double counting of patients who can be included in more than one

		Arm 1 N (127)	Arm 1 (%)	Arm 2 N (129)	Arm 2 (%)	Total N (256)	Total (%)
Prior Surgeries	0	6	(4.72%)	4	(3.10%)	10	(3.91%)
	1	23	(18.11%)	28	(21.71%)	51	(19.92%)
	2	46	(36.22%)	44	(34.11%)	90	(35.16%)
	>=3	52	(40.94%)	52	(40.31%)	104	(40.63%)
	Under Revision			1	(0.78%)	1	(0.39%)

Concomitant therapies

The table below reports data on concomitant medications and procedures per arm, focusing on CMs and CPs received by >10% and >5% of patients (SE population), respectively.

Table 28. Concomitant medications and procedures CPs received by >10% and >5% of patients (SE population)

		Arm 1 (N=122)		Arm 2 (N=124)	
Concomitant medications					
Category	Category				
Total	Analgesics	94	(77.05%)	47	(37.9%)
	Antineoplastic agents	53	(43.44%)	70	(56.4%)
	Antithrombotic agents	52	(42.62%)	37	(0.30%)
	Antiinflammatory and antirheumatic products	48	(39.34%)	13	(0.10%)
	Antibacterials for systemic use	45	(36.89%)	21	(0.17%)
	Agents acting on the renin-angiotensin system	44	(36.07%)	45	(0.36%)
	Drugs for acid related disorders	34	(27.87%)	23	(0.18%)
	Beta blocking agents	29	(23.77%)	28	(0.23%)
	Blood substitutes and perfusion solutions	27	(22.13%)	10	(0.08%)
	Lipid modifying agents	26	(21.31%)	23	(0.18%)
	Thyroid therapy	25	(20.49%)	27	(0.22%)
	Corticosteroids for systemic use	20	(16.39%)	12	(0.10%)
	All other therapeutic products	19	(15.57%)	7	(0.06%)
	Calcium channel blockers	18	(14.75%)	20	(0.16%)
	Diuretics	18	(14.75%)	14	(0.11%)
	Antiemetics and antinauseants	17	(13.93%)	7	(0.06%)
	Drugs used in diabetes	15	(12.30%)	14	(0.11%)
	Psycholeptics	15	(12.30%)	13	(0.10%)
	Anesthetics	14	(11.48%)	11	(0.09%)
	Antihistamines for systemic use	14	(11.48%)	2	(0.02%)
	Vitamins	14	(11.48%)	4	(0.03%)
	Antibiotics and chemotherapeutics for dermatological use	13	(10.66%)	3	(0.02%)
	Mineral supplements	13	(10.66%)	3	(0.02%)

Category	Category		
	Gene mutation analysis	56 (45.90%)	57 (45.97%)
	Tumor excision	26 (21.31%)	32 (25.81%)
	Biopsy	12 (9.84%)	22 (17.74%)
	Surgery	12 (9.84%)	13 (10.48%)
	Lesion excision	11 (9.02%)	5 (4.03%)
	Radiotherapy	11 (9.02%)	15 (12.10%)
	Diagnostic aspiration	9 (7.38%)	5 (4.03%)
	Lymphadenectomy	8 (6.56%)	8 (6.45%)
	Ultrasound	7 (5.74%)	7 (5.65%)

The following table focuses on CMs (including adjuvant therapies) and CPs received by patients after surgery in the FU period.

Table 29. Concomitant medications and procedures CPs received after surgery during follow-up (SE population)

		Arm 1 (N=122)	Arm 2 (N=124)
Concomitant medications			
Category	Category		
	Antineoplastic agents	39 (31.97%)	53 (42.74%)
	BRAF/MEK inhib	8 (6.5%)	13 (10.5%)
	Nivolumab	8 (6.5%)	15 (12.1%)
	Pembrolizumab	20 (16.4%)	25 (20.2%)
	Ipi + Nivo	3 (2.4%)	3 (2.4%)
	T-Vec	-	1 (0.8%)
	Other	-	2 (1.6%)
	Analgesics	27 (22.13%)	23 (18.55%)
	Antiinflammatory and antirheumatic products	18 (14.75%)	5 (4.03%)
	Agents acting on the renin-angiotensin system	13 (10.66%)	19 (15.32%)
	Antibacterials for systemic use	13 (10.66%)	9 (7.26%)
	Antithrombotic agents	12 (9.84%)	13 (10.48%)
	Drugs for acid related disorders	10 (8.20%)	6 (4.84%)
	Thyroid therapy	10 (8.20%)	11 (8.87%)
	Beta blocking agents	9 (7.38%)	11 (8.87%)
Concomitant procedures			
Category	Category		
	Radiotherapy	4 (3.28%)	1 (0.81%)
	Gene mutation analysis	3 (2.46%)	1 (0.81%)

Numbers analysed

The efficacy evaluation is based on the ITT population that includes all 256 randomised patients, except for 10 patients (5 in each group) that were enrolled after the efficacy analysis data cut-off (03-May-2023). All 256 enrolled patients have been included in the re-analysis of the efficacy, performed with a data cut-off 29-Nov-2024.

Outcomes and estimation

Primary Endpoint

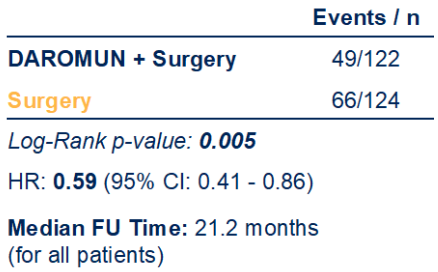
Relapse-free survival (RFS)

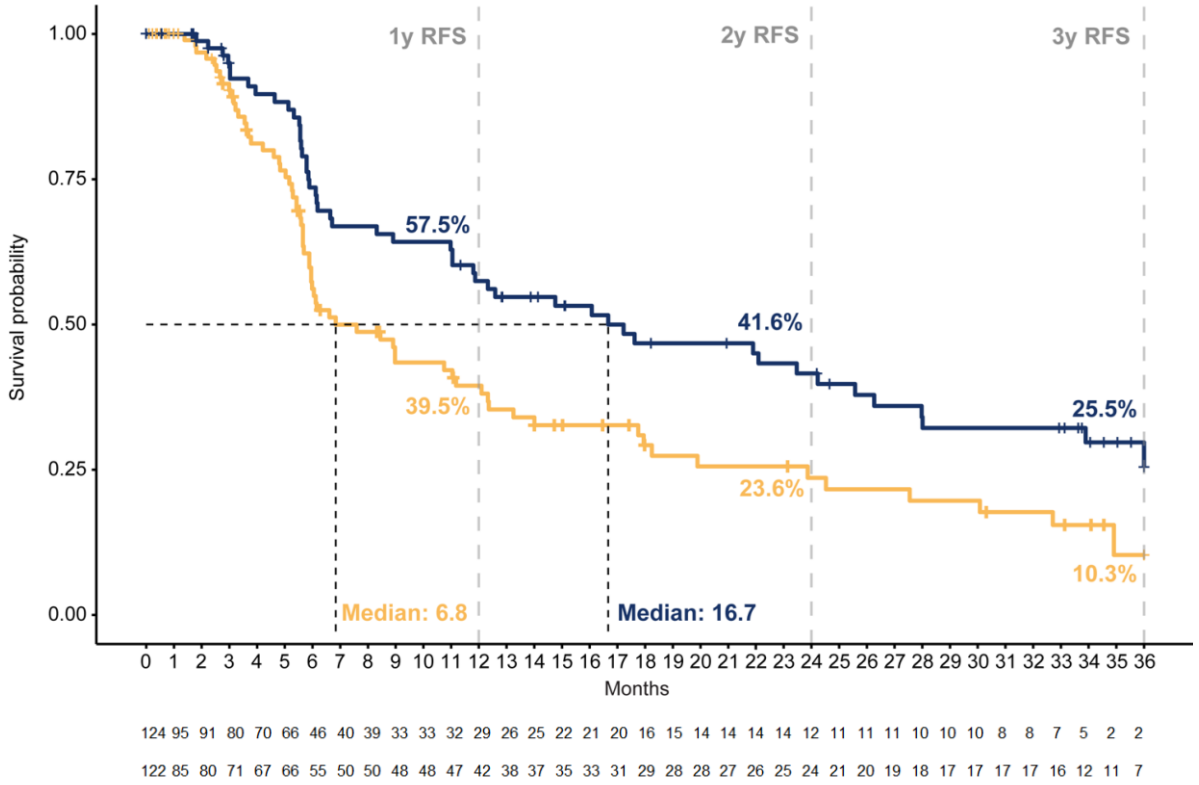
The primary endpoint for this study was relapse-free survival (RFS) assessed by the principal investigators and retrospectively confirmed by Blinded Independent Centralized Review (BICR) of the FDG PET-CT scans.

The analysis of the primary outcome was run when the 95 RFS events foreseen by the clinical protocol and the statistical analysis plan were reached, as assessed by the local investigators. The date of the 95th event (May 3rd, 2023) is considered the cut-off date for all the efficacy analyses. A retrospective BICR of the FDG PET-CT-based RFS events was carried out: after reconciliation of the discordances of BICR and Investigators' assessment, 20 more events (8 in the experimental arm and 12 in the control arm) were identified. Reconciliation rules are described in section 3.3.1.3.1.

The Kaplan-Meier estimator was applied to describe the relapse-free survival behaviour in both arms. The analysis of the curves showed an HR between the RFS of the treatment and control arm of 0.59 [95% CI 0.41-0.86; log-rank p=0.005] as per BICR assessment with 49 events recorded at database cut-off date in the L19IL2/L19TNF arm and 66 events recorded in the control arm. Median RFS was 16.7 months [95% CI 11.8-26.3] in the L19IL2/L19TNF treatment arm as opposed to 6.9 months [95% CI 5.9-12.3] in the control arm with a median duration of follow-up was 21.2 months in both groups.

Figure 5. Recurrence-free survival per BICR assessment





Analysis of efficacy with database cut-off date of May 3rd, 2023, updated with data cleaning as of November 29th, 2024.

Efficacy analysis was repeated, including data with a cut-off date on May 3rd, 2023, but an updated data cleaning as of November 29th, 2024 (99.62% data cleaning).

As for the first efficacy analysis, only patients enrolled before May 3rd, 2023, were included in this analysis (i.e., 122 patients in Arm 1 and 124 patients in Arm 2). This re-analysis shows an HR between the RFS of the treatment and control arm of 0.57 [95% CI 0.39-0.82; log-rank p=0.002] as per BICR assessment, with 49 events recorded in the L19IL2/L19TNF arm and 67 events in the control arm. Median RFS was 17.2 months in the L19IL2/L19TNF treatment arm and 6.8 months in the control arm.

Reasons for Censoring

A table with the number of censored trial participants, censoring type and events for the primary efficacy analysis of RFS by arm and occurrence before or after surgery at the 3 May 2023 database lock date is shown below.

Table 30. Status of trial participants before or after surgery

STATUS OF PATIENTS	No Surgery			With Surgery			Total N=246 (100%)
	Arm 2 N = 7 (100%)	Arm 1 N = 18 (100%)	Overall N = 25 (100%)	Arm 2 N = 117 (100%)	Arm 1 N = 104 (100%)	Overall N = 221 (100%)	
Administrative Censoring	**3 (43%)**	**5 (28%)**	**8 (32%)**	**25 (21%)**	**31 (30%)**	**56 (25%)**	**64 (26%)**
RFS Completed	0 (0%)	0 (0%)	0 (0%)	6 (5.1%)	15 (14%)	21 (9.5%)	21 (9%)
RFS Ongoing	3 (43%)	5 (28%)	8 (32%)	19 (16%)	16 (15%)	35 (16%)	43 (17%)
Non Administrative Censoring	**4 (57%)**	**13 (72%)**	**17 (68%)**	**25 (21%)**	**24 (23%)**	**49 (22%)**	**66 (27%)**
IC withdrawal	3 (43%)	4 (22%)	7 (28%)	7 (6.0%)	3 (2.9%)	10 (4.5%)	17 (6.9%)
Not Eligible for PD	1 (14%)	4 (22%)	5 (20%)	0 (0%)	1* (1.0%)	1 (0.5%)	6 (2.4%)
Undetected Stage IV	0 (0%)	2 (11%)	2 (8.0%)	1 (0.9%)	2 (1.9%)	3 (1.4%)	5 (2%)
Toxicity	0 (0%)	3 (17%)	3 (12%)	0 (0%)	0 (0%)	0 (0%)	3 (1.2%)
Other Diagnosis	0 (0%)	0 (0%)	0 (0%)	6 (5.1%)	0 (0%)	6 (2.7%)	6 (2.4%)
Not-Ned	0 (0%)	0 (0%)	0 (0%)	8 (6.8%)	15 (14%)	23 (10%)	23 (9.3%)
Other	0 (0%)	0 (0%)	0 (0%)	1 (0.9%)	1 (1.0%)	2 (0.9%)	2 (0.8%)
Other Therapies	0 (0%)	0 (0%)	0 (0%)	2 (1.7%)	2 (1.9%)	4 (1.8%)	4 (1.6%)
RFS Event	**0 (0%)**	**0 (0%)**	**0 (0%)**	**67 (57%)**	**49 (47%)**	**116 (52%)**	**116 (47%)**

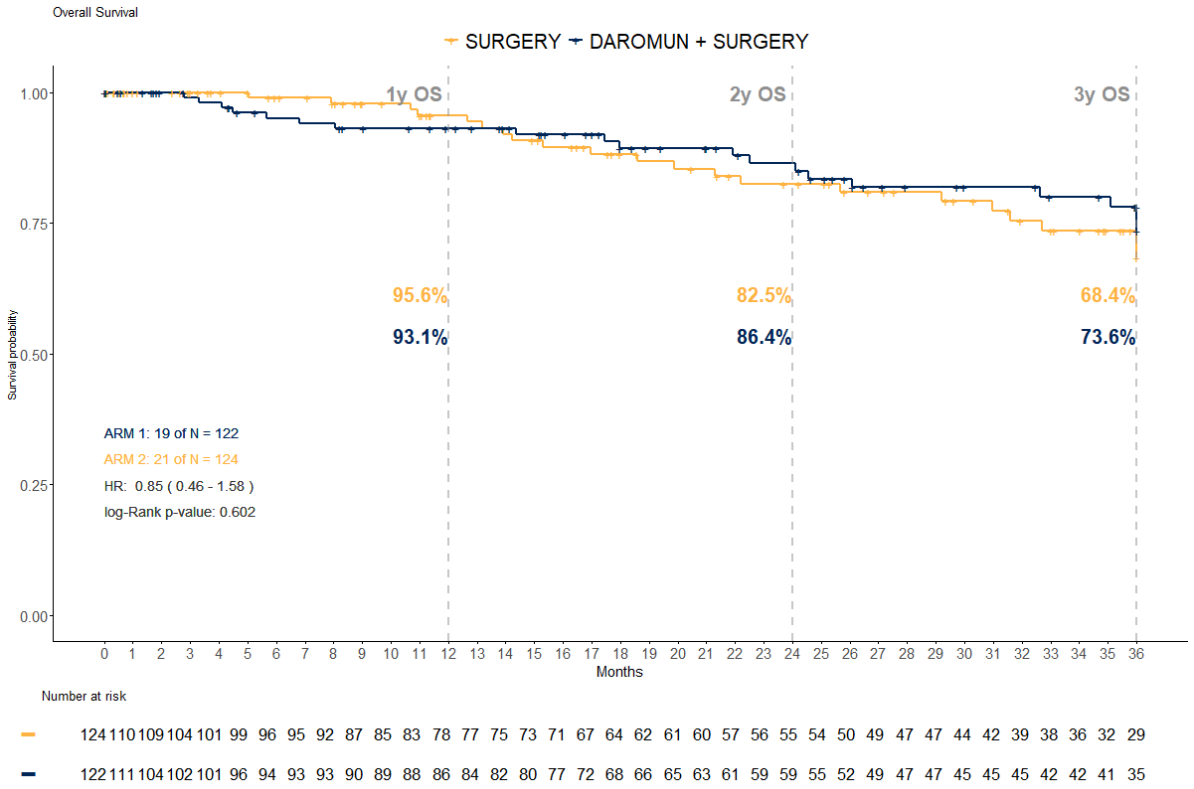
**The patient had progressive disease before surgery which was undetected*

Secondary Endpoints

Overall Survival (OS)

OS was a key secondary endpoint. The number of overall survival events is low, with 19 and 21 events in Arm 1 and Arm 2, respectively. The HR is 0.85 [95% CI 0.46-1.58, log-rank p=0.602].

Figure 6. Overall Survival assessment with database cut-off date on 3 May 2023



Distant metastasis-free survival (DMFS)

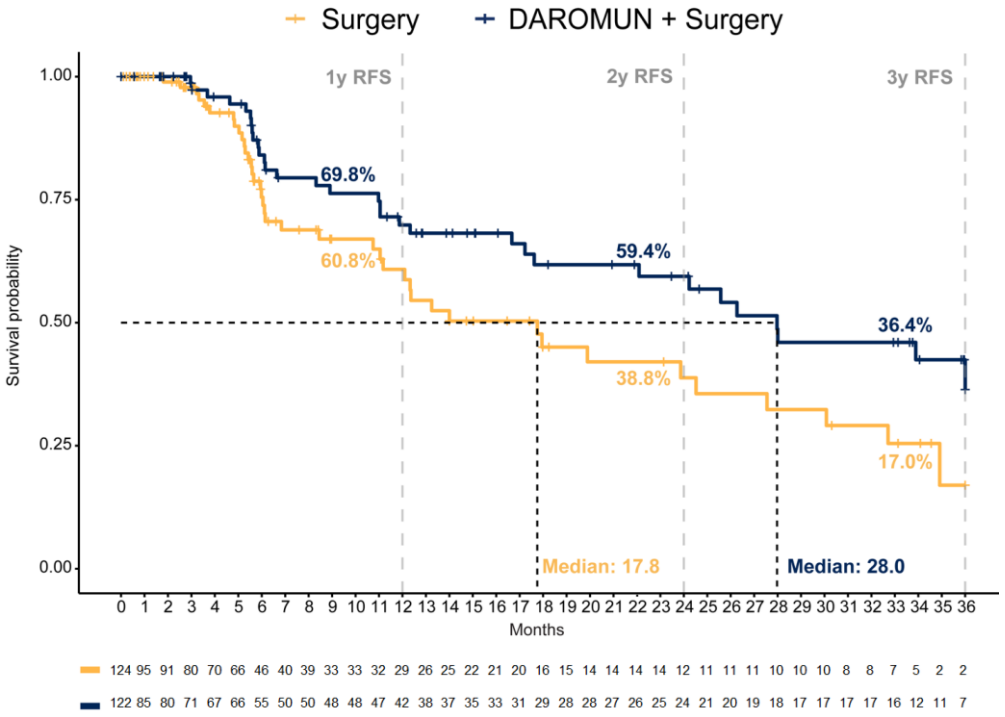
DMFS, as per BICR assessment, showed a 40% reduction in the risk of distant recurrence or death in the experimental arm vs the control arm (HR = 0.60 [95% CI 0.37-0.95; log-rank p=0.03]) with 32 events at database cut-off date in experimental arm and 40 events recorded in the control arm. Median DMFS was 27.9 months [95% CI 22.1-NR] in the L19IL2/L19TNF treatment arm as opposed to 17.7 months [95% CI 11.2-30.1] in the control arm.

Figure 7. Distant metastasis-free survival per BICR assessment

	Events / n
DAROMUN + Surgery	32/122
Surgery	40/124

Log-Rank p-value: ***0.029***

HR: **0.60** (95% CI: 0.37 - 0.95)



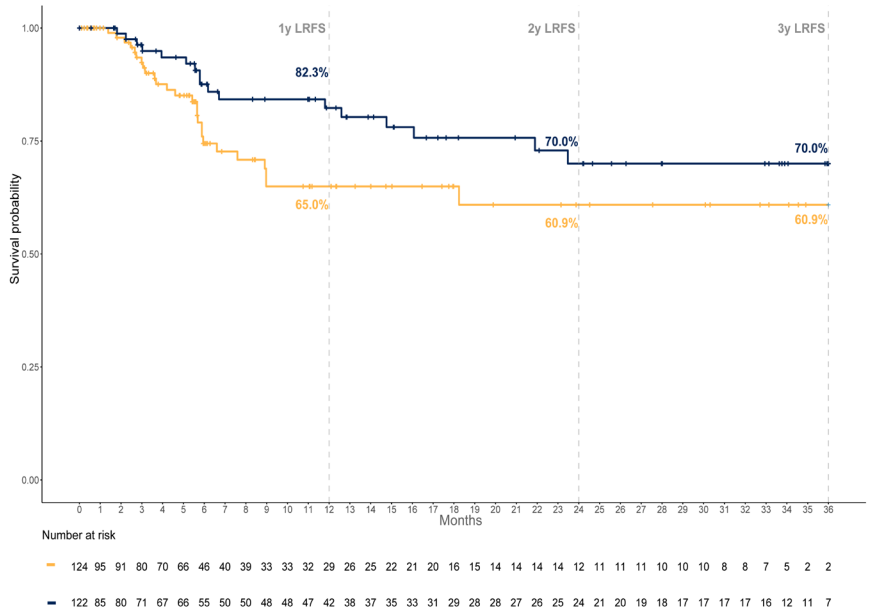
The re-analysis of DMFS as per BICR assessment with database cut-off date on May 3rd, 2023, and data cleaning as per November 29th, 2024, shows an HR of 0.55 [95% CI 0.34-0.87; log-rank p=0.009] and median DMFS of 28.0 months and 13.2 months for Arm 1 and Arm 2, respectively. The number of DMFS events that have occurred at the time of the first database cut-off date were 32 and 43 for Arm 1 and Arm 2, respectively.

Local recurrence-free survival (LRFS)

The LRFS analysis, as per BICR assessment, showed an HR of 0.58 between the LRFS of the treatment and the control arm [95% CI 0.31-1.08; log-rank p=0.08] with 17 events recorded at database cut-off date in the Nidlegy arm and 26 events recorded in the control arm. Median LRFS per BICR analysis was not reached in either arm of the study.

Figure 8. Local recurrence-free survival per BICR assessment

	Events / n
DAROMUN + Surgery	17/122
Surgery	26/124
Log-Rank p-value: ***0.081***	
HR: **0.58** (95% CI: 0.31 – 1.08)	



Re-analysis of LRFS as per BICR assessment with database cut-off date on May 3rd, 2023, and data cleaning as per November 29th, 2024, shows an HR of 0.61 [95% CI 0.33-1.14, log-rank p=0.12], with 17 events and 24 events for Arm 1 and Arm 2, respectively. Median LRFS for both arms were not reached in the re-analysis.

Patients with pathological responses (Arm 1 only)

The analysis of the proportion of patients with pathological responses in the experimental arm was based on standard institutional pathology reports collected after surgery: At the time of writing draft version 2 of this CSR, 25 patients out of the 112 patients (22.3%) in the treatment arm - for whom a pathology report at the time of surgery was available - presented a major pathological response (pCR, 19.6% plus p-nearCR 2.7%). In other 5 patients (4.5%) a partial pathological response was recorded.

As recommended by CHMP, a reanalysis of still available specimens at three German centres (Kiel, Heidelberg, Dresden), which have enrolled cumulatively 44% of all patients enrolled in the study was performed. The re-analysis was run by the local pathologists at the three different centres, according to the criteria described in Tetzlaff et al. Samples were still available for a total of 25 patients of Arm 1 out of the 91 who had had surgery (i.e., IPRs available) at these 3 centres; at the end of the reanalysis, for these 25 patients 7 pCR, 1 pnear-CR, 5 pPR and 12 pNR were recorded. The seven patients who enjoyed a pCR at re-analysis had already been classified as pCR at initial IPR review (i.e., 100% re-test correspondence), one additional patient was newly quantitatively confirmed as near-pCR and 5 were assessed as pPR.

Ancillary analyses

Post-hoc alternative RFS analyses

An alternative RFS analysis using the cutoff 03-May-2023 with updated cleaning of 29-November-2024 was performed defining RFS as the time to first recurrence or death from the date of surgery (i.e. on patients who were disease-free by surgery). Therefore, participants who did not yet had surgery (8 participants), who discontinued before surgery (17 participants) and who were not-NED at surgery (23 participants) were excluded resulting in a total of 198 participants for the analysis.

The number of events, censoring and type of censoring are shown in the table below for both arms.

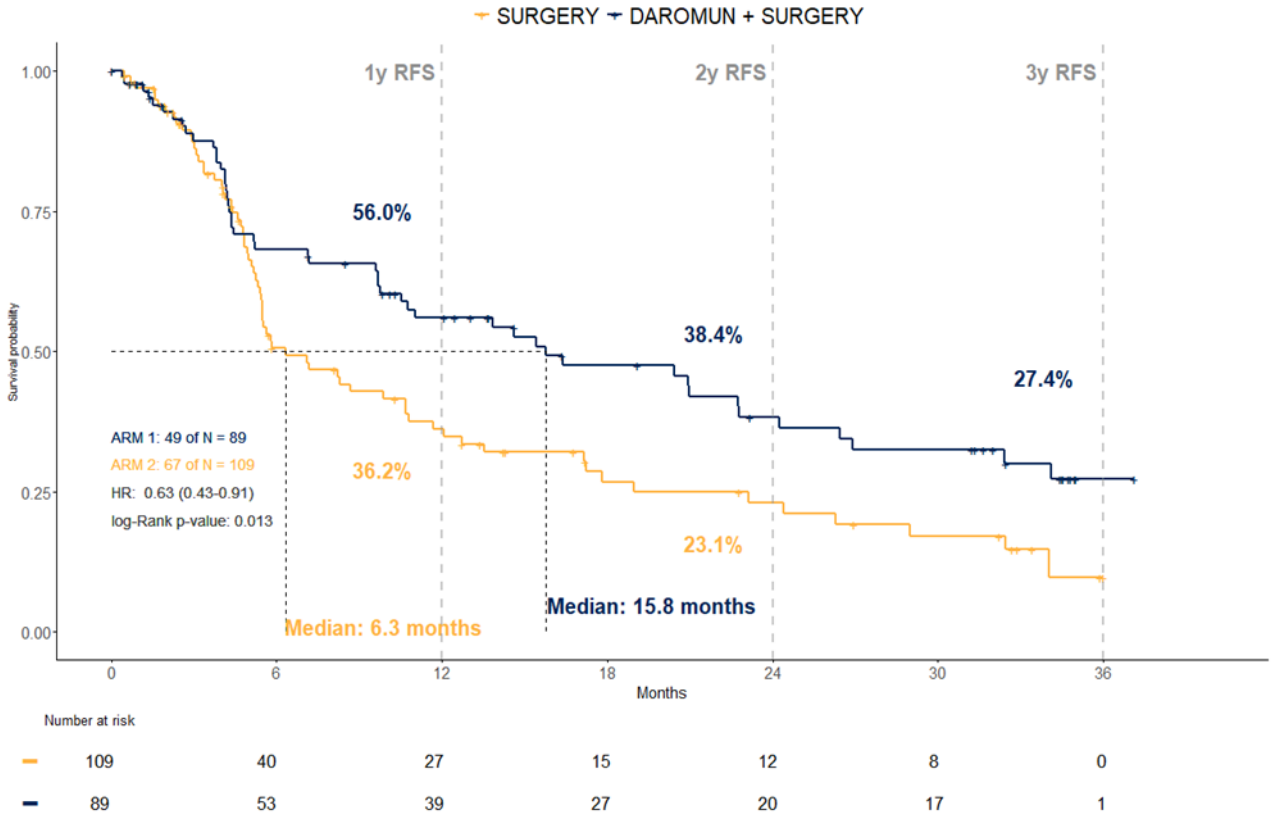
Table 31. Number of censored trial participants, censoring type and events included in the sensitivity analysis of RFS from surgery (Arm 1= experimental arm; Arm 2 = control arm)

STATUS OF PATIENTS NED AFTER SURGERY	**Arm 2 N = 109 (100%)**	**Arm 1 N = 89 (100%)**	**Overall N = 198 (100%)**
Administrative Censoring	**25 (23%)**	**31 (35%)**	**56 (29%)**
RFS Completed	6 (5.5%)	15 (17%)	21 (11%)
RFS Ongoing	19 (17%)	16 (18%)	35 (18%)
Non-Administrative Censoring	**17 (16%)**	**9 (10%)**	**26 (12%)**
IC withdrawal	7 (6.4%)	3 (3.4%)	10 (5.1%)
Not Eligible for PD	0 (0%)	1* (1.1%)	1 (0.5%)
Undetected Stage IV	1 (0.9%)	2 (2.2%)	3 (1.5%)
Other Diagnosis	6 (5.5%)	0 (0%)	6 (3.0%)
Other	1 (0.9%)	1 (1.1%)	2 (1.0%)
Other Therapies	2 (1.8%)	2 (2.2%)	4 (2.0%)
RFS Event	**67 (61%)**	**49 (55%)**	**116 (59%)**

**The patient had progressive disease before surgery which was undetected*

The resulting plot of the Kaplan-Meier curves of RFS from surgery for the two arms are shown in the Figure below.

Figure 9. RFS from surgery: Kaplan-Meier curves by arm



The estimated median time to recurrence or death from any cause was 6.3 months in the control arm and 15.8 months in the experimental arm with a hazard ratio 0.63 (95%CI: 0.43-0.91).

Post-hoc EFS analyses

EFS analyses were performed on the 246 patients enrolled as of May 3, 2023: data cleaning was completed as of November 29, 2024 (99.62% data cleaning). EFS was calculated from the date of randomisation to the date of the event or the date of last observation for censored patients.

The following EFS analyses were performed varying the definition of what constitutes an event:

a) Events were defined as relapse, death from any cause and previously censored for non-administrative reasons. Patients with non-administrative censoring (29 and 37 in the control and treatment arms, respectively) were imputed as events at the time of their last observation for RFS follow-up. The total number of events was 96 in Arm 2 (control arm) and 86 in Arm 1 (experimental arm).

b) Events were defined as relapse, death from any cause, not having surgery (4 in Arm 2 and 13 Arm 1) and no NED at surgery (8 in Arm 2 and 15 Arm). For the last two types of events, the date of occurrence was defined as the date of observation before surgery and the date of surgery. The total number of events was 79 in Arm 2 (control arm) and 77 in Arm 1 (experimental arm).

c) Events were defined as relapse, death from any cause and progression prior to surgery. The total number of events was 68 in Arm 2 (control arm) and 54 in Arm 1 (experimental arm).

d) Events were defined as relapse, death from any cause, cancellation of scheduled surgery, surgery not resulting in NED and initiation of new anticancer therapy. The total number of events was 81 in Arm 2 (control arm) and 79 in Arm 1 (experimental arm).

e) Events were defined as relapse, death from any cause, progression prior to surgery, IC withdrawal, other therapies, undetected Stage IV at randomisation and discontinuation due to other reason. The total number of events was 82 in Arm 2 (control arm) and 68 in Arm 1 (experimental arm).

The results of the analyses are summarised in the table below.

Table 32. Sensitivity EFS analysis with the database lock of 3 May 2023

Analysis type	Number of patients	Number of events	HR	95% CI of HR	Log-Rank p-value
EFS (a)	246	182	0.75	(0.56 – 1.01)	0.057
EFS (b)	246	156	0.81	(0.59 – 1.11)	0.188
EFS (c)	246	122	0.62	(0.43 – 0.89)	0.010
EFS (d)	246	160	0.81	(0.60 – 1.11)	0.195
EFS (e)	246	150	0.67	(0.49 – 0.93)	0.016

EFS Analysis b

EFS was calculated from the date of randomisation to the date of event or the date of last observation for censored patients. Events are defined as relapse, death from any cause, not having surgery (4 in Arm 2 and 13 Arm 1) and no NED at surgery (8 in Arm 2 and 15 Arm). For the last two types of events, the date of occurrence was defined as the date of observation before surgery and the date of surgery. The total number of events was 79 in Arm 2 (control arm) and 77 in Arm 1 (experimental arm). The definition and distribution of events and censored patients for the sensitivity EFS analysis are shown in the table below.

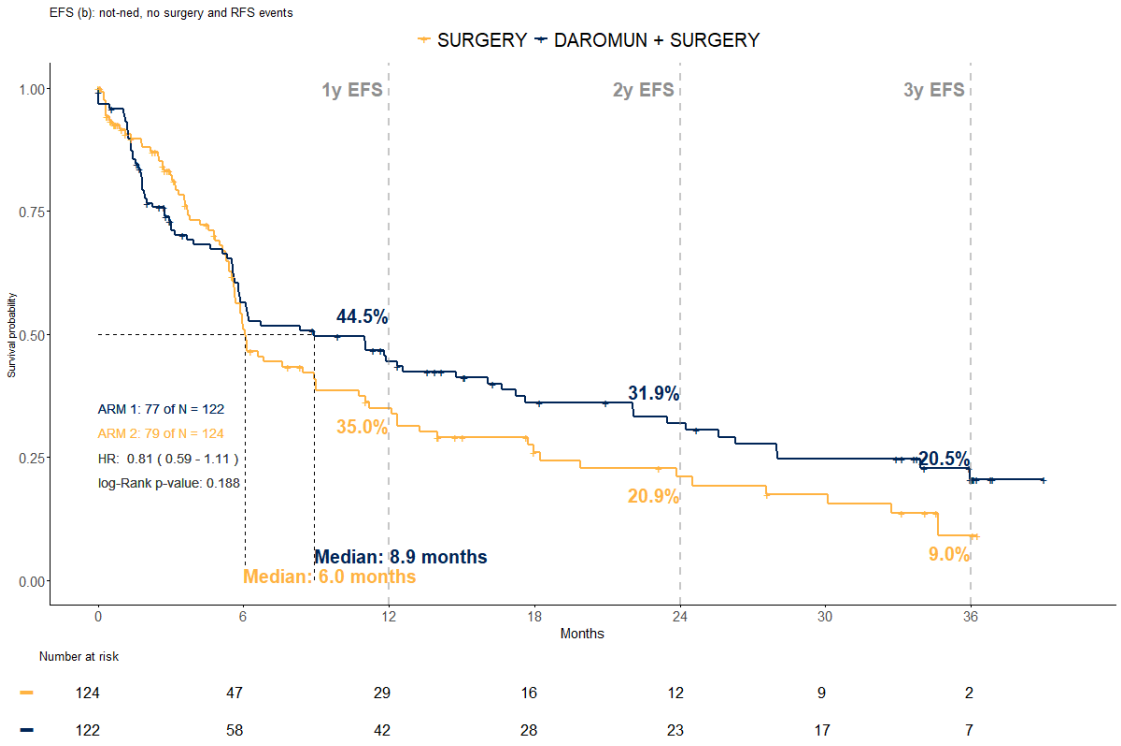
Table 33. Status of trial participants for EFS b

Patient disposal	Arm 2 N = 124 (100%)	Arm 1 N = 122 (100%)	Overall N = 246 (100%)
Counted as EFS event	**79 (64%)**	**77 (63%)**	**156 (63%)**
Death	3 (2.4%)	0 (0%)	3 (1.2%)
Recurrence	64 (52%)	49 (40%)	113 (46%)
Not-NED	8 (6.5%)	15 (12%)	23 (9.3%)

Not having surgery	4 (3.2%)	13 (11%)	17 (6.9%)
Counted as censored patient	**45 (36%)**	**45 (37%)**	**90 (37%)**
Discontinuation (any reason)	15 (12%)	7 (5.7%)	22 (8.9%)
Other Therapies	2 (1.6%)	2 (1.6%)	4 (1.6%)
RFS completed	6 (4.8%)	15 (12%)	21 (8.5%)
RFS ongoing	22 (18%)	21 (17%)	43 (17%)

The resulting Kaplan-Meier curves of both study arms for EFS are shown below.

Figure 10. Kaplan-Meier curve for EFS (Analysis b)



The estimated median time to recurrence or death from any cause was 6.0 months in Arm 2 and 8.9 months in Arm 1. Using a cox model with treatment group as the only covariate, the hazard ratio and 95% confidence interval for the treatment effect is 0.81 (95%CI: 0.59-1.11).

EFS Analysis d

EFS was calculated from the date of randomisation to the date of event or the date of last observation for censored patients. Events are defined as relapse, death from any cause, cancellation of scheduled surgery, surgery not resulting in NED and initiation of new anticancer therapy. The total number of events was 81 in Arm 2 (control arm) and 79 in Arm 1 (experimental arm). The definition and

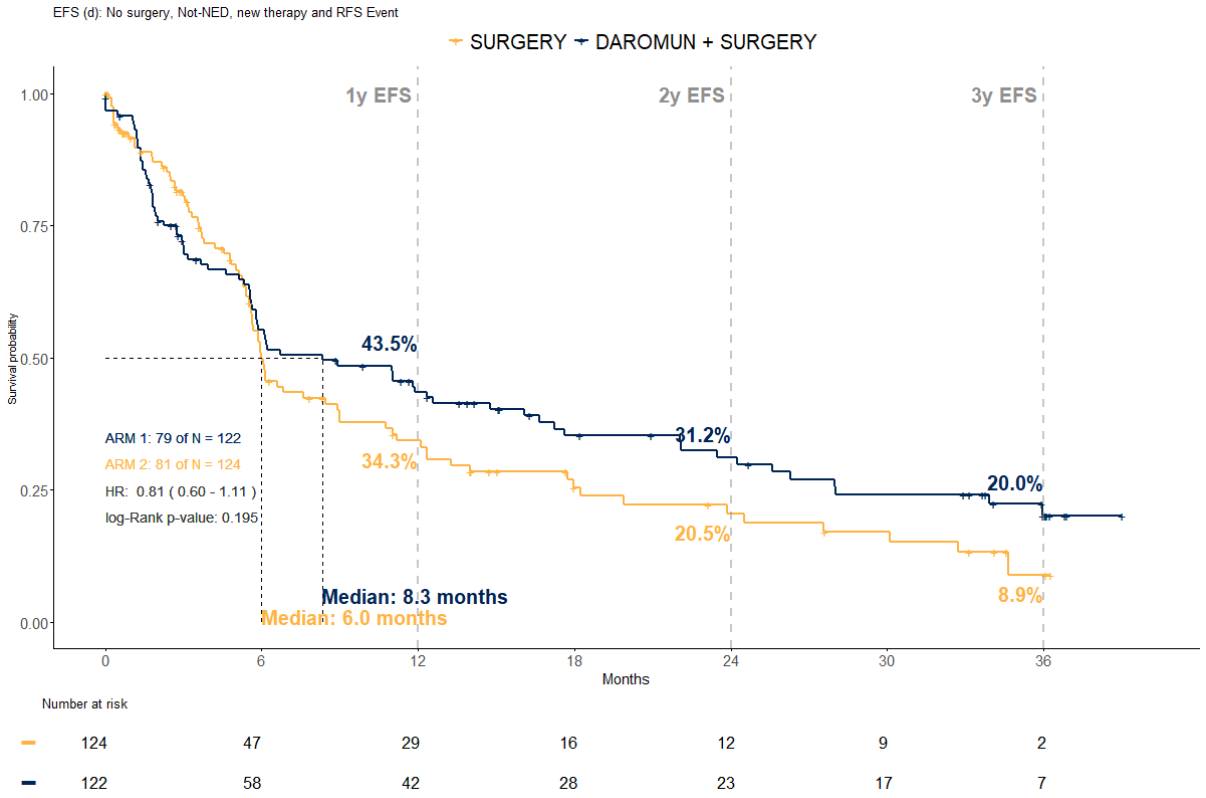
distribution of events and censored patients for the sensitivity EFS analysis are shown in the table below.

Table 34. Status of trial participants for EFS d

Patient disposal	Arm 2 N = 124 (100%)	Arm 1 N = 122 (100%)	Overall N = 246 (100%)
Counted as EFS event	**81 (65%)**	**79 (65%)**	**160 (65%)**
Death	3 (2.4%)	0 (0%)	3 (1.2%)
Recurrence	64 (52%)	49 (40%)	113 (46%)
Not-NED	8 (6.5%)	15 (12%)	23 (9.3%)
Not having surgery	4 (3.2%)	13 (11%)	17 (6.9%)
Other Therapies	2 (1.6%)	2 (1.6%)	4 (1.6%)
Counted as censored patient	**43 (35%)**	**43 (35%)**	**86 (35%)**
Discontinuation (any reason)	15 (12%)	7 (5.7%)	22 (8.9%)
RFS completed	6 (4.8%)	15 (12%)	21 (8.5%)
RFS ongoing	22 (18%)	21 (17%)	43 (17.5%)

The resulting Kaplan-Meier curves of both study arms for EFS are shown below.

Figure 11. Kaplan-Meier curve for EFS (Analysis d)



The estimated median time to recurrence or death from any cause was 6.0 months in Arm 2 and 8.3 months in Arm 1. Using a cox model with treatment group as the only covariate, the hazard ratio and 95% confidence interval for the treatment effect is 0.81 (95% CI: 0.60 – 1.11).

Analysis of efficacy with database cut-off date of November 29th, 2024

Primary Endpoint (RFS)

For the primary endpoint, the re-analysis shows an HR between the RFS of the treatment and control arm of 0.61 [95% CI 0.43-0.86; log-rank p=0.004] as per BICR assessment, with 56 events recorded in the L19IL2/L19TNF arm and 78 events in the control arm. Median RFS at the second cut-off date was 16.7 months [95% CI 11.8-25.6] and 8.9 months [95% CI 6.0-12.3] in the L19IL2/L19TNF arm and control arm, respectively, with a median duration of follow-up was 29.62 months. Re-analysis of RFS per investigators' assessment with data collected up to November 29th, 2024, shows a median RFS of 24.2 months and 11.2 months in the experimental and the control arm, respectively and an HR of 0.63 [95% CI 0.43-0.92, log-rank p=0.016].

Distant metastasis-free survival (DMFS)

The re-analysis of DMFS as per BICR assessment with the database cut-off date on November 29th, 2024, shows an HR of 0.57 [95% CI 0.37-0.88; log-rank p=0.010] and median DMFS of 28.0 months and 14.0 months for Arm 1 and Arm 2, respectively. The number of DMFS events that have occurred at the time of the second database cut-off date were 36 and 51 for Arm 1 and Arm 2, respectively.

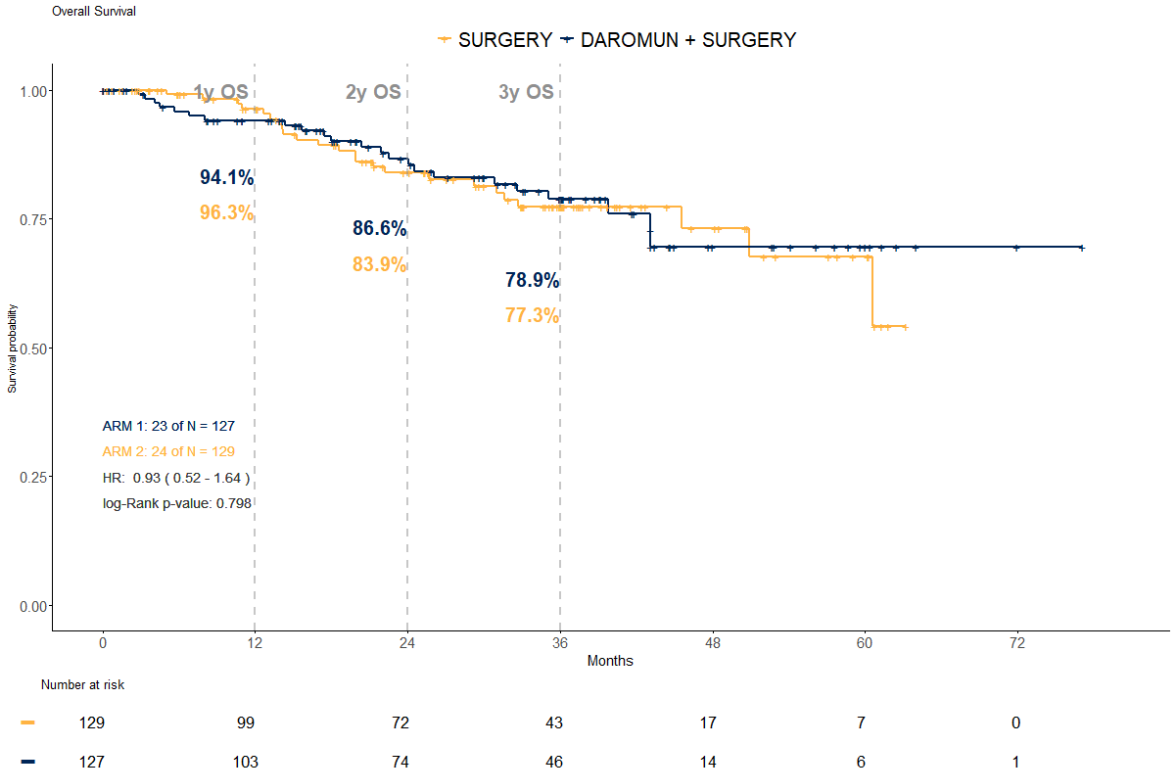
Local recurrence-free survival (LRFS)

Re-analysis of LRFS as per BICR assessment with database cut-off date on November 29th, 2024, shows an HR of 0.68 [95% CI 0.38-1.22, log-rank p=0.196], with 20 events and 27 events for Arm 1 and Arm 2, respectively. Median LRFS for both arms was again not reached in the re-analysis.

Overall survival (OS)

An additional analysis for OS was done upon EMA request after the second database cut-off date (November 29th, 2024). The number of overall survival events was low, with 23 and 24 events in Arm 1 and Arm 2, respectively. The HR is 0.93 [95% CI 0.52-1.64, log-rank p=0.798].

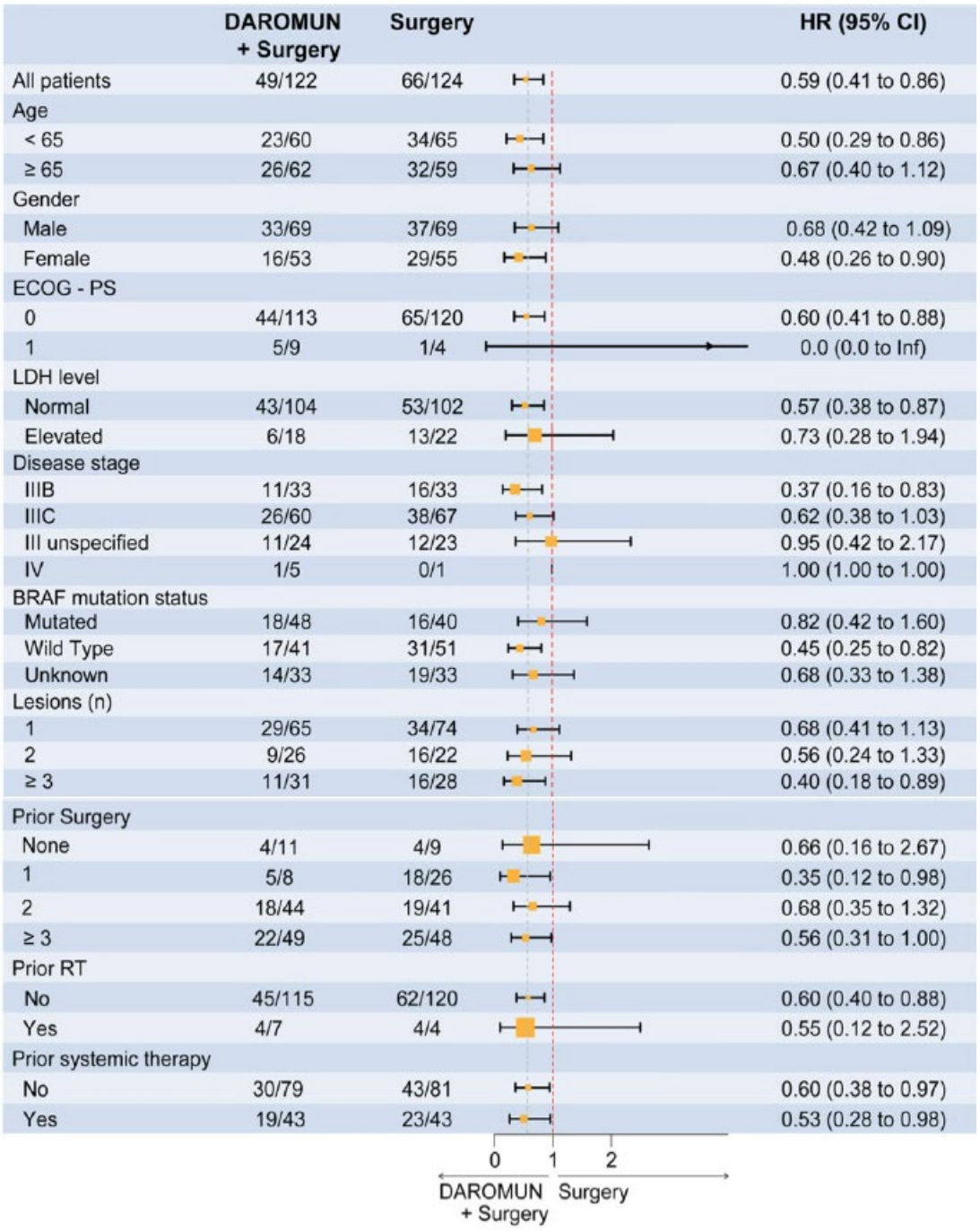
Figure 12. Kaplan-Meier curves for overall survival analysis with database cut-off date on 29 November 2024



Subgroup analyses

The effect of baseline characteristics, such as age, gender, disease stage, primary tumour ulceration, number of lesions, and presence of BRAF V600 mutations, on the primary endpoint RFS was evaluated.

Figure 13. Patient subgroups analysis: forest plot of baseline characteristics and prior treatments, as per database cut-off date on 3 May 2023



Post-surgery treatment with EMA-approved adjuvant therapies was allowed, at discretion of the treating physician in both arms of the study. However, only a proportion of patients in the two arms received post-surgery adjuvant treatments, 31 patients in the neoadjuvant L19IL2/L19TNF arm and 47

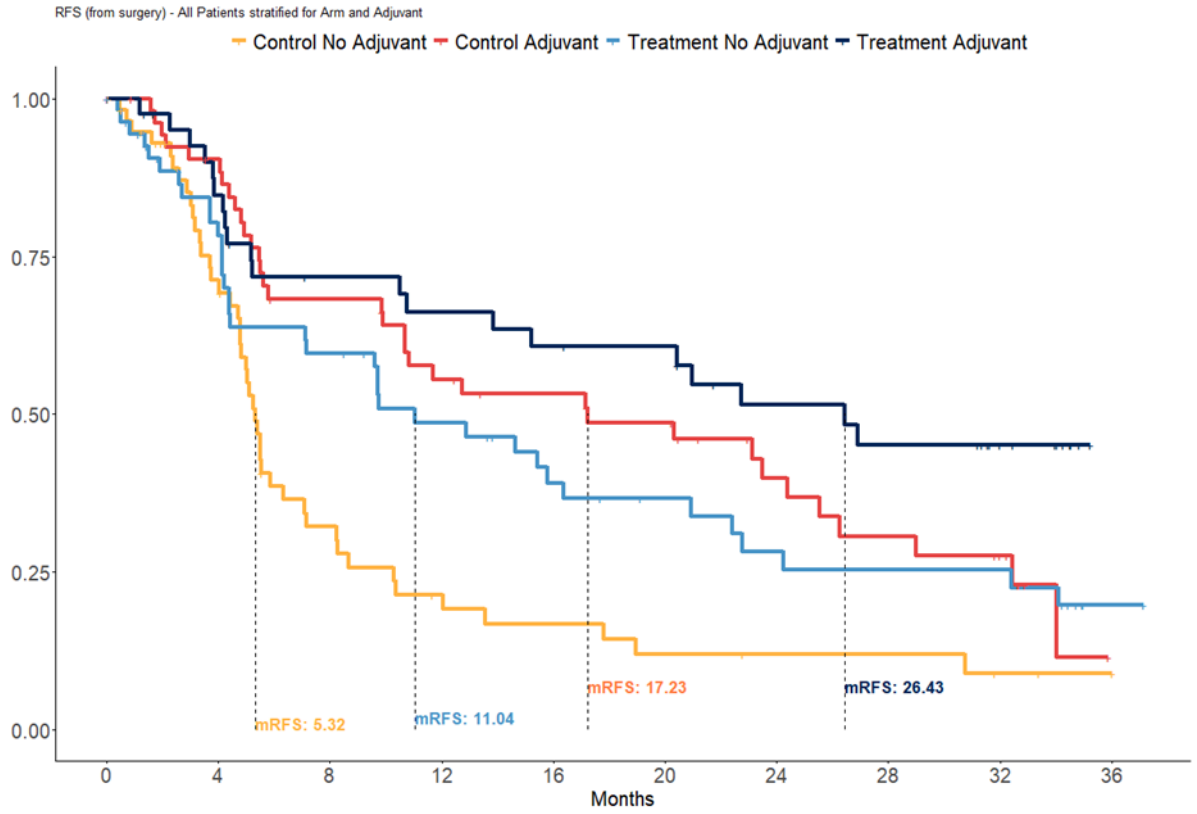
patients in the control arm. Of these, 43 received adjuvant immune checkpoint inhibitors (ICI; 16 in L19IL2/L19TNF arm and 27 in the control arm), 15 received adjuvant targeted therapies (TKI; 7 in L19IL2/L19TNF arm and 8 in the control arm), while 4 patients in the L19IL2/L19TNF arm and 2 in the control arm received adjuvant radiotherapy (RT). Further 14 patients (4 in the L19IL2/L19TNF arm and 10 in the control arm) received different lines of adjuvant therapy at different time points, which included RT + ICIs or RT + TKIs. The table below presents the results of a pre-planned sensitivity analysis with a multivariate Cox model as per database cut-off date on May 3rd, 2023. In this multivariate analysis both neoadjuvant L19IL2/L19TNF treatment and postsurgery adjuvant therapy was significantly and independently affecting RFS.

Table 35. Multivariate analysis of the effect of L19IL2/L19TNF and post-surgery adjuvants as per database cutoff 29 November 2024

Cox Model*	Adjusted HR	95% CI	p-value
Neoadjuvant Treatment: DAROMUN + Surgery vs Surgery	0.6	(0.42 – 0.86)	0.0047
Adjuvant Treatment: any vs none	0.49	(0.34 – 0.69)	< 0.0001
Groups	**Events / n**	**Estimated Median RFS (mo)**	**95% CI**
Surgery, no Adjuvant	44/69	5.33	(4.8 – 7.17)
Surgery + Adjuvant	34/54	17.23	(10.68 – 26.27)
DAROMUN + Surgery, no Adjuvant	36/70	11.04	(7.13 – 22.42)
DAROMUN + Surgery + Adjuvant	20/41	26.43	(13.84 – NA)

*Multivariate Cox Model with two covariates: Neoadjuvant and Adjuvant Treatment

Table 36. Kaplan-Meier curves for RFS – stratified by Arm and Adjuvant treatment



3.3.4.3. Summary of main efficacy results

Table 37. Summary of efficacy for trial PH-L19IL2TNF-02/15

<u>Title:</u> A pivotal Phase III, open label, randomized, controlled multi-center study of the efficacy of L19IL2/L19TNF neoadjuvant intratumoural treatment followed by surgery versus surgery alone in clinical stage III B/C melanoma patients.		
Study identifier	NCT02938299 EudraCT 2015-002549-72 PH-L19IL2TNF-02/15	
Design	This is a Phase III, open label, randomised, controlled, multi-center, comparative efficacy study of the intratumoural neoadjuvant treatment with Nidlegy followed by surgery versus immediate surgery in fully resectable melanoma patients with injectable regional skin and/or nodal metastases. Prior anti-tumour treatments including surgery, radiation therapy (RT) and systemic therapies were allowed. Patients with uveal or mucosal melanoma, metastatic melanoma with unknown primary, or patients with distant metastases (ruled out by FDG PET-CT at screening in all patients) were not eligible. Post-surgery treatment with EMA-approved adjuvant therapies was allowed, at the discretion of the treating physician in both arms of the study. Patients were randomly assigned, in a 1:1 ratio, to receive either up to 4 weekly intratumoural injections of Nidlegy followed by surgery on weeks 5 to 8 (Arm 1) or surgery alone within 4 weeks from randomisation (Arm 2). Randomisation lists were prepared for each study site using permuted block randomisation, with the block size being randomly chosen from two different, pre-specified sizes, random permutation of block size and treatment order within each block and with equal treatment allocation ratio so that patient numbers in the two arms of the study remained reasonably balanced at each center.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	2016-2023 Not applicable Not applicable
Hypothesis	Superiority	
Treatments groups	Arm 1: treatment arm	Neoadjuvant treatment with Nidlegy (13.0 Mio IU of L19IL2 in 1.0 ml + 0.4mg of L19TNF in 1.0 ml) administered intralesionally once weekly for up to 4 weeks followed by surgery on weeks 5 to 8 from randomisation (122 patients).
	Arm 2: control arm	Treatment with surgery alone within 4 weeks from randomisation (124 patients).

Endpoints and definitions	Primary endpoint	Relapse-free survival (RFS)	Defined as the time from the date of randomisation to the date of the documented first disease recurrence or death from any cause. Patients who were without recurrences (disease free) were censored at the time of the last tumour assessment. Recurrences based on FDG PET-CT assessment were confirmed by retrospective Blinded Independent Central Review (BICR) of imaging data. Recurrence (local, regional, distant, new primary melanoma) and death without recurrence before the last planned RFS follow-up were considered events. Patient discontinued from study for any reason other than recurrence or death, or patients receiving new anticancer treatment, or patients with evidence of disease in the surgery specimen (not-NED), or patients who did not receive surgery were censored at the time of randomisation.
	Secondary endpoint	Overall survival (OS)	Defined as the time from randomisation to death from any cause.
	Secondary endpoint	Local recurrence-free survival (LRFS)	Defined as survival free of loco-regional recurrence occurring at any time before systemic recurrence and other events are censored. DMFS is defined as survival free of systemic recurrence, including: 1) systemic recurrence outside the locoregional area at any time before or after loco-regional relapse, 2) systemic recurrence with or without loco-regional relapse, or 3) death from cancer with no information on systemic recurrence
	Secondary endpoint	Distant metastasis-free survival (DMFS)	Defined as survival free of systemic recurrence, including: 1) systemic recurrence outside the locoregional area at any time before or after loco-regional relapse, 2) systemic recurrence with or without loco-regional relapse, or 3) death from cancer with no information on systemic recurrence
	Secondary endpoint	Pathological responses	Only in Arm 1 Histopathological analysis was performed on all lesions removed surgically to determine, for each lesion. The analysis of the proportion of patients with pathological responses in Arm 1 was based on standard institutional pathology reports collected after surgery. Pathological complete response was defined as absence of residual vital tumour cells, in all specimens examined

Database lock	May 3rd, 2023		
Results and Analysis			
Analysis description	**Primary Analysis: RFS**		
Analysis population and time point description	Primary efficacy analysis will be conducted on Intent to treat (ITT) population. ITT population is defined as: all the patients enrolled and randomised to the two different arms of the study (ITT population). Patients lost during the study will be considered as censored to the last available assessment date. Primary efficacy analysis was conducted on 246 patients who had been recruited and randomised by May 3rd, 2023. Ten more patients (5 on each arm) were recruited after the cut-off date who are not included in the efficacy analyses.		
Descriptive statistics and estimate variability	Treatment group	Arm 1: treatment arm neoadjuvant Nidlegy followed by surgery	Arm 2: control arm surgery alone
	Number of subjects	122	124
	RFS according to BICR (median)	16.7 months	6.8 months
	95% confidence interval	11.0 -25.6	5.9 – 11.1
Effect estimate per comparison	RFS	Comparison groups	Arm 1 vs Arm 2
		Hazard risk ratio	0.59
		95% confidence interval	0.41 – 0.86
		P-value (log-rank test)	0.005
	Overall, 28 patients prematurely terminated the study not in accordance with the protocol follow-up rules, 13 in the treatment arm and 15 in the control arm. Arm 1: 8 patients withdrew their consent; 5 patients were retrospectively assessed to have had distant metastases already at screening. Arm 2: 8 patients withdrew their consent; 6 patients were retrospectively assessed to not to have melanoma; 1 patient was retrospectively assessed to have had distant metastases already at screening.		

Analysis description	**Secondary Analysis: DMFS**		
Analysis population and time point description	The analysis was conducted on Intent to treat (ITT) population on 246 patients who had been recruited and randomised by May 3rd, 2023.		
Descriptive statistics and estimate variability	Treatment group	Arm 1: treatment arm neoadjuvant Nidlegy followed by surgery	Arm 2: control arm surgery alone
	Number of subjects	122	124
	DMFS (median)	28.0 months	17.8 months
	95% confidence interval	17.6 - NR	11.0 – 24.5
Effect estimate per comparison	DMFS	Comparison groups	Arm 1 vs Arm 2
		Hazard risk ratio	0.60
		95% confidence interval	0.37 – 0.95
		P-value (log-rank test)	0.029

Analysis description	**Secondary Analyses: LMFS**		
Analysis population and time point description	The analysis was conducted on Intent to treat (ITT) population on 246 patients who had been recruited and randomised by May 3rd, 2023.		
Descriptive statistics and estimate variability	Treatment group	Arm 1: treatment arm neoadjuvant Nidlegy followed by surgery	Arm 2: control arm surgery alone
	Number of subjects	122	124
	LRFS (3-year % LRFS)	70.0%	60.9%
	95% confidence interval	56% - 84%	47% - 75%
Effect estimate per comparison	LRFS	Comparison groups	Arm 1 vs Arm 2
		Hazard risk ratio	0.58
		95% confidence interval	0.31 – 1.08
		P-value (log-rank test)	0.081
Notes	Median LFRS may not yet be estimated as > 50% of the patients are still alive without local relapse at 3 years.		

Analysis description	**Secondary Analyses: OS**		
Analysis population and time point description	Efficacy analyses was conducted on Intent to treat (ITT) population on 246 patients who had been recruited and randomised by May 3rd, 2023.		
Descriptive statistics and estimate variability	Treatment group	Arm 1: treatment arm neoadjuvant Nidlegy followed by surgery	Arm 2: control arm surgery alone
	Number of subjects	122	124
Notes	OS was a key secondary endpoint: however, at the time of data cutoff, only 40 deaths (19 in the experimental arm and 21 in the control arm) had been reported out of the 104 events that would have triggered the survival analysis. No overall survival analysis was thus performed.		

Analysis description	**Secondary Analysis: Pathological Response**	
Analysis population and time point description	Pathological response analysis was conducted only in Arm1 on the 102 patients who had been recruited and randomised by May 3rd, 2023, and for whom a pathological report from the treating Institution was available.	
Descriptive statistics and estimate variability	Treatment group	Arm 1: treatment arm neoadjuvant Nidlegy followed by surgery
	Number of subjects	102
	Complete pathological response % of patients with pCR	20.9%
	95% confidence interval by binomial "exact" calculation	13.2% – 29.7%
Notes	The analysis of the proportion of patients with pathological responses in the experimental arm was based on standard institutional pathology reports collected after surgery. Twenty one patients out of the 102 patients for whom a pathology report at the time of surgery was available in the Nidlegy treatment arm presented a pathological complete response (pCR, 21%) i.e., absence of residual vital tumour cells, in all specimens examined. In other 3 patients (3%) a near-complete pathological response (i.e., ≤ 10% of residual vital tumour cells in the tumour bed of all specimens examined) was recorded.	

3.3.4.4. Clinical studies in special populations

	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	85	59	12

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Non Controlled trials	5	6	0

3.3.4.5. In vitro biomarker test for patient selection for efficacy

Not applicable

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

Not applicable

3.3.4.7. Supportive study(ies)

PH-L19IL2TNF-02/12

A phase II study of intratumoural application of L19IL2/L19TNF in melanoma patients in clinical stage III or stage IV M1a with presence of injectable cutaneous and/or subcutaneous lesions

Table 38. Study identifiers

Study code	PH-L19IL2TNF-02/12
EudraCT number	2012-001991-13
NCT number	NCT02076633
ISRCT number	-
Other identifier(s)	-
Location in eCTD	5.3.5.2.6

The PH-L19IL2TNF-02/12 study was an exploratory phase II open-label, single-arm, multicenter clinical trial aimed at testing the efficacy of Nidlegy (10 Mio IU L19IL2 + 312 µg L19TNF, 4 weekly intralesional injections) in stage III or IV M1a melanoma patients. The primary endpoint of the study was the CR rate in target (injected) lesions at week 12, secondary endpoints included the evaluation of the ORR and DCR in treated and non-treated lesion, as well as the safety and tolerability profile of the tested drug. Patients were to be followed for up to 12 months from treatment's start.

Study population

The study was a mono-national study conducted in two centres in Italy.

Key inclusion criteria:

- Histologically confirmed malignant melanoma of the skin in clinical stage III or stage IV M1a
- Presence of measurable and injectable cutaneous and/or subcutaneous lesions
- Males or females, age > 18 years
- ECOG Performance Status/WHO Performance Status ≤2
- Life expectancy of at least 12 weeks
- Absolute neutrophil count > 1.5 x 10^9/L

Key exclusion criteria:

- Uveal melanoma and mucosal melanoma
- Evidence of visceral metastases and/or active brain metastases at screening
- Previous or concurrent cancer that was distinct in primary site or histology from the cancer being evaluated in this study except cervical carcinoma in situ, treated basal cell carcinoma, superficial bladder tumours (TA, Tis & Ti) or any cancer curatively treated < 5 years prior to study entry
- Presence of active infections (e.g. requiring antimicrobial therapy) or other severe concurrent disease, which, in the opinion of the investigator, would have placed the patient at undue risk or interfere with the study
- Active autoimmune disease
- Planned administration of growth factors or immunomodulatory agents within 7 days before the administration of study treatment
- Patient required or was taking corticosteroids or other immunosuppressant drugs on a long-term basis. Limited use of corticosteroids to treat or prevent acute hypersensitivity reactions was not to be considered an exclusion criterion;

Trial Intervention

The combination of 10 Mio IU of L19-IL2 and 312 µg of L19-TNF was to be administered in an approximate volume of 4.2 ml as a single or multiple intratumoural injection once every week for up to 4 weeks.

L19-IL2 was to be dosed at 10 Mio IU per administration. The amount of L19-TNF per administration should have been 312 µg. However, the dose could be adjusted between 78 and 312 µg per administration according to size and number of lesions and at the investigator's discretion. The total daily dose was to be distributed between all injectable soft-tissue metastases.

Primary Objective

The primary objective of the study was to evaluate the efficacy of L19-IL2/L19-TNF-treated lesions measured as rate of patients with complete response (CR) at week 12 (day 85).

Secondary Objectives

The efficacy of L19-IL2/L19-TNF on treated lesions based on:

- Objective response rate (ORR) (that being, CR and partial response [PR]) and disease control rate (DCR) (that being, CR, PR and stable disease [SD]) of L19-IL2/L19-TNF-treated lesions at week 12, 24 and 36;

- Duration of objective response and disease control of L19-IL2/L19-TNF-treated lesions.

The efficacy of L19-IL2/L19-TNF on treated and non-treated lesions based on:

- Rate of patients with CR, PR and SD of all metastases at week 12, 24 and 36 (ORR and DCR of all metastases according to RECIST v. 1.1);
- Duration of objective response and disease control of all metastases;
- Median overall survival (mOS)

Disposition of participants

Overall, 22 patients (68.75% of screened) were enrolled: 13 (40.63%) at the Milan site and 9 (28.13%) at the Siena site. All 22 enrolled patients were treated: all of them (100.0%) received 1 administration, 20 (90.91%) received 2 and 3 administrations, and 17 (77.27%) received 4 administrations. Seven patients (31.82% of enrolled) completed the study according to protocol, while 15 (68.18%) were discontinued.

Primary Endpoint

CR at week 12 (day 85) in all treated lesions was reported in 2 patients (10.0%). The p value was equal to 0.0003, i.e. the hypothesis of a rate = 50% was rejected.

Table 39. Complete response rate, objective response rate and disease control rate for treated lesions/all lesions by week (EE population)

	ALL LESIONS					
	Week 6 (N=20)	Week 12 (N=20)	Week 24 (N=20)	Week 36 (N=20)	Follow-up 1 (N=18)	Follow-up 2 (N=9)
Complete Response Rate	0 (0.0%)	0 (0.0%)	1 (5.0%)	3 (15.0%)	1 (5.56%)	1 (11.11%)
p value*	NA	NA	<0.0001	0.0017	0.0002	0.0196
Objective Response Rate	9 (45.0%)	12 (60.0%)	8 (40.0%)	8 (40.0%)	5 (27.78%)	2 (22.22%)
p value*	0.6547	0.3711	0.3711	0.3711	0.0593	0.0956
Disease Control Rate	15 (75.0%)	15 (75.0%)	10 (50.0%)	10 (50.0%)	7 (38.89%)	2 (22.22%)
p value*	0.0253	0.0253	1.000	1.000	0.3458	0.0956
	TREATED LESIONS					
	Week 6 (N=20)	Week 12 (N=20)	Week 24 (N=20)	Week 36 (N=20)	Follow-up 1 (N=18)	Follow-up 2 (N=7)
Complete response Rate	0 (0.0%)	2 (10.0%)	1 (5.0%)	3 (15.0%)	1 (5.56%)	1 (14.29%)
p value*	NA	0.0003	<0.0001	0.0017	0.0002	0.0588
Objective Response Rate	9 (45.0%)	12 (60.0%)	8 (40.0%)	7 (35.00%)	5 (27.78%)	2 (28.57%)
p value*	0.6547	0.3711	0.3711	0.1797	0.0593	0.2568
Disease Control Rate	18 (90.0%)	16 (80.0%)	11 (55.0%)	11 (55.0%)	8 (44.44%)	2 (28.57%)
p value*	0.0003	0.0073	0.6547	0.6547	0.6374	0.2568

N=number of evaluable patients; NA=not applicable.

Secondary Endpoints

The results of best overall response in treated lesions based on locoregional tumour response showed that 4 patients (20.0% in the EE population) had CR, 8 (40.0%) had PR, 6 (30.0%) had SD and 2 (10.0%) had PD.

The results of best overall response in all lesions based on RECIST criteria showed that 3 patients (15.0% in the EE population) had CR, 9 (45.0%) had PR, 4 (20.0%) had SD and 4 (20.0%) had PD.

3.3.5. Discussion on clinical efficacy

The applicant is requesting marketing authorisation for Nidlegy (L19IL2/L19TNF), a combination of the immunocytokines bifikafusp alfa (L19IL2) and onfekafusp alfa (L19TNF) administered as intratumoural injection in patients with locally advanced, fully resectable melanoma in the neoadjuvant setting.

The following indication is proposed: "*Nidlegy is indicated for the neoadjuvant treatment of adult patients with locally advanced fully resectable melanoma*".

Efficacy data are currently based on the pivotal phase 3 study **PH-L19IL2TNF-02/15** in patients with stage III melanoma. Results from a single pivotal trial could be sufficient to support marketing authorisation, as long as these are methodologically robust and clinically meaningful and the study is of good quality with robust external and internal validity (refer to ICH E8 and CPMP/EWP/2330/99). Further supportive efficacy data are derived from the phase 2 study **PH-L19IL2TNF-02/12** conducted in stage III/IV melanoma. A second phase 3 study (PH-L19IL2TNF-01/18) is ongoing and efficacy data from PH-L19IL2TNF-01/18 are not available will not be expected during this procedure. The applicant did not seek scientific advice from the EMA on the melanoma development program for L19IL2/L19TNF.

Design and conduct of the clinical studies

PH-L19IL2TNF-02/15 is an ongoing open label, randomised, controlled multi-center phase 3 study to evaluate the efficacy of L19IL2/L19TNF neoadjuvant intratumoural treatment followed by surgery versus surgery alone in patients with clinical stage III B/C melanoma with/without prior therapy and presence of injectable cutaneous and/or subcutaneous or nodal metastases. The primary endpoint was relapse-free survival (RFS). Enrolment is completed and the primary analysis (cut-off 3-May-2023) has been conducted. The follow-up for RFS and OS is still ongoing.

The study enrolled adult patients with stage IIIB and IIIC melanoma. Prior anti-tumour treatment for the primary melanoma lesion (including surgery and approved adjuvant treatments) were allowed. The study started in 2016 and staging was performed according to the AJCC 7th edition. A relatively fit study population was selected with regard to organ function, comorbidities, et cetera. Hence, eligibility criteria should be adequately described in the SmPC. Of the eligibility criteria, inclusion criteria 3 and 6 warranted further discussion. In the second round, the applicant clarified that only one patients received pembrolizumab shortly (8 days) before enrolment. Also, failure to meet IC6 was not a reason for exclusion of participants.

The study was conducted entirely in the European Union with sites located in a few EU countries, namely Italy, Germany, France and Poland. The limited number of sites, however, resulted in a relatively long enrolment time frame (approx. eight years). The study population is relevant for the EU target population, considering the locations of sites.

Patient had to be candidates for intralesional therapy defined as at least one injectable cutaneous, subcutaneous, or nodal melanoma lesion (≥ 10 mm in longest diameter) or having multiple injectable lesions that in aggregate have a longest diameter of ≥ 10 mm).

Patients were randomised (1:1) to receive L19IL2/L19TNF as intralesional injection into all injectable cutaneous, subcutaneous, and nodal tumours once weekly for up to 4 weeks prior to surgical removal of all lesions (Arm 1) or to receive only surgery within 4 weeks (Arm 2). In Arm 1 surgery was planned within 4 weeks from last treatment day with L19IL2/L19TNF while in Arm 2 surgery was planned within four weeks after randomisation. The timing of surgery potentially introduced bias in favour of the experimental treatment for the primary outcome (further discussed below).

Of note, the dose and schedule, as well as the contribution of individual components, have not been thoroughly investigated in earlier clinical trials. Two phase I/II, dose-finding studies (i.e., PH-L19IL2-01/05 and PH-L19TNFα-02/07) were conducted with the mono-components administered intravenously. Thereafter, intralesional application was investigated. In the dossier, the applicant states that the higher local concentration and longer residence time were reasons to exploit a lower dose of intralesional L19IL2/L19TNF. Non-clinical data are still not sufficient to indicate the value of L19 conjugation in the setting of intratumoural administration in human melanomas (refer to non-clinical section). There is also limited clinical evidence to support the theoretical benefits of the product in relation to their untargeted forms. For example, study PH-L19IL2-03/09, in which L19IL2 was administered intralesional, was interrupted prematurely, as the observed ORR was lower than that reported for untargeted IL-2. Hence, this does not support the theoretical advantages of L19IL2 over untargeted IL-2 – although the limitations regarding a cross study comparison are noted. Another study investigated intralesional L19IL2 in combination with L19TNF, but this study did not formally investigate the added benefit of L19TNF to L19IL2. Besides, it remains unclear how the dose of each component was calculated; that is, the translation from systemic to intratumoural. Note that literature is inconclusive with regard to ED-B FN expression in primary melanoma lesions (Kumra et al. Advanced Drug Delivery Reviews. 2016). These issues will not be further pursued.

As per protocol, L19IL2 was to be administered as a fixed dose (13 Mio. IU), while dose adaptations for the L19TNF component between 100 and 400 µg were allowed at the investigator's discretion given the known toxicity of TNF. Dose administration as performed in the study is currently not adequately reflected in the SmPC (see commented SmPC and 3.3.7 Clinical Safety).

Post-surgery treatment with EMA-approved adjuvant therapies (including ipilimumab, nivolumab, pembrolizumab, dabrafenib, and trametinib) were allowed as per protocol amendment (implemented in Feb 2021) at the investigator's discretion. Given the first approvals for adjuvant therapy in 2018, its use was already allowed as per clinical practice. The use of adjuvant therapy was analysed according to the ITT principle. However, the use of adjuvant therapy was introduced via protocol amendment in Feb. 2021 when patients were already enrolled into the study. The impact on the efficacy estimated due to the protocol amendment and the addition of adjuvant remains unclear.

A data and safety monitoring board (DSMB) was appointed, but activities were stopped on Dec 2020 for COVID outbreak and never resumed without any justification. The DSMB minutes provided during the procedure revealed that sponsor facilitators attended the DSMB meetings. This implies that the sponsor employees had knowledge of study results while being involved in the conduct of the trial. Especially since amendments to the protocol are made, data-driven decision-making cannot be excluded. This affects the integrity of the study.

Patients were randomised in a 1:1 ratio. A randomisation sequence was generated by center (using permuted block randomisation with random block size), which is agreed. Generally, factors that are used to stratify the randomisation are expected to be accounted for in the analysis, but given the often limited number of subjects per center in this study, it is accepted that 'center' was not included as a

stratification factor. For the same reason, an assessment of homogeneity of the treatment effect over centres is not feasible. During the procedure, a post-hoc analysis of RFS stratified by country was provided and was consistent with an unstratified test.

The open-label design is not optimal, as it increases the risk of (conscious and unconscious) bias. This is of particular importance for the primary endpoint, as recurrence was determined by investigator and only retrospectively confirmed by blinded independent central review.

The primary endpoint for this study was relapse-free survival (RFS) assessed by the principal investigators and retrospectively confirmed by Blinded Independent Centralized Review (BICR) of the FDG PET-CT scans. RFS was defined as time from randomisation until date of first recurrence (local, lymph nodes, or distant organs). Of note, RFS should have been defined as time from surgery until date of first recurrence. The RFS follow-up time was 3 years. No formal scientific advice on the study design and on the choice of the primary endpoints has been sought by the applicant.

However, RFS as primary endpoint was not ideal for Study PH-L19IL2TNF-02/15 and is not considered to be relevant for the determination of benefit for regulatory decision making. There are two main reasons for this. First, there is a structural difference in the timing of surgery between the two arms (with surgery occurring later in the experimental arm), and as RFS is measured as the time between randomisation and recurrence, a bias in favour of the experimental arm can be expected. Secondly, follow-up for recurrence was only possible if R0 resection is achieved, and R0 resection was not achieved for all participants. Patients who did not achieve R0 were censored at randomisation, along with those who discontinued early or received new anticancer therapy prior to RFS. The rationale for this decision is unclear.

As a result, it is unclear which estimand (EMA/CHMP/ICH/436221/2017) was the target of interest which was not sufficiently provided by the applicant during the procedure. In the original CSR, only the total number of patients who did not undergo surgery or who did not achieve R0 was provided. The applicant was requested to provide additional information by treatment arm on the participants that were withdrawn/censored for not undergoing surgery or not achieving R0 resection. An important imbalance was observed between arms with almost twice as many participants in the experimental arm not achieving R0 resection, leading to the conclusion that informative censoring is present, and that this is likely to be in favour of the treatment arm. Further, as adjuvant therapies are approved in EU and are considered beneficial, not being able to receive adjuvant therapy is considered a loss of chance.

This issue could have been circumvented by using a more suitable endpoint like event-free survival. Several post-hoc analyses of EFS were provided during the assessment procedure. The most relevant EFS analyses, which account for cancellation of surgery and/or non-NED status as an event did not reach statistical significance. Further, reliable results cannot be obtained due to the lack of follow-up for a subset of patients.

Given the uncertainties with the choice of endpoint and censoring rules, the robustness of the current results is questioned.

Secondary endpoints include OS, DMFS, and LRFS. When RFS is used as primary endpoint, it is important that no detrimental effect of therapy on overall survival (OS) as secondary endpoint is observed (EMA/CHMP/205/95 Rev.6). The inclusion of OS as (key) secondary endpoint is therefore supported. PET-CT was performed at screening and every 6 months from randomisation (every 2 follow-up visits starting from follow-up 2). In case of suspect disease recurrence, PET-CT and/or

tumour assessment could be conducted during an unscheduled visit. Scans based on suspected recurrence occurred in 4 participants in arm A (recurrence was confirmed in 0 cases) and 7 participants in arm 2 (recurrence was confirmed in 3 cases). Determining the time of (asymptomatic) distant recurrences may be less exact in the pivotal trial compared to other trials, given that follow-up imaging is typically done every 3 months for at least 2 years (Amaria et al. lancet Oncol. 2019). Additional secondary endpoints include pathological responses assessed on the surgical specimen after tumour removal (Arm 1 only) and changes peripheral T cell subsets (as evaluated per PBMC immunophenotyping). Pathological responses were collected for the surgery arm only. It is remarked that no response criteria were clearly defined in the protocol, whilst a reference guide to pathologic assessment of melanoma specimen exist (Tetzlaff et al. Annals of Oncology 2018).

Conflicting information were provided throughout the study protocol and CSR whether OS was planned to be tested confirmatory. At the time of primary analysis for RFS, an OS interim analysis was planned using the Lan-DeMets alpha spending function. According to the CSR, only 40 deaths had been reported at the time of primary analysis of RFS. Thus, no OS analysis was performed in initial submission, despite this was originally planned. No further explanation on omitting a pre-planned interim analysis was provided after request for clarification. During the procedure the OS analyses were provided which are, however, too immature to enable a conclusion on long-term survival.

During the conduct of the study, several protocol amendments were implemented with two major changes on the primary endpoint: implementation of two interim analyses for RFS (protocol version 4), and change of the primary endpoint from RFS at 1 year to overall RFS (protocol version 5). Both interim analyses did not meet the efficacy criterion to stop early. The choice to include interim analyses was introduced in protocol version 4. During DSMB meeting 5 (January 2019), the motivation for introducing the interim analyses was that "[t]he formal interim analysis would allow Philogen to officially disseminate information about the trial's partial results, increasing awareness about the study and the product and attracting the interest of the scientific community and possible commercial partners". The applicant is requested to confirm that interim results have been handled confidentially. Shortly after the conduct of the second interim analysis, protocol version 5 was implemented including the change of the primary endpoint. At the time the first patient was enrolled in the study, the primary objective was to evaluate the RFS rate in the bifikafusp alfa/onfekafusp alfa plus surgery treatment group (Arm 1) versus surgery alone (Arm 2), "assessed at 1 year after randomization" (of note, given that the proposed analysis was a log-rank test, a more correct formulation of the primary objective would be "a comparison of the RFS rate up to 1 year after randomization"). Per protocol version 5, the objective has been reformulated to simply 'RFS'. The applicant stated that the change in primary objective has been discussed with the EMA. However, only informal feedback have been sought which cannot be considered as valid scientific advice.

To evaluate the impact of the protocol amendment, an RFS analysis as outlined in protocol version 3 (the most recent protocol at the time of the first inclusion) and version 4 was performed; that is, a log-rank test and KM-curves with all subjects censored at 12 months. While the results suggest that the primary endpoint would have been met if the applicant adhered to the original analysis plan, data-driven decision-making cannot be ruled out. Especially, since the provided DSMB minutes indicate that sponsor employees had knowledge of accumulating study results, while being involved in the conduct of the trial. This substantially influences the integrity of the study. Further documentation and earlier versions of the SAP were provided. Nevertheless, the provided information is considered insufficient and further details on the implemented firewalls are requested as the information currently provided indicate that multiple study personnel had access to data.

Per the amendment dated February 02nd, 2021, the possibility to use EMA-approved adjuvant therapies after surgery was explicitly stated in the protocol. Before that, adjuvant therapy was allowed but not defined in the protocol.

PH-L19IL2TNF-02/12 was an exploratory open-label, single-arm, multi-center phase 2 study that aimed to test the efficacy of L19IL2/L19TNF intralesional injections in melanoma patients. The study was conducted before the initiation of the phase 3 study has been completed in March 2015.

The study enrolled adult patients with stage III but also stage IV M1a melanoma. Staging was performed according to the AJCC 7th edition. Patients had to present with measurable and injectable cutaneous and/or subcutaneous melanoma lesions. In this study, the combination treatment with L19IL2/L19TNF was evaluated for the first time, as the prior clinical development only evaluated intralesional L19IL2 administration. As compared to the pivotal study, slightly lower doses of L19IL2 (10 Mio. IU) and L19TNF (312 µg) were used. Dose adaptions for the L19TNF component between 78 and 312 µg were again allowed. L19IL2/L19TNF was administered as intratumoural injection once every week for up to 4 weeks.

The primary endpoint of the study was the Complete Response (CR) rate in target (injected) lesions at week 12. Secondary endpoints included the evaluation of the Objective Response Rate (ORR), defined as the rate of patients with CR and Partial Response (PR) and Disease Control Rate (DCR) at week 12, 24, 36 in patients with treated lesions.

Efficacy data and additional analyses

The efficacy data for **PH-L19IL2TNF-02/15** are based on the data cut-off 03-May-2023 that was triggered when the 95th RFS event was observed. Upon the GCP inspection, the applicant provided updated results based on the data cut-off 03-May-2023 after additional data cleaning until 29-November-2024. The median follow-up is 21.2 months. Additional re-analysis of data based on the cut-off 29-Nov-2024 was provided during the procedure.

Patients were entirely recruited in 20 European study centres located in Germany, France, Italy and Poland. The limited number of sites resulted in a relatively long enrolment time frame (approx. eight years). The first patients was enrolled on 29-Jun-2016; enrolment has been completed with last-patient-first-visit on 20-Jul-2023. The study is ongoing.

A total of 364 patients were screened and 256 were finally randomised (Arm 1: n=122; Arm 2: n=124). There were 256 participants enrolled in the study, which is more than the planned sample size for OS (n=214). This was due to patients who, after surgery, could not be rendered NED (no evidence of disease) and were therefore censored at randomisation and effectively not contributing to the analysis for the primary endpoint.

A total of 96 screen failures were the result of incompliance with eligibility criteria. Approximately half of these were due to distant metastases. Several participants withdrew consent before and after randomisation. Participants were also withdrawn based on investigators decision, which occurred slightly more frequent in the experimental arm. The applicant provided some reflection on these findings, but further discussion is still warranted. A large number of patients did not receive surgery (i.e., more than is graphically displayed in the flow chart). Also, not-NED at surgery was reported for 23 participants; 15 patients in the treatment arm and 8 in the control.

During the 4-week treatment, only 2/3 of patients received the second following doses potentially indicating limited tolerance against L19IL2/L19TNF in some patients. In most cases, this was due to drug interruptions or drug withdrawals due to AEs.

A total of 187 major protocol deviations occurred which is considered rather high. The most common protocol deviation was "IC/EC criteria". There were also several protocol deviations related to the IMP that were in part due to poor instructions provided by the Sponsor. Several issues remain. Namely, the applicant was asked to provide a more thorough/detailed discussion on how the deviations may have affected the completeness, accuracy, and/or reliability of the study data or a subject's rights, safety, or well-being should be provided. Furthermore, the study was performed during the COVID pandemic. Yet, it seems that there is only one deviation as a result of COVID19, which seems unlikely. This will not be further pursued, also considering the major issues that are not resolved at the moment.

The patient's age range was 22 and 91 years, with a median age of 64 years; 138 (56.33%) and 107 (43.67%) female patients were enrolled. 131 patients had melanoma stage IIIC and 69 patients had melanoma stage IIIB. For patients for whom pathological T or N subcategories were not available, a discrimination between stage B and C was not possible. Disease stages are unknown for 12 (4.7%) participants. According to the applicant, some data are still under revision. Nine (3.5%) participants had stage IV disease. The applicant is asked to explain whether patients had stage IV disease at screening or progressed during the trial. The majority of patients (56.5%) presented with a single lesion at screening, 19.5% showed two lesions, while patients with 3 or 4 lesions accounted for ~24% of all patients. A low number of patients (~2%) had a higher number of lesions (up-to 12). The number of lesions was overall balanced between both groups. The majority of patients (n=232; 94.7%) underwent a surgery to treat their tumour prior enrolment in this study. Eighty-four (84) patients (34.3%) received prior systemic therapy and 12 patients (4.9%) received a prior radiotherapy. The most frequently used prior systemic therapy was immunotherapy.

A total of 116 RFS are included in the RFS analysis with data cut-off 03-May-2023 and updated data cleaning. These include the 95 events observed until the efficacy data cut-off, a further event after the updated data cleaning as well as 20 additional RFS events that have been retrospectively included (Arm 1: n=8; Arm 2: n=12), after reconciliation of the discordances of BICR and Investigators' assessment. Reconciliation rules were not pre-specified. The approach to response confirmation has led to a primary analysis that is not entirely based on BICR; thereby, questioning the added value of the BICR assessment. A discordance rate between investigator's and BICR assessment of 26% was observed (67 case out of 256). A reflection on the high discordance rate and the individual analyses (RFS by BICR, RFS by investigator and RFS by the consolidated BICR) is needed as results are expected to differ depending on which assessment is used. Furthermore, the listing of individual assessments by investigator, BICR and the consolidated BICR revealed that only selected participants (all in the control arm) were counted as events in the consolidated BICR due to start of new anti-cancer therapy while others were censored. Thus, when using the consolidated BICR, more events are assigned in the control group and adds further uncertainty to the choice of assessment for the primary analysis and in particular the choice of censoring.

Until the data cut-off 03-May-2023 and updated data cleaning, 49 RFS events have been observed in the treatment arm (arm 1) and 67 RFS events in the control arm (arm 2), resulting in a HR of 0.57 (95% CI 0.39-0.82; p=0.002). Median RFS was 17.2 months (95% CI 11.1-26.3) in the treatment arm compared to 6.8 months (95% CI 5.9-11.2) in the control arm. This corresponds to a gain in median RFS of approximately 10 months for the experimental arm over the control arm. The Kaplan-Meier curves separated after 6 months and remained separated thereafter. The 1-year RFS rate was 59% vs.

39%, the 2-year RFS Rate 42% vs. 23%, and the 3-year RFS rate 27% vs. 10%. As the median follow-up time is currently 21.2 months, interpretation of RFS of longer duration (i.e. beyond 2 years) is limited.

An updated RFS analysis with cutoff 29-November-2024 was provided to obtain more recent data. With the new cutoff, 134 RFS events were observed (56 in the treatment arm and 78 in the control). Median RFS was 16.7 months (95% CI: 11.8-25.6) in the treatment arm compared to 8.9 months (95%CI: 6.0-12.3) in the control arm.

It was questioned during the procedure whether the treatment effect can be adequately characterised and results are sufficiently robust as subjects who receive new anti-cancer therapy, who discontinue treatment/study, who are not-NED at last surgery, or who did not have surgery were censored.

During the procedure, the applicant provided an overview of patients that were censored for non-administrative censoring. 66 patients were censored for non-administrative reasons. These reasons are relevant in the discussion of the treatment effect in the ITT population, as imbalances between arms were noticeable, overall illustrating why RFS is not preferred as primary endpoint in the neoadjuvant setting and does not reflect the treatment effect in the ITT population. Baseline characteristics of study participants who were and were not censored for non-administrative reasons were provided during the procedure. In the experimental arm, 30 percent (37/122) of participants were non-administratively censored, and presented results suggest a possible relation between prognosis at baseline and probability of being non-administratively censored: First, the probability of being non-administratively censored appears greater for participants with ulceration (22/54=0.41) compared to those without (10/51=0.20). Second, the median sum diameters of target lesions is larger for participants who are non-administratively censored (36.75mm, 95%CI: 29.80 - 44.20) than for participants who are not censored (21mm, 95% CI: 17.00 - 27.00). Third, the probability of non-administrative censoring appears greater for participants in AJCC (7th) grade 3B (22/64=0.34) compared to grade 3A (6/36=0.17). While it is acknowledged that sample sizes are limited, taken together, these findings could have resulted in overoptimism in the results for the experimental arm. In the control arm, around 23 percent (29/124) of participants are non-administratively censored, but results do not appear to indicate structural differences in terms of the probability of being non-administratively censored across categories of the presented baseline factors. Without further evaluation (e.g., tipping-point analyses), the impact of the non-administrative censoring on the RFS results remains unclear.

Results from an alternative RFS analysis defined as first recurrence or death from surgery was presented during the procedure. The number of participants reduce from 124 to 109 in the control arm, and from 122 to 89 in the experimental arm. Compared to the original analysis, the KM curves appear to separate later (~5m). The observed difference is statistically significant in favour of the experimental arm. However, currently, it is not clear to what extent this difference represents an actual treatment effect, or is (in part) explained by the fact that a potentially selective subgroup was able to achieve NED status. For instance, it could be envisaged that, in the experimental arm, the delay of surgery resulted in a selection of participants with a more favourable prognosis (because those with worse prognosis did not end up achieving NED). For this reason, baseline tables corresponding to this analysis (i.e., for the subset of patients who achieved NED) were requested, but have not been provided. Additional analyses were performed on the potential influence of informative censoring. However, cherry picking cannot be excluded, meaning that the applicant might have only listed and accounted for reasons of non-administrative censoring that still led to nominal significant result.

Overall, the RFS results can only be considered as supportive evidence – despite it being the primary endpoint. Namely, patients experiencing unsatisfactory treatment/procedure effects before surgery are not taken into account in the RFS analyses.

An EFS analysis is considered more appropriate for the overall evaluation of the treatment effect, and for RFS, an analysis where time is measured from successful surgery (instead of from randomisation), performed only in those subjects who achieved NED status, is considered more informative. EFS was not defined a priori in the pivotal trial, but is a key endpoint in the neoadjuvant setting. In general, it is difficult to determine the optimal analysis to reflect the treatment effect in the ITT. To fully understand the benefit of the product, it is necessary to examine the reasons for non-administrative censoring and the clinical consequences thereof (see discussion on RFS above). It is important to mention that there is no follow-up for patients excluded from the RFS analysis; an issue that cannot be resolved. As there is no harmonised definition of EFS, multiple sensitivity analyses have been performed by the applicant, during the procedure. All EFS analyses performed by the applicant led to a smaller treatment effect compared to the primary RFS analysis and only two reached nominal significance; hence, the robustness of the results is questioned. In essence, it seems that the treatment effect only holds if some – or all – of the negative impacts of treatment are ignored. There seems to be an increased risk of progression or failure to perform an R0 resection due to the delay in surgery in the experimental arm, counteracting the potential favourable effects on RFS. For example, for almost twice as many participants in the experimental arm the outcome was failure to perform an R0 resection, which makes these participants ineligible to receive adjuvant therapy. As adjuvant therapies are approved in EU and are considered beneficial; thereby, not being able to receive adjuvant therapy is considered a loss of chance. From a clinical perspective, an analysis in which non-NED is counted as an event is most appropriate. None of these analyses provided during the procedure reach statistical significance. In addition, the clinical relevance of findings is questioned. The 1-year EFS differences of approx. 10% is observed, which is much smaller than the expected 20% differences assumed for RFS used in the sample size calculation (refer to the protocol). Of note, there was no follow-up for participants classified as not-NED. Moreover, it is entirely unclear if the treatment effect holds in the current treatment landscape, as the study is outdated and the treatment landscape has changed.

A cox proportional-hazards model was used to explore any potential effects of the adjuvant treatment on the primary endpoint. A greater RFS benefit was achieved in patients receiving adjuvant therapy after surgery and treatment with PH-L19/IL2TNF as compared to those treated with surgery and adjuvant therapy only. Nevertheless, results are difficult to interpret, as the use of adjuvant therapy was introduced after protocol version 5 and the choice for post-surgery therapy adjuvant therapy may have been affected by treatment allocation (EMA/CHMP/295050/2013). Kaplan-Meier curves showing the development of RFS in patients with or without adjuvant therapy by treatment arm reveal that participants in the treatment arm without adjuvant therapy may have no benefit in comparison to participants in the control arm with adjuvant therapy. These results do not support the hypothesis that benefit is regardless of adjuvant therapy and therefore, the use of adjuvant therapy and how this reflects current clinical practice, as well as the potential impact of (effective) adjuvant therapies on RFS has remains unclear.

Distant metastasis developed in 32 patients in the treatment arm as opposed to 43 patients in the control arm, resulting in a HR of 0.55 (95% CI 0.34-0.87; p=0.009). Local recurrences occurred in 27 patients in the treatment arm, and in 35 patients in the control arm, resulting in a HR of 0.68 (95% CI 0.41-1.13; p=0.137).

Though an OS interim analysis at the time of RFS primary analysis was pre-specified, no overall survival analysis was performed. However, summarised OS information are available in the dossier but an OS analysis was not provided in the initial submission. Conducting unplanned 'interim' OS analyses (without correction for multiple analyses) and afterwards not conducting a planned interim OS analysis adds to the list of triggers that necessitated the need for a GCP inspection. During the procedure, the applicant provided the requested OS interim analysis based on the 03-May-2023 cutoff and on an updated cutoff 29-November-2024. At the later cutoff 47 participants had died (23 in the treatment and 24 in the control arm) with a HR of 0.93 (95%CI: 0.52-1.64). The OS results are currently considered not mature enough for a sufficient benefit-risk assessment and final OS to evaluate any support for the claim of efficacy.

Additional secondary endpoint data include an analysis of the proportion of patients with pathological responses collected after surgery. In 21/102 a pathological complete response (pCR, 21%) was observed. A near-complete pathological response was recorded in other 3 patients (3%).The applicant performed a reanalysis of available specimens at three German centres (n=25). Confirmation of pCRs in these samples is reassuring. Nevertheless, uncertainty remains, as a large number of samples was not reanalysed and no central review was conducted; thus, confirmation on these complete responses cannot be provided. This uncertainty should be taken into account when interpretating the results on pathological responses.

In **PH-L19IL2TNF-02/12**, the analysis of best overall response in all lesions showed that 3 patients (15.0% of patients in the EE population) had CR, 9 (45.0%) had PR, 4 (20.0%) had SD and 4 (20.0%) had PD. The results of best overall response in treated lesions based on locoregional tumour response were similar to those observed using RECIST criteria: 4 patients (20.0% of patients in the EE population) had CR, 8 (40.0%) had PR, 6 (30.0%) had SD and 2 (10.0%) had PD. The null hypothesis of p = 50% was rejected with a CRR of only 10% indicating the CRR is lower than 50%. Per sample size calculation a CRR of 80% was expected. This is considerable different from the observed 10%. The results of this exploratory study suggested that the concept of intralesional administration with the combination of L19IL2 and L19TNF was associated with anti-tumour activity, also in non-injected lesions, and provided the rationale to conduct the phase 3 study PH-L19IL2TNF-02/15. These results are considered supportive.

Additional expert consultation

Healthcare professional's perspective (EORTC) was provided for this procedure.

With regard to the available treatment and to what extent they cover the indication, it was informed that "Being the intended indication neoadjuvant treatment of adult patients with locally advanced fully resectable melanoma, the neoadjuvant combination of nivolumab and ipilimumab tested in the NADINA trial is likely to cover the intended indication for most new patients without contraindications. There are, however, a small percentage of patients whose melanoma recurs after surgery and neoadjuvant / adjuvant systemic treatment. Both this niche of patients and, also, the ones with contraindication to systemic immunotherapy, remain outside the standard of care adjuvant treatment or the potentially future standard of care neoadjuvant treatment."

New medicinal products "should impact OS", a benefit not yet shown by adjuvant treatments. Investigating health-related quality of life as a key secondary endpoint is crucial, especially when multiple curative approaches exist. Reducing the relapse rate is obviously important, however, patients' well-being and longevity matter at least equally."

Another important perspective was related to pregnancy: "Considering melanoma's impact on young patients, assessing safety for pregnant individuals is essential. An intra-lesional treatment might be an option for pregnant women, if deemed safe.". Regarding this perspective, it is important to note pregnant individuals were not allowed to participate in the study.

3.3.6. Conclusions on clinical efficacy

The efficacy data are based on the pivotal study PH-L19IL2TNF-02/15. In cases where confirmatory evidence is provided by one pivotal study, this study will have to be exceptionally compelling, as described in the Points to Consider (PtC) on applications with one pivotal study (CPMP/EWP/2330/99). Several of the prerequisites from that PtC are not met in this study, with respect to internal and external validity, clinical relevance, consistence. Of note, the study integrity is likely affected by knowledge of interim results. Hence, data-driven decision-making cannot be entirely excluded as important amendments have been made to the protocol after interim results were available.

The robustness of the RFS analysis is largely questioned, given that the choice of endpoint is considered suboptimal in this setting and the selected censoring rules seem to include informative censoring as participants who did not undergo surgery or were not-NED at surgery were censored at randomisation.

EFS is considered of higher value and analyses were provided post-hoc. However, EFS results are not considered to be clinically meaningful and robust. A data-driven decision surrounding the change of the primary endpoint from „1-year RFS" to „RFS" cannot be excluded and thus questions the study integrity. Furthermore, data on OS are currently immature and do not support for the claim of efficacy.

It is also uncertain whether the treatment effect holds in the target population, considering the change in treatment landscape. The results from the ongoing PH-L19IL2THF-01/18 phase 3 study are not expected in short term and it is unclear how the enrolment is going.

Based on these all these critical aspects, the benefit of Nidlegy cannot be determined due to several shortcomings that are unlikely to be resolved.

3.3.7. Clinical safety

All safety data available at the time of submission of the MAA was collected in six clinical trials (two non-controlled single-agent studies with the individual components of Nidlegy administered systemically (L19IL2 or L19TNF), one non-controlled single-agent study with intratumoural L19IL2, one non-controlled study with the intratumoural combination of L19IL2/L19TNF and two controlled, randomised studies with the intratumoural combination of L19IL2/L19TNF).

The main safety data for this MAA to support the safety of L19IL2/L19TNF in the proposed indication for neoadjuvant treatment of adult patients with locally advanced fully resectable melanoma, is considered based on data collected from clinical studies where L19IL2/L19TNF has been administered as combination and intralesional in patients with locally advanced melanoma (studies: PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18).

Table 40. Studies included in the safety database

Study	Number of Participants	Experimental Arm 1 (L19IL2/L19TNF)	Control Arm 2 (Surgery only)
Phase 3 Study PH-L19IL2TNF-02/15	N=246	N=122 <u>Treatment:</u> • L19IL2: 13 Mio IU • L19TNF: up to 400 µg • Up to four weekly intralesional injection • followed by Surgery within 4 weeks from last treatment	N=124 <u>Treatment:</u> surgery within 4 weeks after randomisation
Phase 3 Study PH-L19IL2TNF-01/18	N=99	N= 51 <u>Treatment:</u> • L19IL2: 13 Mio IU • L19TNF: up to 400 µg • Up to four weekly intralesional injection • followed by Surgery within 4 weeks from last treatment	N=48 <u>Treatment:</u> surgery within 4 weeks after randomisation
Phase 2 Study PH-L19IL2TNF-02/12 (uncontrolled)	N=22	N=22 <u>Treatment:</u> • L19IL2: 10 Mio IU • L19TNF: 78 - 312 µg	N=0

		• Up to four weekly intralesional injection	
Overall Population pooled data of study: PH-L19IL2TNF-02/15, PH-L19IL2TNF-01/18, PH-L19IL2TNF-02/12	N=367	N= 195	N= 172

As of the data cut-off date (29 November 2024), the safety database includes a total of 367 melanoma patients (22 patients treated with L19IL2/L19TNF only, 173 patients treated with neoadjuvant L19IL2/L19TNF and surgery, and 172 patients treated with surgery only), which have been enrolled in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. A total of these 195 patients treated with L19IL2/L19TNF received at least one intralesional dose or surgery.

The experimental arms of the randomised, controlled studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, as well as the study PH-L19IL2TNF-02/12, were pooled (Overall population) as the patient population (melanoma) and treatment plan (four weekly intralesional injections) are comparable.

The control arms of the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, which include patients that received surgery within 4 weeks after randomisation, were pooled as they are identical with regard to the study population and the treatment performed.

Table 41. Number of patients per arm for each study included in the safety data

Study	**PH-L19IL2TNF-02/12 (N=22)**	**PH-L19IL2TNF-02/15 (N=246)**		**PH-L19IL2TNF-01/18 (N=99)**		**OVERALL (N=367)**	
Arm	Total (N=22)	Arm 1 (N=122)	Arm 2 (N=124)	Arm 1 (N=51)	Arm 2 (N=48)	Arm 1 (N=195)	Arm 2 (N=172)

At the time of submission of the MAA, the two phase 3 studies, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, were still ongoing. In the pivotal trial (PH-L19IL2TNF-02/15), safety was assessed for the total population of 246 patients at screening, at the surgery visit, and at the follow-up visits every 3 months for up to 3 years. In addition, for patients in experimental arm (Arm 1), safety was also assessed at week 1, 2, 3, 4 and 5 during the pre-surgery treatment period.

The safety data from the non-controlled single-agent clinical trials (study PH-L19IL2-01/05 and PH-L19TNF-02/07, where L19IL2 or L19TNF was administered systemically (i.e. intravenously) and study PH-L19IL2-03/09 with intratumoural administration of L19IL2 alone) are not presented in this document.

Table 42. Clinical studies investigating the safety profile of L19IL2 and L19TNF as single-agents and in combination therapy

Clinical Trial	Type of study	Drug	Mode of administration	Population	#Patients in safety population	Data Cut-Off/ Study completion date	CTCAE version
PH-L19IL2-01/05 Phase I/II Study	Non-controlled	L19IL2	Systemic	Patients with Solid tumours renal cell carcinoma	33	30/04/2008	3.0
PH-L19TNF-02/07 Phase I/II Study	Non-controlled	L19TNF	Systemic	Patients with advanced solid tumours	34	26/05/2011	3.0
PH-L19IL2-03/09 Phase II Study	Non-controlled	L19IL2	Intratumoural	Patients with histopathologically proven malignant melanoma with presence of injectable soft-tissue metastases either in clinical stage III or stage IV M1a	25	28/08/2013	4.02
PH-L19IL2TNF-02/12 Phase II Study	Non-controlled	L19IL2/L19TNF	Intratumoural	Patients with melanoma in clinical stage III or stage IV M1a with presence of injectable cutaneous and/or subcutaneous lesions	22	23/05/2015	4.02

PH-L19IL2TNF-02/15 Pivotal Phase III	Controlled	L19IL2/L19TNF	Intratumoural	Patients with melanoma in clinical stage III B/C with injectable cutaneous and/or subcutaneous and/or nodal lesions with/without prior therapy.	246	29/11/2024	4.03
PH-L19IL2TNF-01/18 Phase III Study	Controlled	L19IL2/L19TNF	Intratumoural	Patients with melanoma in clinical stage III B/C	99	29/11/2024	4.03

Source: 2.7.4 Summary of Clinical Safety, Table 1; 2.5 Clinical Overview, Table 1, individual Study Reports

3.3.7.1. Patient exposure

In the **pivotal study PH-L19IL2TNF-02/15**, for patients in arm 1, a mixture of L19IL2 (13 Mio IU) and L19TNF (up to 400 µg) was administered intratumourally into all injectable lesions once weekly for up to 4 times (or until all injectable tumours have disappeared, or intolerance to study treatment or in the opinion of the investigator immediate surgical resection or any other treatment for melanoma is warranted, whichever occurred first). The whole volume of L19IL2/L19TNF was distributed among all injectable lesions: no injectable lesion was left uninjected. However, the dose of L19TNF could be adjusted between 100 and 400 µg (i.e., 100 µg, 200 µg, 300 µg or 400 µg) per administration according to size and number of lesions and based on the patient's tolerability to the treatment at the investigator's discretion. At each treatment visit, the investigator had to evaluate all known melanoma lesions as injectable or non-injectable. The total daily dose was distributed between all injectable cutaneous, sub-cutaneous and nodal metastases. Surgery was performed within 4 weeks from last treatment.

In the study **PH-L19IL2TNF-01/18** the amount of L19IL2/L19TNF that is injected is dependent on the size of the tumour. The maximum dose to be administered in a single treatment visit is 400 µg of L19TNF and 13MioIU of L19IL2, in a combined total volume of approximately 2mL. In case study drug-related, grade ≥ 3 adverse events are recorded after the first L19IL2/L19TNF dose administration, the L19TNF dose is reduced to 200 µg for the following administrations. The largest injectable lesion is treated first and all other lesions are then prioritised for injection based on decreasing lesion size, until no lesion is left uninjected or until no volume is left to inject (whichever occurs first). Eventual drug leftovers will be discarded. In order to obtain accurate dosing records, for each dosing administration the total L19IL2/L19TNF dose delivered, the volume injected per lesion and the eventual discarded volume will be recorded. The whole volume of Nidlegy will be distributed among all injectable lesions, according to the table below.

Table 43. Volume of Nidlegy injected per lesion diameter

Lesion diameter (cm)	Volume to be injected (ml)
> 3.0	2.0 (or max volume)
> 2.0 to 3.0	1.5
> 1.0 to 2.0	1
> 0.5 to 1.0	0.5
> 0.1 to 0.5	0.25

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In the supportive uncontrolled phase II study PH-L19IL2TNF-02/12, the study treatment consisted of a combination of 10 Mio IU of L19-IL2 and 312 µg of L19-TNF to be administered in an approximate volume of 4.2 ml as a single or multiple intratumoural injection once every week for up to 4 weeks.

L19-IL2 was dosed at 10 Mio IU per administration, the amount of L19-TNF per administration should have been 312 µg. However, the dose could be adjusted between 78 and 312 µg per administration according to size and number of lesions and at the investigator's discretion. The total daily dose was distributed between all injectable soft-tissue metastases. Patients were treated once weekly until all lesions disappeared or for maximal 4 weeks.

The duration of exposure and number of administrations of L19IL2/L19TNF during the study treatment period were summarised with descriptive statistics and by study. The OVERALL population corresponds to the added number of administrations from the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18.

The mean individual weekly dose in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18 was calculated to be 247 ± 101 µg L19TNF and 10 ± 0 Mio IU L19IL2, 342.8 ± 100.2 µg L19TNF and 12.6 ± 1.6 Mio IU L19IL2, and 315.2 ± 90.6 µg L19TNF and 10.2 ± 2.9 Mio IU L19IL2, respectively. This corresponds to a mean relative dose intensity of 81.6 ± 26.5% for L19IL2 and 73.1 ± 30.7% for L19TNF. The median number of L19IL2/L19TNF administrations was four in all three studies, with 69% to 91% of the patients in the individual studies receiving at least three administrations. The mean duration of exposure to L19IL2/L19TNF was 18 ± 7.8 days. The median duration of exposure to L19IL2/L19TNF was 22 days (1-32 days).

Table 44. Exposure to L19IL2/L19TNF by number of administrations for the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18. (Cut-off Date: 29/11/2024)

Number of administrations	PH-L19IL2TNF-02/12 (N=22)		PH-L19IL2TNF-02/15 (N=122)		PH-L19IL2TNF-01/18 (N=51)		OVERALL (N=195)	
0	0		2*	1.64%	0		2	1.03 %
1	2	9.09%	17	13.93%	6	11.76%	25	12.82%
2	0		19	15.57%	9	17.65%	28	14.36%
3	3	13.64%	19	15.57%	7	13.73%	29	14.87%
4	17	77.27%	65	53.28%	29	56.86%	111	56.92%

The column OVERALL corresponds to the added number of administrations from the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18.

*In study PH-L19IL2TNF-02/15, two patients enrolled in arm 1 did not receive treatment with L19IL2/L19TNF, only surgery. The patients are included in the safety population.

Source: 2.7.4 Summary of Clinical Safety, Table 2

Table 45. Study subject drug exposure by dose level of L19TNF and L19IL2 per weekly administration

	PH-L19IL2TNF-02/12				PH-L19IL2TNF-02/15						PH-L19IL2TNF-01/18					OVERALL					
	L19TNF (N=22)			L19IL2 (N=22)	L19TNF (N=122)				L19IL2 (N=122)		L19TNF (N=51)			L19IL2 (N=51)		L19TNF (N=195)				L19IL2 (N=195)	
	Dose Level 1	Dose Level 2	Dose Level 4	Dose Level 1’	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4	Dose Level 1’	Dose Level 2’	Dose Level 2	Dose Level 3	Dose Level 4	Dose Level 1’	Dose Level 2’	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4	Dose Level 1’	Dose Level 2’
Week 1	3	1	18	22	2	3	15	98	6	114		17	33	28	22	5	4	32	149	56	136
Week 2	4	3	13	20	1	5	10	65	4	79	1	8	32	20	21	5	9	18	110	44	100
Week 3	6	1	13	20	2	4	13	66	7	78	2	10	24	18	18	8	7	23	103	45	96
Week 4	5	1	11	17	3	8	14	58	9	75	1	9	24	16	18	8	10	23	93	42	93

The column OVERALL corresponds to the whole population of treated patients in PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/125, and PH-L19IL2TNF-01/18. The dose per week was not available for some patients at data cut-off (29/11/2024) due to ongoing data verification queries in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18.

L19TNF: Dose Level 1: Dose < 100 µg/kg; Dose Level 2: 100 µg/kg ≤ Dose < 200 µg/kg; Dose Level 3: 200 µg/kg ≤ Dose < 300 µg/kg; Dose Level 4: 300 µg/kg ≤ Dose ≤ 400 µg/kg.

L19IL2: Dose Level 1’: Dose ≤ 10 Mio IU; Dose Levels 2’: 10 < Dose ≤ 13 Mio IU.

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 22

3.3.7.2. Adverse events

The adverse events from all studies (PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18) were graded according to the CTCAE version specified in the protocol (PH-L19IL2TNF-02/12: version 4.02, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18: version 4.03) and categorised by medical term using the MedDRA terminology v.27.1. Adverse events, which were described with older versions of the MedDRA terminology were recoded to v.27.1. The number and percentage of patients who reported all AEs and drug-related AEs, SAEs and drug-related SAEs, were summarised by System Organ Class (SOC) and Preferred Term (PT).

The safety database includes a total of 367 melanoma patients (22 patients treated with L19IL2/L19TNF only, 173 patients treated with neoadjuvant L19IL2/L19TNF and surgery, and 172 patients treated with surgery only).

Summary of adverse events

Table 46. Summary of adverse events (AEs) categories by study and arm

	PH-L19IL2-02/12 (N=22)		**PH-L19IL2TNF-02/15 (N=246)**				**PH-L19IL2TNF-01/18 (N=99)**			
	Total (N=22) n %		Arm 1 (N=122) n %		Arm 2 (N=124) n %		Arm 1 (N=51) n %		Arm 2 (N=48) n %	
AEs Categories										
All AEs	21	95.5%	120	98.4%	60	48.4%	46	90.2%	16	33.3%
L19IL2/ L19TNF related AEs	21	95.5%	117	95.9%	0		45	88.2%	0	
SAEs	1	**4.5%**	18	**14.8%**	12	9.7%	10	**19.6%**	2	4.2%
Grade ≥ 3 SAEs	1	4.5%	15	12.3%	9	7.3%	7	13.7%	2	4.2%
L19IL2/ L19TNF related SAEs	1	4.5%	11	**9.0%**	0		8	**15.7%**	0	
Grade ≥ 3 L19IL2/ L19TNF related SAEs	1	4.5%	9	7.4%	0		5	9.8%	0	
Grade ≥ 3 AEs	4	18.2%	41	33.6%	14	11.3%	17	33.3%	2	4.2%

Grade 3 AEs	3	13.6%	36	29.5%	14	11.3%	15	29.4%	2	4.2%
Grade 4 AEs	1	4.5%	4	3.3%	0		2	3.9%	0	
Grade 5 AEs (Deaths)	0		1*	0.8%*	0		0		0	
Drug withdrawal due to AEs	3	13.6%	20	**16.4%**	0		6	**11.8%**	0	
Dose temporarily interrupted due to AEs	4	18.2%	32	**26.2%**	0		9	**17.6%**	0	
Dose reduced due to AEs	0		19	**15.6%**	0		3	**5.9%**	0	
AESI	0		24	**19.7%**	0		20	**39.2%**	0	
IRAE	0		3	2.5%	0		3	5.9%	0	

AESI = Adverse event of special interest, IRAE = Immune-related adverse event. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. Arm 2 corresponds to surgery only as treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. In the table the incidence > 10% has been highlighted (Bold letters).

*This event was not considered to be drug- or surgery-related, but was related to a pulmonary metastasis in a patient with a pulmonary embolism

Source: 2.7.4 Summary of Clinical Safety, Table 4

Table 47. Summary of adverse events (AEs) categories by study for patients who received the maximum recommended dose of L19IL2/L19TNF (400 ug/13 Mio IU) for four weeks

	PH-L19IL2TNF-02/15		**PH-L19IL2TNF-01/18**		**OVERALL**	
	Arm 1 (N=38)		Arm 1 (N=11)		Arm 1 (N=49)	
AEs Categories						
All AEs	37	97.4%	9	81.8%	46	93.9%
Related AEs	36	94.7%	9	81.8%	45	91.8%
SAEs	2	5.3%	2	18.2%	4	8.2%
Related SAEs	1	2.6%	1	9.1%	2	4.1%
AEs with grade 3,4 and 5	8	21.1%	2	18.2%	10	20.4%

AEs with grade 3	6	15.8%	2	18.2%	8	16.3%
AEs with grade 4	1	2.6%	0		1	2.0%
AEs with grade 5 (Deaths)[1]	1	2.6%	0		1	2.0%
AEs Related with grade 3,4 and 5	5	13.2%	2	18.2%	7	14.3%
SAEs with grade 3,4 and 5	2	5.3%	2	18.2%	4	8.2%
SAEs Related with grade 3,4 and 5	1	2.6%	1	9.1%	2	4.1%
Adverse Events of Special Interest	4	10.5%	3	27.3%	7	14.3%
Immune-Related Adverse Events	0		1	9.1%	1	2.0%

Source: Applicant's response document, Applicant's response to question 453b)

Most common adverse events

Table 48. Incidence ≥2% of any adverse event in pooled controlled trials, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, and overall pooled studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18

	PH-L19IL2TNF-02/15 & PH-L19IL2TNF-01/18 (N=345)								**OVERALL (N=367)**							
	Arm 1 (N=173)				**Arm 2 (N=172)**				**Arm 1′ (N=195)**				**Arm 2′ (N=172)**			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
ANY	**166**	**96.0%**	**58**	**33.5%**	**76**	**44.2%**	16	9.3%	**187**	**95.9%**	**62**	**31.8%**	**76**	**44.2%**	16	9.3%
BLOOD AND LYMPHATIC SYSTEM DISORDERS	13	7.5%	0		4	2.3%	0		13	6.7%	0		4	2.3%	0	
ANAEMIA	5	2.9%	0		3	1.7%	0		5	2.6%	0		3	1.7%	0	
LEUKOCYTOSIS	5	2.9%	0		0		0		5	2.6%	0		0		0	
EOSINOPHILIA	4	2.3%	0		0		0		4	2.1%	0		0		0	
CARDIAC DISORDERS	15	8.7%	1	0.6%	4	2.3%	3	1.7%	15	7.7%	1	0.5%	4	2.3%	3	1.7%
TACHYCARDIA	9	5.2%	0		0		0		9	4.6%	0		0		0	
EAR AND LABYRINTH DISORDERS	5	2.9%	0		0		0		9	4.6%	0		0		0	
VERTIGO	4	2.3%	0		0		0		8	4.1%	0		0		0	
GASTROINTESTINAL DISORDERS	**53**	**30.6%**	1	0.6%	8	4.7%	2	1.2%	**60**	**30.8%**	1	0.5%	8	4.7%	2	1.2%
NAUSEA	**37**	**21.4%**	0		4	2.3%	0		**39**	**20.0%**	0		4	2.3%	0	

VOMITING	**20**	**11.6%**	0		2	1.2%	0		**22**	**11.3%**	0		2	1.2%	0	
DIARRHOEA	13	7.5%	0		2	1.2%	0		14	7.2%	0		2	1.2%	0	
ABDOMINAL PAIN UPPER	3	1.7%	0		0		0		4	2.1%	0		0		0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**158**	**91.3%**	**27**	**15.6%**	9	5.2%	1	0.6%	**178**	**91.3%**	**29**	**14.9%**	9	5.2%	1	0.6%
INJECTION SITE REACTION	**103**	**59.5%**	**21**	**12.1%**	0		0		**122**	**62.6%**	**23**	**11.8%**	0		0	
PYREXIA	**85**	**49.1%**	1	0.6%	4	2.3%	0		**99**	**50.8%**	1	0.5%	4	2.3%	0	
CHILLS	**80**	**46.2%**	0		1	0.6%	0		**84**	**43.1%**	0		1	0.6%	0	
FATIGUE	**30**	**17.3%**	1	0.6%	2	1.2%	0		**32**	**16.4%**	1	0.5%	2	1.2%	0	
INFLUENZA LIKE ILLNESS	**20**	**11.6%**	2	1.2%	1	0.6%	0		**22**	**11.3%**	2	1.0%	1	0.6%	0	
INJECTION SITE PAIN	**18**	**10.4%**	0		0		0		19	9.7%	0		0		0	
ASTHENIA	6	3.5%	0		1	0.6%	0		9	4.6%	0		1	0.6%	0	
OEDEMA PERIPHERAL	6	3.5%	0		0		0		8	4.1%	0		0		0	
PAIN	8	4.6%	1	0.6%	1	0.6%	0		8	4.1%	1	0.5%	1	0.6%	0	
AXILLARY PAIN	5	2.9%	0		1	0.6%	0		5	2.6%	0		1	0.6%	0	
OEDEMA	5	2.9%	0		0		0		5	2.6%	0		0		0	
LOCALISED OEDEMA	4	2.3%	0		0		0		4	2.1%	0		0		0	
SWELLING	4	2.3%	0		0		0		4	2.1%	0		0		0	
IMMUNE SYSTEM DISORDERS	7	4.0%	3	1.7%	1	0.6%	1	0.6%	8	4.1%	3	1.5%	1	0.6%	1	0.6%

DRUG HYPERSENSITIVITY	7	4.0%	3	1.7%	1	0.6%	1	0.6%	8	4.1%	3	1.5%	1	0.6%	1	0.6%
INFECTIONS AND INFESTATIONS	**26**	**15.0%**	6	3.5%	16	9.3%	5	2.9%	**27**	**13.8%**	6	3.1%	16	9.3%	5	2.9%
NASOPHARYNGITIS	5	2.9%	0		0		0		5	2.6%	0		0		0	
WOUND INFECTION	1	0.6%	0		4	2.3%	3	1.7%	1	0.5%	0		4	2.3%	3	1.7%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	**25**	**14.5%**	1	0.6%	**26**	**15.1%**	2	1.2%	**27**	**13.8%**	1	0.5%	**26**	**15.1%**	2	1.2%
SEROMA	7	4.0%	1	0.6%	13	7.6%	1	0.6%	8	4.1%	1	0.5%	13	7.6%	1	0.6%
PROCEDURAL PAIN	4	2.3%	0		2	1.2%	0		4	2.1%	0		2	1.2%	0	
RADIATION SKIN INJURY	1	0.6%	0		4	2.3%	0		1	0.5%	0		4	2.3%	0	
INVESTIGATIONS	**42**	**24.3%**	7	4.0%	15	8.7%	0		**42**	**21.5%**	7	3.6%	15	8.7%	0	
ALANINE AMINOTRANSFERASE INCREASED	**18**	**10.4%**	1	0.6%	3	1.7%	0		18	9.2%	1	0.5%	3	1.7%	0	
GAMMA-GLUTAMYLTRANSFERASE INCREASED	13	7.5%	2	1.2%	3	1.7%	0		13	6.7%	2	1.0%	3	1.7%	0	
ASPARTATE AMINOTRANSFERASE INCREASED	10	5.8%	0		2	1.2%	0		10	5.1%	0		2	1.2%	0	
BLOOD ALKALINE PHOSPHATASE INCREASED	5	2.9%	0		1	0.6%	0		5	2.6%	0		1	0.6%	0	
LIPASE INCREASED	5	2.9%	0		0		0		5	2.6%	0		0		0	

BLOOD CREATININE INCREASED	4	2.3%	1	0.6%	1	0.6%	0		4	2.1%	1	0.5%	1	0.6%	0	
METABOLISM AND NUTRITION DISORDERS	15	8.7%	3	1.7%	3	1.7%	1	0.6%	17	8.7%	4	2.1%	3	1.7%	1	0.6%
DECREASED APPETITE	9	5.2%	0		0		0		10	5.1%	0		0		0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	**23**	**13.2%**	1	0.6%	7	4.1%	0		**27**	**13.8%**	1	0.5%	7	4.1%	0	
PAIN IN EXTREMITY	6	3.5%	0		2	1.2%	0		7	3.6%	0		2	1.2%	0	
ARTHRALGIA	4	2.3%	0		4	2.3%	0		6	3.1%	0		4	2.3%	0	
BACK PAIN	6	3.5%	1	0.6%	0		0		6	3.1%	1	0.5%	0		0	
MYALGIA	4	2.3%	0		2	1.2%	0		4	2.1%	0		2	1.2%	0	
NERVOUS SYSTEM DISORDERS	**41**	**23.7%**	5	2.9%	8	4.7%	1	0.6%	**53**	**27.2%**	5	2.6%	8	4.7%	1	0.6%
HEADACHE	**22**	**12.7%**	0		1	0.6%	0		**33**	**16.9%**	0		1	0.6%	0	
DIZZINESS	5	2.9%	0		0		0		5	2.6%	0		0		0	
SYNCOPE	4	2.3%	3	1.7%	0		0		4	2.1%	3	1.5%	0		0	
PSYCHIATRIC DISORDERS	5	2.9%	1	0.6%	1	0.6%	0		6	3.1%	1	0.5%	1	0.6%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	13	7.5%	3	1.7%	2	1.2%	0		16	8.2%	4	2.1%	2	1.2%	0	
DYSPNOEA	5	2.9%	0		0		0		7	3.6%	0		0		0	
COUGH	4	2.3%	0		2	1.2%	0		5	2.6%	0		2	1.2%	0	

SKIN AND SUBCUTANEOUS TISSUE DISORDERS	**44**	**25.4%**	1	0.6%	13	7.6%	0		**49**	**25.1%**	1	0.5%	13	7.6%	0	
ERYTHEMA	11	6.4%	0		1	0.6%	0		13	6.7%	0		1	0.6%	0	
NIGHT SWEATS	7	4.0%	0		0		0		7	3.6%	0		0		0	
PRURITUS	7	4.0%	0		2	1.2%	0		7	3.6%	0		2	1.2%	0	
RASH	3	1.7%	0		1	0.6%	0		6	3.1%	0		1	0.6%	0	
HYPERHIDROSIS	3	1.7%	0		0		0		4	2.1%	0		0		0	
VASCULAR DISORDERS	**30**	**17.3%**	8	4.6%	10	5.8%	5	2.9%	**31**	**15.9%**	8	4.1%	10	5.8%	5	2.9%
HYPOTENSION	12	6.9%	2	1.2%	0		0		12	6.2%	2	1.0%	0		0	
HYPERTENSION	9	5.2%	5	2.9%	5	2.9%	4	2.3%	10	5.1%	5	2.6%	5	2.9%	4	2.3%
LYMPHOEDEMA	5	2.9%	0		4	2.3%	0		5	2.6%	0		4	2.3%	0	
FLUSHING	4	2.3%	0		0		0		4	2.1%	0		0		0	

This table lists the percentage of patients that reported AEs in the different treatment arms. For the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment, while Arm 2 corresponds to surgery only as treatment. In the OVERALL column the adverse events of all three studies are added. Arm 1' corresponds to the total patients in the PH-L19IL2TNF-02/12 study and the patients treated with L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Arm 2' corresponds to the patients in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, who only received surgery as a treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arms across all three studies (OVERALL, Arm 1', Any Grade). In the table the incidence > 10% has been highlighted (bold letters).

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 24

Table 49: Mean incidence of the most common adverse events by grade and by treatment arm (as applicable) in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18.

	OVERALL (N=367)						
	Arm 1 – combined L19IL2/L19TNF therapy (N=195)				Arm 2 - Surgery alone (N=172)		
CTCAE Grade	Any Grade n %		Grade ≥3 n %		Any Grade n %		Grade ≥3 n %
PT							
INJECTION SITE REACTION	122	62.6%	23	11.8%	0		0
PYREXIA	99	50.8%	1	0.5%	4	2.3%	0
CHILLS	84	43.1%	0		1	0.6%	0
NAUSEA	39	20.0%	0		4	2.3%	0
HEADACHE	33	16.9%	0		1	0.6%	0
FATIGUE	32	16.4%	1	0.5%	2	1.2%	0
VOMITING	22	11.3%	0		2	1.2%	0
INFLUENZA LIKE ILLNESS	22	11.3%	2	1.0%	1	0.6%	0

Arm 1 corresponds to the total patients in the PH-L19IL2TNF-02/12 study and the patients treated with L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Arm 2 corresponds to the patients in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, who only received surgery as a treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024.

Source: 2.7.4 Summary of Clinical Safety, Table 5

Table 50: Incidence (≥5%) of any adverse events in patients who received the maximum recommended dose of L19IL2/L19TNF (13 MioIU/400 µg) over four weeks in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18

	PH-L19IL2TNF-02/15				PH-L19IL2TNF-01/18				OVERALL			
	Arm 1 (max. dose) (N=38)				Arm 1 (max. dose) (N=11)				Overall (max. dose) (N=49)			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
ANY	**37**	**97.4%**	**8**	**21.1%**	**9**	**81.8%**	**2**	**18.2%**	**46**	**93.9%**	**10**	**20.4 %**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	**4**	**10.5%**	0		1	9.1%	0		**5**	**10.2%**	0	
ANAEMIA	1	2.6%	0		1	9.1%	0		2	4.1%	0	
EOSINOPHILIA	2	5.3%	0		0		0		2	4.1%	0	
CARDIAC DISORDERS	2	5.3%	0		**2**	**18.2%**	1	9.1%	4	8.2%	1	2.0%
ANGINA PECTORIS	0		0		1	9.1%	0		1	2.0%	0	
CARDIAC FAILURE	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
PALPITATIONS	0		0		1	9.1%	0		1	2.0%	0	
GASTROINTESTINAL DISORDERS	**7**	**18.4%**	0		**2**	**18.2%**	1	9.1%	**9**	**18.4%**	1	2.0%
NAUSEA	**4**	**10.5%**	0		**2**	**18.2%**	0		**6**	**12.2%**	0	
VOMITING	**4**	**10.5%**	0		1	9.1%	0		**5**	**10.2%**	0	

DIARRHOEA	1	2.6%	0		1	9.1%	0		2	4.1%	0	
COLITIS	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**35**	**92.1%**	2	5.3%	**9**	**81.8%**	1	9.1%	**44**	**89.8%**	3	6.1%
INJECTION SITE REACTION	**20**	**52.6%**	2	5.3%	**4**	**36.4%**	1	9.1%	**24**	**49.0%**	3	6.1%
CHILLS	**19**	**50.0%**	0		**4**	**36.4%**	0		**23**	**46.9%**	0	
PYREXIA	**18**	**47.4%**	0		**5**	**45.5%**	0		**23**	**46.9%**	0	
FATIGUE	2	5.3%	0		**3**	**27.3%**	0		**5**	**10.2%**	0	
INFLUENZA LIKE ILLNESS	**4**	**10.5%**	0		1	9.1%	0		**5**	**10.2%**	0	
INJECTION SITE PAIN	**4**	**10.5%**	0		1	9.1%	0		**5**	**10.2%**	0	
AXILLARY PAIN	1	2.6%	0		**2**	**18.2%**	0		3	6.1%	0	
PAIN	3	7.9%	0		0		0		3	6.1%	0	
ASTHENIA	2	5.3%	0		0		0		2	4.1%	0	
LOCALISED OEDEMA	1	2.6%	0		1	9.1%	0		2	4.1%	0	
OEDEMA PERIPHERAL	1	2.6%	0		1	9.1%	0		2	4.1%	0	
SWELLING	1	2.6%	0		1	9.1%	0		2	4.1%	0	
FACE OEDEMA	0		0		1	9.1%	0		1	2.0%	0	
INJECTION SITE ERYTHEMA	0		0		1	9.1%	0		1	2.0%	0	
NON-CARDIAC CHEST PAIN	0		0		1	9.1%	0		1	2.0%	0	

TEMPERATURE REGULATION DISORDER	0		0		1	9.1%	0		1	2.0%	0	
IMMUNE SYSTEM DISORDERS	**4**	**10.5%**	1	2.6%	0		0		4	8.2%	1	2.0%
DRUG HYPERSENSITIVITY	**4**	**10.5%**	1	2.6%	0		0		4	8.2%	1	2.0%
INFECTIONS AND INFESTATIONS	**5**	**13.2%**	1	2.6%	1	9.1%	0		**6**	**12.2%**	1	2.0%
MASTITIS	0		0		1	9.1%	0		1	2.0%	0	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	**9**	**23.7%**	0		1	9.1%	1	9.1%	**10**	**20.4%**	1	2.0%
SEROMA	3	7.9%	0		1	9.1%	1	9.1%	4	8.2%	1	2.0%
PROCEDURAL PAIN	2	5.3%	0		0		0		2	4.1%	0	
INVESTIGATIONS	**9**	**23.7%**	2	5.3%	**3**	**27.3%**	0		**12**	**24.5%**	2	4.1%
ALANINE AMINOTRANSFERASE INCREASED	3	7.9%	1	2.6%	**2**	**18.2%**	0		**5**	**10.2%**	1	2.0%
ASPARTATE AMINOTRANSFERASE INCREASED	3	7.9%	0		1	9.1%	0		4	8.2%	0	
GAMMA-GLUTAMYLTRANSFERASE INCREASED	2	5.3%	0		1	9.1%	0		3	6.1%	0	
BLOOD CREATININE INCREASED	1	2.6%	0		1	9.1%	0		2	4.1%	0	
ACTIVATED PARTIAL THROMBOPLASTIN TIME PROLONGED	0		0		1	9.1%	0		1	2.0%	0	
BLOOD GLUCOSE INCREASED	0		0		1	9.1%	0		1	2.0%	0	
INTERNATIONAL NORMALISED RATIO INCREASED	0		0		1	9.1%	0		1	2.0%	0	
METABOLISM AND NUTRITION DISORDERS	**4**	**10.5%**	0		1	9.1%	0		**5**	**10.2%**	0	
DECREASED APPETITE	3	7.9%	0		0		0		3	6.1%	0	

HYPERURICAEMIA	0		0		1	9.1%	0		1	2.0%	0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	**6**	**15.8%**	0		1	9.1%	0		**7**	**14.3%**	0	
BACK PAIN	2	5.3%	0		0		0		2	4.1%	0	
MYALGIA	1	2.6%	0		1	9.1%	0		2	4.1%	0	
NERVOUS SYSTEM DISORDERS	**10**	**26.3%**	1	2.6%	**2**	**18.2%**	0		**12**	**24.5%**	1	2.0%
HEADACHE	**5**	**13.2%**	0		1	9.1%	0		**6**	**12.2%**	0	
DIZZINESS	1	2.6%	0		1	9.1%	0		2	4.1%	0	
SYNCOPE	2	5.3%	1	2.6%	0		0		2	4.1%	1	2.0%
PSYCHIATRIC DISORDERS	0		0		1	9.1%	0		1	2.0%	0	
ANXIETY	0		0		1	9.1%	0		1	2.0%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1	2.6%	0		**2**	**18.2%**	**2**	**18.2%**	3	6.1%	2	4.1%
DYSPNOEA	0		0		1	9.1%	0		1	2.0%	0	
HYPOXIA	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
PULMONARY EMBOLISM	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	**10**	**26.3%**	0		**2**	**18.2%**	0		**12**	**24.5%**	0	
ERYTHEMA	**4**	**10.5%**	0		0		0		4	8.2%	0	
NIGHT SWEATS	3	7.9%	0		0		0		3	6.1%	0	
ACTINIC KERATOSIS	2	5.3%	0		0		0		2	4.1%	0	

HYPERHIDROSIS	1	2.6%	0		1	9.1%	0		2	4.1%	0	
RASH MACULO-PAPULAR	0		0		1	9.1%	0		1	2.0%	0	
VASCULAR DISORDERS	2	5.3%	1	2.6%	0		0		2	4.1%	1	2.0%

This table lists the percentage of patients that reported AEs in the population of patients that received the maximum recommended dose of L19IL2/L19TNF over four weeks in studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events of all patients who were treated with the maximum dose of L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are added. The two studies are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence across all three studies (OVERALL, Any Grade)

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 37

Most common related adverse events

Table 51. Incidence ≥1% of any adverse event related to L19IL2/L19TNF treatment in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18

	PH-L19IL2TNF-02/12				PH-L19IL2TNF-02/15				PH-L19IL2TNF-01/18				OVERALL			
	Total (N=22)				Arm 1 (N=122)				Arm 1 (N=51)				Arm 1' (N=195)			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
ANY	**21**	**95.5%**	**2**	**9.1%**	**117**	**95.9%**	**33**	**27.0%**	**45**	**88.2%**	**15**	**29.4%**	**183**	**93.6%**	**50**	**25.6%**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	0		0		9	7.4%	0		3	5.9%	0		12	6.2%	0	
LEUKOCYTOSIS	0		0		4	3.3%	0		1	2.0%	0		5	2.6%	0	
ANAEMIA	0		0		2	1.6%	0		2	3.9%	0		4	2.1%	0	
EOSINOPHILIA	0		0		3	2.5%	0		1	2.0%	0		4	2.1%	0	
CARDIAC DISORDERS	0		0		11	9.0%	0		2	3.9%	0		13	6.7%	0	
TACHYCARDIA	0		0		8	6.6%	0		1	2.0%	0		9	4.6%	0	
PALPITATIONS	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
EAR AND LABYRINTH DISORDERS	**4**	**18.2%**	0		5	4.1%	0		0		0		9	4.6%	0	
VERTIGO	**4**	**18.2%**	0		4	3.3%	0		0		0		8	4.1%	0	
ENDOCRINE DISORDERS	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	

HYPOTHYROIDISM	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
GASTROINTESTINAL DISORDERS	**6**	**27.3%**	0		**31**	**25.4%**	0		**11**	**21.6%**	1	2.0%	**48**	**24.6%**	1	0.5%
NAUSEA	2	9.1%	0		**25**	**20.5%**	0		**10**	**19.6%**	0		**37**	**19.0%**	0	
VOMITING	1	4.5%	0		**14**	**11.5%**	0		4	7.8%	0		19	9.7%	0	
DIARRHOEA	1	4.5%	0		8	6.6%	0		2	3.9%	0		11	5.6%	0	
ABDOMINAL PAIN UPPER	1	4.5%	0		2	1.6%	0		0		0		3	1.5%	0	
ANAL INCONTINENCE	1	4.5%	0		0		0		0		0		1	0.5%	0	
COLITIS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
ODYNOPHAGIA	0		0		0		0		1	2.0%	0		1	0.5%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**20**	**90.9%**	2	9.1%	**114**	**93.4%**	**17**	**13.9%**	**43**	**84.3%**	**9**	**17.6%**	**177**	**90.8%**	**28**	**14.4%**
INJECTION SITE REACTION	**19**	**86.4%**	2	9.1%	**82**	**67.2%**	**15**	**12.3%**	**21**	**41.2%**	**6**	**11.8%**	**122**	**62.6%**	**23**	**11.8%**
PYREXIA	**14**	**63.6%**	0		**61**	**50.0%**	1	0.8%	**24**	**47.1%**	0		**99**	**50.8%**	1	0.5%
CHILLS	**4**	**18.2%**	0		**59**	**48.4%**	0		**21**	**41.2%**	0		**84**	**43.1%**	0	
FATIGUE	2	9.1%	0		**18**	**14.8%**	1	0.8%	**11**	**21.6%**	0		**31**	**15.9%**	1	0.5%
INFLUENZA LIKE ILLNESS	2	9.1%	0		**14**	**11.5%**	0		3	5.9%	1	2.0%	19	9.7%	1	0.5%
INJECTION SITE PAIN	1	4.5%	0		7	5.7%	0		**10**	**19.6%**	0		18	9.2%	0	
ASTHENIA	2	9.1%	0		4	3.3%	0		2	3.9%	0		8	4.1%	0	
PAIN	0		0		4	3.3%	0		3	5.9%	1	2.0%	7	3.6%	1	0.5%

OEDEMA PERIPHERAL	1	4.5%	0		3	2.5%	0		2	3.9%	0		6	3.1%	0	
OEDEMA	0		0		2	1.6%	0		3	5.9%	0		5	2.6%	0	
AXILLARY PAIN	0		0		2	1.6%	0		2	3.9%	0		4	2.1%	0	
LOCALISED OEDEMA	0		0		1	0.8%	0		3	5.9%	0		4	2.1%	0	
SWELLING	0		0		3	2.5%	0		1	2.0%	0		4	2.1%	0	
INJECTION SITE ERYTHEMA	1	4.5%	0		1	0.8%	0		1	2.0%	0		3	1.5%	0	
INJECTION SITE INFLAMMATION	0		0		1	0.8%	0		2	3.9%	0		3	1.5%	0	
CHEST PAIN	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
FEELING COLD	0		0		2	1.6%	0		0		0		2	1.0%	0	
INDURATION	0		0		0		0		2	3.9%	0		2	1.0%	0	
INFLAMMATION	0		0		2	1.6%	0		0		0		2	1.0%	0	
TEMPERATURE REGULATION DISORDER	0		0		0		0		2	3.9%	0		2	1.0%	0	
CHEST DISCOMFORT	0		0		0		0		1	2.0%	0		1	0.5%	0	
FACE OEDEMA	0		0		0		0		1	2.0%	0		1	0.5%	0	
INJECTION SITE RASH	0		0		0		0		1	2.0%	0		1	0.5%	0	
INJECTION SITE SWELLING	0		0		0		0		1	2.0%	0		1	0.5%	0	
INJECTION SITE ULCER	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
MALAISE	0		0		0		0		1	2.0%	0		1	0.5%	0	

NON-CARDIAC CHEST PAIN	0		0		0		0		1	2.0%	0		1	0.5%	0	
PUNCTURE SITE PAIN	0		0		0		0		1	2.0%	0		1	0.5%	0	
ULCER	0		0		0		0		1	2.0%	0		1	0.5%	0	
IMMUNE SYSTEM DISORDERS	1	4.5%	0		7	5.7%	3	2.5%	0		0		8	4.1%	3	1.5%
DRUG HYPERSENSITIVITY	1	4.5%	0		7	5.7%	3	2.5%	0		0		8	4.1%	3	1.5%
INFECTIONS AND INFESTATIONS	1	4.5%	0		7	5.7%	0		3	5.9%	2	3.9%	11	5.6%	2	1.0%
CELLULITIS	0		0		0		0		2	3.9%	1	2.0%	2	1.0%	1	0.5%
INJECTION SITE INFECTION	0		0		2	1.6%	0		0		0		2	1.0%	0	
HERPES ZOSTER	1	4.5%	0		0		0		0		0		1	0.5%	0	
LOCALISED INFECTION	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1	4.5%	0		6	4.9%	0		2	3.9%	0		9	4.6%	0	
SEROMA	1	4.5%	0		1	0.8%	0		0		0		2	1.0%	0	
WOUND DEHISCENCE	0		0		2	1.6%	0		0		0		2	1.0%	0	
WOUND SECRETION	0		0		2	1.6%	0		0		0		2	1.0%	0	
CONTUSION	0		0		0		0		1	2.0%	0		1	0.5%	0	
SKIN ABRASION	0		0		0		0		1	2.0%	0		1	0.5%	0	
INVESTIGATIONS	0		0		**29**	**23.8%**	5	4.1%	4	7.8%	1	2.0%	**33**	**16.9%**	6	3.1%
ALANINE AMINOTRANSFERASE	0		0		12	9.8%	1	0.8%	3	5.9%	0		15	7.7%	1	0.5%

INCREASED																
GAMMA-GLUTAMYLTRANSFERASE INCREASED	0		0		9	7.4%	2	1.6%	1	2.0%	0		10	5.1%	2	1.0%
ASPARTATE AMINOTRANSFERASE INCREASED	0		0		7	5.7%	0		2	3.9%	0		9	4.6%	0	
BLOOD ALKALINE PHOSPHATASE INCREASED	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
BODY TEMPERATURE INCREASED	0		0		3	2.5%	0		0		0		3	1.5%	0	
LIPASE INCREASED	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
AMYLASE INCREASED	0		0		1	0.8%	0		1	2.0%	1	2.0%	2	1.0%	1	0.5%
BLOOD CREATININE INCREASED	0		0		2	1.6%	0		0		0		2	1.0%	0	
C-REACTIVE PROTEIN INCREASED	0		0		2	1.6%	0		0		0		2	1.0%	0	
TRANSAMINASES INCREASED	0		0		2	1.6%	1	0.8%	0		0		2	1.0%	1	0.5%
METABOLISM AND NUTRITION DISORDERS	1	4.5%	0		11	9.0%	2	1.6%	0		0		12	6.1%	2	1.0%
DECREASED APPETITE	1	4.5%	0		8	6.6%	0		0		0		9	4.6%	0	
HYPOPHOSPHATAEMIA	0		0		2	1.6%	1	0.8%	0		0		2	1.0%	1	0.5%
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	**3**	**13.6%**	0		**13**	**10.7%**	1	0.8%	4	7.8%	0		**20**	**10.2%**	1	0.5%

ARTHRALGIA	2	9.1%	0		2	1.6%	0		2	3.9%	0		6	3.1%	0	
BACK PAIN	0		0		4	3.3%	1	0.8%	1	2.0%	0		5	2.6%	1	0.5%
PAIN IN EXTREMITY	1	4.5%	0		4	3.3%	0		0		0		5	2.6%	0	
MYALGIA	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
NERVOUS SYSTEM DISORDERS	**9**	**40.9%**	0		**27**	**22.1%**	3	2.5%	**11**	**21.6%**	0		**47**	**24.1%**	3	1.5%
HEADACHE	**8**	**36.4%**	0		**16**	**13.1%**	0		**6**	**11.8%**	0		**30**	**15.4%**	0	
DIZZINESS	0		0		2	1.6%	0		3	5.9%	0		5	2.6%	0	
MIGRAINE	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
SYNCOPE	0		0		2	1.6%	2	1.6%	0		0		2	1.0%	2	1.0%
TREMOR	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
MUSCLE CONTRACTIONS INVOLUNTARY	1	4.5%	0		0		0		0		0		1	0.5%	0	
MYOCLONUS	1	4.5%	0		0		0		0		0		1	0.5%	0	
PRESYNCOPE	1	4.5%	0		0		0		0		0		1	0.5%	0	
PSYCHIATRIC DISORDERS	0		0		2	1.6%	0		2	3.9%	1	2.0%	4	2.1%	1	0.5%
ANXIETY	0		0		0		0		2	3.9%	0		2	1.0%	0	
DEPRESSION	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1	4.5%	0		5	4.1%	0		1	2.0%	1	2.0%	11	5.6%	1	0.5%
DYSPNOEA	1	4.5%	0		2	1.6%	0		1	2.0%	0		4	2.1%	0	

COUGH	0		0		3	2.5%	0		0		0		3	1.5%	0	
DYSPHONIA	0		0		0		0		1	2.0%	0		1	0.5%	0	
EPISTAXIS	0		0		0		0		1	2.0%	0		1	0.5%	0	
HYPOXIA	0		0		0		0		1	2.0%	0		1	0.5%	0	
OROPHARYNGEAL PAIN	0		0		0		0		1	2.0%	0		1	0.5%	0	
PULMONARY EMBOLISM	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	**4**	**18.2%**	0		**28**	**23.0%**	1	0.8%	**12**	**23.5%**	0		**44**	**22.6%**	1	0.5%
ERYTHEMA	1	4.5%	0		7	5.7%	0		4	7.8%	0		12	6.2%	0	
NIGHT SWEATS	0		0		6	4.9%	0		1	2.0%	0		7	3.6%	0	
PRURITUS	0		0		3	2.5%	0		3	5.9%	0		6	3.1%	0	
RASH	2	9.1%	0		3	2.5%	0		0		0		5	2.6%	0	
HYPERHIDROSIS	1	4.5%	0		2	1.6%	0		1	2.0%	0		4	2.1%	0	
BLISTER	0		0		0		0		2	3.9%	0		2	1.0%	0	
RASH MACULO-PAPULAR	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
ERYTHEMA MULTIFORME	0		0		0		0		1	2.0%	0		1	0.5%	0	
SKIN NECROSIS	0		0		0		0		1	2.0%	0		1	0.5%	0	
SKIN ULCER	0		0		0		0		1	2.0%	0		1	0.5%	0	
VASCULAR DISORDERS	1	4.5%	0		**14**	**11.5%**	3	2.5%	**6**	**11.8%**	2	3.9%	**21**	**10.8%**	5	2.6%

HYPOTENSION	0		0		6	4.9%	1	0.8%	5	9.8%	1	2.0%	11	5.6%	2	1.0%
HYPERTENSION	1	4.5%	0		4	3.3%	2	1.6%	1	2.0%	1	2.0%	6	3.1%	3	1.5%
FLUSHING	0		0		3	2.5%	0		1	2.0%	0		4	2.1%	0	
SUPERFICIAL VEIN THROMBOSIS	0		0		0		0		1	2.0%	0		1	0.5%	0	

This table lists the percentage of patients that reported drug-related AEs in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events in patients treated with L19IL2/L19TNF in all three studies are added. Arm 1' corresponds to the total patients in the PH-L19IL2TNF-02/12 study and the patients treated with L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arms across all three studies (OVERALL, Arm 1', Any Grade). In the table the incidence > 10% has been highlighted (bold letters).

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 25

3.3.7.3. Serious adverse events, deaths, and other significant events

Serious adverse events

Table 52. Incidence ≥1% of any serious adverse event by SOC and PT in individual studies separated by different treatment arms, if applicable

	PH-L19IL2TNF-02/12 (N=22)				PH-L19IL2TNF-02/15 (N=246)						PH-L19IL2TNF-01/18 (N=99)						OVERALL (N=367)					
	Total (N=22)				Arm 1 (N=122)				Arm 2 (N=124)		Arm 1 (N=51)				Arm 2 (N=48)		Arm 1' (N=195)				Arm 2' (N=172)	
CTCAE Grade	**Any**		**≥3**		**Any**		**≥3**		**Any**	**≥3**	**Any**		**≥3**		**Any**	**≥3**	**Any**		**≥3**		**Any**	**≥3**
ANY	1	4.5%	1	4.5%	**18**	**14.8%**	**15**	**12.3%**	12	9.7%	9	7.3%	**10**	**19.6%**	7	**13.7%**	2	4.2%	2	4.2%	**29**	**14.9%**	**23**	**11.8%**	14	8.1%	11	6.4%
CARDIAC DISORDERS	0		0		1	0.8%	0		3	2.4%	3	2.4%	1	2.0%	1	2.0%	0		0		2	1.0%	1	0.5%	3	1.7%	3	1.7%
ANGINA PECTORIS	0		0		0		0		0		0		1	2.0%	0		0		0		1	0.5%	0		0		0	
CARDIAC FAILURE	0		0		0		0		0		0		1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
GASTROINTESTINAL DISORDERS	0		0		1	0.8%	0		1	0.8%	1	0.8%	0		0		1	2.1%	1	2.1%	1	0.5%	0		2	1.2%	2	1.2%
NAUSEA	0		0		0		0		0		0		0		0		1	2.1%	1	2.1%	0		0		1	0.6%	1	0.6%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	1	4.5%	1	4.5%	6	4.9%	3	2.5%	1	0.8%	0		**6**	**11.8%**	3	5.9%	0		0		13	6.7%	7	3.6%	1	0.6%	1	0.6%

INJECTION SITE REACTION	1	4.5%	1	4.5%	3	2.5%	3	2.5%	0		0		5	9.8%	2	3.9%	0		0		9	4.6%	6	3.1%	0		0	
PYREXIA	0		0		3	2.5%	0		0		0		0		0		0		0		3	1.5%	0		0		0	
INFLUENZA LIKE ILLNESS	0		0		0		0		0		0		1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
IMMUNE SYSTEM DISORDERS	0		0		3	2.5%	3	2.5%	0		0		0		0		1	2.1%	1	2.1%	3	1.5%	3	1.5%	1	0.6%	1	0.6%
DRUG HYPERSENSITIVITY	0		0		3	2.5%	3	2.5%	0		0		0		0		1	2.1%	1	2.1%	3	1.5%	3	1.5%	1	0.6%	1	0.6%
INFECTIONS AND INFESTATIONS	0		0		4	3.3%	4	3.3%	2	1.6%	1	0.8%	2	3.9%	2	3.9%	1	2.1%	1	2.1%	6	3.1%	6	3.1%	3	1.7%	2	1.2%
POSTOPERATIVE WOUND INFECTION	0		0		2	1.6%	2	1.6%	0		0		0		0		0		0		2	1.0%	2	1.0%	0		0	
CELLULITIS	0		0		0		0		0		0		1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
LOCALISED INFECTION	0		0		0		0		0		0		1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
WOUND INFECTION	0		0		0		0		2	1.6%	1	0.8%	0		0		1	2.1%	1	2.1%	0		0		3	1.7%	2	1.2%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	0		0		0		0		4	3.2%	2	1.6%	2	3.9%	1	2.0%	0		0		2	1.0%	1	0.5%	4	2.3%	2	1.2%
INCISION SITE HAEMATOMA	0		0		0		0		0		0		1	2.0%	0		0		0		1	0.5%	0		0		0	
SEROMA	0		0		0		0		1	0.8%	1	0.8%	1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	1	0.6%	1	0.6%
INVESTIGATIONS	0		0		1	0.8%	1	0.8%	0		0		1	2.0%	1	2.0%	0		0		2	1.0%	2	1.0%	0		0	

AMYLASE INCREASED	0		0		0		0		0		0	1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
NERVOUS SYSTEM DISORDERS	0		0		1	0.8%	1	0.8%	1	0.8%	1	0.8%	1	2.0%	1	2.0%	0		0		2	1.0%	2	1.0%	1	0.6%	1	0.6%
APHASIA	0		0		0		0		0		0	1	2.0%	1	2.0%	0		0		1	0.5%	1	0.5%	0		0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	0		0		0		0		0		0	3	5.9%	3	5.9%	0		0		3	1.5%	3	1.5%	0		0	
PULMONARY EMBOLISM	0		0		0		0		0		0	2	3.9%	2	3.9%	0		0		2	1.0%	1	0.5%	0		0	
DYSPNOEA	0		0		0		0		0		0	1	2.0%	0		0		0		1	0.5%	1	0.5%	0		0	
HYPOXIA	0		0		0		0		0		0	1	2.0%	1	2.0%	0		0		1	0.5%	2	1.0%	0		0	
VASCULAR DISORDERS	0		0		3	2.5%	1	0.8%	1	0.8%	1	0.8%	0		0		0		0		3	1.5%	1	0.5%	1	0.6%	1	0.6%
HYPOTENSION	0		0		3	2.5%	1	0.8%	0		0		0		0		0		0		3	1.5%	3	1.5%	0		0	

This table lists the percentage of patients that reported SAEs in the different treatment arms of studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. For the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment, while Arm 2 corresponds to surgery only as treatment. In the OVERALL column the serious adverse events of all three studies are added. Arm 1' corresponds to the total patients in the PH-L19IL2TNF-02/12 study and the patients treated with L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Arm 2' corresponds to the patients in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, who only received surgery as a treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arms across all three studies (OVERALL, Arm 1', Any Grade). In the table the incidence > 10% has been highlighted (bold letters).

This data was compiled based on the listings and summaries on SAEs reported in patients in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. The source data can be found in the corresponding CSRs.

Source: 2.7.4.7 Appendix Summary of Clinical Safety, Table 26

Table 53: Incidence of serious adverse events in patients who received the maximum recommended dose of L19IL2/L19TNF (13 MioIU/400 µg) over four weeks in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18

	PH-L19IL2TNF-02/15				PH-L19IL2TNF-01/18				OVERALL			
	Arm 1 (max. dose) (N=38)				Arm 1 (max. dose) (N=11)				Overall (max. dose) (N=49)			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
ANY	2	5.3%	2	5.3%	**2**	**18.2%**	**2**	**18.2 %**	4	8.2%	4	8.2%
CARDIAC DISORDERS	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
ANGINA PECTORIS	0		0		1	9.1%	0		1	2.0%	0	
CARDIAC FAILURE	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
INJECTION SITE REACTION	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
IMMUNE SYSTEM DISORDERS	1	2.6%	1	2.6%	0		0		1	2.0%	1	2.0%
DRUG HYPERSENSITIVITY	1	2.6%	1	2.6%	0		0		1	2.0%	1	2.0%
INFECTIONS AND INFESTATIONS	1	2.6%	1	2.6%	0		0		1	2.0%	1	2.0%
PNEUMONIA	1	2.6%	1	2.6%	0		0		1	2.0%	1	2.0%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
SEROMA	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	0		0		**2**	**18.2%**	**2**	**18.2 %**	2	4.1%	2	4.1%
DYSPNOEA	0		0		1	9.1%	0		1	2.0%	0	
HYPOXIA	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%
PULMONARY EMBOLISM	0		0		1	9.1%	1	9.1%	1	2.0%	1	2.0%

This table lists the percentage of patients that reported SAEs in the population of patients that received the maximum recommended dose of L19IL2/L19TNF over four weeks in studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. . In the OVERALL column the adverse events of all patients who were treated with the maximum dose of L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are added. The two studies are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence across all three studies (OVERALL, Any Grade)

Source: 2.7.4.7 Appendix Summary of Clinical Safety, Table 38

Drug-related serious adverse events (SAEs)

Table 54. Incidence of serious adverse events related to L19IL2/l19TNF treatment in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18

	PH-L19IL2TNF-02/12				**PH-L19IL2TNF-02/15**				**PH-L19IL2TNF-01/18**				**OVERALL**			
	Total (N=22)				**Arm 1 (N=122)**				**Arm 1 (N=51)**				**Arm 1' (N=195)**			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
ANY	1	4.5%	1	4.5%	**11**	**9.0%**	**9**	**7.4%**	**8**	**15.7%**	**5**	**9.8%**	**20**	**10.3%**	**15**	**7.7%**
EYE DISORDERS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
DIPLOPIA	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%

GASTROINTESTINAL DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
NAUSEA	0		0		1	0.8%	0		0		0		1	0.5%	0	
VOMITING	0		0		1	0.8%	0		0		0		1	0.5%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	1	4.5%	1	4.5%	6	4.9%	3	2.5%	**6**	**11.8%**	**3**	**5.9%**	**13**	**6.7%**	7	3.6%
INJECTION SITE REACTION	1	4.5%	1	4.5%	3	2.5%	3	2.5%	**5**	**9.8%**	2	3.9%	9	4.6%	6	3.1%
PYREXIA	0		0		3	2.5%	0		0		0		3	1.5%	0	
CHILLS	0		0		1	0.8%	0		0		0		1	0.5%	0	
INFLUENZA LIKE ILLNESS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
IMMUNE SYSTEM DISORDERS	0		0		3	2.5%	3	2.5%	0		0		3	1.5%	3	1.5%
DRUG HYPERSENSITIVITY	0		0		3	2.5%	3	2.5%	0		0		3	1.5%	3	1.5%
INFECTIONS AND INFESTATIONS	0		0		0		0		2	3.9%	2	3.9%	2	1.0%	2	1.0%
CELLULITIS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
LOCALISED INFECTION	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
INVESTIGATIONS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
AMYLASE INCREASED	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
NERVOUS SYSTEM DISORDERS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
SYNCOPE	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
RESPIRATORY, THORACIC AND	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%

MEDIASTINAL DISORDERS																
PULMONARY EMBOLISM	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
VASCULAR DISORDERS	0		0		2	1.6%	1	0.8%	0		0		2	1.0%	1	0.5%
HYPOTENSION	0		0		2	1.6%	1	0.8%	0		0		2	1.0%	1	0.5%

This table lists the percentage of patients that reported drug-related SAEs in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events in patients treated with L19IL2/L19TNF in all three studies are added. Arm 1' corresponds to the total patients in the PH-L19IL2TNF-02/12 study and the patients treated with L19IL2/L19TNF in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arms across all three studies (OVERALL, Arm 1', Any Grade). In the table the incidence > 5% has been highlighted (bold letters).

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 27

Deaths

In the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18 no deaths have been collected during the period of drug exposure or within a period up to 30 days following the discontinuation of the drug. Further, no deaths have been recorded that occurred later than 30 days but resulted from adverse events that had an onset during exposure or during the 30 days follow up period.

Drug-related deaths

Not applicable.

Adverse events of special interest

The AEs of Special Interest (AESI) have been determined for L19IL2/L19TNF based on pre-clinical and/or clinical safety data (safety profile of IL2 (Proleukin), TNF (Beromun) and the analysis of drug-related AEs collected from the previous studies conducted with L19IL2 and L19TNF, both for systemic and intratumoural delivery applications), labelling and/or regulatory authority interest for immunotherapy for PH-L19IL2TNF-02/15 (Phase III) and PH-L19IL2TNF-01/18 (Phase III) studies, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate (ICH E2F: Development Safety Update Report).

Table 55. **AEs of special interest per SOC**

System Organ Class	**Preferred Term**	**Severity**
Cardiac Disorders	Tachycardia	≥3
Endocrine disorders	Adrenocortical insufficiency	Any grade
	Autoimmune thyroiditis	Any grade
General disorders and administration site conditions	Injection site reaction	≥3
	Oedema peripheral	≥3
Immune system disorders	Drug hypersensitivity	≥3
	Cytokine Release Syndrome	≥3
Infections and Infestations	Infection	Any grade
Investigations	Blood creatinine increased	Any grade
Metabolism and nutrition disorders	Hypocalcaemia	≥3
	Hypercalcaemia	≥3
	Hypokalaemia	≥3

System Organ Class	Preferred Term	Severity
	Hyperkalaemia	≥3
Respiratory and mediastinal disorders	Dyspnoea	Any grade
Vascular disorders	Hypotension	≥3
	Thrombosis	≥3
	Embolism	≥3

The study protocol PH-L19IL2TNF-02/12 did not foresee any AESI collection. Therefore, no adverse events of special interest were collected in study PH-L19IL2TNF-02/12.

Adverse Events Special Interest (AESI) was recorded and summarised by CTCAE grade.

AESIs were reported in 24 patients (19.7%) of study PH-L19IL2TNF-02/15 and in 20 patients (39.2%) of study PH-L19IL2TNF-01/18.

The most common AESI is CTCAE G3 Injection Site Reaction.

Table 56. Incidence of adverse events of special interest in the study PH-L19IL2TNF-02/15

	PH-L19IL2TNF-02/15			
	Arm 1 (N=122)			
CTCAE Grade	**Any Grade**		**Grade ≥3**	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	15	**12.3%**	15	**12.3%**
INJECTION SITE REACTION	15	**12.3%**	15	**12.3%**
IMMUNE-MEDIATED DISORDERS	3	2.5%	3	2.5%
DRUG HYPERSENSITIVITY	3	2.5%	3	2.5%
INVESTIGATIONS	2	1.6%	0	
BLOOD CREATININE INCREASED	2	1.6%	0	
METABOLISM AND NUTRITION DISORDERS	1	0.8%	1	0.8%
HYPOKALAEMIA	1	0.8%	1	0.8%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2	1.6%	0	
DYSPNOEA	2	1.6%	0	

VASCULAR DISORDERS	1	0.8%	1	0.8%
HYPOTENSION	1	0.8%	1	0.8%

This table lists the percentage of patients that reported AEs of special interest in study PH-L19IL2TNF-02/15. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. The study PH-L19IL2TNF-02/15 is still ongoing, and data is presented up to the cut-off date 29/11/2024. In the table the incidence >10% has been highlighted (bold letters).

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 35

Table 57. Incidence of adverse events of special interest in the study PH-L19IL2TNF-01/18

	PH-L19IL2TNF-01/18			
	Arm 1 (N=51)			
CTCAE Grade	**Any Grade**		**Grade ≥3**	
CARDIAC DISORDERS	1	2.0%	0	
TACHYCARDIA	1	2.0%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	9	**17.6%**	7	**13.7%**
INJECTION SITE REACTION	6	**11.8%**	6	**11.8%**
CHEST PAIN	1	2.0%	0	
NON-CARDIAC CHEST PAIN	1	2.0%	0	
PAIN	1	2.0%	1	2.0%
INFECTIONS AND INFESTATIONS	3	5.9%	2	3.9%
CELLULITIS	2	3.9%	1	2.0%
LOCALISED INFECTION	1	2.0%	1	2.0%
INVESTIGATIONS	3	5.9%	0	
ALANINE AMINOTRANSFERASE INCREASED	3	5.9%	0	
ASPARTATE AMINOTRANSFERASE INCREASED	2	3.9%	0	
GAMMA-GLUTAMYLTRANSFERASE INCREASED	1	2.0%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2	3.9%	1	2.0%

DYSPNOEA	1	2.0%	0	
PULMONARY EMBOLISM	1	2.0%	1	2.0%
VASCULAR DISORDERS	5	9.8%	1	2.0%
HYPOTENSION	5	9.8%	1	2.0%

This table lists the percentage of patients that reported AEs of special interest in study PH-L19IL2TNF-01/18. Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. The study PH-L19IL2TNF-01/18 is still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arm (Arm 1, Any Grade) In the table the incidence >10% has been highlighted (bold letters).

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 36

Evaluation of particular adverse effects

Particular adverse effects resulting from clinical experience were identified as those events that might require closer evaluation and/or recommendations to prevent and/or manage events.

- Injection site reactions

In the pivotal study **L19IL2TNF-02/15 (Phase III)** injection site reactions were the most frequent adverse reactions associated with the intralesional administration of L19IL2/L19TNF and were graded according to the following criteria:

☐ Grade 1: tenderness with or without associated symptoms (e.g., warmth, erythema, itching)

☐ Grade 2: pain, lipodystrophy, oedema, phlebitis

☐ Grade 3: ulceration or necrosis, severe tissue damage, operative intervention indicated.

☐ Grade 4: life-threatening consequences or urgent intervention indicated.

So far, no injection site reaction has been collected with severity grade higher than Grade 3. Tumour necrosis and recruitment of mediators of inflammation at tumour site is a major driver of the anticancer effect of L19IL2/L19TNF. It is important to evaluate if the necrosis and inflammation is localised exclusively within the tumour tissue (sign of efficacy) or if also healthy tissue is involved. As wide locoregional injection site reactions could mimic loco-regional infections it may be necessary to carefully assess differential diagnosis between "sterile" IL2-and TNF- mediated inflammation and infection.

Hypersensitivity Reactions

Episodes of allergic reactions/drug hypersensitivity possibly associated with L19IL2/L19TNF have been reported in 8 patients (4.3%). In 3 out of 8 patients the reaction was characterised by severity grade CTCAE G3 (1.6%) and considered serious since it required hospitalisation. The non-serious episodes of hypersensitivity to L19IL2/L19TNF included one case of CTCAE G2 (0.5%) allergic reaction characterised by jugular constriction and leading to permanent discontinuation and 4 patients with CTCAE G1 episodes of drug hypersensitivity (2.1%).

Immune-related Adverse Events (irAEs)

A retrospective analysis showed that 6 out of 195 patients treated with L19IL2/L19TNF (3.1%) experienced possible irAEs: immune-mediated thyroiditis (0.5%), colitis (0.5%), dermatitis (0.5%), erythema multiforme (0.5%), two rash maculopapular (1.0%) and one superficial vein thrombosis (0.5%). The irAE of colitis was severe (CTCAE G3) and the irAE of rash maculopapular and superficial vein thrombosis was CTCAE G2. All the others were characterised by mild severity (CTCAE G1). Moreover, it should be considered that 3 out 5 patients had been exposed to treatment with ICIs, thus a possible role of these drugs cannot be completely ruled out.

No immune-related adverse events were observed in the study PH-L19IL2TNF-02/12.

3.3.7.4. Laboratory findings

Tables of treatment-emergent shifts of CTCAE grade in haematology, coagulation and blood chemistry have been provided and are presented below. The treatment-emergent shifts of CTCAE grade in haematology, coagulation and blood chemistry were evaluated for the pooled population of studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, since in study PH-L19IL2TNF-02/12 a different grading system was used.

Table 58. Treatment-emergent shifts in haematology, coagulation and blood chemistry pooled for the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18

		Treatment (N=173)								Follow-Up 1 (N=173)							
		Intensity of Shift								Intensity of Shift							
Test	**Max Grade at Baseline**	**+1**		**+2**		**+3**		**+4**		**+1**		**+2**		**+3**		**+4**	
Haematology																	
Haemoglobin Decreased	0	36	20.8%							3	1.7%						
	1	2	1.2%														
Leukocytes Decreased	0	4	2.3%														
Lymphocytes Decreased	0	3	1.7%	2	1.2%												
Lymphocytes Increased	0			2	1.2%	1	0.6%										
Neutrophil Decreased	0	2	1.2%					1	0.6%	2	1.2%						
Platelets Decreased	0	2	1.2%					1	0.6%								
Coagulation																	
INR Decreased	0	1	0.6%			1	0.6%			1	0.6%						
	1			1	0.6%												
INR Increased	0	1	0.6%														
Blood Chemistry																	

ALT Increased	0	39	22.5%	2	1.2%	2	1.2%			3	1.7%			1	0.6%			
AST Increased	0	24	13.9%	1	0.6%	1	0.6%											
Albumin Decreased	0	13	7.5%															
ALP Increased	0	28	16.2%							3	1.7%							
Total Bilirubin Increased	0	1	0.6%															
Creatine Kinase Increased	0	2	1.2%	1	0.6%													
	1	2	1.2%															
Calcium Decreased	0	11	6.4%							1	0.6%							
Calcium Increased	0	6	3.5%															
Creatine Increased	0	10	5.8%					1	0.6%	1	0.6%							
	1	1	0.6%															
GGT Increased	0	38	22.0%	7	4.1%	1	0.6%			5	2.9%							
	1	3	1.7%	1	0.6%													
Glucose Decreased	0	8	4.6%	2	1.2%					1	0.6%	1	0.6%					
Glucose Increased	0	33	19.1%	1	0.6%					7	4.1%							
	1	10	5.8%							1	0.6%							
	2	3	1.7%							1	0.6%							
Magnesium Decreased	0	7	4.0%															
Magnesium Increased	0	3	1.7%															

Phosphorus Decreased	0	7	4.0%	12	6.9%	3	1.7%											
	1	3	1.7%															
	2	3	1.7%															
Potassium Decreased	0	7	4.0%															
	1		0.0%	2	1.2%													
Potassium Increased	0	22	12.7%			1	0.6%			2	1.2%							
Sodium Decreased	0	9	5.2%							1	0.6%							
Sodium Increased	0	4	2.3%															
Uric Acid Increased	0	8	4.6%					2	1.2%							1	0.6%	
	1					1	0.6%											

This table lists the percentages of patients who experienced a shift in CTCAE grade of abnormalities in haematology, coagulation and blood chemistry after L19IL2/L19TNF treatment and in follow-up compared to maximum grade at baseline values for the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Baseline refers to laboratory values measured at screening and at the first treatment visit (before L19IL2/L19TNF injection). Treatment refers to any laboratory values obtained after the first L19IL2/L19TNF administration until surgery visit. For follow-up, only the laboratory values of patients who experienced a shift during treatment and only the first follow-up visit were considered. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024.

This data was compiled based on the summaries on treatment-emergent shifts of clinical laboratory values for patients in studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. The source data can be found in the corresponding CSRs

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 39

3.3.7.5. In vitro biomarker test for patient selection for safety

Not applicable.

3.3.7.6. Safety in special populations

Age

Out of 366 patient, 199 patients were <65 years old (arm1: n=104, arm 2: n=95), 90 patients were between 65 and 74 years (arm1: n=47, arm 2: n=43), 65 patients were between 75-84 years (arm1: n=38, arm 2: n=27) and 12 patients were 85 years or older (arm1: n=5, arm 2: n=7).

In the L19IL2/L19TNF treatment arm, most reported adverse events by PT like pyrexia (58.7% vs 42,2%), chills (45.2% vs 41,1%), injection site pain (11.5% vs. 7.8%), influenza like illness (12.5% vs 10.0%), alanine aminotransferase increased (11.5% vs. 6.67%), gamma-glutamyltransferase increased (10.6% vs 2.2%), headache (21.2% vs 12.2%) and hypotension (9.6% vs 2.2%) occurred more frequently in patients < 65 years.

The incidence of injection site reaction (65.6% [n=59] vs. 60.6% [n=63]), nausea (21.1% [n=19] vs 19.2% [n=20]), vomiting (12.2% [n=11] vs 10.6% [n=11]), diarrhoea (10.0% [n=9] vs 4.8% [n=5]) and fatigue (18.9% [n=17] vs 14.4% [n=15]) was slightly higher in patients ≥65 years compared to patients <65 years in the L19IL2/L19TNF treatment arm.

Table 59. Summary of adverse events categories by treatment arm and age

	Age (years)															
	Arm 1 (N=194)*								**Arm 2 (N=172)**							
	< 65 (N=104)		**65 ≤ age < 75 (N=47)**		**75 ≤ age < 85 (N=38)**		**≥85 (N=5)**		**< 65 (N=95)**		**65 ≤ age < 75 (N=43)**		**75 ≤ age < 85 (N=27)**		**≥85 (N=7)**	
All AEs	99	95.2%	45	95.7%	37	97.4%	5	100.0%	38	40.0%	20	46.5%	14	51.9%	4	57.1%
Related AEs	98	94.2%	44	93.6%	36	94.7%	5	100.0%	0		0		0		0	
SAEs	11	10.6%	10	21.3%	6	15.8%	2	40.0%	5	5.3%	2	4.7%	5	18.5%	2	28.6%

Related SAEs	9	8.7%	6	12.8%	3	7.9%	2	40.0%	0		0		0		0	
AEs with grade 3,4 and 5	28	26.9%	18	38.3%	13	34.2%	2	40.0%	6	6.3%	3	7.0%	5	18.5%	2	28.6%
Grade 3	26	25.0%	15	31.9%	10	26.3%	2	40.0%	6	6.3%	3	7.0%	5	18.5%	2	28.6%
Grade 4	2	1.9%	2	4.3%	3	7.9%	0		0		0		0		0	
Grade 5 (Deaths)[1]	0		1	2.1%	0		0		0		0		0		0	
AEs Related with grade 3,4 and 5	24	23.1%	13	27.7%	11	28.9%	2	40.0%	0		0		0		0	
SAEs with grade 3,4 and 5	7	6.7%	9	19.1%	5	13.2%	2	40.0%	3	3.2%	2	4.7%	4	14.8%	2	28.6%
SAEs Related with grade 3,4 and 5	5	4.8%	5	10.6%	3	7.9%	2	40.0%	0		0		0		0	0.0%
SAEs that lead to																
Hospitalisation	3	2.9%	4	8.5%	4	10.5%	2	40.0%	3	3.2%	2	4.7%	2	7.4%	1	14.3%
Life-threatening	0		0		1	2.6%	0		0		0		0		0	
Disability/incapacity	0		1	2.1%	1	2.6%	0		0		0		0		0	
Other SAEs (medically significant)	3	2.9%	2	4.3%	1	2.6%	1	20.0%	0		0		0		0	
SAEs Congenital anomaly/birth defect	0		0		0		0		0		0		0		0	
AEs that lead to Drop-Out[2]	2	1.9%	1	2.1%	2	5.3%	0		0		0		0		0	
PULMONARY EMBOLISM	0		0		1	2.6%	0		0		0		0		0	

INJECTION SITE REACTION	1	1.0%	1	2.1%	1	2.6%	0		0		0		0		0	
DRUG HYPERSENSITIVITY	1	1.0%	0		0		0		0		0		0		0	
Psychiatric disorders	4	3.8%	0		2	5.3%	0		0		0		1	3.7%	0	
Nervous system disorders	32	30.8%	11	23.4%	9	23.7%	0		5	5.3%	2	4.7%	0		1	14.3%
Accidents and injuries	13	12.5%	9	19.1%	5	13.2%	0		14	14.7%	8	18.6%	2	7.4%	2	28.6%
Cardiac disorders	8	7.7%	6	12.8%	1	2.6%	0		0		1	2.3%	3	11.1%	0	
Vascular disorders	21	20.2%	7	14.9%	3	7.9%	0		5	5.3%	4	9.3%	1	3.7%	0	
Cerebrovascular disorders	0		0		0		0		0		0		0		0	
Infections and infestations	14	13.5%	5	10.6%	7	18.4%	1	20.0%	9	9.5%	3	7.0%	4	14.8%	0	
Anticholinergic syndrome	0		0		0		0		0		0		0		0	
Quality of life decreased	0		0		0		0		0		0		0		0	
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	12	11.5%	2	4.3%	1	2.6%	0		0		0		0		0	
Most common AEs (≥10%) in older patients																
CHILLS	47	45.2%	20	42.6%	16	42.1%	1	20.0%	0		1	2.3%	0		0	
DIARRHOEA	5	4.8%	4	8.5%	5	13.2%	0		1	1.1%	0		1	3.7%	0	
FATIGUE	15	14.4%	7	14.9%	7	18.4%	3	60.0%	2	2.1%	0		0		0	

HEADACHE	22	21.2%	8	17.0%	3	7.9%	0		1	1.1%	0		0		0	
INFLUENZA LIKE ILLNESS	13	12.5%	5	10.6%	3	7.9%	1	20.0%	0		1	2.3%	0		0	
INJECTION SITE REACTION	63	60.6%	33	70.2%	23	60.5%	3	60.0%	0		0		0		0	
NAUSEA	20	19.2%	10	21.3%	9	23.7%	0		1	1.1%	2	4.7%	1	3.7%	0	
PYREXIA	61	58.7%	20	42.6%	16	42.1%	2	40.0%	1	1.1%	2	4.7%	1	3.7%	0	
VOMITING	11	10.6%	5	10.6%	6	15.8%	0		0		0		2	7.4%	0	

This table lists the percentage of patients that reported AEs in different categories by age across the three studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Arm 1 corresponds all patients treated with L19IL2/L19TNF. Arm 2 corresponds to surgery only as treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. AESI = Adverse event of special interest, IRAE = Immune-related adverse event. *For one patient in Arm 1 (study PH-L19IL2TNF-01/18), the age is unknown. This patient experienced one adverse event (grade 3, nervous system disorder) and no SAEs.

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 41

Gender

Table 60. Summary of any adverse events (incidence ≥5%) by system organ class, preferred term and gender

	Gender							
	Male				**Female**			
	Arm 1 (N=111)		**Arm 2 (N=99)**		**Arm 1 (N=84)**		**Arm 2 (N=73)**	
ANY	**105**	**94.6%**	**44**	**44.4%**	**82**	**97.6%**	**32**	**43.8%**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	7	6.3%	4	4.0%	6	7.1%	0	
CARDIAC DISORDERS	7	6.3%	3	3.0%	8	9.5%	1	1.4%
TACHYCARDIA	3	2.7%	0		6	7.1%	0	
EAR AND LABYRINTH DISORDERS	7	6.3%	0		2	2.4%	0	
VERTIGO	6	5.4%	0		2	2.4%	0	
ENDOCRINE DISORDERS	1	0.9%	2	2.0%	2	2.4%	1	1.4%
GASTROINTESTINAL DISORDERS	**22**	**19.8%**	3	3.0%	**38**	**45.2%**	5	6.8%
NAUSEA	**13**	**11.7%**	1	1.0%	**26**	**31.0%**	3	4.1%
VOMITING	10	9.0%	1	1.0%	**12**	**14.3%**	1	1.4%
DIARRHOEA	7	6.3%	1	1.0%	7	8.3%	1	1.4%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**101**	**91.0%**	4	4.0%	**77**	**91.7%**	5	6.8%

INJECTION SITE REACTION	**66**	**59.5%**	0		**56**	**66.7%**	0	
PYREXIA	**50**	**45.0%**	1	1.0%	**49**	**58.3%**	3	4.1%
CHILLS	**48**	**43.2%**	1	1.0%	**36**	**42.9%**	0	
FATIGUE	**15**	**13.5%**	1	1.0%	**17**	**20.2%**	1	1.4%
INFLUENZA LIKE ILLNESS	**15**	**13.5%**	1	1.0%	7	8.3%	0	
INJECTION SITE PAIN	**12**	**10.8%**	0		7	8.3%	0	
IMMUNE SYSTEM DISORDERS	2	1.8%	1	1.0%	6	7.1%	0	
DRUG HYPERSENSITIVITY	2	1.8%	1	1.0%	6	7.1%	0	
INFECTIONS AND INFESTATIONS	**14**	**12.6%**	7	7.1%	**13**	**15.5%**	**9**	**12.3%**
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	**15**	**13.5%**	**17**	**17.2%**	**12**	**14.3%**	**9**	**12.3%**
SEROMA	3	2.7%	8	8.1%	5	6.0%	5	6.8%
INVESTIGATIONS	**21**	**18.9%**	8	8.1%	**21**	**25.0%**	7	9.6%
ALANINE AMINOTRANSFERASE INCREASED	9	8.1%	2	2.0%	**9**	**10.7%**	1	1.4%
GAMMA-GLUTAMYLTRANSFERASE INCREASED	6	5.4%	2	2.0%	7	8.3%	1	1.4%
ASPARTATE AMINOTRANSFERASE INCREASED	5	4.5%	1	1.0%	5	6.0%	1	1.4%
METABOLISM AND NUTRITION DISORDERS	9	8.1%	2	2.0%	8	9.5%	1	1.4%
DECREASED APPETITE	6	5.4%	0		4	4.8%	0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	**13**	**11.7%**	5	5.1%	**14**	**16.7%**	2	2.7%

NERVOUS SYSTEM DISORDERS	**27**	**24.3%**	6	6.1%	**26**	**31.0%**	2	2.7%
HEADACHE	**16**	**14.4%**	0		**17**	**20.2%**	1	1.4%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	8	7.2%	0		8	9.5%	2	2.7%
DYSPNOEA	6	5.4%	0		1	1.2%	0	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	**28**	**25.2%**	7	7.1%	**21**	**25.0%**	6	8.2%
ERYTHEMA	6	5.4%	1	1.0%	7	8.3%	0	
NIGHT SWEATS	6	5.4%	0		1	1.2%	0	
PRURITUS	6	5.4%	2	2.0%	1	1.2%	0	
VASCULAR DISORDERS	**18**	**16.2%**	5	5.1%	**13**	**15.5%**	5	6.8%
HYPOTENSION	6	5.4%	0		6	7.1%	0	
HYPERTENSION	6	5.4%	3	3.0%	4	4.8%	2	2.7%

This table lists the percentage of patients that reported AEs by gender across the three studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. Arm 1 corresponds all patients treated with L19IL2/L19TNF. Arm 2 corresponds to surgery only as treatment. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence in the L19IL2/L19TNF treatment arms across all three studies. In the table the incidence > 10% has been highlighted (Bold letters).

Source: 2.7.4 Summary of Clinical Safety, Appendix 2.7.4.7, Table 42

ECOG status

In general, more patients with an ECOG status PS0 (n=345) than PS1 (n=22) were include in the clinical trials. Thereof, 178 patients with an ECOG status PS0 and 17 patients with an ECOG status PS1 were included in the L19IL2/L19 treatment arm. The occurrence of most adverse events by ECOG status is equally distributed between patients with an ECOG status of 0 and 1 in the L19IL2/L19 treatment arm. Adverse events by PT, which occurred in more than 10% of patients treated with L19IL2/L19TNF and which showed a more than 5% increased frequency in patients with ECOG status of 1 compared to patients

with ECOG status of 0 were injection site reaction (70.6% [n=12] vs 61.8% [n=110]), chills (52.9% [n=9] vs 42.1% [n=75]), fatigue (35.3% [n=6] vs 14.6% [n=26]), headache (23.5% [n=4] vs 16.3% [n=29]), diarrhoea (PS1: 17.6% [n=3] vs PS0: 6.2% [n=11]), axillary pain (17.6% [n=3] vs 1.1% [n=2]), seroma (17.6% [n=3] vs 2.8% [n=5]), gastroesophageal reflux disease (11.8% [n=2] vs 0% [n=0]), oedema peripheral (11.8% [n=2] vs 3.4% [n=6]), lipase increase (11.8% [n=2] vs 1.7% [n=3]), cough (11.8% [n=2] vs 1.7% [n=3]) rash maculo-papular (11.8% [n=2] vs 0% [n=0]).

Hepatic and renal impairment

Table 61: AEs inpatients with hepatic and renal impairment

	ARM1 (L19IL2/L19TNF + surgery)		ARM2 (surgery alone)	
MedDRA Terms	**Hepatically impaired N=0**	**Renally impaired N=2**	**Hepatically impaired N=2**	**Renally impaired N=2**
Total AEs	-	2 (100%)	2 (100%)	2 (100%)
Total AEs G3	-	-	2 (100%)	-
Total AEs related to L19IL2/L19TNF	-	2 (100%)	-	
Total AEs related to surgery	-	-	1 (50%)	1 (50%)
Serious AEs - Total	-	-	2 (100%)	-
Serious AEs – Total G3	-	-	-	-
Total SAEs related	-	-	-	-
Total SAEs related surgery	-	-	1 (50%)	-
Fatal	-	-	-	-
Hospitalisation/prolong existing hospitalisation	-	-	2 (100%)	-

Source: 2.7.4 Summary of Clinical Safety, Table 20

3.3.7.7. Immunological events

Please refer to AR section 3.3.1 "Clinical pharmacology".

3.3.7.8. Safety related to drug-drug interactions and other interactions

No clinical studies designed to evaluate potential drug-drug or drug-food interactions were performed. No clinical effects of drug-drug or drug-food interactions have been observed in any of the clinical studies

performed with the monotherapies of L19IL2 and L19TNF, as well as the combination therapy L19IL2/L19TNF.

3.3.7.9. Discontinuation due to adverse events

Table 62. Summary of reasons for dose reduction, discontinuation and interruption per time-point by study (PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18) and overall for Arm 1

	PH-L19IL2TNF-02/15 Arm 1 (N=122)				PH-L19IL2TNF-01/18 Arm 1 (N=51)				PH-L19IL2TNF-02/15 & PH-L19IL2TNF-01/18 Arm 1 (N=173)			
Week	1	2	3	4	1	2	3	4	1	2	3	4
Reason for Drug Interruption (total):	0	23	16	8	1	5	8	3	1	28	24	11
Drug interruption due to AEs		22	14	8		5	6	3	0	27	20	11
Drug interruption due to Patient Decision		1	2						0	1	2	0
Drug interruption due to localisation and/or size of lesion							1		0	0	1	0

Under revision					1		1		1	0	1	0
Reason for Drug Withdrawn (total):	2	13	6	9	0	5	2	7	2	18	8	16
Drug withdrawn due to AEs		9	6	7		4	2	4	0	13	8	11
eCRF entry incomplete			2			1	2	1		1	4	1
Drug withdrawn due to Patient Decision	1	3		1		1			1	4	0	1
Drug withdrawn due to Localisation and/or size of lesion	1							3	1	0	0	3
Drug withdrawn due to No more lesion		1							0	1	0	0
Under revision				1					0	0	0	1
Reason for Drug Reduced (total):	24	26	32	29	2	3	5	2	26	29	37	31

Patient decision	1	1	1						1	1	1	0
Protocol deviation					1	1	2	1	1	1	2	1
Wrong dose reduction procedure	1	1	1	1					1	1	1	1
Adverse event		12	11	14			1	1	0	12	12	15
Maximum volume obtained in the extraction with the syringe						1			0	1	0	0
Technical problem	3	4	4	2					3	4	4	2
Tissue issue	2		1	1					2	0	1	1
Investigator decision	7	5	5	3					7	5	5	3
Localisation and/or size of lesion	2	1	4	3					2	1	4	3
Under revision	8	2	5	5	1	1	2		9	3	7	5

Adverse events leading to permanent withdrawal of study drug

Table 63. Summary of adverse events that led to drug withdrawal by primary system organ class, preferred term and study

	PH-L19IL2TNF-02/12				PH-L19IL2TNF-02/15				PH-L19IL2TNF-01/18				OVERALL			
	Total (N=22)				Arm 1 (N=122)				Arm 1 (N=51)				Overall (N=195)			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
CARDIAC DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
TACHYCARDIA	0		0		1	0.8%	0		0		0		1	0.5%	0	
EYE DISORDERS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	0	
DIPLOPIA	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	0	
GASTROINTESTINAL DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
NAUSEA	0		0		1	0.8%	0		0		0		1	0.5%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**2**	**9.1%**	1	4.5%	**15**	**12.3%**	5	4.1%	**3**	**5.9%**	1	2.0%	**20**	**10.3%**	7	3.6%
INJECTION SITE REACTION	**2**	**9.1%**	1	4.5%	**13**	**10.7%**	5	4.1%	**3**	**5.9%**	1	2.0%	**18**	**9.2%**	7	3.6%
PYREXIA	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
FATIGUE	0		0		1	0.8%	0		0		0		1	0.5%	0	
INJECTION SITE PAIN	0		0		1	0.8%	0		0		0		1	0.5%	0	
IMMUNE SYSTEM DISORDERS	1	4.5%	0		2	1.6%	2	1.6%	0		0		3	1.5%	0	

DRUG HYPERSENSITIVITY	1	4.5%	0		2	1.6%	2	1.6%	0		0		3	1.5%	0	
INFECTIONS AND INFESTATIONS	0		0		1	0.8%	0		2	3.9%	2	3.9%	3	1.5%	2	1.0%
CELLULITIS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
INJECTION SITE INFECTION	0		0		1	0.8%	0		0		0		1	0.5%	0	
LOCALISED INFECTION	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
INVESTIGATIONS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
AMYLASE INCREASED	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
METABOLISM AND NUTRITION DISORDERS	1	4.5%	0		0		0		0		0		1	0.5%	0	
HYPOKALAEMIA	1	4.5%	0		0		0		0		0		1	0.5%	0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
BACK PAIN	0		0		1	0.8%	0		0		0		1	0.5%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
PULMONARY EMBOLISM	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	1	4.5%	0		0		0		0		0		1	0.5%	0	
RASH	1	4.5%	0		0		0		0		0		1	0.5%	0	
VASCULAR DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
EMBOLISM	0		0		1	0.8%	0		0		0		1	0.5%	0	

This table lists the percentage of patients that reported AEs that led to drug withdrawal in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. For the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events of all patients who were treated with L19IL2/L19TNF in the three studies are added. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence across all three studies (OVERALL, Any Grade). In the table the incidence > 5% has been highlighted (Bold letters).

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 28

Adverse events leading to dose reduction of study drug

Table 64: Summary of adverse events that led to dose reduction by primary system organ class, preferred term and study

	PH-L19IL2TNF-02/12		PH-L19IL2TNF-02/15		PH-L19IL2TNF-01/18		OVERALL	
	Total (N=22)		Arm 1 (N=122)		Arm 1 (N=51)		Overall (N=195)	
CTCAE Grade	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	0	0	2 1.6%	0	0	0	2 1.0%	0
LEUKOCYTOSIS	0	0	1 0.8%	0	0	0	1 0.5%	0
THROMBOCYTOSIS	0	0	1 0.8%	0	0	0	1 0.5%	0
CARDIAC DISORDERS	0	0	1 0.8%	0	0	0	1 0.5%	0
SINUS TACHYCARDIA	0	0	1 0.8%	0	0	0	1 0.5%	0
GASTROINTESTINAL DISORDERS	0	0	3 2.5%	0	0	0	3 1.5%	0
NAUSEA	0	0	3 2.5%	0	0	0	3 1.5%	0

VOMITING	0		0		3	2.5%	0		0		0		3	1.5%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	0		0		**14**	**11.5%**	3	2.5%	**3**	**5.9%**	**3**	**5.9%**	**17**	**8.7%**	6	3.1%
INJECTION SITE REACTION	0		0		**8**	**6.6%**	2	1.6%	2	3.9%	2	3.9%	**10**	**5.1%**	4	2.1%
CHILLS	0		0		3	2.5%	0		0		0		3	1.5%	0	
PYREXIA	0		0		3	2.5%	1	0.8%	1	2.0%	0		4	2.1%	1	0.5%
CHEST PAIN	0		0		1	0.8%	0		0		0		1	0.5%	0	
INFLUENZA LIKE ILLNESS	0		0		1	0.8%	0		0		0		1	0.5%	0	
INJECTION SITE PAIN	0		0		1	0.8%	0		0		0		1	0.5%	0	
INJECTION SITE ULCER	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
OEDEMA PERIPHERAL	0		0		0		0		1	2.0%	0		1	0.5%	0	
INJECTIONS AND INFESTATIONS	0		0		1	0.8%	0		0		0		1	0.5%	0	
INJECTION SITE INFECTION	0		0		1	0.8%	0		0		0		1	0.5%	0	
INVESTIGATIONS	0		0		2	1.6%	0		0		0		2	1.0%	0	
ALANINE AMINOTRANSFERASE INCREASED	0		0		1	0.8%	0		0		0		1	0.5%	0	
ASPARTATE AMINOTRANSFERASE INCREASED	0		0		1	0.8%	0		0		0		1	0.5%	0	
BLOOD ALKALINE PHOSPHATASE INCREASED	0		0		1	0.8%	0		0		0		1	0.5%	0	

BODY TEMPERATURE INCREASED	0		0		1	0.8%	0		0		0		1	0.5%	0	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
PAIN IN EXTREMITY	0		0		1	0.8%	0		0		0		1	0.5%	0	
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	0		0		1	0.8%	0		0		0		1	0.5%	0	
TUMOUR PAIN	0		0		1	0.8%	0		0		0		1	0.5%	0	
NERVOUS SYSTEM DISORDERS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
SYNCOPE	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
ERYTHEMA	0		0		1	0.8%	0		0		0		1	0.5%	0	
VASCULAR DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
FLUSHING	0		0		1	0.8%	0		0		0		1	0.5%	0	

This table lists the percentage of patients that reported AEs that led to dose reduction in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. For the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events of all patients who were treated with L19IL2/L19TNF in the three studies are added. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence across all three studies (OVERALL, Any Grade). In the table the incidence > 5% has been highlighted (Bold letters).

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 30

Adverse events leading to interruption of study drug

Table 65: Summary of adverse events that led to drug interruption by primary system organ class, preferred term and study

	PH-L19IL2TNF-02/12				PH-L19IL2TNF-02/15				PH-L19IL2TNF-01/18				OVERALL			
	Total (N=22)				Arm 1 (N=122)				Arm 1 (N=51)				Overall (N=195)			
CTCAE Grade	**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**		**Any Grade**		**Grade ≥3**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS	0		0		1	0.8%	0		0		0		1	0.5%	0	
ANAEMIA	0		0		1	0.8%	0		0		0		1	0.5%	0	
CARDIAC DISORDERS	0		0		1	0.8%	0		1	2.0%	1	2.0%	2	1.0%	1	0.5%
ARRHYTHMIA	0		0		1	0.8%	0		0		0		1	0.5%	1	0.5%
CARDIAC FAILURE	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	0	
EAR AND LABYRINTH DISORDERS	1	4.5%	0		0		0		0		0		1	0.5%	0	
VERTIGO	1	4.5%	0		0		0		0		0		1	0.5%	0	
GASTROINTESTINAL DISORDERS	0		0		2	1.6%	0		0		0		2	1.0%	0	
DIARRHOEA	0		0		1	0.8%	0		0		0		1	0.5%	0	
NAUSEA	0		0		1	0.8%	0		0		0		1	0.5%	0	
VOMITING	0		0		1	0.8%	0		0		0		1	0.5%	0	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**4**	**18.2%**	0		**21**	**17.2%**	2	1.6%	5	9.8%	1	2.0%	**30**	**15.4%**	3	1.5%

INJECTION SITE REACTION	**3**	**13.6%**	0		**21**	**17.2%**	2	1.6%	3	5.9%	1	2.0%	**27**	**13.8%**	3	1.5%
OEDEMA	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
INDURATION	0		0		0		0		1	2.0%	0		1	0.5%	0	
INFLUENZA LIKE ILLNESS	1	4.5%	0		0		0		0		0		1	0.5%	0	
MALAISE	1	4.5%	0		0		0		0		0		1	0.5%	0	
INFECTIONS AND INFESTATIONS	0		0		3	2.5%	0		1	2.0%	0		4	2.1%	0	
CELLULITIS	0		0		0		0		1	2.0%	0		1	0.5%	0	
ERYSIPELAS	0		0		1	0.8%	0		0		0		1	0.5%	0	
INJECTION SITE INFECTION	0		0		1	0.8%	0		0		0		1	0.5%	0	
NASOPHARYNGITIS	0		0		1	0.8%	0		0		0		1	0.5%	0	
INVESTIGATIONS	0		0		5	4.1%	4	3.3%	1	2.0%	0		6	3.1%	4	2.1%
ALANINE AMINOTRANSFERASE INCREASED	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
ASPARTATE AMINOTRANSFERASE INCREASED	0		0		2	1.6%	0		1	2.0%	0		3	1.5%	0	
GAMMA-GLUTAMYLTRANSFERASE INCREASED	0		0		2	1.6%	2	1.6%	1	2.0%	0		3	1.5%	2	1.0%
BLOOD ALKALINE PHOSPHATASE INCREASED	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
BLOOD CREATININE INCREASED	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
TRANSAMINASES INCREASED	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%

NERVOUS SYSTEM DISORDERS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
SYNCOPE	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
PSYCHIATRIC DISORDERS	1	4.5%	0		0		0		0		0		1	0.5%	0	
ANXIETY	1	4.5%	0		0		0		0		0		1	0.5%	0	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	0		0		1	0.8%	0		1	2.0%	1	2.0%	2	1.0%	1	0.5%
DYSPNOEA	0		0		1	0.8%	0		0		0		1	0.5%	0	
HYPOXIA	0		0		0		0		1	2.0%	1	2.0%	1	0.5%	1	0.5%
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	1	4.5%	0		2	1.6%	1	0.8%	1	2.0%	0		4	2.1%	1	0.5%
BLISTER	0		0		0		0		1	2.0%	0		1	0.5%	0	
RASH	1	4.5%	0		0		0		0		0		1	0.5%	0	
RASH ERYTHEMATOUS	0		0		1	0.8%	1	0.8%	0		0		1	0.5%	1	0.5%
RASH MACULO-PAPULAR	0		0		1	0.8%	0		0		0		1	0.5%	0	
VASCULAR DISORDERS	0		0		1	0.8%	0		1	2.0%	0		2	1.0%	0	
EMBOLISM	0		0		1	0.8%	0		0		0		1	0.5%	0	
SUPERFICIAL VEIN THROMBOSIS	0		0		0		0		1	2.0%	0		1	0.5%	0	

This table lists the percentage of patients that reported AEs that led to drug interruption in studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 individually and overall. For the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18, Arm 1 corresponds to neoadjuvant L19IL2/L19TNF followed by surgery as treatment. In the OVERALL column the adverse events of all patients who were treated with L19IL2/L19TNF in the three studies are added. The studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 are still ongoing, and data is presented up to the cut-off date 29/11/2024. The adverse events by PT are ordered (within the corresponding SOC) by frequency of incidence across all three studies (OVERALL, Any Grade). In the table the incidence > 5% has been highlighted (Bold letters).

Source: 2.7.4 Summary of Clinical Safety, 2.7.4.7 Appendix, Table 32

3.3.7.10. Post marketing experience

Not applicable. L19IL2/L19TNF is not marketed in any country in the world. All safety data available to date was collected in clinical trials with intralesional L19IL2/L19TNF in patients with locally advanced melanoma.

3.3.8. Discussion on clinical safety

This initial application seeks marketing authorisation for bifikafusp alfa (L19TNF) and onfekafusp alfa (L19IL2) as neo-adjuvant intralesional treatment for adult patients with locally advanced, fully resectable melanoma (stage III disease).

All safety data available at the time of submission of the MAA was collected in six clinical trials.

However, the main safety data set of L19IL2/L19TNF for the marketing authorisation are considered based on data collected from clinical studies where L19IL2/L19TNF has been administered as combination intralesional in patients with locally advanced melanoma and are presented from study PH-L19IL2TNF-02/15 (phase 3), study PH-L19IL2TNF-01/18 (phase 3) and study PH-L19IL2TNF-02/12 (phase 2). The safety data from the non-controlled single-agent clinical trials (study PH-L19IL2-01/05 and PH-L19TNF-02/07, where L19IL2 or L19TNF was administered systemically (i.e. intravenously) and study PH-L19IL2-03/09 with intratumoural administration of L19IL2 alone) are considered only as supportive data.

As of the data cut-off date (29/11/2024), the safety database for the marketing authorisation includes a total of 367 melanoma patients (22 patients treated with L19IL2/L19TNF only, 173 patients treated with neoadjuvant L19IL2/L19TNF and surgery (treatment arm 1), and 172 patients treated with surgery only (control arm 2).

In total 195 patients treated with L19IL2/L19TNF, were included in the safety analysis set. Thereof, 122 patients received L19IL2/L19TNF in the pivotal study PH-L19IL2TNF-02/15, 51 patients received L19IL2/L19TNF in the study PH-L19IL2TNF-01/18, and 22 patients received L19IL2/L19TNF in the study PH-L19IL2TNF-02/12.

The experimental arms of the randomised, controlled phase 3 studies PH-L19IL2TNF-02/15 (N=122) and PH-L19IL2TNF-01/18 (N=51), as well as the uncontrolled phase 2 study PH-L19IL2TNF-02/12 (N=22), were pooled (overall population: N=195) as the patient population (melanoma) and treatment plan (four weekly intralesional injections) are comparable. In general, the **exposure data** submitted might be appropriate to inform on the safety profile. The exposure in days was calculated as difference between the last date of visit/treatment and the date of first treatment. However, it should be noted that the maximum L19-IL2 concentration and the maximum amount of L19-TNF per administration was lower in the uncontrolled phase 2 study compared to the controlled phase 3 studies (L19-IL2: 10 Mio IU vs. 13 IU; L19TNF: intended to be 312 µg vs. 400 µg). In general, only data corresponding to the same dosage should be combined and can be compared. However, in this specific case, as the amount of L19IL2/L19TNF were administered intralesional, depending on size and number of lesions, a combination of the data might be acceptable. The same recommended dose of 13 MioIU L19IL2 and 400µg L19TNF per administration, as a single or multiple intratumoural injection once every week for up to 4 weeks, applies in both the Phase III study PH-L19IL2TNF-02/15 and the Phase III PH-L19IL2TNF-01/18 study, and the total daily dose was distributed between all injectable cutaneous, sub-

cutaneous and nodal metastases. However, the amount of Nidlegy that was injected in the phase III PH-L19IL2TNF-01/18 study was dependent on the size of the tumour whereas in the pivotal phase III study PH-L19IL2TNF-02/15 the dose of L19TNF could be adjusted between 100 and 400 µg (i.e., 100 µg, 200 µg, 300 µg or 400 µg) per administration according to size and number of lesions and based on the patient's tolerability to the treatment at the investigator's discretion. Thus, differences in the dose administration procedure and dose adjustment exist between the studies. Especially, in the pivotal study the dosage distribution seems to be based solely on the decision of the investigator. This can result in heterogeneity in the treatment of the patients within the study and across the studies and makes it difficult to compare the data.

Overall, it appears that different dose levels have been administered, and not all study participants received the same dose level, the same number of administrations and specifically not all received the proposed recommended dose of L19IL2 (13 Mio IU) and L19TNF (400 µg) for four weeks. In total only 49 patients received the maximum recommended dose of L19IL2/L19TNF (400 ug/13 Mio IU) for four weeks. Therefore, it is currently still unclear, under which circumstances which dose of L19TNF or L19IL2 was administered in the pivotal study. Moreover, the exact administered dose of L19TNF or L19IL2 is unclear. It appears that patients were exposed to individual L19TNF and L19IL2 concentrations, both within a study and across the studies and thus results in a different exposure to L19TNF and L19IL2. In summary, although, the dose distribution for patients with multiple lesions within and between the studies is not expected to have a major impact on the safety results, in case the same entire dose was administered, it might have an impact on the comparability of the data and the efficacy results (please refer to the efficacy AR section). Therefore, further discussion/clarification, how comparability of the data in/between the clinical studies was ensured should be provided (OC). In this context, further discussion/clarification as well as data should be provided whether in the pivotal study the administration procedure was performed in a similar way (lesion size/volume) as in study PH-L19IL2TNF-01/18. Moreover, it should be clearly presented how the dose of L19TNF was adjusted between 100 and 400 µg (i.e., 100 µg, 200 µg, 300 µg or 400 µg) per administration by the investigator in the pivotal phase III study PH-L19IL2TNF-02/15. In addition, the dose intensity data as the actual cumulative administrated dose / cumulative planned dose of the four administrations should be provided.

No information on exposure >12 months are available, as L19IL2/L19TNF doses were administered only up to four times, once a week, before surgical treatment followed. This is considered acceptable.

Based on the demographic and other characteristics provided, no clear imbalances in distribution of demographic characteristics could be observed between the studies and between patients enrolled in the L19IL2/L19TNF arm and the control arm within the respective studies, according to the applicant. The mean age in all three studies was reported to be 62±14 years. Most patients (94%) were classified with an ECOG status of 0. 210 of 367 patients (57.2%) were male, while 157 of 367 patients (42.8%) were female. In the three studies, melanoma patients with clinical stage III (or stage IV only in PH-L19IL2TNF-02/12 and PH-L19IL2TNF-02/15) with presence of injectable cutaneous and/or subcutaneous lesions were enrolled and treated. The most common stage was IIIc, which was observed in 181 patients (48.3%). Most patients (84.9%) presented normal-low lactate dehydrogenase levels (not assessed in PH-L19IL2TNF-02/12 study). However, the analysis of the narratives raised some concern of the included patient population. Patients with previous or concurrent cancer that is distinct in primary site or histology from the cancer being evaluated in this study were to be excluded except: cervical carcinoma in situ, treated basal cell carcinoma, superficial bladder tumours (Ta, Tis & T1), second primary melanoma in situ or any cancer curatively treated ≥ 5 years prior to study entry.

Based on the narratives several patients have been included with previous or concurrent cancer that is distinct in primary site or histology from the cancer being evaluated in the pivotal trial. Therefore, further information on the Major Protocol Deviations are still required.

Adverse events were mostly presented by SOC and PT for the individual studies by treatment arms and for the overall population (pooled data from the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18). The treatment arm (L19IL2/L19TNF [Arm1]) and the control arm (surgery only [Arm2]) of the pooled data sets were presented side by side. However, it should be noted, that the phase 2 study was uncontrolled and thus had no control arm. Although only 22 patients treated with L19IL2/L19TNF were included in the uncontrolled phase 2 study, the data of the phase 2 study extend the safety set for L19IL2/L19TNF and thus provided more safety data for the target population.

Differences of an incidence ≥5% of AEs, L19IL2/L19TNF related AEs, L19IL2/L19TNF related SAEs, AEs dose temporarily interrupted due to AEs, dose reduced due to AEs and AESI were observed in the L19IL2/L19TNF treatment arm between the two phase III studies. Especially a higher number of AESIs occurred in PH-L19IL2TNF-01/18 (39.2%) compared to the number of AESIs reported in PH-L19IL2TNF-02/15 (19.7%). This observation should be discussed and clarified whether anything was done differently between the three studies.

The **most common AEs (incidence ≥ 15%) by PT** in the overall population were injection site reaction (Arm 1: 62.6% vs. Arm 2: 0%), pyrexia (Arm 1: 50.8% vs. Arm 2: 2.3%), chills (Arm 1: 43.1% vs. Arm 2: 0.6%), nausea (Arm 1: 20.0% vs. Arm 2: 2.3%), headache (Arm 1: 16.9% vs. Arm 2: 0.6%) and fatigue (Arm 1: 16.4% vs. Arm 2: 1.2%).

No AE (any grade) with an incidence of ≥ 15% of patients was reported in the control arm.

The **most common AE (incidence ≥ 3%) by PT in the control arm** (pooled data from the phase 3 studies [PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18]) was seroma (7.6%).

Most of the reported **AEs** in the L19IL2/L19TNF treatment arm were considered **related to L19IL2/L19TNF**.

In the **overall population,** a total of 183 patients (93.6%) treated with L19IL2/L19TNF experienced an AE related to L19IL2/L19TNF.

The **most common AEs related to L19IL2/L19TNF (incidence ≥ 5%)** presented **by PT** were injection site reaction (62.6%), pyrexia (50.8%), chills (43.1%), nausea (19.0%), fatigue (15.9%), headache (15.4%), vomiting (9.7%), influenza like illness (9.7%), injection site pain (9.2%), alanine aminotransferase increase (7.7%), erythema (6.2%), hypotension (5.6%), diarrhoea (5.6%) and gamma-glutamyltransferase increased (5.1%).

None of the reported AEs possibly related to L19IL2/L19TNF were characterised by a severity grade higher than grade 3. The most common **AEs of grade 3 considered related to L19IL2/L19TNF (incidence ≥ 1%)** in the overall population were injection site reaction (11.8%), drug hypersensitivity (1.5%), hypertension (1.5%), syncope (1.0%), gamma-glutamyltransferase increased (1.0%) and hypotension (1.0%).

Serious adverse events were reported in about 4.5%-19.6% of patients treated with L19IL2/L19TNF across all three studies compared to 4.2%-9.7% in the control arm, in which the patients received surgery only. However, it is noticed that the number of SAEs reported in patients of study PH-L19IL2TNF-01/18 treated with L19IL2/L19TNF was higher (19.6%) compared to the study PH-

L19IL2TNF-02/15 (14.8%) and the phase 2 study PH-L19IL2-02/12 (4.5%). This numerical difference observed appears to be mostly triggered by the occurrence of injection side reactions. Even though, this difference observed could be a chance finding, due to the low number of patients included in arm1 of study PH-L19IL2TNF-01/18 (n=51).

In the **overall population** a total of 29 patients (14.9%) treated with L19IL2/L19TNF and 14 patients (8.1%) enrolled in the control arm experienced at least one SAE.

The most common SAEs (incidence ≥ 1%) by PT in the overall population was injection site reaction (Arm 1: 4.6% vs. Arm 2: 0%), pyrexia (Arm 1: 1.5% vs. Arm 2: 0%), drug hypersensitivity (Arm 1: 1.5% vs. Arm 2: 0.6%), postoperative wound infection (Arm 1: 1.0% vs. Arm 2: 0%), wound infection (Arm 1: 0% vs. Arm 2: 1.7%), pulmonary embolism (Arm 1: 1.0% vs. Arm 2: 0%), and hypotension (Arm 1: 1.5% vs. Arm 2: 0%).

Only one **AE leading to death** has been reported in the L19IL2/L19TNF arm of the study PH-L19IL2TNF-02/15 (refer to the table "summary of adverse events (AEs) categories by study and arm"). However, this event was not considered to be drug- or surgery-related, but was related to a pulmonary metastasis in a patient with a pulmonary embolism.

Serious adverse events related to L19IL2/L19TNF treatment were reported in about 4.5%-15.7% of patients treated with L19IL2/L19TNF across all three studies. However, it is noticed that the number of SAEs related to L19IL2/L19TNF treatment reported in study PH-L19IL2TNF-01/18 was higher (15.7%) compared to the pivotal study PH-L19IL2TNF-02/15 (9.0%) and the phase 2 study PH-L19IL2-02/12 (4.5%). This numerical difference appears to be mostly triggered by the occurrence of injection side reaction and could be a chance finding, due to the low number of patients included in arm1 of study PH-L19IL2TNF-01/18 (n=51).

In the **overall population**, a total of 20 patients (10.3%) treated with L19IL2/L19TNF experienced at least one SAE related to L19IL2/l19TNF treatment.

The **most common SAEs (incidence ≥ 1%) related to L19IL2/L19TNF in the overall population presented by PT** was injection site reaction (4.6%), pyrexia (1.5%), drug hypersensitivity (1.5%), and hypotension (1.0%).

No **death related to the L19IL2/l19TNF** treatment has been reported.

Adverse events of special interest (AESIs) were reported in 24 patients (19.7%) of study PH-L19IL2TNF-02/15 and in 20 patients (39.2%) of study PH-L19IL2TNF-01/18. The higher number of AESIs reported in PH-L19IL2TNF-01/18 (39.2%) compared to the number of AESIs reported in PH-L19IL2TNF-01/18 (19.7%) should be discussed. In addition, it was recognised that in the updated safety data set the number of reported AESIs in study PH-L19IL2TNF-02/15 was reduced from 27% to 19.7% compared to the previous one. This need to be further clarified.

The most frequently reported AESI (any grade and ≥grade 3) by PT in arm 1 was injection site reaction in both studies, PH-L19IL2TNF-02/15 (any grade: 12.3%, n=15; ≥grade 3: 12.3%, n=15) and PH-L19IL2TNF-01/18 (any grade: 11.8%; ≥grade 3: 11.8%), and the incidence was similar. Drug hypersensitivity was reported in study PH-L19IL2TNF-02/15 for three patients (2.5%), but was not reported in study PH-L19IL2TNF-01/18. Injection site reaction and drug hypersensitivity are reflected in the SmPC under section 4.4. special warnings and precautions for use.

At the data cut-off date (29/11/2024), in total 29 patients (14.9%) experienced an **AE that led to discontinuation of L19IL2/L19TNF**. Of these 29 patients, 3 patients (13.6%) were enrolled in study

PH-L19IL2TNF-02/12, 20 patients (16.4%) in the pivotal study phase III (PH-L19IL2TNF-02/15) and 6 patients (11.8%) in the study PH-L19IL2TNF-01/18. Therefore, the number of subjects with at least one event leading to permanent discontinuation of study drug were slightly higher in the pivotal study compared to the other two studies. However, the numbers of patients who experienced an AE that led to discontinuation of L19IL2/L19TNF were low in the phase II study PH-L19IL2TNF-02/12 and in the phase III study PH-L19IL2TNF-01/18. The most reason for drug withdrawn in the phase III studies was due to AEs and occurred from the second week onwards. The number of patients that had a drug discontinuation due to AEs was more or less similar from the second to the fourth week.

The **most common AEs (incidence ≥ 1%) leading to discontinuation of L19IL2/L19TNF by PT** in the overall population were injection site reaction (9.2%), pyrexia (1.5%) and drug hypersensitivity (1.5%).

22 patients (11.3%) in the overall population experienced an **AE that led to dose reduction of L19IL2/L19TNF.** Of these 22 patients, 19 patients (15.6%) in the study PH-L19IL2TNF-02/15 and 3 patients (5.9%) in the study PH-L19IL2TNF-01/18 experienced an AE that led to dose reduction of L19IL2/L19TNF. Thus, the number of subjects with at least one event leading to dose reduction of study drug were higher in the study PH-L19IL2TNF-02/15 compared to the studies PH-L19IL2TNF-02/12 and PH-L19IL2TNF-01/18. The higher occurrence of any AE leading to dose reduction is mostly driven by the PT injection site reactions (study PH-L19IL2TNF-02/15: n=8 [6.6%] and PH-L19IL2TNF-01/18: n=2 [3.9%]). In study PH-L19IL2TNF-02/12 no AEs leading to dose reduction. The most reason for drug reduction in the phase III studies was due to AEs and occurred from the second week onwards. The number of patients that had a dose reduction due to AEs was similar from the second to the fourth week.

The **most common AE (incidence ≥1%) leading to dose reduction of L19IL2/L19TNF by PT** in the **overall population** was injection site reaction (5.1%), pyrexia (2.1%), nausea (1.5%), vomiting (1.5%) and chills (1.5%).

45 patients (23.1%) experienced an **AE that led to interruption of L19IL2/L19TNF.** Of these 45 patients (23.1%), 4 patients (18.2%) were enrolled in study PH-L19IL2TNF-02/12, 32 patients (26.2%) in the pivotal study phase III (PH-L19IL2TNF-02/15) and 9 patients (17.6%) in the study PH-L19IL2TNF-01/18.

The most reason for drug reduction in the phase III studies was due to AEs and occurred from the second week onwards. The number of patients that had a drug interruption due to AEs decreased slightly from the second to the fourth week. **The most common AEs (incidence ≥1%) leading to interruption of L19IL2/L19TNF by PT in the overall population** was injection site reaction (13.8%), gamma-glutamyltransferase increased (1.5%), alanine aminotransferase increased (1.5%), aspartate aminotransferase increased (1.5%), blood alkaline phosphatase increased (1.0%) and oedema (1.0%),

However, the circumstances under which a dose was discontinued, reduced, or interrupted, e.g. the grade defined for dose interruption, for resumption of therapy or for permanent discontinuation, is currently still unclear for the pivotal study PH-L19IL2TNF-02/15 and need to be further clarified.

Moreover, since the number of patients that received the maximum recommended dose of L19IL2/L19TNF (400 ug/13 Mio IU) for four weeks is low (n=49) and although, no major safety concern has currently been identified in patients that received the maximum recommended dose of L19IL2/L19TNF (400 ug/13 Mio IU) for four weeks compared to the overall population based on the

provided safety data set, a trend exist that the maximum dose (400 ug/13 Mio IU) per administration is apparently not so well tolerated, as the most reasons for drug interruptions, drug withdrawn and drug reduction were due to AEs. Therefore, the applicant is requested to further discuss and justify the recommended dose of L19IL2/L19TNF (400 ug/13 Mio IU) for up to four weeks.

Upon request data of patients that had a delay of surgery (n=18; 9 patients in each arm) or where surgery was not possible overall (n=15 in arm 1) has been provided for the study PH-L19IL2TNF-02/15. However, according to the applicant, a specific reason for delayed surgery is only available for 7 out of these 18 patients (Arm 1: 3 patients, Arm2: 4 patients; most reason for surgical delay was logistic reasons) and a specific reason why surgery was not possible is only available for 3 out of these 15 patients. Based on this very limited data, no final conclusion can be drawn whether neoadjuvant therapy with L19IL2/L19TNF might have an impact on resectability /complications of surgery.

Summary tables of **adverse events for the special population** (age, gender, ECOG status) have been provided.

Adverse events by age

The occurrence of any adverse event by age is equal in arm 1 between patients with an age of < 65 years (95.2%) and those with an age between 65 to 74 years (95.7%), whereas a slight increase in the total occurrence of any adverse events in the L19IL2/L19TNF arm was observed in patients with an age of 75 – 84 years (arm 1: 97.4% [n=37]) and above (arm 1: 100% [n=5]). However, the number of patients that were 85 years or older was very limited. In the control arm an increase in the total occurrence of any adverse event was identified by increasing age (< 65 years: 40% [n=38], 65 - 74 years: 46.5% [n=20], 75 – 84 years: 51.9% [n=14] and ≥85 years: 57.1% [n=4]).

In addition, in the L19IL2/L19TNF treatment arm, a trend appears that the AEs related with grade 3, 4 and 5 (< 65 years: 23.1% [n=24], 65 - 74 years: 27.7% [n=13], 75 – 84 years: 28.9% [n=11] and ≥85 years: 40% [n=2]), and SAEs that lead to hospitalisation (< 65 years: 2.9% [n=3], 65 - 74 years: 8.5% [n=4], 75 – 84 years: 10.5% [n=4] and ≥85 years: 40% [n=2]) increase with increasing age. In addition, SAEs occurred more often in patients 65 years and older compared to patients that were younger than 65 years (< 65 years: 10.6% [n=11], 65 - 74 years: 21.3% [n=10], 75 – 84 years: 15.8% [n=6] and ≥85 years: 40% [n=2]). Therefore, further information is requested.

Based on the available data there is a potential trend that patients ≥85 years exhibit lower tolerance to the treatment compared to younger patients. The most frequently reported AEs by PT in patients ≥85 years that received L19IL2/L19TNF were injection site reaction (60.0% [n=3]), pyrexia (40% [n=2]) and fatigue (60% [n=3]). Especially fatigue was more frequently reported in patients ≥85 years compared to the other age groups. All other reported adverse events by PT in the L19IL2/L19TNF treatment arm for patients ≥85 years (chills, influenza like illness, oedema peripheral, drug hypersensitivity, upper respiratory tract infection, lipase increased, amylase increased and rash maculo-papular) occurred only once (n=1). However, it is important to consider that the sample size of these patients is very limited (n=5), and older individuals typically present with comorbidities and a generally poorer overall health status. Data for patients aged 85 years and above are too limited to draw conclusions.

Adverse events by gender

In general, more male (n=210) than female (n=157) participants were include in the clinical trials. Thereof, 111 male participants and 84 female participants received L19IL2/L19 treatment. The incidence of any adverse event was more or less similar between male (94.6%) and female (97.6%) in arm 1. Higher frequencies with > 5% difference of nausea (31.0% [n=26] vs 11.7% [n=13]), vomiting (14.3% [n=12] vs. 9.0% [n=10)], injection site reaction (66.7% [n=56] vs 59.5% [n=66]), pyrexia (58.3% [n=49] vs 45.0% [n=50]), fatigue (20.2% [n=17] vs 13.5% [n=15]), drug hypersensitivity (7.1% [n=6] vs 1.8% [n=2]) and headache (20.2% [n=17] vs 14.4% [n=16]) were reported in the female subgroup, whereas higher frequencies of influenza like illness (8.3% [n=7] vs 13.5% [n=15]) were observed in the male subgroup. In addition, tachycardia was reported in twice as many females (7.1% [n=6] as males (2.7% [n=3]).

Based on the currently provided safety data by gender, a trend appears that females have a higher safety risk than males. This finding and possible reasons for observed differences between male and female participants should be provided.

Adverse events by ECOG performance status

In general, of the most common adverse events (incidence ≥15%) by PT of patients treated with L19IL2/L19TNF across all studies (injection site reaction, pyrexia, chills, nausea, headache and fatigue), injection site reaction, chills, fatigue and headache were reported more often in patients with ECOG status 1 compared to those with ECOG status 0. Based on the currently provided safety data by ECOG status, a potential effect that patients with ECOG status PS1 might have a higher safety risk than patients with ECOG status PS0 cannot be excluded so far. However, it is important to consider that the sample size of these patients with ECOG status PS1 (n=22) is very limited, and patients with ECOG status PS1 have generally a poorer overall health status. Based on the limited data available for patients with ECOG status PS1 (n=22) no final conclusion can be drawn.

In addition, clinical data in patients with renal or hepatic impairment has been provided.

A total of four patients with renal impairment (stage CKD stage 3b) have been included in the clinical trials PH-L19IL2TNF-01/18 and PH-L19IL2TNF-02/15. Two of those patients were enrolled in arm 1 (L19IL2/L19TNF + surgery) and two patients were enrolled in arm 2 (surgery only). Based on the applicant, no SAE, AESI, IRAE, grade ≥3 AEs or AEs leading to drop-out occurred in those patients.

A total of two patients with hepatic impairment were included in the PH-L19IL2TNF-02/15 trial. Both patients were included in arm 2 (surgery only). Based on the applicant, both patients experienced grade 3 adverse events, serious adverse events as well as events that lead to hospitalisation or prolonged hospitalisation, but no AESI, IRAE, grade 4 or 5 AE or AE leading to drop-out occurred in those patients.

Based on the mode of action on the locally applied therapy, duration of therapy and the provided data no further actions are considered required on renal and hepatic impairment.

Information about the laboratory findings like abnormalities of haematology, urinalysis and serum chemistry parameters has been provided for study PH-L19IL2TNF-02/12 and in study PH-L19IL2TNF-02/15. Upon request a summary table of treatment-emergent shifts in haematology, coagulation and blood chemistry pooled for the studies PH-L19IL2TNF-02/15 and in study PH-L19IL2TNF-01/18 has been provided. Study PH-L19IL2TNF-02/12 was not included, since a different grading system was used. Thus, for Study PH-L19IL2TNF-02/12 only a description of how the number of patients with

abnormalities in laboratory parameters before and after the (first) drug administration changed was available.

The most common abnormalities of clinical laboratory parameters (>10% in intensity shift +1) were haemoglobin decreased, potassium increased, GGT increased, glucose increased, AST increased, ALP increased and ALT increased. The number of treatment emergent shifts≥ +3 from baseline were low. Treatment emergent shifts ≥+3 from baseline in more than one patient has been observed for ALT increased (n=3 [1.7%]), phosphorus decreased (n=3 [1.7%]) and uric acid increased (n=2 [1.2%]. The shifts in laboratory parameters during treatment were mostly mild and transient and did not raise any major safety concerns. In addition, the rate and type of abnormalities in coagulation, haematology, serum chemistry, and urinalysis observed in the studies PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18 were in line with the known safety profile of L19IL2, L19TNF, and L19IL2/L19TNF. The treatment with L19IL2/L19TNF appears to be well tolerated based on the evaluation of vital signs and physical findings. Overall, the treatment with L19IL2/L19TNF appears to be well tolerated based on the evaluation of clinical laboratory parameters.

A description/information of the selection of ADRs has been provided and is considered acceptable. The selected ADRs are in line with the expected safety profile for a locally intratumorally administrated immunostimulatory therapy.

In summary, the safety data considered related to L19IL2/L19TNF treatment, reflects on one hand the local side effects and on the other hand the systemic side effect of the L19IL2/L19TNF treatment. The local side effects of the L19IL2/L19TNF treatment were for example presented by skin and subcutaneous tissue disorders, like drug hypersensitivity, erythema, injection side pain, injection site reactions and rash. The systemic side effects of the L19IL2/L19TNF treatment were for example presented by general (like pyrexia, chills, headache, fatigue), gastrointestinal (like, decrease appetite, diarrhoea, nausea, vomiting) or renal-hepatic-pancreatic (like, alanine aminotransferase increased, aspartate aminotransferase increased and gamma-glutamyltransferase increased) impairment and impairment of the vascular system (like flushing, hypertension and hypotension). Most of the reported AEs are injection site reactions, which could be well controlled with standard interventions and most of the common adverse events related to L19IL2/L19TNF were grade 1 or grade 2. None of the most common AEs possibly related to L19IL2/L19TNF were characterised by a severity grade higher than grade 3. In addition, no treatment-related deaths have been observed.

Therefore, the available safety database with a total of 367 melanoma patients might be sufficient for this MAA. No new safety signals for L19IL2/L19TNF have been identified. The safety profile of L19IL2/L19TNF in patients with melanoma appears to be consistent with the known L19IL2 (e.g. injection site reactions, fatigue and pyrexia), L19TNF (e.g. chills, pyrexia, and vomiting) and L19IL2/L19TNF safety profile. The AE profile does not show unexpected AEs for the locally administered therapy based on the activities of the two cytokine moieties, IL-2 or TNF and the antibody moiety (L19) binding to the alternatively spliced EDB domain of fibronectin. The safety profile of L19IL2/L19TNF appears to be tolerable and manageable in patients with locally advanced fully resectable melanoma. However, there are still some concerns that should be addressed by the applicant and the SmPC needs to be revised.

Additional expert consultation

Not applicable.

Assessment of paediatric data on clinical safety

Not applicable.

The EMA Paediatric Committee granted a full paediatric on class waiver (amongst others, the class of immunomodulatory cytokine medicinal products for treatment of neuroendocrine malignant neoplasms, skin malignant neoplasms, myeloproliferative neoplasms and mature B, T and NK cell neoplasms).

3.3.9. Conclusions on clinical safety

The safety data for L19IL2/L19TNF (Nidlegy) for the treatment of patients with resectable melanoma generally appears to reflect the known toxicity profile of L19IL2, L19TNF and L19IL2/L19TNF as well as from other drugs with a similar mode of action (cytokines). No deaths have been reported during the period of drug exposure or within a period up to 30 days following in the studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15, and PH-L19IL2TNF-01/18 and no new safety concerns have been identified. The safety profile of L19IL2/L19TNF (Nidlegy) appears to be tolerable. Based on the available data, the safety profile of bifikafusp alfa and onfekafusp alfa (Nidlegy) administered in combination intralesionally over a period of four weeks in the neoadjuvant setting, can be considered acceptable for the treatment of patients with resectable melanoma. However, there are still uncertainties at present which need to be addressed adequately and the SmPC needs to be revised.

3.4. Risk management plan

3.4.1. Safety Specification

Summary of safety concerns

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

Table 66. Summary of safety concerns

Summary of safety concerns	
Important identified risks	Injection site reaction
Important potential risks	None
Missing information	Use in pregnant or lactating woman

3.4.1.1. Discussion on safety specification

From the Rapporteur's point of view, the presentation of the safety specification in the RMP appears to be in line with the clinical assessment in general and the presentation in the RMP is generally considered to be acceptable.

The patient population not enrolled in clinical trials listed in the RMP have now been discussed by the applicant, and it can be considered acceptable that the discussed diverse exclusion criteria are not

considered as missing information in the RMP. However, the use in pregnant or lactating woman, currently listed as missing information, should be removed from the summary of safety concerns as the product is not recommended during pregnancy and lactation. Nevertheless, it should be monitored through routine PhV measures.

The updated section "Part II: Module SVII - Identified and potential risks" of the Risk Management Plan can be considered acceptable in general. However, injection site reaction should not be included as important identified risk, as this risk is characterised and described in the SmPC. Nevertheless, it should be monitored through routine PhV measures.

3.4.1.2. Conclusions on the safety specification

Having considered the data in the safety specification, it is considered that the following should not be a safety concern:

- Injection site reaction should not be included as important identified risk, as this risk is characterised and described in the SmPC. This risk should be followed in PSURs with routine PhV measures.
- Use in pregnant or lactating woman should not be included as missing information. This risk should be followed in PSURs with routine PhV

3.4.2. Pharmacovigilance plan

The MAH considers that routine pharmacovigilance activities are sufficient for the safety monitoring of the product without the need for additional actions. Routine pharmacovigilance activities allow to monitor and follow-up any concern which may arise and modify and/or plan further actions than the hereby detailed.

3.4.2.1. Routine pharmacovigilance activities

Routine pharmacovigilance activities are sufficient for the safety monitoring of the product without the need for additional actions.

3.4.2.2. Summary of additional PhV activities

There are no planned additional pharmacovigilance activities for the concerned product.

3.4.2.3. Overall conclusions on the PhV Plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

The PRAC Rapporteur also considered that routine PhV remains sufficient to monitor the effectiveness of the risk minimisation measures.

3.4.2.3. Summary of Post authorisation efficacy development plan

Not applicable. There are no planned imposed post-authorisation efficacy studies concerning Nidlegy product.

3.4.3. Risk minimisation measures

3.4.3.1. Routine Risk Minimisation Measures

The routine risk minimisation measures are considered sufficient for the safe use of the product.

3.4.3.2. Summary of additional risk minimisation measures

Not applicable.

Table 67. Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important Identified Risk: Injection site reaction	*Routine risk communication:* • SmPC Section 4.2 Posology and method of administration • SmPC Section 4.4 Special warnings and precautions for use • SmPC Section 4.8 Undesirable effects The PL of the concerned products is in line with the information contained in the SmPC previously described. Such information is given in the following section of the PL: • PL Section 2 What you need to know before Nidlegy is used • PL Section 4 Possible side effects *Legal status:* • Hospital use Only Medicine • Prescription Only Medicine. *Additional risk minimisation measures:* None.	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:* None *Additional pharmacovigilance activities:* None
Missing Information: Use in Pregnant or lactating women	*Routine risk minimisations measures:* • SmPC Section 4.4 Special warnings and precautions for use • SmPC Section 4.6 Fertility, pregnancy and lactation The PL of the concerned products is in line with the information contained in the SmPC	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:* None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	previously described. Such information is given in the following section of the PL: • PL Section 2 What you need to know before Nidlegy is used *Legal status:* • Hospital use Only Medicine • Prescription Only Medicine.	*Additional pharmacovigilance activities:* None

3.4.3.3. Overall conclusions on risk minimisation measures

The PRAC Rapporteur having considered the data submitted was of the opinion that:

The proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s). However, since the safety concerns which need to be included to the RMP may be revised, the RMM may need to be updated too.

3.4.4. Summary of the risk management plan

The public summary of the RMP may require revision.

3.4.5. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.4 could be acceptable if the applicant implements the changes to the RMP as detailed in the endorsed Rapporteur assessment report and in the list of questions in section 6.3.

The applicant is reminded that in case of a Positive Opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

3.5. Pharmacovigilance

3.5.1. Pharmacovigilance system

It is considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

3.5.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required.

4. Non-conformity with agreed Paediatric Investigation Plan

Not applicable.

5. Benefit risk assessment

5.1. Therapeutic context

5.1.1. Disease or condition

The following indication is claimed:

> *Nidlegy is indicated for the neoadjuvant treatment of adult patients with locally advanced fully resectable melanoma.*

According to the current definition for melanoma staging by the American Joint Committee on Cancer (AJCC) (8th edition) this includes melanoma with stage III B, C, and D.

Patients with stage IIIB disease have a reported 10-year survival rate of 77%. The majority of the patients in the pivotal trial had stage IIIC disease. Patients with stage IIIC disease have a reported 10-year survival rate of 60% (CA Cancer J Clin 2017; 67(6):472-492).

The neoadjuvant treatment with Nidlegy (L19IL2/L19TNF) aims to provide an additional treatment option for patients with resectable melanoma prior surgical removal of the tumour and adjuvant therapy. The product will be given as intratumoural injection prior to surgery.

The rationale for neoadjuvant therapy with L19IL2/L19TNF is to provide immune stimulation, while the tumour is still present, potentially resulting in a more robust antitumour immune response. The aim of the new treatment is to enhance relapse-free survival and ultimately overall survival in the targeted melanoma population.

5.1.2. Available therapies and unmet medical need

According to the most recent ESMO guideline (Michielin et al.; 2019), current standard treatment for locally advanced melanoma consists of upfront surgery followed by adjuvant systemic therapy which includes PD-1 inhibitors (nivolumab and pemprolizumab) as well as targeted therapies including the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib in patients with a BRAF V600 mutation. The International Neoadjuvant Melanoma Consortium Informs that "patients with clinical stage III disease remain at a high risk of recurrence even with these adjuvant therapy advances. Therefore, improving existing therapies, innovating new therapeutic drugs, and investigating new combination regimens is greatly needed in the neoadjuvant setting (i.e., drug is given before definitive resection) for patients with high-risk clinical stage III melanoma" (Amaria et al. Lancet Oncol. 2019).

Patients with lymph-node involvement (stage III) and/or in-transit/satellite metastases, have a high risk of local and distant recurrence after surgery, with 5-year survival dropping down to 39-70% (Balch et al.; 2009). Despite the availability of adjuvant therapy, relapse rates remain high ranging between 30% and 90% (Michielin et. al.; 2020; Rutkowski et al.; 2022).

With regard to neoadjuvant therapy, several approaches have already been investigated, such neoadjuvant immunotherapy (e.g., pembrolizumab, the combination of relatlimab and nivolumab, and the combination of nivolumab and ipilimumab) and intralesional treatment (e.g., T-VEC). Currently, no neoadjuvant therapies for locally advanced, resectable melanoma are authorised by the European Commission, but encouraging results have recently been published for nivolumab plus ipilimumab as neoadjuvant therapy (Blank et al. NEJM. 2024) and already referred to in literature as the new standard of care (O'Leary. Nature Medicine. Research Highlight. 2024).

5.1.3. Main clinical studies

Efficacy data are based on the results from the single pivotal phase 3 study PH-L19IL2TNF-02/15. This was an open-label, randomised, controlled, multi-center study to evaluate the efficacy of L19IL2/L19TNF neoadjuvant intratumoural treatment (L19IL2: 13 Mio IU; L19TNF: 400 µg) followed by surgery (n=127) versus surgery alone (n= 129) in patients with clinical stage III B/C melanoma with/without prior therapy and presence of injectable cutaneous and/or subcutaneous or nodal metastases. The primary efficacy analysis has been conducted with a data cut-off 03-May-2023. The follow-up for RFS and OS is still ongoing.

Safety data are derived from studies PH-L19IL2TNF-02/12, PH-L19IL2TNF-02/15 and PH-L19IL2TNF-01/18. The safety database includes a total of 367 melanoma patients.

5.2. Favourable effects

A difference in median Relapse Free Survival (RFS) was observed with 17.2 months (95%CI: 11.1-26.3) in the treatment arm and 6.8 months (95%CI: 5.9-11.2) in the control arm (HR: 0.57; 95%CI: 0.39-0.82; log-rank p=0.002).

A post-hoc analysis of Event-Free Survival (EFS) resulted in a median EFS of 8.9 months in the treatment arm and 6 months in the control arm (HR: 0.81; 95%CI:0.59-1.11; log-rank p=0.188).

5.3. Uncertainties and limitations about favourable effects

Efficacy data are based on a single pivotal controlled trial. The treatment effects observed have not been replicated. A second phase 3 study (PH-L19IL2TNF-02/18) in the same indication and with similar design is ongoing; however, results from this study are not expected in short term and it is unclear how the enrolment is going.

The study was open label; thereby, the risk of bias was not minimised. The study integrity is likely affected by knowledge of interim results. Hence, data-driven decision-making cannot be entirely excluded as important amendments (i.e.. change of the primary endpoint) have been made to the protocol after interim results were available.

The usefulness of the RFS analysis for the determination of benefit is questioned, given that the choice of endpoint is considered suboptimal in this setting and the selected censoring rules seem to include informative censoring as participants who did not undergo surgery or were not-NED at surgery were censored at randomisation.

EFS is considered of higher value and analyses were provided post-hoc. However, EFS results are not considered to be clinically meaningful and robust. All EFS analyses performed by the applicant led to a smaller treatment effect compared to the primary RFS analysis and only two reached nominal significance; hence, the robustness of the results is questioned. It seems that the treatment effect only holds if some – or all – of the negative impacts of treatment are ignored. There seems to be an increased risk of progression or failure to perform an R0 resection due to the delay in surgery in the experimental arm, counteracting the potential favourable effects on RFS.

The provided OS results are currently too immature and final OS data are needed to evaluate any support for the claim of efficacy.

Adjuvant therapy was allowed at investigator's discretion. Due to the enrolment time frame (approx. eight years) and the change in treatment landscape, it remains questionable whether the use adjuvant therapy reflects current clinical practice.

5.4. Unfavourable effects

The incidence of adverse events and serious adverse events was higher in the L19IL2/L19TNF treatment arm 1 compared to the control arm 2 (AEs: 95.9% vs. 44.2%; SAEs: 14.9% vs. 8.1%). However, this was to be expected, as the patients in the control arm only underwent surgery, due to the selected application route (intratumoural) of L19IL2/L19TNF. Therefore, no drug related adverse events were reported for the control arm.

The key safety findings reported by **PT** in the overall safety population (N=367) included the following:

- **Any AEs** (incidence≥ 15%) were injection site reaction (Arm 1: 62.6% vs. Arm 2: 0%), pyrexia (Arm 1: 50.8% vs. Arm 2: 2.3%), chills (Arm 1: 43.1% vs. Arm 2: 0.6%), nausea (Arm 1: 20.0% vs. Arm 2: 2.3%), headache (Arm 1: 16.9% vs. Arm 2: 0.6%) and fatigue (Arm 1:16.4% vs. Arm 2: 1.2%).
- **Any SAEs** (incidence ≥ 1%) were injection site reaction (Arm 1: 4.6% vs. Arm 2: 0%), pyrexia (Arm 1: 1.5% vs. Arm 2: 0%), drug hypersensitivity (Arm 1: 1.5% vs. Arm 2: 0.6%), hypotension (Arm 1: 1.5% vs. Arm 2: 0%), postoperative wound infection (Arm 1: 1.0% vs. Arm 2: 0%), wound infection (Arm 1: 0% vs. Arm 2: 1.7%) and pulmonary embolism (Arm 1: 1.0% vs. Arm 2: 0%).

The key safety findings reported by **PT** in the overall safety population related to L19IL2/L19TNF (N=195) included the following:

- **AEs related to L19IL2/L19TNF** (any grade, incidence≥ 10%) were injection site reaction (62.2%), pyrexia (50.8%), chills (43.1%), nausea (19.0%), fatigue (15.9%) and headache (15.4%).
- **AEs of grade 3 related to L19IL2/L19TNF** (incidence ≥ 1%) were injection site reaction (11.8%), drug hypersensitivity (1.5%), hypertension (1.5%), gamma-glutamyltransferase increased (1.0%), syncope (1.0%) and hypotension (1.0%).
- **SAEs related to L19IL2/L19TNF** (incidence ≥ 1%) was injection site reaction (4.6%), pyrexia (1.5%), drug hypersensitivity (1.5%), and hypotension (1.0%).
- **AEs leading to discontinuation of L19IL2/L19TNF** (incidence ≥ 1%) were injection site reaction (9.2%), drug hypersensitivity (1.5%) and pyrexia (1.5%).

5.5. Uncertainties and limitations about unfavourable effects

- Uncertainties regarding the drug exposure, dose selection/administration, dose reduction and discontinuation during the pivotal study. More information is required.
- It currently appears that females have a higher safety risk than males. This should be discussed and further information is required.
- Safety data of patients ≥85 years is very limited.

5.6. Effects table

Table 68. Effects table for Nidlegy for the neoadjuvant treatment of locally advanced fully resectable melanoma (data cut-off: efficacy: 3 May 2023; safety: 29 Nov 2024)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
RFS	Time from randomisation until date of first recurrence.	M	17.2 (11.1 – 26.3)	6.8 (5.9 – 11.2)	HR: 0.57 (0.39 – 0.82) p=0.005 Based on one single pivotal study. Immature OS data Adequacy of censoring rules for RFS is questioned: patients not-NED at surgery and cancellation of surgery censored (see EFS). Change of primary endpoint after 2 interim analyses potentially data driven.	PH-L19IL2TNF-02/15

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
EFS	Time from randomisation until date of first recurrence, death, no NED at surgery or cancellation of surgery	M	8.9	6.0	HR: 0.81 (0.59 – 1.11) P = 0.188 Post-hoc analysis The robustness of EFS is unclear as results are highly dependent on chosen censoring rules. Clinical relevance of result unclear.	
Unfavourable Effects Applicant's overall population (N=367)*						
Tolerability						
	All cause AE	N, %	187/195, 95.9%	76/172, 44.2%	Uncertainties related to drug exposure, dose selection/administration, reduction and discontinuation during the pivotal study	2.7.4 SCS, Appendix Table 24 Table 25 Table 26 Table 27 Table 28
	All cause SAE	N, %	29/195, 14.9%	14/172, 8.1%		
	AEs related to L19IL2/L19TNF	N, %	183/195, 93.6%	-		
	SAEs related to L19IL2/L19TNF	N, %	20/195, 10.3%	-		
	AEs leading to discontinuation **L19IL2/L19TNF related**	N, %	29/195, 14.9%	-		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Injection site reaction	All cause **L19IL2/L19TNF related** - Grade ≥3 - Serious - Led to drug discontinuation - Led to dose reduction - Led to dose interruption	N, %	122/195, 62.2% - 23/195, 11.8% - 9/195, 4.6% - 18/195, 9.2% - 10/195, 5.1% - 27/195, 13.8%	- - - - - -		2.7.4 SCS, Appendix Table 24 Table 25 Table 27 Table 28 Table 30 Table 32

Abbreviations: ADR, adverse drug reaction; AE, adverse events; DMFS, Distant metastasis-free survival; EFS, Event-Free-Survival, HR, Hazard ratio; N, number of participants; RFS, Relapse-free survival; SAEs, serious adverse events;

Notes: Safety results are shown as submitted with the updated dossier (data cut-off 29/11/2024).

* overall population: pooled data from the studies PH-L19IL2TNF-02/12 (uncontrolled phase 2 study), PH-L19IL2TNF-02/15 (pivotal phase 3 study) and PH-L19IL2TNF-01/18 (phase 3 study).

5.7. Benefit-risk assessment and discussion

5.7.1. Importance of favourable and unfavourable effects

Based on the pre-specified primary RFS analysis, treatment with L19IL2/L19TNF administered as intratumoural injection in the neoadjuvant setting prior to surgery resulted in a 10-month improvement in the recurrence-free survival in patients with locally advanced melanoma. Additional data from secondary endpoints include analysis of LRFS, DMFS, Pathological complete responses.

Despite this favourable effect observed in the primary analysis. The usefulness of the RFS analysis for the determination of benefit is questioned, given that the choice of endpoint is considered suboptimal in this setting as the primary definition of RFS censors participants who did not undergo surgery or were not-NED at surgery at randomisation. An EFS analysis is considered of higher value and analyses were provided post-hoc during the procedure. However, provided EFS results are not considered to be clinically meaningful nor robust. Moreover, a data-driven decision surrounding the change of the

primary endpoint from „1-year RFS" to „RFS" cannot be excluded, which questions the study integrity. Additionally, data on OS are currently too immature to enable conclusions on long-term benefit.

The efficacy data provided in the single pivotal trial PH-L19IL2TNF-02/15, are overall are lacking sufficient internal and external validity, clinical relevance, and consistency. It is also uncertain whether the treatment effect would hold in the target population, considering the change in treatment landscape. The results from the second ongoing phase 3 study (PH-L19IL2THF-01/18) may provide further clarity. Study results, however, are currently not available, not expected in short term, and it is unclear how the enrolment is overall going.

Based on all these shortcomings, the benefit of Nidlegy cannot be determined.

The most important safety concern appears to be injection site reaction. The adverse events appear generally manageable and acceptable in this disease population. However, there are still some uncertainties with respect to patient's exposure, how the dose selection/administration and dose adjustment in the pivotal study was performed and how comparability of the data in the clinical studies was ensured, which need to be clarified.

5.7.2. Balance of benefits and risks

As outlined above the benefit of Nidlegy cannot be determined. The risks are considered acceptable for a life-threatening disease such as melanoma. Moreover, numerous quality major objections remain unsolved at this stage.

5.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

N/A

Marketing authorisation under exceptional circumstances

N/A

Health care provider engagement

The European Organisation for Research and Treatment of Cancer (EORTC) shared their view on the marketing application for Nidlegy during the initial phase of the assessment.

The medical need for stage III melanoma patients, despite the availability of adjuvant treatments was agreed. The potential of neoadjuvant therapy to provide additional benefit as compared to surgery and adjuvant therapy alone was highlighted. The proposed 4-week duration L19IL2/L19TNF treatment appeared appropriate for the EORTC. Given this short duration of treatment patients are likely to find the grade 3 side effects (e.g., injection site reactions) manageable.

The importance of benefit in Overall Survival as important outcome to be ultimately achieved for new melanoma treatments was emphasised. Improvements in health-related quality of life were considered as equally important besides reductions in melanoma recurrence rates.

5.8. Conclusions

The overall benefit /risk balance of Nidlegy is negative.