Programmed death ligand 1 (PD-L1)-PET imaging in patients with (Diffuse) Large B-cell Lymphoma who are treated with CD19-directed CAR T-cell therapy: a pilot study.

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PROTOCOL TITLE:

Programmed death ligand 1 (PD-L1)-PET/CT imaging in patients with (Diffuse) Large B-cell Lymphoma who are treated with CD19-directed CAR T-cell therapy.

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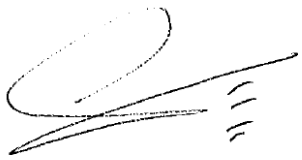
Principal investigator: T van Meerten, MD, PhD		12/01/2022

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

^{89}Zr	Zirconium-89
ALK	Anaplastic Lymphoma Kinase
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
ANC	Absolute neutrophil count
ANCA	Anti-neutrophil cytoplasmic antibody
anti-dsDNA	anti-double stranded DNA
AUC	Area under the concentration-time curve
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BA CRO	Bioanalytical contract research organization.
BUN	Blood urea nitrogen
CBC	Complete blood count
CD	Cluster of differentiation
C_{max}	Maximum serum concentration
CNS	Central nervous system
C_{PET}	Serum concentration at the time of the ^{89}Zr-atezolizumab-PET scan
(LD-)CT	(Low dose-) Computed Tomography
CTLA4	cytotoxic T-lymphocyte antigen-4
CTCAE	Common Terminology Criteria for Adverse Events
DNA	Deoxyribonucleic acid

DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EudraCT	European drug regulatory affairs Clinical Trials
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HER2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
IC	Immune checkpoint inhibitor
IHC	Immunohistochemistry
IMPD	Investigational Medicinal Product Dossier
INR	International normalized ratio
irAE	Immune related adverse event
IST	Investigator sponsored trial
LDH	Lactate dehydrogenase
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NSCLC	Non-small-cell lung cancer

ORR	Overall response rate
PBMCs	Peripheral blood mononuclear cells
PD	Pharmacodynamics
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PET	Positron emission tomography
PK	Pharmacokinetics
RBC	Red blood count
RNA	Ribonucleic acid
(S)AE	(Serious) Adverse Event
SST	Serum separator tube
SUSAR	Suspected Unexpected Serious Adverse Reaction
SUV	Standardized uptake value
Tmax	Time to reach maximum serum concentration
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
WBC	White blood count
WFI	Water for Injection
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

1. SUMMARY

1.1 Rationale:

Anti-CD19 Chimeric Antigen Receptor (CAR)-T cell therapy has changed the treatment landscape of patients with (Diffuse) Large B-cell Lymphoma (LBCL) and other types of Non-Hodgkin Lymphoma (NHL). Patients with LBCL who do not respond to first-line therapy, have a relapse within 6 months (primary refractory), or after second-line therapy, including high-dose chemotherapy and autologous stem cell rescue, have a poor prognosis and only 6% of these patients have a long-term survival. The recent results of 3 pivotal studies with 3 different anti-CD19 CAR T-cell products administered to patients with relapsed/refractory (R/R) LBCL resulted in high response rates and long-term remissions in almost half of the patients. Unfortunately, some patients do not respond to CAR T-cell therapy and their prognosis is extremely poor.

Possible mechanisms of non-responsiveness include loss of CD19 expression on the tumor cells, upregulation of immune checkpoint proteins such as programmed death-ligand 1 (PD-L1, CD274) or impaired T-cell function due to an immunosuppressive microenvironment. However the role each of these mechanisms play in the case of a diminished or non-response to CAR T-cell therapy is not yet defined.

Engagement of the PD-1/PD-L1 pathway is known to result in the reduction of T-cell activation, proliferation, and survival. Moreover, PD-L1 expression can be altered by radiotherapy, used as bridging therapy in CAR T-cell therapy. Hypothetically, patients with high or upregulated PD-L1 expression are more likely to be non-responders to CAR T-cell therapy and these patients might benefit from additional PD-1/PDL-1 blocking therapy. However, the exact role of PD-L1 expression in CAR T-cell therapy is not yet defined.

Currently, predicting tumor PD-L1 expression is not optimal, because it requires invasive sequential biopsies that are often subject to the errors and limitations of invasive tissue collection. Recently, positron-emission tomography computed topography (PET/CT) imaging with ^{89}Zr-atezolizumab (formerly known as ^{89}Zr-MPDL3280A), a PET-labeled antibody against PD-L1, was found to correlate better with response to immune checkpoint inhibition than immunohistochemistry- or RNA-sequencing-based predictive biomarkers in different solid tumors. By performing a ^{89}Zr-atezolizumab-PET/CT scan prior to CD19-directed CAR T-cell therapy PD-L1 expression could be defined in a non-invasive manner.

Additionally, repeated ^{89}Zr-atezolizumab-PET/CT scan in the case of a suspected relapse or non-response might distinguish between lymphoma activity and a treatment-related inflammatory reaction without the need to take invasive biopsies. Fluor-18-deoxyglucose (^{18}F-FDG)-PET/CT scans are currently used for the diagnosis, staging, and evaluation of response in patients with NHL. However, response assessment with ^{18}F-FDG-PET/CT may regularly be false-positive as a result of pseudo-progression or local immune activation (histiocytic or sarcoid-like reactions). Most often these reactions are mediated by macrophages/histiocytes which are known to express PD-L1 at high levels on their cell surface compared to lower levels on malignant B-cells. Therefore, a ^{89}Zr-atezolizumab could be used to differentiate between a relapse/non-response or a treatment-related inflammatory reaction.

1.2 Objectives:

Primary objectives: The first primary objective is to study the expression of PD-L1 in normal tissue and lymphoma lesions before CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate the pretreatment ^{89}Zr-atezolizumab distribution to the

response to CD19-directed CAR T-cell therapy thereby identify clinically relevant PD-L1 expression.

The second primary objective is to study whether the amount of ^{89}Zr-atezolizumab uptake, measured by the intensity of ^{89}Zr-atezolizumab PET/CT imaging (SUV), can be used to differentiate between lymphoma activity and a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) in patients with an end-of-treatment ^{18}F-FDG-positive PET/CT signal.

Secondary objectives:

a) To correlate the pretreatment ^{89}Zr-atezolizumab distribution to CAR T-cell peak expansion and persistence.

b) To correlate the pretreatment ^{89}Zr-atezolizumab uptake to CAR T-cell therapy related grade 1-5 adverse events (cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity (ICANS)).

c) To correlate tumor ^{89}Zr-atezolizumab uptake with tumor and immune cell PD-L1-expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy.

d) To compare the ^{89}Zr-atezolizumab distribution in irradiated versus non-irradiated lymphoma lesions in patients who require radiotherapy as a bridging strategy prior to CAR T-cell infusion. If possible, these results will be compared to tumor and immune cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy of an irradiated lymphoma lesion.

e) To determine the incidence of a treatment-related inflammatory signal on ^{18}F-FDG-PET/CT scan (histiocytic/sarcoid-like reaction) after CAR T-cell therapy.

1.3 Study design:

This is a single-center, single-arm pilot trial designed to evaluate the expression of PD-L1 in patients with LBCL and its role in non-responsiveness to CAR T-cell therapy in a non-invasive manner. Moreover, we aim to study the possibility of PD-L1 PET/CT imaging to distinguish between lymphoma activity and a treatment-related inflammatory signal (histiocytic/sarcoid-like reaction) in case of a relapse or non-response after CAR T-cell therapy.

1.4 Study population:

Patients with relapsed/refractory LBCL after 2 prior lines of therapy and who qualify for CD19-directed CAR T-cell therapy. Eligibility for CAR T-cell therapy is based on the criteria posed by the Dutch Immune Effector Cell working group tumor board.

1.5 Intervention:

In this imaging trial, the purpose is to explore the feasibility of anti-PD-L1 PET/CT imaging in patients to gain insights into clinically relevant PD-L1 expression in the setting of CD19-directed CAR T-cell therapy. Patients with R/R LBCL after 2 prior lines of therapy will receive standard of care CD19-directed CAR T-cell therapy according to the eligibility criteria as formed by the Dutch Immune Effector Cell tumor board.

1.6 Main study parameters/endpoints:

Primary endpoints:

- to study the expression of PD-L1 in normal tissue and lymphoma lesions before CD19-directed CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate pretreatment ^{89}Zr-atezolizumab uptake to response to CD19-directed CAR T-cell therapy and thereby identify clinically relevant PD-L1 expression. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.
- To study whether the amount of ^{89}Zr-atezolizumab uptake, measured by the intensity of ^{89}Zr-atezolizumab PET/CT imaging (SUV), can be used to differentiate between lymphoma activity and treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) in patients with an end-of-treatment ^{18}F-FDG-positive PET/CT signal.

Secondary endpoints:

- To correlate the pretreatment 89Zr-atezolizumab distribution to CAR T-cell peak expansion and persistence.
- To correlate the pretreatment 89Zr-atezolizumab uptake to CAR T-cell therapy related grade 1-5 adverse events (cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS)).
- To correlate tumor 89Zr-atezolizumab uptake with tumor and immune cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy.
- To compare the 89Zr-atezolizumab distribution in irradiated versus non-irradiated

lymphoma lesions in patients who require radiotherapy as a bridging strategy prior to CAR T-cell infusion. If possible, these results will be compared to tumor and immune cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy of an irradiated lymphoma lesion.

- To determine the incidence of a treatment-related inflammatory signal on 18F-FDG-PET/CT scan (histiocytic/sarcoid-like reaction) after CAR T-cell therapy.

1.7 Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

For this imaging study, patients have to make a maximum of 6 extra visits to the clinic for screening, ^{89}Zr-atezolizumab injection, a PET/CT-scan visit and a biopsy taken within 7 days of the PET-scan visit. In case of an end-of-treatment positive ^{18}F-FDG PET/CT signal two visits are needed for the second ^{89}Zr-atezolizumab injection and the PET/CT-scan. The biopsy taken afterwards is part of standard procedure of care.

In case of bridging with radiotherapy and depending on the accessibility of the tumor lesions, patients will be asked if they are willing to undergo an extra biopsy. This extra biopsy will not be obligatory to participate in the study. In practice, most procedures will be combined with visits to the hospital in the context of clinical care, to minimize patient burden.

The intravenous tracer injection ^{89}Zr-atezolizumab is between day -19 to day -10 of the CAR T-cell infusion (depending on time needed for bridging strategy). All patients will be observed for at least 30 minutes after ^{89}Zr-atezolizumab injection to monitor for possible acute infusion related adverse events. The subsequent ^{89}Zr-atezolizumab PET/CT imaging scan is 4 or 7 days (day -15 to day -6). The PET/CT-imaging is followed by a biopsy within 7

days, but the biopsy will always take place before day -5. Optimal time point for the ^{89}Zr-atezolizumab-PET/CT-scan will be determined in the first 3 patients, as they will receive a ^{89}Zr-atezolizumab-PET/CT-scan on day 4 and 7.

In case of an end-of-treatment ^{18}F-FDG-positive PET/CT signal another ^{89}Zr-atezolizumab injection will be given followed by a ^{89}Zr-atezolizumab PET/CT imaging and a biopsy within 7 days (standard procedure).

The radiation burden following administration of 37 MBq of ^{89}Zr-atezolizumab is 18.1 mSv, in addition to 1 mSv per low-dose attenuation correction CT-scan. Thus, patients will receive 37 or 74 MBq doses of ^{89}Zr-atezolizumab and undergo to 1 or 2 low-dose CT-scans. The radiation exposure will be 19.1 mSv per imaging round. Besides PET/CT imaging, patients will be asked to provide 12 blood samples (108 mL), which are taken in combination with standard clinical and outpatient care. The easiest and safest accessible tumor lesion will be biopsied within 7 days of the ^{89}Zr-atezolizumab PET/CT scans. Based on a literature review, the risk of tumor biopsies is considered low with a small risk of significant or major complications or death. The risk associated with the ^{89}Zr-atezolizumab is considered acceptable based on extensive preclinical testing of separate components and clinical safety data from the first-in-human ^{89}Zr-atezolizumab-study where only one low-grade adverse event (pruritus, grade 1) has been reported. Although patients do not directly benefit from this study, results from this study will be valuable for our understanding of the tumor immune response and will guide further prospective research and hopefully, treatment decisions. After participation within the imaging trial, eligible patients will proceed with CD19-directed CAR T-cell treatment, provided they continue to meet the eligibility criteria to receive CD19-directed CAR T-cells.

2. INTRODUCTION AND RATIONALE

2.1 Disease background:

(Diffuse) Large B-cell Lymphoma (LBCL) is an aggressive cancer and the most common B-cell Non-Hodgkin Lymphoma (NHL) and if left untreated, fatal within months. Patients with LBCL who do not respond to first-line therapy, have a relapse within 6 months (primary refractory), or after second-line therapy, including high-dose chemotherapy and autologous stem cell rescue, have a poor prognosis with long term survival in only 6% (1).

2.2 Anti-CD19 CAR T-cell therapy, principle

CD19-directed Chimeric Antigen Receptor (CAR) T-cell therapy is a novel cellular treatment approach that has been studied in patients with relapsed/refractory LBCL. CD19-directed CAR T-cells are generated from autologous T-cells collected during apheresis during a multi-step process. The collected white blood cells will be enriched for T-cells and via viral transduction the genomic code for the anti-CD19-CAR and a costimulatory domain, CD28, will be introduced into the T-cells. Subsequently, these generated CD19-directed CAR T-cells are multiplied, cryopreserved and shipped to the treatment site, the place where the cells are infused, in a single infusion, into the patient. The process of collection of cells, the genetic modification, expansion, quality check, transports, and finally, the infusion of cells may take up to 4 weeks (2).

Prior to CAR T-cell infusion (day 0), the patients will receive a lympho-depleting chemotherapy regimen (fludarabine-cyclophosphamide) to create a more favorable environment for the CAR T-cells and to minimize the risk of premature CAR T-cell elimination by the endogenous T-cells (day -5 to day -3). Thus, after CAR T-cell infusion, the expansion of T-cells is considered to be the expansion of only CAR T-cells (3).

2.3 CD19-directed CAR T-cell therapy, results

Recently, the results of 3 pivotal studies with 3 different CD19-directed CAR T-cell products administered to patients with relapsed/refractory DLBCL were presented. Patients received a single injection of CAR T-cells, and all studies reported long-term remissions and disease-free survival for more than 2 years in about half of the patients (2, 4, 5). For example, in the ZUMA-1 study, an infusion of 2×10^6 CD19-directed CAR T-cells per kg of body weight resulted in a complete response (CR) rate of 58%. Of those who achieved a CR at month 3, 72% have a progression-free survival (PFS) of more than 2-years (2, 6). This milestone is of high importance, as the 2-year PFS in this disease is a surrogate for the overall survival (7).

2.4 Bridging therapy

As the process of CAR T-cell manufacturing and infusion may take up to 4 weeks some patients (with highly aggressive disease or bulky masses) might require bridging therapy to be able to receive the CAR T-cells. Radiation therapy is an acknowledged approach as a bridge to CAR T-cell therapy (8, 9). In general, after apheresis, patients may receive radiotherapy (usually 20Gy in 5 fractions) to reduce total tumor burden, on bulky or symptomatic sites, or highly proliferative/progressive lesions (near vital organs), while preserving lymphoma lesions that are not directly necessary to irradiate. In this way, the remaining lymphoma lesions will ascertain the necessary antigen exposure for CAR T-cell activation and proliferation. In case of clinical need, there might be a possibility that a patient will be bridged with systemic therapy like chemotherapy or steroids or a combination of systemic therapy and radiotherapy.

2.5 Mechanisms of CAR T-cell therapy failure

Unfortunately, half of the patients do not respond to CD19-directed CAR T-cell therapy and their prognosis is extremely poor. (10) Several mechanisms of resistance to CAR T-cell therapy have been identified, namely loss of CD19 expression, impaired T-cell function due to lost death receptor signaling and a T-cell unattractive tumor microenvironment related to M2 macrophages. (11,12,13)

In addition, the existence of a number of immunosuppressive pathways can restrict the full potential of CAR T-cell therapy,as increased expression of these pathways can limit the duration and strength of the adaptive T-cell response(14, 15, 16) This includes increased expression of inhibitory immune receptors following T-cell activation such as T-cell membrane protein-3 (TIM-3), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and/or programmed cell death-1 (PD-1) or its ligand programmed cell death ligand 1 (PD-L1) on T-cells. Moreover, PD-L1 expression can be altered by radiotherapy, nowadays used as bridging strategy before CAR T-cell therapy, through the mechanism of enhanced interferon signaling of CD8+ T-cells. (17) However, the role of the PD-1/PD-L1 axis in CAR T-cell therapy is not yet defined.

In general, the PD-1/PD-L1 axis is upregulated on many different tumor types. Blocking this pathway using antibodies and thereby restoring natural immune response against cancer cells has shown to be a promising therapy in patients with advanced melanoma, non-small cell lung, renal cell, as well as hematologic malignancies. (18, 19, 20) Response to blockade therapy and level of PD-L1 expression on tumor cells are significantly correlated. (21)

Moreover, Zolov et al. recently demonstrated that the upregulation of the PD-1/PD-L1 axis leads to diminished cytokine production and proliferative capacity of CD28-costimulated

CAR T-cells. Thereby emphasizing the possible contribution of the PD-1/PD-L1 pathway to unresponsiveness to CAR T-cell therapy. (22)

To identify patients who are likely to benefit from PD-L1 blockade therapy accurate diagnostics of expression levels are needed. Nowadays immunohistochemistry is used to quantify these levels, but unfortunately it is subjected to errors and limitations of invasive tissue collection.

Positron-emission tomography computed tomography (PET/CT) imaging with Zirconium-89-labeled anti-PD-L1 antibodies (^{89}Zr-atezolizumab) is a non-invasive approach that might identify patients with R/R DLBCL with clinically relevant PD-L1 expression. (23)

In addition to the identification of patients with relevant PD-L1 expression, PET/CT PD-L1 imaging might also identify patients with false positive ^{18}F-FDG PET scans after CAR T-cell therapy. ^{18}F-FDG-PET/CT scans are the gold standard for staging and response assessment in DLBCL, but ^{18}F-FDG-PET/CT scans are inaccurate in differentiating between lymphoma activity or a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction), as these nonmalignant events also consume high levels of glucose. Evaluation of biopsies taken from ^{18}F-FDG-avid lesions of two patients did not show lymphoma activity, but inflammatory changes compatible with a histocytic/sarcoid-like reaction and demonstrate a change to very high and intense PD-L1 staining compared to pre-CAR T-cell therapy biopsies. (data not published). Therefore, ^{89}Zr-atezolizumab PET/CT imaging could be used to distinguish between lymphoma activity or a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction).

2.6 Rationale for molecular imaging

As mentioned above, accurate quantification of PD-L1 expression can identify patients who will benefit from blockade therapy during CAR T-cell therapy and patients who develop a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) after CAR T-cell therapy.

However, the approved diagnostics are revealed to be inaccurate for predicting response to blockade therapy and require an invasive biopsy to perform quantification. In several cases lesions are not applicable for biopsies, causing a gap of essential information in developing an effective treatment strategy.

Recently, pre-treatment PD-L1 PET/CT imaging with ^{89}Zr-atezolizumab was found to better correlate with response than immunohistochemistry-or RNA-sequencing-based predictive biomarkers in different solid tumors. (23) Moreover, molecular imaging may be able to noninvasively monitor whole-body systemic and intratumoral PD-L1 expression serially before and during therapy, preventing misinterpretation due to tumor heterogeneity or sampling error. This would be an essential step in quantification of PD-L1 expression in a noninvasive manner, increasing the potential of identifying patients with a lower to non-response to CAR T-cell therapy due to PD-L1 expression and being able to provide this patient blockade therapy. Moreover, this imaging approach could be able to distinguish malignant tissue from inflammatory tissue and reduce the frequency of biopsies.

2.7 Anti-PD-L1 PET imaging

^{89}Zr-atezolizumab constitutes of the monoclonal anti-PD-L1 antibody atezolizumab labeled with the positron-emitter Zirconium-89, allowing whole-body PET-imaging of PD-L1 expression. The separate components Zirconium-89 (for tumor PET-imaging) and

atezolizumab (in higher dosage for the treatment of several cancers) have already been used in various studies and regular health care.

A first-in-human study showed safe injection of ^{89}Zr-atezolizumab, with only one related low-grade adverse event (pruritus, grade 1). The optimal tracer protein dosage was determined to be 10 mg (unlabeled atezolizumab in combination with 2,5 mg (37 MBq) of ^{89}Zr-atezolizumab), corresponding with a serum atezolizumab dosage 100-fold lower than reached with the recommend treatment dosage. Optimal imaging time points are 4 and 7 days after tracer injection.

Evaluation of ^{89}Zr-atezolizumab biodistribution showed high uptake in intestines, liver and kidney, reflecting antibody metabolism and elimination. Moreover, lymphoid tissue, including the spleen, uptake was high. All tumor localizations of non-small cell lung cancer, triple-negative breast cancer and bladder cancer showed evident uptake of ^{89}Zr-atezolizumab. (23)

3. OBJECTIVES

4.1 Primary objective:

- to study the expression of PD-L1 in normal tissue and lymphoma lesions before CD19-directed CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate pretreatment ^{89}Zr-atezolizumab uptake to response to CD19-directed CAR T-cell therapy and thereby identify clinically relevant PD-L1 expression. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.
- to study whether the amount of ^{89}Zr-atezolizumab uptake, measured by the intensity of ^{89}Zr-atezolizumab PET/CT imaging (SUV), can be used to differentiate lymphoma activity and treatment-related inflammatory signal (histiocytic/sarcoid-like reaction) in patients with an end-of-treatment ^{18}F-FDG-positive PET/CT signal. Confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of the positive FDG-PET lesion or in case of non-accessibility of the lesion by an experienced clinician via a wait-and-see strategy with repeated FDG-PET scans.

4.2 Secondary Objectives

- to correlate the pretreatment ^{89}Zr-atezolizumab distribution, as measured by the uptake of ^{89}Zr-atezolizumab on PET/CT imaging in lymphoma, to CAR T-cell peak expansion and persistence as measured in peripheral blood.
- To correlate the pretreatment ^{89}Zr-atezolizumab uptake to CAR T-cell therapy related

grade 1-5 adverse events (cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS)). Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring SUV on the ^{89}Zr-atezolizumab PET/CT scan. CAR-T cell therapy related toxicities such as CRS and ICANS will be assessed using 2018 ASBMT Consensus Grading (24).

- to correlate tumor ^{89}Zr-atezolizumab uptake with tumor and tumor infiltrating cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy.
- To compare the ^{89}Zr-atezolizumab distribution in irradiated versus non-irradiated lymphoma lesions in patients who require radiotherapy as a bridging strategy prior to CAR T-cell infusion. If possible, these results will be compared to tumor and tumor infiltrating cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy of an irradiated lymphoma lesion.
- To determine the incidence of a treatment-related inflammatory reactions (histiocytic/sarcoid-like reaction) after CAR T-cell therapy.

4. STUDY DESIGN

4.1 CAR T-cell therapy standard of care

The treatment of patients with a relapse or refractory DLBCL after 2 prior lines of therapy with CAR T-cell therapy (eg axicabtagene ciloleucel; Yescarta) is considered to be standard of care. The infusion of CAR T-cells is defined as ***Day 0***. Therefore, apheresis is performed ***Day -28***, as the manufacturing, quality controls and logistics of the autologous CAR T-cells will take up to 28 days. In case patients have bulky, symptomatic and/or high proliferative disease, debulking/bridging therapy (radiotherapy, systemic therapy (e.g. chemotherapy or steroids) or a combination) is allowed after apheresis (***Day -27*** to ***Day -10***). Prior to lympho-depleting chemotherapy with fludarabine and cyclophosphamide (***Day -5 to Day -3***), a standard ^{18}F-FDG PET/CT scan will be performed to determine the active disease status prior to the CAR T-cell therapy. Standard ^{18}F-FDG PET/CT response assessment will be performed at Months +1, +3, +6, and +12.

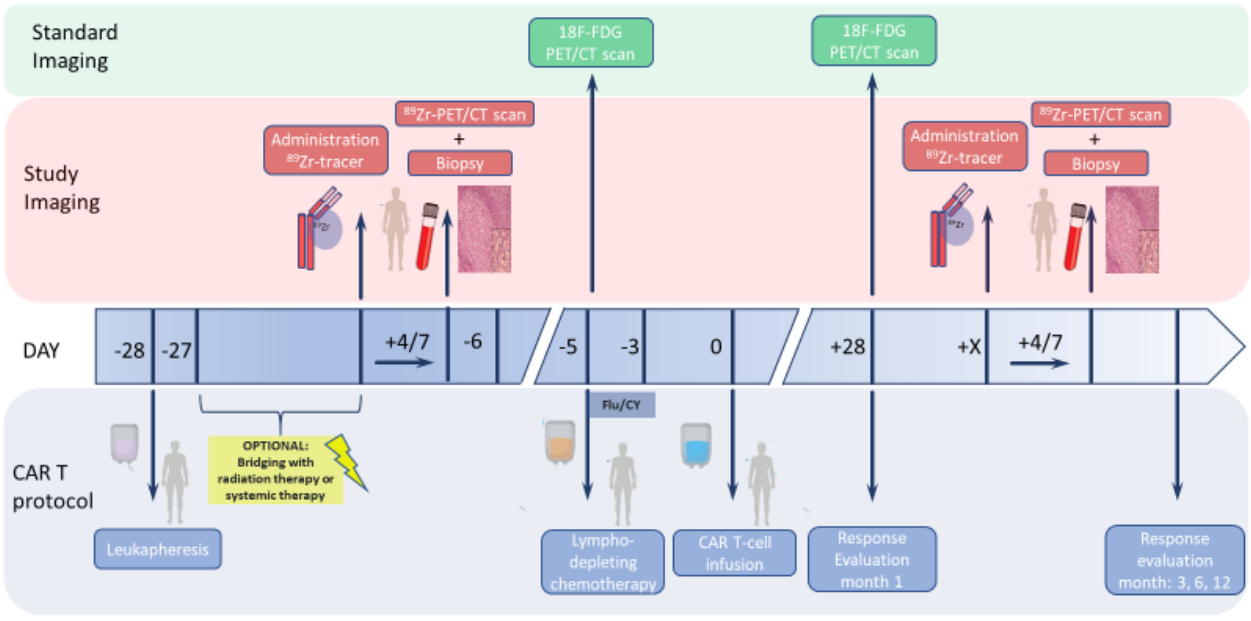

Figure 1. Overview of standard CAR T-cell therapy and the study procedures.

4.2 ^{89}Zr-atezolizumab PET/CT Imaging

The study will include ~20 patients that will be imaged with ^{89}Zr-atezolizumab PET/CT imaging. Patients will be imaged with ^{89}Zr-atezolizumab PET/CT imaging before CAR T-cell therapy (see figure 1). Patients who do have an end-of-treatment ^{18}F-FDG-positive signal will undergo a second ^{89}Zr-atezolizumab PET/CT scan.

In order to determine the PD-L1 expression, we first inject the tracer consisting of 10mg total protein tracer dosage (37 MBq (1 mCi)/~2,5mg ^{89}Zr-atezolizumab and unlabeled atezolizumab) at ***day -19 to day -10*** (depending on time needed for optional bridging therapy) subsequently the whole-body ^{89}Zr-atezolizumab PET scan will be acquired at ***day -15 to day -6*** *(*(4 or 7 days after tracer injection). The optimal time point of PET imaging scan 4 or 7 days after tracer injection will be determined in the first 3 patients.

SUVmax and SUVpeak (on tumor lesion) and the geometric SUVmax, SUVpeak (sum of all

tumor samples) will be determined by using spherical VOIs with predefined sizes.

Within 7 days of the ^{89}Zr-atezolizumab PET/CT scan but no later than day -6, a tumor biopsy will be obtained. For revealing tracer distribution at the microscopic level, formalin-fixed tumor sections (10 µM) of post-tracer tumor biopsies will be exposed to a phosphorimaging screen for 24 hr and will be scanned with a Cyclone phosphorimager. Subsequent sections of the same tumor tissue will be stained for hematoxylin and eosin, PD-L1, and T-cell and macrophage markers.

Safety is assessed according to CTCAEv5.0, CRS and ICANS grading will be evaluated according to the ASBMT criteria (24). CAR T-cell treatment response is assessed based on the

Lugano criteria (with ^{18}F-FDG-PET CT scans) at day 28, month 3, month 6, and at month 12 (standard procedure).

4.3 CAR T-cell expansion in peripheral blood

In addition, in these patients, CAR T-cell expansion in peripheral blood will be measured with peak levels at day 5-7, and persistence at day 7, 14, 28 with an anti-CAR T-cell monoclonal antibody. T-cell subsets (CD4 / CD8 ratio, different T-cell phenotypes) will be determined on day 5. Cytokines (IL-2, IL-6, IL-10, IFNγ) will be measured in serum at days -28, 0, 5, 7, 14, and 28 to determine the pro-inflammatory and immune-modulating cytokines in response to CAR T-cell therapy. Samples will be collected during venipuncture as part of the standard procedure, thereby minimizing patient burden.

4.4 Tumor Biopsy

As mentioned above tumor biopsies will be taken within 7 days of the ^{89}Zr-atezolizumab PET/CT scan. Moreover, in case of bridging with radiotherapy and depending on the accessibility of the tumor lesion, patients will be asked if they are willing to undergo an extra biopsy. This biopsy will be used to compare ^{89}Zr-atezolizumab uptake in a irradiated lesion on PET-imaging to PD-L1 expression measured via immunohistochemistry. This extra biopsy will not be obligatory to participate in the study.

PD-L1 expression in post-tracer tumor biopsies will be assessed centrally. We will evaluate PD-L1 staining on tumor cells (TC) and on tumor infiltrating immune cells (IC). PD-L1 expression will be scored as negative (IC0 or TC0: staining on <1% of IC or TC, respectively; IHC score 0) or positive (IC1/2/3 or TC1/2/3: staining on ≥1% of IC or TC; IHC score 1/2/3 depending on the highest staining for either IC or TC). In addition, we will evaluate T-cell infiltration as measured by CD8 and CD4 expression and will determine the polarization of macrophages and histiocytes. RNA from post-tracer tumor biopsies will be isolated for gene

expression analysis by TruSeq RNA Access RNA-seq (Q2Labsolutions).

Autoradiography of the biopsies will be performed using a phosphorimaging screen for 24 hr and will be then scanned with a Cyclone phosphorimager.

5. STUDY POPULATION

5.1 Population

Patients with R/R LBCL after two or more lines of therapy who fulfill the eligibility criteria for CD19-directed CAR T-cell therapy according the Immune Effector Cell Working Group Tumorboard.

5.2 Inclusion criteria

In order to be eligible to participate in this study, subject must meet the following:

1. Histologically confirmed DLBCL and associated subtypes, defined by WHO 2016 classification: DLBCL not otherwise specified (NOS), High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements with DLBCL histology (DHL/THL) and FL3B, Aggressive B-cell lymphoma, T-cell/histiocyte rich B-cell lymphoma, Primary mediastinal B-cell lymphoma, EBV+ DLBCL, transformed lymphoma (e.g. transformed follicular or marginal zone lymphoma)
2. Tumor lesion(s) of which a histological biopsy can safely be obtained according to standard clinical care procedures.
3. Measurable disease, as defined by *Lugano criteria* (see section 11.1)
4. If has history of CNS disease, then must have
    a. No signs or symptoms of CNS disease
    b. No active disease on magnetic resonance imaging (MRI)
    c. Absence of large cell lymphoma in cerebral spinal fluid (CSF) on cytospin preparation and flow cytometry, regardless of the number of white blood cells.

5. If has history of cerebral vascular accident (CVA)
    a. The CVA event must be >12 months prior to apheresis
    b. Any neurological deficits must be stable
6. Signed informed consent.
7. Age ≥18 at the time of signing informed consent.
8. Life expectancy ≥12 weeks.
9. Eastern Cooperative Oncology Group (ECOG) performance status 0-1
10. Ability to comply with the protocol.

5.3 Exclusion criteria

A potential subject, who meets any of the following criteria, will be excluded from participation in this study:

1. Signs or symptoms of active infection within 2 weeks prior to ^{89}Zr-atezolizumab injection, unless treated to resolution.
2. Prior CD19-directed CAR T-cell therapy or other bi-specific antibodies targeting CD19 receptor (e.g. blinatumomab).
3. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins.
4. Any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of ^{89}Zr-atezolizumab, or that may affect the interpretation of the results or render the patient at high risk from complications.

5.4 Sample size calculation

This exploratory study will be a proof-of-concept, open-label, single-center study to explore the expression of PD-L1 in normal tissue and lymphoma lesions before CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate these expression levels to response to CAR T-cell therapy. Thereby this study aims to define clinically relevant PD-L1 expression in CAR T-cell therapy. In addition, we will investigate the ability of ^{89}Zr-atezolizumab PET-imaging to differentiate between lymphoma activity and a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) in case of a suspected relapse or non-response.

All patients must have measurable disease at the start of apheresis as determined by ^{18}F-FDG PET/(diagnostic)CT scan. In case, radiation as bridging therapy is required, at least 1 non-irradiated lesion must remain, according to the general practice of CAR T-cell therapy, which will allows for ^{89}Zr-atezolizumab PET/CT imaging in both non-irradiated and irradiated lesions.

Based on previous studies, we estimate that approximately 50% of the patients will have a CR upon CAR T-cell therapy, and the vast majority of the patients will reach this CR within 3 months. Non-response or relapses will in general also occur within 3 months. We, therefore, consider inclusion of 20 patients (with ~10 relapses/non-responders) to yield sufficient information for this proof-of-concept study.

With an expected accrual rate of 1 patient a month, the study could be completed within 2 years.

6. TREATMENT OF SUBJECTS

6.1 Investigational treatment

For extensive information about the investigational product ^{89}Zr-atezolizumab see IMPDs *"IMPD ^{89}Zr-atezolizumab, version 8".*

In this study, patients will receive a defined dose of radioactivity of 37 MBq ^{89}Zr-atezolizumab and defined dose of approximately 10 mg of protein (sum of ^{89}Zr-atezolizumab and unlabeled atezolizumab).

^{89}Zr-atezolizumab conjugation and radiolabeling is performed in the radiopharmacy unit of the department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen. Previous results showed efficient radiolabeling with preservation of antigen-binding capacity. No radioactive impurities were detected. For further details, see the IMPD of ^{89}Zr-atezolizumab, version 8.

The ^{89}Zr-atezolizumab in combination with the unlabeled atezolizumab (total protein dosage 10mg, 37 MBq) will be injected as a continuous intravenous bolus injection followed by physiologic saline to flush the line (infusion rate will be comparable to the infusion rate of administration of a treatment of dose of atezolizumab (250ml/hr)).

The ^{89}Zr-atezolizumab PET/CT scan will be performed before CAR T-cell therapy using 2,5 mg of ^{89}Zr-atezolizumab (see Figure). In patients with a relapse or non-response after CAR T-cell therapy, a second ^{89}Zr-atezolizumab PET/CT series will be performed in the same manner.

6.2 CAR -T-cell therapy

The treatment of patients with a relapse or refractory DLBCL after 2 prior lines of therapy with CAR T-cell therapy (e.g., axicabtagene ciloleucel, Yescarta). The infusion of CAR T cells is

defined as ***Day 0***. Therefore, apheresis is performed ***Day -28***, as the manufacturing, quality controls and logistics of the autologous CAR T-cells will take up to 28 days. In case patients have bulky and/or high proliferative disease, debulking/bridging therapy (radiotherapy, systemic therapy (eg chemotherapy or steroids) or a combination) is allowed after apheresis (***Day -27*** to ***Day -10***). Prior to lymphodepleting chemotherapy with fludarabine and cyclophosphamide (***Day -5 to Day -3***), a standard ^{18}F-FDG PET/CT scan will be performed to determine the active disease status prior to the CAR T-cell therapy. Standard ^{18}F-FDG PET/CT response assessment will be performed at months +1, +3, +6, and +12.

6.3 Use of co-intervention

Female patients of childbearing potential and male patients with partners of childbearing potential must agree (by patient and/or partner) to use a highly effective form of contraception (e.g., surgical sterilization, a reliable barrier method, birth control pills, or contraceptive hormone implants)

6.4 Escape medication

Not applicable

7. INVESTIGATIONAL PRODUCT

All information on the investigational product ^{89}Zr-atezolizumab (^{89}Zr-MPDL3280A) can be found in the investigational medicinal product dossier (IMPD) of ^{89}Zr-atezolizumab : *"IMPD [89Zr]-Atezolizumab, version 8".*

7.1 Name and description of investigational product(s)

In this Clinical Trial Application, ^{89}Zr-atezolizumab will be administered in combination with unlabeled atezolizumab as an injection. The ^{89}Zr-atezolizumab injection contains a maximum amount of 2.5 mg ^{89}Zr-atezolizumab, supplemented with unlabeled atezolizumab to obtain a total protein dose of 10 mg.

Atezolizumab is a humanized IgG1 monoclonal antibody which targets human PD-L1 on antigen-presenting cells or tumor cells. It is provided as a solution of 60 mg/ml in 20 mM Histidine Acetate, 120 mM Sucrose, 0.04% PS20, pH 5.8.

^{89}Zr-atezolizumab is a radiolabeled monoclonal antibody directed against PD-L1. [89Zr] is a positron emitter with a physical half-life of 3.27 days. The ^{89}Zr-atezolizumab drug product is a clear, colorless liquid solution for iv administration which is free of visible particles. The product is sterile and free of endotoxins, with a pH of 6-7. The target radioactivity for ^{89}Zr-atezolizumab is 37 MBq at injection time, with a targeted volume of 2.5 mg.

7.2 Summary of findings from non-clinical studies

No toxicology or pharmacokinetics studies are performed on ^{89}Zr-atezolizumab based on abundant safety data available on the separate components. Several studies with ^{89}Zr-labeled antibody tracers have demonstrated safe use of the isotope. Safety studies have been performed on atezolizumab and showed safe use in patients. It is recently

registered for the treatment of urothelial carcinoma and non-small cell lung cancer. A summary of non-clinical studies is provided below. For more details, refer to the IMPD of ^{89}Zr-atezolizumab.

Binding studies of atezolizumab showed similar affinity to human and cynomolgus monkey T-cells. Therefore cynomolgus monkey was selected as primary and relevant nonclinical model. Furthermore, it was defined that atezolizumab binds to target with high affinity and completely blocks binding to receptors B7-1 (PD-L1) and PD-1.

Toxicology studies of atezolizumab were perfomed in mice and cynomolgus monkeys. The 8-week *in vivo* toxicity and toxicokinetic evaluation in Cynomolgus monkeys did not result in any local or systemic adverse effects, including no effects on safety pharmacology or clinical, immunologic and anatomic pathology endpoints.

In vitro internalization rates of ^{89}Zr-atezolizumab were determined in two tumor cell lines and healthy volunteers peripheral blood mononuclear cells (PBMCs). Tumor cells showed a high internalization rate, whereas lower rates were found in human PBMCs and T-cells. (23)

7.3 Summary of findings from clinical studies

First-in-human study, performed in the UMCG, to assess the feasibility and biodistribution of imaging with ^{89}Zr-atezolizumab included 25 patients. Tracer injection was safe, with only one related low-grade adverse event (pruritus, grade 1). The optimal tracer injection protocol was the combination of unlabeled atezolizumab administration with the radioactive labeled ^{89}Zr-atezolizumab (total protein dosage 10 mg, 2.5 mg ^{89}Zr-atezolizumab (37 MBq)) , and the optimal moment for PET scanning was 4 or 7 days after tracer administration. Pharmacokinetic analysis confirmed that the circulating tracer

dose corresponded with a serum concentration of atezolizumab reached with 0,1-0,3 mg/kg atezolizumab, which is almost 100-fold lower than that reached with recommend treatment dose. Day 4 blood pool, liver and kidney ^{89}Zr-atezolizumab uptake was comparable to the results of other ^{89}Zr-labeled antibody tracers.

The tracer showed high uptake in lymphoid tissue, at sites of inflammation and at all main metastatic sites. Low uptake was observed in the brain, subcutaneous tissue, muscle, compact bone and lung. No signs of toxicity were observed.

Moreover, the internalization rates of ^{89}Zr-atezolizumab in PBMCs of patients receiving ^{89}Zr-atezolizumab tracer injection were determined to be 0.3% and 0.4% on day 4 (n=2) and 0.6% on day 7(n=1).

^{89}Zr-atezolizumab tumor uptake, based on PET/CT imaging, was correlated to patient response to atezolizumab therapy (anti-PD-L1 blockade therapy) and to target lesion size change during atezolizumab therapy. Furthermore, the geometric mean SUVmax of ^{89}Zr-atezolizumab was significantly associated with progression free survival and overall survival even after adjustment of tumor type and tumor load, whereas PD-L1 IHC assays of the same patients showed moderate to poor discrimination for patient outcome. In conclusion, It has been shown that ^{89}Zr-atezolizumab is capable of detecting clinical significant PD-L1 expression. (23)

7.4 Summary of known and potential risks and benefits

Measuring the PD-L1 expression in patient tumors may help to understand the influence of PD-L1 expression in the effectiveness of CAR T-cell therapy and evaluate the possible diagnostic tool to identify a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) in patients with potential relapse after CAR T-cell

therapy. The potential future benefit of ^{89}Zr-atezolizumab is to enable systemic assessments of PD-L1 distribution at baseline to identify patients who will potentially benefit from PD-1/PD-L1 blockade therapy and after CAR T-cell therapy to identify treatment-related inflammatory reactions (histiocytic/sarcoid-like reaction) without the need for an invasive biopsy.

The potential risk associated with ^{89}Zr-atezolizumab administration is considered acceptable based on the non-clinical assessments and the clinical experience obtained so far. ^{89}Zr-atezolizumab administration has showed safety in the first-in-human study in het UMCG (See Section 7.3). Potential risks may include infusion-related reactions and injection site reactions. An additional risk associated with this study involves the exposure to radiation that is associated with all positron-emitting agents used in PET imaging studies. To date, several ^{89}Zr-labeled antibodies have been evaluated in vivo in animals and in clinical studies in patients without any sign of toxicity.

As there is radioactivity involved for tumor detection, the patient is exposed to ionizing radiation. The radioactive dose caused by a single administration of 37 MBq of ^{89}Zr-atezolizumab will be 18.1 millisieverts (mSv) based on the available dosimetric data. As a comparison, the radiation dose from an ^{18}fluorodeoxyglucose (^{18}F-FDG) PET/CT scan is usually in the range of 5.7-11.4 mSv, corresponding to 300-600 MBq. Generally, if ^{18}F-FDG PET/CT scans are part of the pharmacological response assessment in a clinical study, each patient would undergo at least 3 (or even more) ^{18}F-FDG PET/CT scans, corresponding to a similar or even higher cumulative radiation dose than the total radiation dose patients will receive in this imaging study. Of note, based on the median age of the patients at diagnosis of LBCL, it is expected that most patients who will

participate in the study will be aged over 60 years. The risk of adverse effects from the anticipated exposure to ionizing radiation is therefore considered minimal.

The tracer doses used in this study and the experience of the investigators in the execution of PET studies in humans, in addition to the potential benefit of the ^{89}Zr-atezolizumab PET/CT for diagnostic and pharmacodynamic assessments make the relative risk/benefit acceptable for the proposed study.

7.5 Description and justification of route of administration and dosage

The proposed total protein tracer injection dose of 10 mg is mainly based on imaging and pharmacokinetic considerations as described previously. (23) This dosage provides sufficient PET-signal without any high-grade adverse events or toxicity. Circulating tracer dose corresponds with a serum atezolizumab concentration 100-fold lower than reached with the recommend treatment dose.

7.6 Dosages, dosage modifications and method of administration

Based on the study in the UMCG in patients with solid cancer, the tracer formulation with ^{89}Zr-atezolizumab (37 MBq, 10 mg total protein dosage) is optimal. (23)

7.7 Preparation and labeling of Investigational Medicinal Product

Atezolizumab is provided as a solution of 60 mg/ml in 20 mM Histidine Acetate, 120 mM Sucrose, 0.04% PS20, pH 5.8. The final ^{89}Zr-MPDL3280A product is a clear light opalescent, colourless to light yellow liquid contained in a 10 mL sterile syringe for i.v. injection, containing ~2,5mg ^{89}Zr-atezolizumab (37 mBq) in 0,9% NaCl.

7.8 Drug accountability

Atezolizumab is shipped at 2-8˚C. Upon receipt at the UMCG, the product is released by the QP for administration as injection and for use in the manufacturing of the ^{89}Zr-atezolizumab drug product. If applicable, the product will be destroyed according to local regulations.

^{89}Zr-atezolizumab drug product is manufactured by the UMCG. The product is released by the QP for administration as injection. ^{89}Zr-atezolizumab is stored at 2-8˚C. If applicable, product will be destroyed according to local regulations.

8. NON-INVESTIGATIONAL PRODUCT

All information about the non-investigational product 18F-FDG PET/CT-scan can be found in het UMCG FDC SmPC (Version 5) included as attachment.

All information about the non-investigational product “Yescarta” (axicabtagene ciloleucel), in this protocol referred to as CAR T-cell therapy, can be found in the SmPC of the European Medicines Agency.

8.1 Name and description of non-investigational product(s)

UMCG-FDG is indicated for imaging in patients as part of oncologic diagnostic procedures, using fludeoxyglucose (^{18}F) in combination with positron emission tomography (PET). Fludeoxyglucose is a glucose analogue which is accumulate in all cells using glucose as primary energy source. Therefore it is accumulate in tumours with a high glucose turnover.

Yescarta is a CD19-directed genetically modified autologous T-cell immunotherapy. Patient’s own T-cells are harvested and genetically modified ex vivo to express a chimeric antigen receptor (CAR), consisting of an anti-CD19 single chain variable fragment linked to a CD28 co-stimulatory domain and CD3-zeta signalling domain (second generation CAR). The CAR T-cells are expanded and infused into the patient.

8.2 Dosages, dosage modifications and method of administration

The recommend activity for an adult weighing 70kg is 100 to 400 MBq (depending on body weight, type of camera and acquisition mode) and is administered by direct intravenous injection. The activity of fludeoxyglucose has to be measured with activimeter immediately prior to injection.

Each patient specific single infusion bag of Yescarta contains a dispersion of CAR T-cells in approximately 68 mL for a target dose of 2 x10^6 CAR T-cells/kg body weight (range 1x10^6-2 x10^6), with a maximum of 2 x10^6 CAR T-cells. No dose adjustment is required in patients ≥ 65 year. Yescarta is administered via intravenous infusion after a lymphodepleting chemotherapy regimen consisting of cyclophosphamide 500 mg/m^2 intravenous and fludarabine 30 mg/m^2 intravenous on the 5th, 4th, 3rd day before infusion of Yescarta. Yescarta infusion should begin within 30 minutes of thaw completion and the total Yescarta infusion time should not exceed 30 minutes.

8.3 Preparation and labelling of Non Investigational Medicinal Product

UMCG-FDG is manufactured at the department of Nuclear Medicine and Molecular Imaging of the UMCG. A part of the quality control is done by the department of Hospital Pharmacy of the UMCG. UMCG-FDG is provided in a 25 ml glass vial containing 25 mL of the solution which corresponds to 600 MBq per mL at calibration time. The product should be stored a temperature below 25 °C for a maximum of 10 hours after production time.

Yescarta is provided from Kite Pharma EU B.V. in an ethylene-vinyl acetate cryostorage bag with sealed addition tube and two available spike ports, containing approximately 68 mL of cell dispersion. Yescarta is stable for 1 year when stored frozen in the vapour phase of liquid nitrogen (≤-150 °C). Thawed product should not be refrozen.

9. METHODS

9.1 Study parameters/endpoints

9.2 Main study parameters/endpoints

- to study the expression of PD-L1 in normal tissue and lymphoma lesions before CD19-directed CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate pretreatment ^{89}Zr-atezolizumab uptake to response to CD19-directed CAR T-cell therapy and thereby identify clinically relevant PD-L1 expression. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.
- to study whether the amount of ^{89}Zr-atezolizumab uptake, measured by the intensity of ^{89}Zr-atezolizumab PET/CT imaging (SUV), can be used to differentiate between lymphoma activity and treatment-related inflammatory signal (histiocytic/sarcoid-like reaction) in patients with an end-of-treatment ^{18}F-FDG-positive PET/CT signal. Confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of the positive FDG-PET lesion or in case of non-accessibility of the lesion by an experienced clinician via a wait-and-see strategy with repeated ^{18}F-FDG PET/CT scans.

9.3 Secondary study parameters/endpoints

- to correlate the pretreatment ^{89}Zr-atezolizumab distribution, as measured by the uptake of ^{89}Zr-atezolizumab on PET/CT imaging in lymphoma, to CAR T-cell peak expansion and persistence as measured in peripheral blood.
- To correlate the pretreatment ^{89}Zr-atezolizumab uptake to CAR T-cell related grade 1-5 adverse events (cytokine release syndrome (CRS) and immune effector cell

associated neurotoxicity syndrome (ICANS)). Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring SUV on the ^{89}Zr-atezolizumab PET/CT scan. CAR-T cell therapy related toxicities such as CRS and ICANS will be assessed using 2018 ASBMT Consensus Grading (24).

- to correlate tumor ^{89}Zr-atezolizumab uptake with tumor and tumor infiltrating cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.
- To compare the ^{89}Zr-atezolizumab distribution in irradiated versus non-irradiated lymphoma lesions in patients who require radiotherapy as a bridging strategy prior to CAR T-cell infusion. If possible, these results will be compared to tumor and tumor infiltrating cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy of an irradiated lymphoma lesion.
- To determine the incidence of a treatment-related inflammatory signal on ^{18}F-FDG PET/CT scan (histiocytic/sarcoid-like reaction) after CAR T-cell therapy. Confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of the positive ^{18}F-FDG-PET/CT lesion or in case of non-accessibility of the lesion by an experienced clinician via a wait-and-see strategy with repeated ^{18}F-FDG-PET/CT scans.

9.4 Randomization, blinding and treatment allocation

This is a single arm, non-blinded study. Patients will therefore not be randomized. Treatment with CD19-directed CAR T-cell therapy is standard of care.

9.5 Study procedures

Flow charts of scheduled study assessments are provided in 15.3.

9.6 Screening

Patients with R/R LBCL after 2 or more lines of treatment who qualify for CD19-directed CAR T-cell therapy will be asked to participate in this imaging trial. Written informed consent for participation in the imaging trial, must be obtained before performing any study specific screening tests or evaluations. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrollment. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

9.7 Assessment of the medical history and demographics

Medical history includes clinically significant diseases, surgery/operations, cancer history (including prior cancer therapies and procedures), reproductive status, and all medication (e.g. prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to the screening visit. A history of pleural or pericardial effusion or of ascites requiring intervention should be entered in the medical history. Demographic data will include age, sex, and self-reported race/ethnicity. Results of prior analysis of tumor characteristics will be recorded in the electronic case record form. If applicable, immunohistochemistry on archival tissue will be repeated as formulated in the CD19-

directed CAR T-cell treatment protocol, and results need to be available before ^{89}Zr-atezolizumab injection.

9.8 Physical examination and vital signs

A complete physical examination should include evaluating the head, eyes, ears, nose, and throat and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. Any abnormality identified at baseline should be recorded. The performance score, height and weight should be measured and recorded. Vital signs will include measurements of pulse rate, blood oxygen saturation, respiratory rate, systolic and diastolic blood pressures while the patient is in a seated position, and temperature.

9.9 Laboratory assessments

Samples for hematology, serum chemistries, coagulation, urinalysis, and the pregnancy test will be analyzed at the study site's local laboratory.

Local laboratory assessments will include the following:

- Hematology (CBC, including RBC count, hemoglobin, hematocrit, WBC with differential [neutrophils, eosinophils, lymphocytes, monocytes, basophils, and other cells], and platelet count)
- Serum chemistries (glucose, urea, creatinine, sodium, potassium, magnesium, chloride, bicarbonate, calcium, phosphorus, total bilirubin, ALT, AST, alkaline phosphatase, LDH, CRP, total protein, CRP, and albumin)
- Coagulation (aPTT, PT, fibrinogen, D-dimeer)

- Urinalysis: Urine assessment will include the following: leukocytes, nitrite, haem, pH, protein (including total amount of protein), glucose, ketones, bilirubin, urobilinogen, erythrocytes and bacteria.

9.10 Tumor evaluation

Screening assessments must include diagnostic ^{18}F-FDG-PET and contrast-enhanced CT scans of the neck, chest, abdomen, and pelvis. A ^{18}F-FDG-PET/CT scan, which has been performed >14 days but ≤ 42 days before ^{89}Zr-atezolizumab injection, can be used for assessment of the eligibility criteria, if no systemic or local treatment has been applied in between. The ^{18}F-FDG-PET/CT scan used for baseline assessment of Lugano criteria measurements must be performed ≤14 days before ^{89}Zr-atezolizumab injection, otherwise, a new scan will be performed with the ^{89}Zr-atezolizumab PET/CT scan.

9.11 Cardiac and pulmonary function tests

- Electrocardiogram (ECG): Twelve-lead ECG is required at screening and as clinically indicated. ECGs should be obtained on the same machine whenever possible. Lead placement should be as consistent as possible. ECG recordings should be performed after the patient has been resting in a supine position for at least 10 minutes. For safety monitoring purposes, the investigator must review, sign, and date all ECG tracings. Paper copies of ECG tracings will be kept as part of the patient's permanent study file at the site. Any morphologic waveform changes or other ECG abnormalities must be documented.
- Echocardiograms: Echocardiograms or MUGA scan is considered standard of

care and is required as vital CAR T-cell therapy eligibility criterium.

9.12 ^{89}Zr-atezolizumab injection

^{89}Zr-atezolizumab injection will be administered as described in section 6.1 (see chapter "Investigational treatment"). Sequentially, a defined dose (10 mg) of unlabeled atezolizumab will be followed by the radioactive dose of ^{89}Zr-atezolizumab (37 MBq [~2,5 mg]). After ^{89}Zr-atezolizumab injection, patients will be observed for 30 minutes for safety reasons. Patient's vital signs (pulse rate, respiratory rate, blood pressure, and temperature) will be evaluated. Patients will be discharged when clinically stable after the observation period (30 minutes) or otherwise on investigators discretion.

All patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms. As clinically indicated, on the days of PET scanning or otherwise, limited, symptom-directed physical examinations will be performed. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events.

9.13 ^{89}Zr-atezolizumab PET/CT procedure

All scans will be obtained in total body mode (trajectory mid-thigh-skull vertex), using low-dose (LD) CT for attenuation correction and localization purposes. For all PET scans, acquisition will comprise approximately 14 bed positions. For ^{89}Zr-atezolizumab PET/CT imaging, the harmonization procedures, comparable to the EARL PET/CT accreditation and European Association of Nuclear Medicine guidelines, as described by Makris et al. (26) will be applied. PET/CT imaging of the first 3 patients will be performed on day 4

and 7 to determine the optimal time point. Afterwards PET/CT imaging will be performed on the determined (4 or 7 days) time point.

9.14 Venous blood sampling

CAR T-cell expansion in peripheral blood will be measured with expected peak levels at days 5-7, and persistence at day 7, 14, 28 with an anti-CAR T-cell monoclonal antibody. T-cell subsets (CD4 / CD8 ratio, different T-cell phenotypes) will be determined on day 5. Cytokines (IL-2, IL-6, IL-10, IFNγ) will be measured in serum at days -28, 0, 5, 7, 14, and 28 to determine the pro-inflammatory and immune-modulating cytokines in response to CAR T-cell therapy. Samples will be collected during venipuncture as part of the standard procedure, thereby minimizing patient burden.

9.15 Biopsy

All patients participating in the imaging trial will undergo at least one mandatory tumor biopsy. Biopsies will be taken, preferably directly but within 7 days after the ^{89}Zr-atezolizumab-PET scan. In case there is a suspicion of a relapse or refractory disease after CAR T-cell therapy based on ^{18}F-FDG PET/CT-scan a second tumor biopsy will be obtained within 7 days of the second ^{89}Zr-atezolizumab. Moreover, in case of bridging with radiotherapy and depending on the accessibility of the tumor lesion, patients will be asked if they are willing to undergo an extra biopsy. This biopsy will be used to compare ^{89}Zr-atezolizumab uptake in a irradiated lesion on PET/CT imaging to PD-L1 expression measured via immunohistochemistry. This extra biopsy will not be obligatory to participate in the study.

Determination of the biopsy site will be based on tumor localization on previous

conventional imaging and will be performed according to current safety criteria. The location of the biopsy site should be determined before ^{89}Zr-atezolizumab PET/CT examination to minimize the bias. The biopsy will be performed according to standard clinical care. Fine-needle aspiration, brushing, cell pellets from pleural effusion, and lavage samples are not acceptable. Acceptable samples include core needle biopsies for deep tumor tissue or excisional, incisional, punch, or forceps biopsies for cutaneous, subcutaneous, or mucosal lesions. For core needle biopsy specimens, at least 2-3 cores should be obtained for molecular evaluation. Biopsies will be taken by the intervention-radiologist guided by CT or ultrasound.

9.16 End of study

According standard procedure of care patients will be regularly followed after CAR T-cell therapy (day +28, 3/6/12 mth.). Study follow-up will take place during these visits. Patients will be eligible for a second ^{89}Zr-atezolizumab PET/CT scan within 1 year after CAR T-cell administration.

Adverse events related to the ^{89}Zr-atezolizumab administration will be followed 30±2 days after last ^{89}Zr-atezolizumab injection, or until the last (serious) adverse event related to the ^{89}Zr-atezolizumab administration or other study procedure (venipuncture, biopsy) has resolved.

9.17 Molecular analysis

On-site, UMCG laboratories will perform the histology, CD8, PD-L1, and additional biomarker assessment on tumor tissues, and assessments of autoradiography on tumor

tissues.

9.18 Tumor tissue analysis

Representative tumor specimens in paraffin blocks (preferred) or at least 15 unstained slides from freshly cut, serial sections, should be available at the study site. Tumor tissue should be of good quality based on total and viable tumor content. Tissue samples will be collected to evaluate the relationship between tumor ^{89}Zr-atezolizumab uptake and tumor tissue PD-L1 expression before CAR T-cell treatment. Moreover, in case of a suspicion of relapse or refractory disease tissue samples will be obtained to evaluate the risk between ^{89}Zr-atezolizumab uptake and a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction). In addition, in case of bridging with radiotherapy and depending on the accessibility of the tumor lesion, patients will be asked if they are willing to undergo an extra biopsy. This biopsy will be used to compare ^{89}Zr-atezolizumab uptake in a irradiated lesion on PET/CT imaging to PD-L1 expression measured via immunohistochemistry. This extra biopsy will not be obligatory to participate in the study.

Analyses include but are not restricted to PD-L1 expression on tumor cells and tumor infiltrating lymphocytes, as well as the evaluation of T-cell infiltration, measured by CD8 en CD4 expression, and the determination of histiocytes and macrophages. RNA from post-tracer biopsies will be isolated for gene expression analysis by TruSeq RNA Acces RNA-seq (Q2Labsolutions). Furthermore, the samples might be used to develop biomarker or diagnostic assays and establish the performance characteristics of these assays. Autoradiography on obtained tumor tissue will be performed to evaluate the relationship between marker expression and local radioactivity by tracer accumulation

within a tissue sample.

9.19 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. If the patient wants to leave the trial, it will be enough if he/she informs one of the investigators of the decision. The decision will be documented in the (electronic) patient dossier. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

9.20 Specific criteria for withdrawal

Reasons for withdrawal from the study are the following:

- Patient withdrawal of consent at any time.
- Any medical condition that the principal investigator determines may jeopardize the patient's safety if he or she continues in the study.
- Investigator or Sponsor determines it is in the best interest of the patient.
- Patient non-compliance.

9.21 Replacement of individual subjects after withdrawal

Subjects that withdraw from the study before the ^{89}Zr-atezolizumab PET/CT scan before CAR T-cell therapy will be replaced. Subjects with an indication for a second ^{89}Zr-atezolizumab PET/CT scan that that withdraw from the study before this scan will not be substituted.

9.22 Follow-up of subjects withdrawn from the study

Every effort should be made to obtain information on patients who withdraw from the study. However, patients will not be followed, besides the standard follow-up after CAR T-cell treatment, for any reason after consent has been withdrawn.

9.23 Premature termination of the study

Premature termination will be applied when serious unforeseen adverse events are detected, which could be clearly attributed to the investigational product, and which prompt a premature termination of the study.

10. SAFETY REPORTING

10.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care of all subjects being kept informed.

10.2 AEs, SAEs and SUSARs

10.2.1 *Adverse events (AEs)*

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the ^{89}Zr-atezolizumab injection. All (serious and non-serious) adverse events, whether reported by the patient or noted by authorized study personnel, will be recorded. For each AE and SAE recorded on the AE CRF, the investigator will make an assessment of seriousness, severity and causality. The AE grading (severity) scale found in the NCI CTCAE v5.0 will be used for assessing AE severity.

In the context of AEs related to CAR T-cell treatment as the standard of care parameter, see section 4.1.

10.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);

- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- Any other important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

SAE will be monitored until 30 (±2) days after the injection of the tracer.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above.

Causality is initially assessed by the investigator. For serious adverse events, causality can be one of 2 possibilities:

- No (unrelated; equals not drug related).
- Yes (remotely, possibly, probably or definitely drug related).

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events. SAEs that result in death or are life-threatening should be

reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

10.2.3 Suspected unexpected serious adverse reactions (SUSARs)

An unexpected Adverse Event is one of which nature or severity is not consistent with the applicable product information.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorized medicinal product;
    - Investigator's Brochure for an unauthorized medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;

- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States. The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life-threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

10.2.4 Adverse events of special interest

Not applicable.

10.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

10.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study, as defined in the protocol.

10.5 Data Safety Monitoring Board (DSMB)/Safety Committee

An independent DSMB has been established for reviewing all participants in this imaging trial. The DSMB will perform safety reviews for this study that includes a review of all AEs and SAEs reported for each patient for the period as defined in section 10.2.2. The DSMB will receive reports every six months on all AEs and SAEs reported for this trial. The DSMB will minimally consist of the following independent members: 1, prof. dr. Andor Glaudemans, nuclear medicine specialist 2) prof dr. E Buskens, epidemiologist and 3) dr. R. Fehrmann, internist-medical oncologist. The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

11. STATISTICAL ANALYSIS

Data of the CD19-directed CAR T-cell trial with ^{89}Zr-atezolizumab PET/CT imaging will be analyzed in a pre-planned joined analysis. The statistics will be mainly descriptive due to the small sample size. Correlations between imaging variables, response measures and pathological assessments will be assessed through data tabulations and graphical techniques. Sample size permitting, statistical measures of correlations such as Spearman's rho coefficient may also be calculated.

11.1 Primary study parameters

The first primary endpoint of this study is to study the expression of PD-L1 in normal tissue and lymphoma lesions before CD19-directed CAR T-cell therapy in LBCL patients by ^{89}Zr-atezolizumab PET/CT imaging and to correlate the pretreatment ^{89}Zr-atezolizumab uptake to response to CD19-directed CAR T-cell therapy and thereby identify clinically relevant PD-L1 expression. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.

The second primary endpoint is to study whether the amount of ^{89}Zr-atezolizumab uptake differentiates between lymphoma activity and treatment-related inflammatory signal (histiocytic/sarcoid-like reaction) in patients with an end-of-treatment ^{18}F-FDG-positive PET/CT signal. Confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of the positive FDG-PET lesion or in case of non-accessibility of the lesion by an experienced clinician via a wait-and-see strategy with repeated FDG-PET

scans. Heterogeneity of ^{89}Zr-atezolizumab uptake will be evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan.

Response assessment will be performed according to the Lugano criteria. (i.e., revised criteria of the International Working Group, rIWG) using local assessments at 4 weeks, 12 weeks, 6 and 12 months after CD19-directed CAR T-cell infusion. Criteria are summarized in 12.1.3-12.1.8.

11.1.1 Measurement of ^{89}Zr-atezolizumab *uptake*

^{89}Zr-atezolizumab tumor uptake will be assessed, evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scans 4 or 7 days after ^{89}Zr-atezolizumab injection. The optimal time point will be determined in the first 3 patients (4 or 7 days).

^{89}Zr-atezolizumab tumor uptake and organ distribution will be scored visually and quantitatively. Qualitative assessment of ^{89}Zr-atezolizumab uptake in non-malignant lymphoid tissue identified in the PET/CT scan will also be performed. Quantification of ^{89}Zr-atezolizumab distribution will be performed using AMIDE software (Stanford University, Palo Alto, CA) and Accurate software (developed by R. Boellaard). ^{89}Zr-atezolizumab uptake will be corrected for body weight and injected dose and be quantitatively assessed as standardized uptake value, which is calculated using the formula: [tissue activity concentration (MBq/g)]/[(injected dose (MBq)/body weight (g)]. The SUV of all tumor lesions and in relevant normal tissues will be calculated on all ^{89}Zr-atezolizumab PET scans.

11.1.2 Methods of assessments (Lugano Criteria (20))

Efficacy

Response assessment will be performed at time points as given in the visit schedule. Assessment of response includes a careful physical exam, evaluation of the peripheral blood, bone marrow histology if clinically indicated and either CT or MRI in cases of CT IV contrast contraindication or FDG-PET-CT. Response is assessed based on Lugano criteria. (27)

The Lugano criteria are summarized in the following.

11.1.3 General definitions

The following categories of response are defined: CR, PR, SD, relapse and progression (PD). In the case of SD, follow-up assessments must have met the SD criteria at least once after entry to that step at a minimum interval of eight weeks.

The following guidelines are to be used for establishing tumor measurements at the baseline of each treatment step and for subsequent comparison:

The six largest measurable nodes or extranodal masses must be identified as target lesions at baseline. If there are 6 or fewer measurable nodes and extranodal masses, all must be listed as Target Lesions.

If there are more than 6 involved measurable nodes or extranodal masses, the 6 largest nodes or extranodal masses should be selected as Target Lesions according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved. When there are more than 6 involved measurable nodes or extranodal masses, any lesions

that are not included within these 6 Target Lesions will be considered non- measured lesions.

Non-measured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed, measured or followed by imaging.

Measurements for all target lesions will be reported at baseline of each treatment step. Measurements for non-measured lesions are not required.

The lymph nodes or extranodal masses selected as target lesions for measurement should be measured in two perpendicular diameters, one of which is the longest perpendicular diameter. The lymph nodes should be measured in centimeters to the nearest one-tenth of a centimeter (e.g., 2.0 cm, 2.1 cm, 2.2 cm, etc.). A measurable node must have a longest diameter (LDi) >1.5 cm. Measurable extranodal disease (e.g., hepatic nodules) may be included in the six representative, measured lesions. A measurable extranodal lesion should have an LDi greater than 1.0 cm.

The two measured diameters of each target lesion should be multiplied, giving a product for each nodal site or extranodal mass. The product of each site should be added, yielding the

sum of products of the diameters (SPD). The SPD will be used in determining the definition of response for those who have less than a complete response.

Tumor assessment response with standard FDG-PET-CT-based evaluation should use the following 5 point scale: PET 5 point scale: 1, no uptake above background; 2, uptake ≤mediastinum; 3, uptake >mediastinum but ≤liver; 4, uptake moderately >liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

11.1.4 Complete Response

Complete disappearance of all detectable clinical evidence of disease, and disease-related symptoms if present prior to therapy.

^{18}F-FDG PET/CT Based Criteria

- Complete metabolic response with a 5-point scale score of 1, 2 or 3, with or without a residual mass.
- In patients with bone marrow involvement before treatment there must be no residual FDG uptake in the marrow.
- In patients with a typically FDG-avid lymphoma with no pre-treatment PET scan, or for lymphomas for which the FDG-PET scan was positive prior to therapy: a post-treatment residual mass of any size is permitted as long as it is FDG-PET-negative.

CT Based Criteria

• For variably FDG-avid lymphomas without a pretreatment FDG-PET scan, or if a pretreatment PET scan was negative: all lymph nodes and extranodal masses must have regressed on CT to normal size (≤1.5 cm in their greatest transverse diameter for nodes >1.5 cm prior to therapy). Previously involved nodes that were 1.1-1.5 cm in their long axis and >1.0 cm in their short axis prior to treatment must have decreased to ≤1 cm in their short axis after treatment.

• The spleen and/or liver, if considered enlarged prior to therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination, and nodules related to lymphoma should disappear. However, no normal size can be specified because of the difficulties in accurately evaluating splenic and hepatic size and involvement. E.g., a spleen considered normal size may contain lymphoma, whereas an enlarged spleen may not necessarily reflect the presence of lymphoma, but variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes.

• If the bone marrow was involved by lymphoma prior to treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of >20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but demonstrating a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in subject outcome.

Of note, the definitions of CR (as given in this section) and of PR (as given in Section 11.1.5) eliminate the category of complete remission/unconfirmed.

11.1.5 Partial Response

The designation of PR requires all of the following:

PET-CT Based Criteria

- Partial metabolic response with reduced uptake compared with baseline AND a 5-point scale score of 4 or 5.
- For a typically FDG-avid lymphoma with no pretreatment FDG-PET scan or one that was FDG-PET-positive prior to therapy, the post-treatment FDG-PET should be positive at any previously involved sites.
- In patients with bone marrow involvement before treatment, residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan.

CT Based Criteria

- For variably FDG-avid lymphomas/FDG-avidity unknown, without a pretreatment PET scan, or if a pretreatment FDG- PET scan was negative, CT scan criteria should be used.
- A ≥50% decrease in sum of the product of the diameters (SPD) of up to 6 of the largest target lesions. These nodes or masses should be selected according to the following: (a) they should be clearly measurable in at least 2 perpendicular dimensions; if possible, they should be from disparate regions of the body; (b) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase in the size of other nodes, liver or spleen.

• Bone marrow assessment is irrelevant for the determination of a PR if the sample was positive prior to treatment. However, if positive, the cell type should be specified, e.g., large-cell lymphoma or small cleaved cell lymphoma.

• No new sites of disease

• Patients who achieve CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered PR

• When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered PR

11.1.6 Stable Disease

PET/CT Based Criteria:

• Absence of metabolic response, with a score of 4 or 5 AND no significant change from baseline at interim or end of treatment.

• In patients with bone marrow involvement before treatment, there must be no change from pre-treatment FDG-PET scan.

• No new areas of FDG uptake

CT Based Criteria

• For variably FDG-avid lymphomas/FDG-avidity unknown: for patients without pretreatment FDG-PET scan or if the pre-treatment FDG-PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

• Less than 50% decrease from baseline in SPD of up to 6 target lesions

• No increase in organ enlargement and non-measurable lesions compatible with PD

11.1.7 Progression and relapse

PET/CT Based Criteria

Progressive metabolic disease:

- Individual target nodes and nodal masses must present increase intensity of uptake from baseline, with a 5-point score of 4 or 5, or
- Extranodal lesions with new FDG-avid foci consistent with lymphoma at interim or end of treatment assessment, or
- New FDG-avid foci consistent with lymphoma rather than another etiology (e.g. infection, inflammation). If uncertain regarding the etiology of new lesions, a biopsy or repeat imaging scan should be considered.

CT Based Criteria

- For determination of relapsed and progressive disease, lymph nodes should be considered abnormal if the long axis is >1.5 cm, regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if the short axis is >1 cm. Lymph nodes ≤1 cm x ≤1 cm will not be considered as abnormal for relapse or progressive disease.
- At least a 50% increase from the nadir in the SPD of any previously involved target lesions, or in a single involved node or extranodal mass, or the size of other lesions (e.g., splenic or hepatic nodules).
- To be considered PD, a lymph node or extranodal mass with a diameter of the long or short axis of ≤2.0 cm must have increased by at least 0.5 cm; lesions >2.0 cm must have increased by at least 1.0 cm.

- In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase from baseline. If no prior splenomegaly, must increase by at least 2.0 cm from baseline.
- New lesions: Regrowth of previously resolved lesions; or a new lymph node >1.5 cm in any axis; or a new extranodal site >1.0 cm in any axis (new extranodal disease <1.0 cm in any axis, can be considered PD if its presence is unequivocal and attributable to lymphoma).
- New or recurrent bone marrow involvement
- Clinical PD can be determined using the following criteria:
    - ECOG PS of at least 3
    - Patient unable to have follow-up radiologic assessment due to performance status decline
    - Symptomatic decline deemed related to metastatic disease or disseminated disease (not toxicity from therapy or concurrent illness)

11.1.8 Endpoint definitions

Best Overall Response (BOR)

This is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Duration of response (DOR)

Duration of response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the time of the last assessment of response before the first date that SD, or recurrent or progressive disease is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

Overall survival (OS)

This is defined as the date of enrollment to the date of death.

Progression-free survival (PFS)

PFS is defined as the time from IMP infusion until PD or death from any cause. PFS reflects tumor growth and, therefore, occurs prior to the endpoint of OS. PFS is not confounded by the administration of subsequent therapy. Progression is defined as the first date of documentation of a new lesion or enlargement of a previous lesion, or the date of the scheduled clinic visit immediately after radiologic assessment has been completed. If there is missing information, censoring of the data may be defined as the last date at which progression status was adequately assessed.

11.1.9 Treatment-related inflammatory reaction

^{18}F-FDG-PET/CT scans will be performed regularly after CAR T-cell treatment (standard procedure follow-up day +28, 3/6/12 mth). In case of a positive ^{18}F-FDG-PET signal a second ^{89}Zr-atezolizumab PET/CT scan and a biopsy will be performed. Confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of the

positive ^{18}F-FDG-PET lesion or in case of non-accessibility of the lesion by an experienced clinician via a wait-and-see strategy with repeated ^{18}F-FDG-PET/CT scans.

11.2 Secondary study parameter(s)

11.2.1 Correlative expression analysis between ^{89}Zr-atezolizumab-distribution and CAR T-cell peak expansion and persistence

Heterogeneity of ^{89}Zr-atezolizumab tumor uptake will be assessed, evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scans 4 or 7 days after ^{89}Zr-atezolizumab injection. Optimal time point will be determined in the first 3 patients, who will receive a ^{89}Zr-atezolizumab PET/CT scan on day 4 and 7. Distribution will be correlated to CAR T-cell peak expansion and persistence as measured in peripheral blood with an anti-CAR T-cell monoclonal antibody. Peak levels will be determined at day +5 (±2 d). Persistence levels will be determined at day 0, 5,28, 90, 365.

11.2.2 *Correlative expression analysis between ^{89}Zr-atezolizumab uptake and CAR T-cell therapy related grade 1-5 adverse events (cytokine release syndrome and immune effector cell associated neurotoxicity syndrome).*

Heterogeneity of ^{89}Zr-atezolizumab tumor uptake will be assessed, evaluated by measuring standardized uptake value (SUV) on the ^{89}Zr-atezolizumab PET/CT scan 4 or 7 days after ^{89}Zr-atezolizumab injection. CAR-T cell therapy related toxicities such as cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS) will be assessed using 2018 ASBMT Consensus Grading. (24) Data will be derived from the electronic medical dossier in a prospectively observational way, assessed by investigators or treating physician's initiative and graded.

Definitions of adverse events

Adverse events (AEs)

An AE is defined as any untoward medical occurrence in a patient administered with CAR T-cell administration and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal clinically significant laboratory finding), symptom, or disease temporarily associated with the CAR T-cell administration, whether or not causally related to the (CAR T-cell administration.

Treatment-emergent adverse event (TEAE)

TEAEs are undesirable events not present prior to medical treatment, or an already present event that worsens either in intensity or frequency following the introduction of the investigational therapy. TEAE will be defined as any AE with onset on or after the first dose of conditioning chemotherapy and including the MB-CART2019.1 cell infusion, the 28 post-infusion follow up period, and up to 90 days post-infusion period.

Adverse Reactions (ARs)

ARs include all untoward and unintended responses to the CAR T-cell administration or to the lymphodepletion regimen related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having a causal relationship of possibly, probably, or definitely related to the IMP or the lymphodepletion regimen qualify as ARs.

All ARs are judged by the reporting investigator and the sponsor as having a reasonable causal relationship to a medicinal product. The expression “reasonable causal relationship” means to convey in general that there is evidence or argument to suggest a causal

relationship.

Events for that no causality is given or that are classified as “not assessable” are considered as AR until enough information is provided to allow a proper assessment of the causal relationship.

Serious Adverse Events (SAEs)

An SAE is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. A serious adverse drug reaction (SAR) is a serious AR.

In addition, medical and scientific judgment should be exercised in deciding whether other conditions should also be considered serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient’s safety or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Definition of ‘life-threatening’:

An AE is life-threatening if the patient was at immediate risk of death from the event as it occurred; i.e., it does not include a reaction that if it had occurred in a more serious form, it might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-

threatening even though drug-induced hepatitis can be fatal.

Definition of 'disability/incapacitating':

An AE is incapacitating or disabling if the event results in a substantial and/or permanent disruption of the patient's ability to carry out normal life functions.

Suspected unexpected serious adverse reaction (SUSAR)

Suspected unexpected serious adverse reactions are serious adverse reactions in patients given a drug, that may or may not be dose-related, but are unexpected, as they are not consistent with current information (e.g., Investigator's Brochure of the IMP, product information for approved drugs).

Assessment of Severity

CAR-T cell therapy related toxicities such as CRS and ICANS will be assessed using 2018 ASBMT Consensus Grading (24).

Safety evaluations will be made based on NCI CTCAE Version 5. CTCAE grades help to assess the severity of the AE:

Grade 1	mild AE
Grade 2	moderate AE
Grade 3	severe AE
Grade 4	life-threatening AE
Grade 5	death related to AE

In case the CTCAE are not applicable, the maximum intensity should be assigned to one of the following categories:

Mild: For example, an AE which is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

Moderate: For example, an AE which is sufficiently discomforting to interfere with normal everyday activities.

Severe: For example, an AE which is incapacitating and prevents normal everyday activities.

Causality of Adverse Events

The causality of AEs refers to the relationship of the AE to trial treatment. When completing the eCRF, the investigator will be asked to assess the causality of the event as 'IMP-related', 'concomitant medication-related or 'other'. Causality will then be categorized by using a simple binary decision for causality according to the following criteria for all three parameters as applicable:

Not related:

An AE for which there is no reasonable possibility of a causal relationship with the IMP, with the concomitant medication or other causes specified by the investigator.

Related:

An AE for which there is a reasonable possibility of a causal relationship with the IMP concomitant medication or other causes specified by the investigator. This means that there are facts (evidence) or arguments to suggest a causal relationship.

The assessment of whether there is a reasonable possibility of a causal relationship is usually made by the investigator. The causality assessment given by the investigator should not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both, the investigator and the sponsor, should be provided with the report. The following points should be considered during causality assessment of AEs:

Timing of the event between administration of the drug and the onset of the AE Drug levels and evidence, if any, of overdose.

The expectedness assessment will be done by the sponsor (or delegate).

11.2.3 Correlation of tumor ^{89}Zr-atezolizumab uptake and tumor cell or tumor infiltrating cell PD-L1-expression as assessed by a fresh contemporaneous tumor biopsy

PD-L1 expression will be scored based on immunohistochemistry as negative (IC0 or TC0: staining on <1% of IC or TC, respectively; IHC score 0) or positive (IC1/2/3 or TC1/2/3: staining on ≥1% of IC or TC; IHC score 1/2/3 depending on the highest staining for either IC or TC). These IHC results will be compared with tumor ^{89}Zr-atezolizumab uptake.

In addition, we will evaluate T-cell infiltration as measured by CD8 and CD4 expression and will determine the polarization of macrophages and histiocytes. RNA from post-tracer tumor biopsies will be isolated for gene expression analysis by TruSeq RNA Access RNA-seq (Q2Labsolutions). Autoradiography of the biopsies will be performed using a phosphorimaging screen for 24h and will be then scanned with a Cyclone phosphorimager and will be compared with PD-L1 expression in adjacent tissue biopsy samples.

11.2.4 Comparison of ^{89}Zr-atezolizumab distribution in irradiated vs non-irradiated lymphoma lesions

In case a patient requires bridging therapy between leukapheresis and standard lympho-depleting chemotherapy and the subsequent CAR T-cell infusion, radiation therapy might be applied. Only lymphoma lesions that are highly proliferative and are at risk for vital organs or causes discomfort will be irradiated, while preserving lymphoma lesions that are not directly necessary to irradiate. In this way, the remaining lymphoma lesions will ascertain the necessary antigen exposure for CAR T-cell activation and proliferation. ^{89}Zr-atezolizumab injection will be after the final irradiation day. Heterogeneity of ^{89}Zr-atezolizumab tumor uptake in irradiated and non-irradiated lymphoma lesions will be assessed, evaluated by measuring SUV parameters on the ^{89}Zr-atezolizumab PET/CT scans 4 or 7 days after ^{89}Zr-atezolizumab injection. Depending on the accessibility of the tumor lesion, patients will be asked if they are willing to undergo an extra biopsy, these results will be compared to tumor and tumor infiltrating cell PD-L1 expression as assessed by immunohistochemistry on a fresh contemporaneous tumor biopsy of an irradiated lymphoma lesion. This extra biopsy is not mandatory for participation in the study.

11.2.5 Determination of the incidence of a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) after CAR T-cell therapy .

Standard procedure for patients with an end-of-treatment positive ^{18}F-FDG PET/CT scan consists of a biopsy if suspected lesion is accessible or a wait-and-see strategy with repeated ^{18}F-FDG PET/CT scans. Hence, confirmation of the presence of treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) will be determined by an experienced pathologist on a fresh contemporaneous biopsy of this lesion or in case of non-accessibility determined by an experienced clinician via a wait-and-see strategy with

repeated ^{18}F-FDG PET/CT scans, which in case of a treatment-related inflammatory signal will show regression of the lesion over time. Descriptive statistics will be used to determine the incidence of treatment-related inflammatory signals (histiocytic reaction/sarcoid-like reaction).

12. ETHICAL CONSIDERATIONS

12.1 Regulation statement

The study will be conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki (64, October 2013, Fortaleza, Brazil) and with Dutch law, in accordance with the WMO and according to “Besluit Stralingsbescherming (BS 2000), artikel 64 – en Nota van Toelichting (Staatsblad 2001, 397)".

12.2 Recruitment and consent

All patients referred to the UMCG for CAR T-cell therapy are possible candidates for this study. Patients will be recruited by the treating physician and be informed by the PI or CI. Before they agree to participation in this trial, all patients will be provided with written information in the form of a Patient Information Sheet. This document will be submitted for approval to the METC along with the protocol. A statement of approval should be provided before commencement of the study. Furthermore, the subjects will be informed about the CAR T-cell treatment each patient will be given the opportunity to ask questions and will be informed about the right to withdraw from the study at any time without prejudice. The formal written consent for this trial of a patient must be obtained before initiation of any study-specific procedure. Patients must be given adequate opportunity to read the information and enquire about the details of the study before consent is given. The informed consent procedure takes place according to the ICH guidelines on Good Clinical Practice. This

implies that the written informed consent form will be signed and personally dated by the patient. The informed consent statement will be signed and dated by the investigator afterward and the patient will receive a copy. The general physician of each patient will be informed about the enrolment of the patient to the study.

Subjects are free to decide whether or not to participate in this trial. Non-participation will not have any consequences concerning their treatment. If the patient agrees to participate in the trial, it will be documented in the (electronic) patient dossier.

12.3 Objection by minors or incapacitated subjects

Not applicable.

12.4 Benefits and risks assessment, group relatedness

Benefits

Molecular imaging with an antigen-targeted PET scan is an upcoming technique for obtaining non-invasive whole-body information about specific molecular targets. In this study, we want to evaluate the uptake and distribution of ^{89}Zr-atezolizumab in patients with R/R LBCL who are eligible for CD19-directed CAR T-cell therapy. We will correlate the results with the response to CAR T-cell therapy. The ^{89}Zr-atezolizumab distribution might predict which patients may have a diminished response to CAR T-cell therapy and will possibly benefit from CAR T-cell therapy in combination with PD-1/PD-L1 blockade therapy.

In addition, in case of an end-of-treatment ^{18}F-FDG-positive PET/CT signal after CAR T-cell therapy the ^{89}Zr-atezolizumab uptake might differentiate between a treatment-related inflammatory reaction (histiocytic/sarcoid-like reaction) and lymphoma activity in a non-

invasive manner. The possible benefits of this study, however, do not directly apply to the participants of this study because no decisions will be made based on ^{89}Zr-atezolizumab results in this study. Nevertheless, their participation will help to get more insight into the tumor immune response and will guide further prospective research.

Risks of ^{89}Zr-atezolizumab

Recent first-in-human study showed safe administration of ^{89}Zr-atezolizumab in patients with solid malignancies, as only one infusion-relation adverse event (pruritus, grade 1) was reported (see IMPD). (23) Pharmacokinetic analysis confirmed that the circulating tracer dose corresponded with a serum concentration of atezolizumab reached with administration of 0,1-0,3 mg/kg, which is almost 100-fold lower than reached with the recommend treatment dose.

Both atezolizumab as ^{89}Zr-labeled antibodies al already used abundantly in various studies and/or standard care. Therefore, it already is established that atezolizumab binds to its target with high affinity. Moreover, preclinical safety assessments of atezolizumab in Cynolmolgus monkeys showed no local or systemic adverse effects in a 8-week in vivo toxicity evaluation.

All patients will be informed about the possibility and symptoms of adverse events related to infusion of ^{89}Zr-atezolizumab, including an allergic reaction and pain at the site of infusion. First clinical study showed safe injection of ^{89}Zr-atezolizumab, with only one related low-grade adverse event. All patients will be observed for at least 30 minutes after ^{89}Zr-atezolizumab injection to monitor for possible acute infusion related adverse events.

Radiation burden

Since ^{89}Zr-atezolizumab is a radioactive compound, it will cause radiation burden to the patient. The projected effective dose after receiving 37 MBq of the ^{89}Zr-atezolizumab PET scan is 18.1 mSv and 1 mSv for each low dose attenuation correction CT scan. The total effective dose for patients will be 19.1 mSv. In case of the need for a second ^{89}Zr-atezolizumab PET/CT scan because of a suspicion of relapse of refractory disease after CD19-directed CAR T-cell therapy, the effective dose for those patients will be in total 38.2mSv.

Risks of biopsy and blood sampling

Besides PET/CT imaging, patients will be asked to give in total, 12 blood samples (108ml), which will be collected during venipuncture as part of the standard procedure. Thereby minimizing patient burden. In addition, patients will undergo a fresh tumor biopsy. Patients will only be eligible for the study if they have a safely accessible lesion as assessed by the treating physician and the physician performing the biopsy. Based on a literature review, the risk of tumor biopsies is considered low with a small risk on significant/major complications (0 to 1.6%) or death (0 to 0.48%). The results of this literature review are depicted in the Appendix (section 15.1).

Time investment

Whenever possible, to minimize the burden, intervention and patients' visits are preferably planned on the same day. For this study, patients will make maximal 4 extra visits to the hospital: 1) Screening visit, 2) ^{89}Zr-atezolizumab injection 3) ^{89}Zr-atezolizumab PET/CT scan 4

or 7 days after ^{89}Zr-atezolizumab injection, 4) Biopsy visit within 7 days after ^{89}Zr-atezolizumab PET/CT scan. In case of a suspicion of a relapse or refractory disease after CD19-directed CAR T-cell therapy, 2 extra visits to the hospital have to be made: 1) ^{89}Zr-atezolizumab injection 2) ^{89}Zr-atezolizumab PET/CT scan 4 or 7 days after ^{89}Zr-atezolizumab injection. Biopsy will be obtained within 7 days after ^{89}Zr-atezolizumab PET/CT scan, but is considered to be part of standard care procedure.

In conclusion, the risk associated with ^{89}Zr-atezolizumab appears acceptable and although patients do not directly benefit from this study, the results of this study will be valuable for our understanding of the tumor immune response and will guide further prospective research.

12.5 Compensation for injury

The UMCG as sponsor/investigator has a liability insurance, which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance, which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

12.6 Incentives

For each day of patient-related study procedures, the subjects will receive compensation for traveling expenses (€ 0.19/km) and a ticket for free parking.

13. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

13.1 Handling and storage of data and documents

As a result of the regulations for radiation exposure and safety, the information on the administered radioactivity will be stored (non-anonymously) in the patients' files. Images of experimental PET/CT scans that are obtained at the PET/CT center (Department of Nuclear Medicine and Molecular Imaging) will internally be stored non-anonymously. However, personal information will only be accessible for medical personnel affiliated to that department and/or (delegated) members of the research team and handled confidentially. Data analysis will be performed on anonymized scan images.

For other data management, study subjects will receive a code. The key to the code (number linked to the patient) is safeguarded by the investigator. The study code assigned to the patients will also be used in the collection of all the study results by the investigator who will perform the data-analysis. An overview of all data and data-analysis is made according to this code so that the final results cannot be traced back to the patients by another person than the investigators involved in the study (in compliance with the EU General Data Protection Regulation Data will be stored for a maximum period of 15 years after the study is finished. In case a patient give permission to store their personal information and samples for a langer period of time and to use it in future research in the field of CAR T-cell therapy, data will be stored for a maximum period of 25 years.

The handling of personal data will comply with the EU General Data Protection Regulation, in Dutch often referred to as the Algemene Verordering Gegevensbescherming (AVG).

13.2 Monitoring and Quality Assurance

On-site monitoring will take place conform the NFU (Nederlandse Federatie van Universitaire Medisch Centra)-guideline "Kwaliteitsborging van mensgebonden onderzoek 2020" by the appointed monitor. Monitoring will take place to assure the quality and validity of the research data. The monitor will perform source data verification on the research data by comparing the data entered into the CRF with the available source documentation and other available documents. Source documents are defined as the patient's hospital medical records, clinician notes, laboratory print outs, digital and hard copies of imaging, memos, electronic data etc.

The monitor will verify the following items: Patient flow (inclusion speed and dropout rate); Informed consent forms (presence, dates, signatures); Trial Master File and Investigator Files (presence of all documents), in-/exclusion criteria (using source documents); SAEs/SUSARs (number, missed, reporting procedures); study product (administration, accountability). After each control the monitor will send a written report to the sponsor (including a summary; quality assessment; summary of findings, deviations and shortcomings; possible solutions to warrant compliance with the protocol; final conclusion).

13.3 Auditing and inspections

Participation in this study implies acceptance of potential inspection by national health authorities.

13.4 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

13.5 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

13.6 End of study report

The sponsor will notify the accredited METC and the competent authority (CA) of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the CA.

13.7 Public disclosure and publication policy

The sponsor of this investigator driven study is the University Medical Centre Groningen.

This study is financially supported by PUSH call 2020 and the Cancer research fund UMCG.

All publication manuscripts will be reviewed by the investigating group before submission.

14. STRUCTURED RISK ANALYSIS

14.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Mechanism of action of the investigational products, atezolizumab and ^{89}Zr-atezolizumab relies on the binding to the PD-L1 receptor on antigen-presenting cells or tumor cells. Further information about mechanism of action can be obtained from the IMPD of^{89}Zr-atezolizumab (version 8) .

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

This study in which ^{89}Zr-atezolizumab will be applied in patients with R/R LBCL who are eligible for CD19-directed CAR T-cell therapy will be the first study with ^{89}Zr-atezolizumab imaging in this specific patient category. Previous study with ^{89}Zr-atezolizumab imaging in patients with active solid malignancies were noted to be safe (see IMPD). In addition, several studies with ^{89}Zr-labeled antibodies have demonstrated that the isotope used (^{89}Zr) is safe for human use.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

See the "*IMPD ^{89}Zr-atezolizumab, version 8".* In addition, several preclinical studies with ^{89}Zr-labeled antibodies demonstrate the feasibility and safety of PET imaging in cynomolgus monkeys.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

^{89}Zr-atezolizumab binds selectively to human programmed death-ligand 1 (PD-L1) on antigen-presenting cells or tumor cells.

f. Pharmacokinetic considerations

Pharmacokinetic analysis in a recent first-in-human study showed good correlation with the blood pool on PET with a total tracer protein dose of 10 (unlabeled atezolizumab with ~2,5mg ^{89}Zr-atezolizumab). ^{89}Zr-atezolizumab uptake was comparable to results of other ^{89}Zr-labeled antibodies. For further information see the "*IMPD ^{89}Zr-atezolizumab, version 8".*

g. Study population

For information about the study population, see section 1.4.

h. Interaction with other products

The dose of ^{89}Zr-atezolizumab is unlikely to cause any interactions.

i. Predictability of effect

In this study no pharmacological effect will be pursued with the investigational product, so no biomarkers are used to predict a clinical effect.

j. Can effects be managed?

Subjects will be carefully monitored for side effects of ^{89}Zr-atezolizumab. A protocol for intervention in case of infusion reactions is available and known by clinical care staff.

14.2 Synthesis

For a full synthesis of risk associated with the investigational product and burden for the patient please refer to section 12.4.

15. APPENDIX

15.1 Lymphoma biopsy: Safety

The mandatory biopsy will be taken within 7 days of the ^{89}Zr-atezolizumab PET/CT scan before CAR T-cell treatment start.

In UMCG, histologic and mutation analysis of tumors is currently standard practice. Therefore, biopsies are necessary to guide the best treatment. Patients are involved in this decision as part of good clinical practice and the procedure is discussed for safety reasons in the weekly multidisciplinary board or together with biopsy performing specialist.

Histological biopsy of a lymphoma lesion will be performed by an interventional-radiologist under CT or ultrasound guidance. Registration is needed on which lymphoma lesion the biopsy has been performed.

All biopsy site(s) will be chosen based on safety aspects. Review of the literature shows that in general, tumor biopsies can be performed with only minor complications and acceptable risks (Table 1). Although biopsies are generally considered to be safe, the location of the tumor is an important determinant of the risk associated with the biopsy. The risk assessment of liver biopsies shows that overall mortality of ultrasound-guided large-core needle biopsy of liver tumors is very small, i.e., < 0.5 %. Large-core needle biopsies of liver lesions are performed with an 18G biopsy needle and the use of a 17G guiding needle, which is positioned into the target lesion. In general, 2-3 biopsy samples (specimen length 22 mm) per lesion are obtained. Complications are generally limited when precautions are taken into account (e.g., adequate coagulation status). Bleeding risk, in general, is considered to be lower than 10%. This percentage is much lower when CT of the biopsied lesion is carefully imaged and evaluated whether vessels are present in the biopsy track. In contrast, biopsies

of lung metastases have a risk of causing pneumothorax, whereas biopsies of subcutaneous metastases are considered to be very safe.

Overall, tumor biopsies provide important information and pose a minor burden to the patient's well-being. Biopsies are conducted according to the local clinical protocol. All patients will be observed after biopsy according to the local protocol for a full recovery.

Author	Study design	No. of patients (pts)	No. of biopsies	Overall mortality	Mortality in patients with malignancy	Significant or major complications	Pain	Bleeding	Complications associated with malignancy
Meyers, 2008[25]	Retrospective review of records (Percutaneous liver biopsies)	3627	4275	6 pts (0.17% of pts; 0.14% of bps)	6/? (total no. of pts with malignancies NR)	32 pts[b] (0.75% of all biopsies)	22/3627 (0.51%) requiring admission	15/3627[c] (0.35%)	15/32 (47%; HCC n=5; metastases n=10) 8/15 (53%) bleeding had malignancy
Appelbaum, 2008[26]	Retrospective study (FNAB)	208	208 (408 passes)	0	0	0	10/208 (4.8%) requiring analgesics	0[d]	0 (176 Malignant tumors of which 128 metastases)
Thanos, 2005[27]	Prospective study (18-gauge automated biopsy gun)	767	(2351 passes)	0	0	0	8/767 (1.0%) had mild pain during biopsy	1[e]	NR (736/767 malignant tumors; of which 193 metastases)
Terjung, 2003[28]	Retrospective review of records	574	629	3/629 (0.48%)	0	10/629 (1.6%)	NR	72/629[f] (11.4%)	7/79 (8.9%; HCC n=7/26, metastases n=0/53)
McGill, 1990[29]	Prospective recorded data	9212	9212	10/9212 (0.11%)	7/1766 (0.4%)	22/9212 (0.24%) hemorrhage	NR	22/9212 (0.24%) significant bleeding	0.57% non-fatal hemorrhage in cancer pts (2-6 x higher risk of bleeding than without cancer)
Piccinino, 1986[30]	Retrospective multicenter study	NR	68276	6 pts	3 pts (total no. with malignancy NR)	NR	NR	42/68276 (0.06%)	5/1755 (0.28%)

NR = not reported; [a] five pts died due to massive bleeding and one due to aspiration pneumonia and congestive heart failure; [b] 15/32 (47%) had malignancy; [c] 8/15 (53%) had malignancy; [d] not checked for subclinical hemorrhage; [e] one perihepatic hematoma; [f] symptomatic and asymptomatic

Table 1 Safety of tumor biopsies

(from CPCT – 02 biopsy protocol, *Development of a platform for next-generation DNA sequencing-based personalized for cancer patients: Protocol to obtain biopsies from patients with locally advanced (incurable) or metastatic cancer*, ToetsingOnline nr.: NL35781.041.11)

15.2 Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living[a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living[b,c]
4	Life-threatening consequences or urgent intervention indicated[d]
5	Death related to adverse event[d]

NCI CTCAE= National Cancer Institute Common Terminology Criteria for Adverse Events.

Note: Based on the version of NCI CTCAE v4.0, which can be found at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Examples of self-care activities of daily living include bathing, dressing and undressing, feeding one's self, using the toilet, and taking medications, as performed by patients who are not bedridden.

[c] If an event is assessed as a "significant medical event," it must be reported as a serious adverse event, per the definition of serious adverse event in 9.2.2.

[d] Grade 5 events must be reported as serious adverse events, per the definition of serious adverse event in Section 10.2.2.

15.3 Timeline and procedures

Study flowchart

Study flowchart	**Screening**	**Apheresis**	**Radiation therapy (if applicable)**	**Infusion of tracer**	**^{89}Zr - PET scan**	**Biopsy**	**FDG-PET scan 1**	**Infusion of CAR T-cells**	**FDG-PET scan 2**	**Visit**	**Infusion of tracer ****	**^{89}Zr - PET scan****	**Biopsy****
Window of assessments	D-35 to D-30	D-28	D-27 to -10	D-19 to - 10	D-12 to -6	D-12 to -6	D-6	D0	**D28**	D30±2	D …	D …	D ….
				D X	D X + 4-7*						D X	D X+ 4-7*	DX+ 11-14
Signed Informed Consent Forms[1]	X												
Review of eligibility criteria (both CAR T-cell therapy and anti-PD-L1 imaging)	X												
Medical, surgical, and cancer histories, including demographic information[2]	X												
Concomitant medication[3]	X	X	X		X				X	X		X	
Physical examination[4]	X	X	X		X				X	X		X	
Vital signs[5]	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG performance status	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight and height	X									X			
HIV, HBV, and HCV serology[6]	X												
CRP and ferritin[7]	X	X	X					X	X				
Blood laboratory tests (including cytokines)[8]	X						X	X	X			X	
Tumor assessment (FDG-PET CT)[9]	X						X		X				
12-lead electrocardiogram[10]	X												
Echocardiogram/MUGA scan[11]	X												
Infusion of ^{89}Zr-atezolizumab				X							X		
^{89}Zr-atezolizumab -PET scan[12]					X							X	
Fresh tumor biopsy[13]						X							X
Archive tumor tissue	X												
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X

* Exact moment of ^{89}ZR-PET scan (4 or 7 days after tracer injection) will be determined in the first 3 patients.

** In case of suspicion of a relapse or pseudoprogression based on a FDG-PET scan.

[1]Written informed consent can be obtained up to 30 days prior to study entry and is required for performing any study-specific tests or procedures. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study entry may be used for screening assessments rather than repeating such tests.

[2] Cancer history includes stage, date of diagnosis, and prior anti-tumor treatment. Demographic information includes sex, age, and self-reported race/ethnicity. A history of pleural or pericardial effusion or of ascites requiring intervention should be entered in the medical history Reproductive status and smoking history should also be captured.

[3] Concomitant medications include any prescription medications or over-the-counter medications. At screening, any medications the patient has used within the 7 days prior to the screening visit should be documented. At subsequent visits, changes to current medications or medications used since the last documentation of medications will be recorded.

[4] Complete physical examination is defined in Section 9.8. A limited physical examination will be performed at screening visit and on every other visit if clinically indicated.

[5] Vital signs at screening visit include pulse rate, blood oxygen saturation, respiratory rate, systolic and diastolic blood pressures while the patient is in a seated position, and temperature. On the day of injection, the patient's vital signs (pulse rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before, directly after (± 10 minutes) and 30 (± 10) minutes after injection of the

unlabeled dose, as well as directly before and 60 minutes (± 15 minutes) after the infusion dose (pulse rate, blood pressure, and temperature only). Additional vital signs should be determined during the infusion if clinically indicated.

[6] All patients will be tested for HIV prior to the inclusion into the study and HIV-positive patients will be excluded from the clinical trial. Hepatitis B surface antigen, anti-HBc antibody, anti-HBs antibody, and anti-HCV antibody should be collected during screening. In patients who have positive serology for the anti-HBc antibody, HBV DNA should be collected prior to ^{89}Zr-atezolizumab injection.

[7] CRP and ferritin testing will be performed as part of the screening assessments and if clinically indicated.

[8] Hematology consists of CBC, including RBC count, hemoglobin, hematocrit, WBC count with automated differential (neutrophils, lymphocytes, eosinophils, monocytes, basophils, and other cells), and platelet count. A manual differential can be done if clinically indicated. Serum chemistry includes urea, creatinine, sodium, potassium, magnesium, chloride, bicarbonate, calcium, phosphorus, glucose, total bilirubin, ALT, AST, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, and C-reactive protein. Urine analysis includes specific gravity, pH, glucose, protein, ketones, and blood. Furthermore, only at screening, a serum pregnancy test will be performed and TSH, free T3, free T4 will be assessed. Serum pregnancy test (for women of childbearing potential, including women who have had a tubal ligation) must be performed and documented as negative within 14 days prior to ^{89}Zr-atezolizumab injection. Cytokines include IL-2, IL-6, IL-10, IFNγ.

[9] Tumor assessments (18F-FDG PET/CT scan of chest, abdomen and pelvis) must be performed ≤14 days before injection, otherwise, a new diagnostic CT scan needs to be performed with one of the ^{89}Zr-atezolizumab-PET scans. A CT or MRI, which has been performed >14 days but ≤

42 days before injection, can be used for assessment of the eligibility criteria if no systemic or local treatment has been applied in-between. All measurable and evaluable lesions should be assessed and documented at the screening visit.

[10] ECG recordings will be obtained during screening. Patients should be resting and in a supine position for at least 10 minutes prior to ECG collection.

[11] Required at screening. MUGA is an acceptable modality for this assessment. In such patients, echocardiograms will be performed at screening and clinically indicated. Patients who develop new pericardial effusions while on study must be followed by echocardiography.

[12] In part A every patient will undergo 4 mandatory PET scans. Depending on the findings in part A, in part B study PET scans will be performed according to the schedule as assessed in part A.

[13] All patients will undergo a mandatory tumor biopsy sample collection, if clinically feasible as determined by the study investigator, preferably after the PET scan procedures. Acceptable samples include core needle biopsies for deep tumor tissue or lymph nodes or excisional, incisional, punch, or forceps biopsies for cutaneous, subcutaneous, or mucosal lesions. For core needle biopsy specimens, at least 2-3 cores should be submitted for evaluation.

15.4 CAR T-cell inclusion criteria

Verwijsformulier CAR T-celbehandeling DLBCL/ALL buiten studieverband

Info verwijzer:	**Info patiënt:**
Naam arts: Klik of tik om tekst in te voeren. **Naam centrum:** Klik of tik om tekst in te voeren. **Ingevuld door:** Klik of tik om tekst in te voeren. **Datum:** Klik of tik om een datum in te voeren.	**Achternaam & initialen:** Klik of tik om tekst in te voeren. **Geboortedatum:** Klik of tik om een datum in te voeren.

*Let op:

- Dit formulier biedt geen garantie dat de patiënt daadwerkelijk voor CAR-T cel behandeling in aanmerking komt.
- Bij afwijkende waarden overleggen met het CAR T-cel centrum.
- Voor CAR T-cel behandeling in studieverband gelden aanvullende in- & exclusiecriteria.
- Dit formulier wordt beoordeeld door de landelijke CAR T-cel tumorboard en naar aanleiding hiervan wordt binnen 3 werkdagen contact met de verwijzer opgenomen. Als de patiënt aan de voorwaarden lijkt te voldoen én er een slot beschikbaar is voor behandeling zal de patiënt binnen een week worden opgeroepen voor screening.

- Dit formulier graag zo compleet mogelijk invullen, en samen met verwijsbrief en recente/relevante verslagen (lab/beeldvorming/pathologie/etc.) mailen naar het regionale CAR T-celcentrum en naar het landelijke email adres: cart-nl@amsterdamumc.nl.

- Recente/relevante beeldvorming ook graag zsm op CDrom opsturen naar het regionale CAR T-celcentrum

- Tevens patiënt informed consent laten tekenen voor opname van de geanonimiseerde verwijsgegevens in de “Database CAR T-celbehandeling Nederland” en informed consent meesturen.

Indicatie:

☐ **DLBCL : na 2 eerdere lijnen systemische behandeling (DLBCL, PMBL, transformed FL) (Yescarta)**

☐ **ALL: Pediatrische en jongvolwassen patiënten tot 25 jaar, refractair of met een recidief na transplantatie of met tweede/later recidief (Kymriah)**

Patiëntgegevens	
Diagnose: Lengte: Gewicht: Reisafstand ≤ 1 uur tot het CAR T-celcentrum: Alleenwonend:	Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren.kg ☐ JA / ☐ NEE ☐ JA / ☐ NEE

Diagnose: Lengte: Gewicht: Reisafstand ≤ 1 uur tot het CAR T-celcentrum: Alleenwonend:	Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren.kg ☐ JA / ☐ NEE ☐ JA / ☐ NEE

Voorgeschiedenis	
Hematologische VG	Klik of tik om tekst in te voeren.
Niet-hematologische VG	Klik of tik om tekst in te voeren.

Diagnostiek	
Beeldvorming (CT, PET/CT, Cerebrale MRI of CT)	Klik of tik om tekst in te voeren.
Pathologie (lymfeklier-, beenmergbiopt, liquor)	Klik of tik om tekst in te voeren.

Eerdere behandellijnen	Start- & stopdatum	Respons
1e lijn: Klik of tik om tekst in te voeren.	Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.	Klik of tik om tekst in te voeren.
2de lijn: Klik of tik om tekst in te voeren.	Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.	Klik of tik om tekst in te voeren.
3de lijn: Klik of tik om tekst in te voeren.	Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.	Klik of tik om tekst in te voeren.
4de lijn: Klik of tik om tekst in te voeren.	Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.	Klik of tik om tekst in te voeren.
≥5de lijn: Klik of tik om tekst in te voeren.	Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.	Klik of tik om tekst in te voeren.
Toxiciteit na eerdere behandelingen ≤ graad 1 m.u.v. klinisch niet relevante afwijkingen zoals haaruitval.	☐ JA / ☐ NEE Klik of tik om tekst in te voeren.	

Kwalificatie		
Leeftijd ≥ 18	☐ JA / ☐ NEE	Klik of tik om een datum in te voeren.
WHO-PS 0 - 2	☐ JA / ☐ NEE	Klik of tik om een datum in te voeren.
Meetbare ziekte (LNN of extranodale laesie ≥ 15 mm)	☐ JA / ☐ NEE	Klik of tik om een datum in te voeren.
Hematologische parameters: - ANC ≥ 1000/µL (1x10e9/L) - Trombocyten ≥ 75,000/µL (75x10e9/L) - ALC ≥ 100/µL (0.1x10e9/L)	 ☐ JA / ☐ NEE ☐ JA / ☐ NEE ☐ JA / ☐ NEE	 Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.

Adequate nierfunctie: - Kreatinine ≤ 1.5xULN - Kreatinineklaring > 40 ml/min (Cockcroft)	 ☐ JA / ☐ NEE ☐ JA / ☐ NEE	 Klik of tik om een datum in te voeren. Klik of tik om een datum in te voeren.
Adequate leverfunctie: - ALAT/ASAT ≤ 3xULN - Totaal Bilirubine < 2 mg/dl (34 µmol/L) tenzij syndroom van Gilbert	 ☐ JA / ☐ NEE ☐ JA / ☐ NEE	 Klik of tik om een datum in te voeren.
CRP < 100 mg/L Alternatieve verklaring indien verhoogd?	☐ JA / ☐ NEE	Klik of tik om een datum in te voeren. Klik of tik om tekst in te voeren.
Adequate hartfunctie: - Ejectiefractie ≥ 40% en klinisch goede LV functie - Significant pericardvocht - Klinisch significante ECG afwijkingen	 ☐ JA / ☐ NEE ☐ JA / ☐ NEE ☐ JA / ☐ NEE	 Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren.
Adequate longfunctie: - Significant pleuravocht - Baseline saturatie ≥ 92% bij kamerlucht	 ☐ JA / ☐ NEE ☐ JA / ☐ NEE	 Klik of tik om tekst in te voeren. Klik of tik om tekst in te voeren.
HIV/HepB/HepC infectie	☐ JA / ☒ NEE	Klik of tik om tekst in te voeren.
Overige actieve infectie	☐ JA / ☐ NEE	Klik of tik om tekst in te voeren.
Verdenking of bewezen CZS lokalisatie lymfoom	☐ JA / ☐ NEE	Klik of tik om tekst in te voeren.
Neurologische afwijkingen	☐ JA / ☐ NEE	Klik of tik om tekst in te voeren.
Auto-immuunziekte	☐ JA / ☐ NEE	Klik of tik om tekst in te voeren.
Andere significante aandoeningen	☐ JA / ☐ NEE	Klik of tik om tekst in te voeren.
Opmerkingen: Klik of tik om tekst in te voeren.		

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